Spectroscopy
Lecture 4

Applications of UV–VIS Spectroscopy

Measurement of the concentration of an analyte in solution

- According to the Beer-Lambert law, the absorbance of a material in solution is directly dependent on the concentration of that material.
- If the absorption coefficient is known for the absorbing species, the concentration can be calculated after experimental measurement of the absorbance of the solution.

Absorbance measurements on very small sample volumes

- Biomolecules are often expensive, difficult to isolate, and not always available in these large sample sizes.
- Recent advances in the development of spectrophotometers now make available instruments for rapidly quantifying and analyzing micro-volume samples.
- For example, NanoDrop UV-VIS spectrophotometers can measure samples as small as 0.5µl in about 5 seconds.
- Measurements can be made on any biomolecules that absorb in the wavelength range. This includes proteins, DNA, RNA, nucleotides, cofactors, fluorescent substances, and many others.

Identification of unknown biomolecules by spectrophotometry

- The UV-VIS spectrum of a biomolecule reveals much about its molecular structure.
- Therefore, a spectral analysis is one of the first experimental measurements made on an unknown biomolecule.
- Natural molecules often contain chromophoric (color-producing) functional groups that have characteristic spectra.
- The procedure for obtaining a UV-VIS spectrum begins with the preparation of a solution of the species under study.
- A standard solution should be prepared in an appropriate solvent. An aliquot of the solution is transferred to a cuvette and placed in the sample chamber of a spectrophotometer.
- A cuvette containing solvent is placed in the reference holder.
- The spectrum is scanned over the desired wavelength range and an absorption coefficient is calculated for each major wavelength maximum.

Kinetics of biochemical reactions

Spectrophotometry is one of the best methods available for measuring the rates of biochemical reactions. Consider a general reaction given as below.

$$A+B \leftrightarrow C+D$$

- If reactants A or B absorb in the UV-VIS region of the spectrum at some wavelength, the rate of the reaction can be measured by monitoring the decrease of absorbance due to loss of A or B.

Alternatively,

- If products C or D absorb at a specific wavelength the kinetics of the reaction can be evaluated by monitoring the absorbance increase due to C and D. According to the Beer-Lambert law, the absorbance change of a reactant or product is proportional to the concentration change of that species occurring during the reaction. This method is widely used to assay enzyme catalyzed processes. Since the rates of chemical reactions vary with temperature, the sample cuvette containing the reaction mixture must be held in a thermostated chamber.