Ultra-violet and visible spectroscopy

Samples must always be clear solutions as particles will scatter the incident light and therefore give erroneous results. Filter if necessary, but remember that if the molar absorptivity (formerly known as molar extinction coefficient) , is to be quoted, the exact mass of solid *dissolved* is required.

A reference solution is always required, although it may not be used after the initial background scan has been recorded (as in the single beam instruments). Double beam instruments require reference cells during the scanning time.

Cell selection and handling:
There are three types of cells used routinely in this department. All are manufactured with precision dimensions giving a solution path length of exactly 1 cm. Two sides are highly polished and should not be touched by hands. The light beam should pass through these two windows.

Glass is the common choice but the cells are not cheap at about $35 each. They can be used down to wavelengths of 350 nm.

Polystyrene cells are significantly cheaper (about 10 cents each) but can only be used for aqueous solutions. Their range is good to about 300 nm.

Quartz cells are manufactured with different grades of silica. Typically, the range extends down to the lower limit of the instruments available (200 nm). Their cost is much higher at about $85 each.

The large price differential between the cells will require that you only use the quartz cells when it is necessary to observe in the uv region (below 350 nm). If you are uncertain about the type of cell, run a quick check at 290 nm. Glass will absorb strongly at this wavelength.

Code	Material	Guaranteed Transmittance
G	Optical glass	80% at 365 nm
QG, H	Standard silica, Herasil	80% at 250 nm
I	IR grade silica	80% at 220 nm
Q, S	Far UV silica, Suprasil	80% at 200 nm
P	Polystyrene	80% at 400 nm

How much sample to use:
The quantity of sample to use will depend largely on the results desired and expected. It is strongly advisable to make a few calculations in advance. The absorbance is given by the Beer-Lambert law of $A = \varepsilon bc$, where ε is the molar absorptivity (molar extinction coefficient), b is the path length in cm and c is the concentration in $mol.L^{-1}$. The absorbance range observable is in the range 0 to 2 A

for reasonable accuracy, but some instruments will give “data” to 10 A. The path length is always 1 cm (special path-length cells are rarely used now that data can be manipulated by computer). The value of , can vary enormously. Highly coloured species with charge transfer bands can have values of 10^5 while d-d transitions may be a more modest 10^1. In uv spectroscopy, there is no colour guide. In the absence of literature values, several test runs may be necessary before an accurate spectrum may be recorded. Remember also that there may be more than one band present, and the second band may differ in , so much from that of the first, that the band may not be observable in the first scan.

Solvent selection:
The solvent must obviously be able to dissolve a sufficient amount of the compound to be studied. Since the sample must contain no particles, it is important to make sure that everything does dissolve. Sonication with a small ultrasound bath can sometimes help, as can grinding the crystals before putting them into the solvent.

The solvent (spectroscopic grade) must be optically clear in the region of study. This means that absorbance due to the solvent is less than 1A.

Sample Preparation for determination of ,:
Although this may be tedious, care and attention to detail will produce good results. It will be necessary to accurately weigh a sample of the compound. Since you will often be measuring materials in the milligram range, use a balance that is accurate in that range - the present electronic top-loading balances are not.

Transfer the sample to a volumetric flask. You should not use a flask larger than 25 mL to avoid wasting solvent. Many spectroscopic grade solvents are very expensive, and it is senseless to use 100 mL of solvent when only 3.5 mL are required to fill the cell. Dissolve the sample in the solvent of choice, and make the solution up to the mark. Run the spectrum.

It is possible (likely?) that the solution will be too concentrated. If that is the case, estimate a dilution factor necessary to bring the band into the absorbance range desired. Dilute a portion of your sample solution, again using a flask no larger than 25 mL. Rerun the spectrum. At this point, you may wish to "fine tune" your dilution to produce a reasonable size band, although this has become less of a necessity as computer graphics can redraw weak peaks to a suitable scale.

This method will allow you to calculate , from a single point. To get a more accurate determination of ,, several spectra at different concentrations will need to be run, and a plot of A *vs* c will give , as the slope.

Filling the cells:
As mentioned above it is important that each solution be free of particulate matter.

The light beam must pass through exactly 1 cm of solution so be sure there are no air bubbles inside the cell, or that no solution has run down the outside of the cell. Wipe the cell before putting it into the spectrometer.

It is not necessary to fill the cells to the top. The light beam passes through the lower 2.5 cm of the cell and therefore filling the cell to within 1 cm of the top is adequate.

Data reporting:

A routine uv/vis spectrum is normally reported by indicating the position of each peak by its wavelength at maximum height (8_{max}) and the molar absorptivity , in parentheses. The solvent should also be mentioned.