**Principle of UV-Vis Spectroscopy**

The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. [Spectroscopy](https://byjus.com/chemistry/spectroscopy/) is based on the interaction between light and matter. When the matter absorbs the light, it undergoes excitation and de-excitation, resulting in the production of a spectrum.

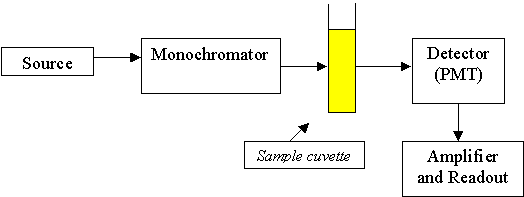
When matter absorbs ultraviolet radiation, the electrons present in it undergo excitation. This causes them to jump from a ground state (an energy state with a relatively small amount of energy associated with it) to an excited state (an energy state with a relatively large amount of energy associated with it). It is important to note that the difference in the energies of the ground state and the excited state of the electron is always equal to the amount of ultraviolet radiation or visible radiation absorbed by it.

**UV-Visible Spectroscopy and the Beer-Lambert Law**

**The statement of the Beer-Lambert law** can be written as follows:

**When a beam of monochromatic light is made incident on a solution that contains a substance that absorbs the monochromatic light, the rate at which the intensity of the beam decreases along the thickness of the solution is directly proportional to the concentration of the absorbing substance in the solution and is also directly proportional to the intensity of the incident monochromatic radiation.**

As per the Beer-Lambert law, the greater the number of absorbing molecules (that have the ability to absorb light of a specific wavelength), the greater the extent of absorption of the radiation.



**Applications:**

UV‑Vis has found itself applied to many uses and situations including but not limited to:

### DNA and RNA analysis

Quickly verifying the purity and concentration of RNA and DNA is one particularly widespread application. A summary of the wavelengths used in their analysis and what they indicate are given in Table 1. When preparing DNA or RNA samples, for example for downstream applications such as sequencing, it is often important to verify that there is no contamination of one with the other, or with protein or chemicals carried over from the isolation process.

The 260 nm/280 nm absorbance (260/280) ratio is useful for revealing possible contamination in nucleic acid samples, summarized in Table 2.Pure DNA typically has a 260/280 ratio of 1.8, while the ratio for pure RNA is usually 2.0. Pure DNA has a lower 260/280 ratio than RNA because thymine, which is replaced by uracil in RNA, has a lower 260/280 ratio than uracil. Samples contaminated with proteins will lower the 260/280 ratio due to higher absorbance at 280 nm.

### Pharmaceutical analysis

One of the most common uses of UV-Vis spectroscopy is in the pharmaceuticals industry. In particular, processing UV-Vis spectra using mathematical derivatives allows overlapping absorbance peaks in the original spectra to be resolved to identify individual pharmaceutical compounds.

### Bacterial culture

UV-Vis spectroscopy is often used in bacterial culturing. OD measurements are routinely and quickly taken using a wavelength of 600 nm to estimate the cell concentration and to track growth.[18](https://doi.org/10.1038/srep38828)600 nm is commonly used and preferred due to the optical properties of bacterial culture media in which they are grown and to avoid damaging the cells in cases where they are required for continued experimentation.

### Beverage analysis

The identification of particular compounds in drinks is another common application of UV Vis spectroscopy. Caffeine content must be within certain legal limits,1,[19](https://www.doi.org/10.11648/j.ajac.20160402.14) for which UV light can facilitate quantification. Certain classes of colored substances, such as anthocyanin found in blueberries, raspberries, blackberries, and cherries, are easily identified by matching their known peak absorbance wavelengths in wine for quality control using UV Vis absorbance.

UV‑Vis spectroscopy is also qualitatively useful in some more specialized research. Tracking changes in the wavelength corresponding to the peak absorbance is useful in examining specific structural protein changesand in determining battery composition. Shifts in peak absorbance wavelengths can also be useful in more modern applications such as characterization of very small nanoparticles. The applications of this technique are varied and seemingly endless.