

Brooklyn College
Department of Chemistry

Instrumental Analysis (Chem 42/790)

UV/VIS Spectroscopy

These notes provide an introduction to the use of the Hitachi Model U-2001 UV/VIS Spectrophotometer. They are oriented towards the experiment to be done in the instrumental analysis course, but should provide a useful introduction for anyone using the instrument for the first time.

Procedure

1. Remove any cells from the sample and reference beam compartments of the U-2001 UV/VIS Spectrophotometer.
2. Turn the instrument on, using the ON/OFF switch at the front of the instrument. Check if the cartridge is installed inside the printer. Turn on the printer.

At power-up the INITIALIZATION frame appears on the display and the instrument performs a self-diagnosis and automatic wavelength calibration.

During self-diagnosis the U-2001 UV/Vis Spectrophotometer checks the following items:

- ROM
- RAM
- Wavelength drive mechanism
- WI lamp (tungsten iodide)
- D2 lamp (deuterium)

As each item is checked, the message "OK" will display if the item's function is normal or the message "ERROR" will display if the item's function is abnormal.

When all items are diagnosed normal, the INITIALIZATION screen automatically changes to the MAIN MENU screen.

If the message "ERROR" displays for any item discussed above, the system stops. If this occurs, ask your instructor for help.

3. MAIN MENU

After initialization, the Main Menu screen appears on the display. You can access a desired option from it in one of two ways:

- a. press the numeric key corresponding to the desired measurement item, then press [ENTER]; or
- b. use the arrow keys to move the background highlight to the desired measurement item, then press [ENTER].

4. WAVELENGTH SCANNING

This section describes how to set up the instrument to collect a spectrum using Wavelength Scan.

a. Select wavelength scan from the Main Menu;

Use the arrow keys to move the background highlight to the wavelength option (WL Scan) and press [ENTER]; or press [2] on the keyboard, followed by pressing [ENTER].

b. Enter the Data Acquisition Mode

Select the DATA MODE function and press [ENTER]. The display screen is now split in half and a highlight appears over DATA MODE.

Select the ABS mode by pressing [1] on the keyboard and then press [ENTER].

(To return to the DATA MODE line or to any other line on the WL SCAN, press [RETURN] and then use the arrow keys to move the highlight to the desired line.)

c. Enter the Wavelength Scan Test Setup Variables

Select the TEST SETUP. Press [ENTER].

Use the arrow keys to highlight the appropriate parameter from the list on the right side of the screen.

Select the range/option by entering appropriate numbers from the keyboard. Then press [ENTER].

(After you enter each value, the background highlight immediately steps to the next field.)

Use the following experimental parameters:

Start WL (nm):	520
Stop WL (nm):	300
UP Scale:	2.500
LO Scale:	- 0.300
Scan Speed (nm/min):	400
Init Delay:	0
Num Cycles:	1
Cycle Time:	0
Display Format:	Sequential

d. Enter a Test Name

Highlight Test Name in the menu option list, press [ENTER].

Type a name for your spectra (*e.g.*, NiSO4-1), using the letter and number keys on the keyboard.

(To convert each key to its alphabetic equivalent, hold down [SHIFT] as you type in your letters. To delete the last character, press [CLEAR].)

e. Select the Instr Setup and its variables

Use the following parameters:

Baseline:	User
Response:	Medium
Lamp Change WL (nm):	340
WI Lamp:	ON
D2 Lamp:	ON
Graph Print:	OFF
Text Print:	OFF
List Interval (nm):	10.0
Print Format (data/line)	1

f. User Baseline Variables

User baseline uses the same parameters as selected in the TEST SETUP and INSTR SETUP.

g. Collecting a User baseline.

Place sample cuvettes containing a reference (water) and blank solution (water) in both the reference (back) and sample (front) positions.

Highlight User Baseline in the WL Scan submenu list. Press [ENTER].

Press [START] to begin collection of the background spectrum.

(These data are stored in the baseline memory location and are used in all subsequent experiments until you enter a new User baseline to replace the current one.)

h. Collecting a Spectrum in Wavelength Scan

Fill the sample cuvette with your sample solution, place the sample cuvette in the front cuvette holder and the reference cuvette (water) in the back holder.

Press [FORWARD] on the keyboard. The measurement screen will appear, with axes dimensioned for the impending scan and the selected options listed at the bottom of the screen.

(The parameters are checked automatically at this time. An error message appears at the bottom of the screen to inform you if a parameter must be modified.)

