# 2.5: Applications

## Is UV/VIS spectroscopy useful as a qualitative tool?

The answer depends in part of what type of system you are examining. Ultraviolet-absorbing organic molecules usually involve n-$\pi^*$ and $\pi$-$\pi^*$ transitions. Since UV/VIS absorption spectra are usually recorded at room temperature in solution, collisional broadening leads to a blurring together of all of the individual lines that would correspond to excitations to the different vibrational and rotational states of a given electronic state. As such, UV/VIS absorption spectra of organic compounds are not all that different and distinct from each other. Whereas we can reliably assign unique structures to molecules using the spectra that are obtained in NMR spectroscopy, the spectra in UV/VIS spectroscopy do not possess enough detail for such an analysis. Therefore, UV/VIS spectroscopy is not that useful a tool for qualitative analysis of organic compounds.

However, a particular organic compound does have a specific UV/VIS absorption spectrum as seen in the various examples provided above. If the spectrum of an unknown compound exactly matches that of a known compound (provided both have been recorded under the same conditions – in the same solvent, at the same pH, etc.), it is strong evidence that the compounds are the same. However, because of the featureless nature of many UV/VIS spectra, such a conclusion must be reached with caution. The use of a UV-diode array detector as a liquid chromatographic detection method is quite common. In this case, the match of identical spectra with the match in retention time between a known and unknown can be used to confirm an assignment of the identity of the compound.

Many transition metal ions have distinct UV/VIS absorption spectra that involve d-d electron transitions. The position of peaks in the spectra can vary significantly depending on the ligand, and there is something known as the spectrochemical series that can be used to predict certain changes that will be observed as the ligands are varied. UV/VIS spectroscopy can oftentimes be used to reliably confirm the presence of a particular metal species in solution. Some metal species also have absorption processes that result from a charge transfer process. In a charge transfer process, the electron goes from the HOMO on one species to the LUMO on the other. In metal complexes, this can involve a ligand-to-metal transition or metal-to-ligand transition. The ligand-to-metal transition is more common and the process effectively represents an internal electron transfer or redox reaction. Certain pairs of organic compounds also associate in solution and exhibit charge-transfer transitions. An important aspect of charge transfer transitions is that they tend to have very high molar absorptivities.

## Is UV/VIS spectroscopy useful as a quantitative tool?

We have the ability to sensitively measure UV/VIS radiation using devices like photomultiplier tubes or array detectors. Provided the molar absorptivity is high enough, UV/VIS absorption is a highly sensitive detection method and is a useful tool for quantitative analysis. Since many substances absorb broad regions of the spectrum, it is prone to possible interferences from other components of the matrix. Therefore, UV/VIS absorption spectroscopy is not that selective a method. The compound under study must often be separated from other constituents of the sample prior to analysis. The coupling of liquid chromatography with ultraviolet detection is one of the more common analysis techniques. In addition to the high sensitivity, the use of UV/VIS absorption for quantitative analysis has wide applicability, is accurate, and is easy to use.

## If you were using UV spectroscopy for quantitative analysis, what criteria would you use in selecting a wavelength for the analysis?

The best wavelength to use is the one with the highest molar absorptivity ($\lambda_{max}$), provided there are no interfering substances that absorb at the same wavelength. If so, then there either needs to be a separation step or it may be possible to use a different wavelength that has a high enough molar absorptivity but no interference from components of the matrix.

## What variables influence the recording of UV/VIS absorption spectra and need to be accounted for when performing qualitative and quantitative analyses?

We have discussed several of these already in the unit. The solvent can have an effect and cause bathochromic and hypsochromic shifts. Species in the matrix that may form dipole-dipole interactions including hydrogen bonds can alter the spectra as well. Metal ions that can form donor-acceptor complexes can have the same effect. Temperature can have an effect on the spectrum. The electrolyte concentration can have an effect as well. As discussed above, the possibility that the sample has interferences that absorb the same radiation must always be considered.

Finally, pH can have a pronounced effect because the spectrum of protonated and deprotonated acids and bases can be markedly different from each other. In fact, UV/VIS spectroscopy is commonly used to measure the pKa of new substances. The reaction below shows a generalized dissociation of a weak acid (HA) into its conjugate base.

$$\mathrm{HA} + \mathrm{H_2O} = \mathrm{A^-} + \mathrm{H_3O^+}$$

## Provided the UV/VIS absorption spectra of HA and $A^-$ differ from each other, describe a method that you could use to measure the pKa of the acid.

This rate of dissociation represented above is slow on the time scale of absorption (the absorption of a photon occurs over the time scale of $10^{-14}$ to $10^{-15}$ seconds). Because the reaction rate is slow, this means that during the absorption of a photon, the species is only in one of the two forms (either HA or $A^-$). Therefore, if the solution is at a pH where both species are present, peaks for both will show up in the spectrum. To measure the pKa, standards must first be analyzed in a strongly acidic solution, such that all of the species is in the HA form, and a standard curve for HA can be generated. Then standards must be analyzed in a strongly basic solution, such that all of the species is in the $A^-$ form, to generate a standard curve for $A^-$. At intermediate pH values close to the pKa, both HA and $A^-$ will be present and the two standard curves can be used to calculate the concentration of each species. The pH and two concentrations can then be substituted into the Henderson-Hasselbalch equation to determine the pKa value.

$$\mathrm{pH} = \mathrm{pKa} + \log\left(\frac{[\mathrm{A^-}]}{[\mathrm{HA}]}\right)$$