Press [START] on the keyboard.

(Options 1 - 6 (Post-processing commands) listed at the bottom of this screen are functional only when a spectrum is present. They are selected by pressing the appropriate numerical key on the keyboard and then pressing [ENTER].)

REMEMBER: The STOP key is always active. If you have initiated a data acquisition you want to terminate, simply press [STOP].

i. Saving a Spectrum

The spectrum you acquire is stored in a memory location labeled SCAN0. If you intend to process the data, you **MUST** relocate the file to another memory location before you

acquire the next spectrum. Remember: the new spectrum is stored automatically in the SCAN0 location, overwriting the previous spectrum.

Select option (6) FILES from Post-Run Processing options, then press [ENTER].

A list of selectable FILE options will appear at the bottom of the screen.

To save current spectra, select (2) SAVE SCAN0. This option transfers the data in SCAN0 to another file location. You can save up to 12 spectra, specifying any file number from SCAN1 through SCAN12.

Write down all files numbers and appropriate scan names (you are going to run 9 spectra).
(The display scaling in the SCAN0 area will be preserved intact.)

j. Displaying a Spectrum

You can display specified file data once you enter a file number from 1 to 12. The specified file's data will appear on the screen display in place of the currently displayed spectrum. Select FILE from the post-processing options, then select (3) DISPLAY option, and (1) SCAN option. Select the file number (e.g., SCAN2).

k. Overlaying Spectra

You can display more than one spectrum at a time. After displaying a specified spectrum, select OVERLAY from FILE options. Now you can specify any file from SCAN1 to SCAN12 as the file you wish to lay over the current display. The graph axes of the file you call up will be applied to the overlaid presentation, therefore, the resulting display may be misleading if both spectra do not have the same spectral range or axes.

l. Listing Existing Files

You can print out a directory list of currently stored data. Select LIST (1) from the FILE options.

m. Rescaling a Spectrum

You can rescale the axes of a selected spectrum. Select (3) RESCALE from the post-processing options, then press [ENTER]. The system will display a series of options at the bottom of the screen.

n. Tracing a Spectrum Using the Crosshair(+) Cursor

Use the crosshair cursor to trace a spectrum. Select (1) TRACE from the post-processing options, press [ENTER]. A crosshair cursor will appear. Use the arrow keys to move the crosshair cursor to the left or right on the spectrum. The cursor moves faster if you hold down. The data that appear in the cursor windows indicate the X-axis and Y-axis values for the current cursor location.

(Use this option to determine absorbance at I_{max}^1 for nickel and I_{max}^2 for dichromate).

o. Printing a Spectrum

After selecting and displaying appropriate spectrum (spectra) you can print it.

Select (4) PRINT from the Post-Run Processing options, then select (1) Printer.

(Use one page to print two sets of spectra).

Analysis of a potassium dichromate / nickel sulfate solution.

In this experiment you will obtain data for nickel sulfate and potassium dichromate solutions and will analyze an unknown solution containing a mixture of the two components. Record all spectra from 520 nm to 300 nm.

Baseline check - as describe in section **g**.

Rinse the cuvette with small portions of the nickel sulfate solution provided. Fill the cuvette and obtain a spectrum from 520 nm to 300 nm. If necessary, rescale the spectrum (section **m**). Save this spectrum as SCAN1 (section **i**). Use the crosshair cursor to determine the absorbance and wavelength (λ_{\max}^1) of the strong peak (section **n**).

Thoroughly rinse the cuvette with deionized water. Then rinse and fill it with the dichromate solution. Obtain a dichromate spectrum. Save it as SCAN2. Determine its absorbance at λ_{\max}^2 and also at the λ_{\max}^1 determined earlier for nickel sulfate. Now display the nickel sulfate spectrum SCAN1 (section **j**) and determine the absorbance at the λ_{\max}^2 for dichromate.

Obtain the spectrum of the unknown solution - no dilutions are required for the unknown. Save this spectrum as SCAN3. Measure and record the absorbance values at both wavelengths, λ_{\max}^1 and λ_{\max}^2 , for this solution.

Overlay three spectra, for a dichromate, nickel and unknown solution (section **k**). Print these spectra.

Using the absorbance at λ_{\max} for each solution, prepare three dilutions of each solution (dichromate and nickel standard solutions). In general, dilutions should be such that the absorbance of the strong peak does not go below 0.1 ABS. Obtain spectra for each of these