

Info Note 804: UV-VIS Nomenclature and Units

Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry (UV/VIS) involves the spectroscopy of photons in the UV-visible region. It uses light in the visible and adjacent near ultraviolet (UV) and near infrared (NIR) ranges. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

UV/VIS is based on absorbance. In spectroscopy, the absorbance A is defined as:

$$A_{\lambda} = \log_{10}(I_0/I) \quad (1)$$

where I is the intensity of light at a specified wavelength λ that has passed through a sample (transmitted light intensity) and I_0 is the intensity of the light before it enters the sample or incident light intensity. Absorbance measurements are often carried out in analytical chemistry, since the absorbance of a sample is proportional to the thickness of the sample and the concentration of the absorbing species in the sample, in contrast to the transmittance I / I_0 of a sample, which varies exponentially with thickness and concentration. The Beer-Lambert law is used for concentration determination.

The term absorption refers to the physical process of absorbing light, while absorbance refers to the mathematical quantity. Also, absorbance does not always measure absorption: if a given sample is, for example, a dispersion, part of the incident light will in fact be scattered by the dispersed particles, and not really absorbed. Absorbance only contemplates the ratio of transmitted light over incident light, not the mechanism by which light intensity decreases. Despite this fact, absorbance can still be used to determine concentrations (of particles) in such cases.

Although absorbance does not have true units, it is quite often reported in "Absorbance Units" or AU.

Optical density, or OD, is the absorbance per unit length, i.e., the absorbance divided by the thickness of the sample, although it is sometimes used as a synonym for the absorbance with a base-10 logarithm. Optical density is given as:

$$OD_{\lambda} = \frac{A_{\lambda}}{l} = -\frac{1}{l} \log_{10} T = \frac{1}{l} \log_{10} \left(\frac{I_0}{I} \right) \quad (2)$$

Where:

L = the distance that light travels through the sample (the sample thickness), measured in cm

A_{λ} = the absorbance at wavelength λ

T = the per-unit transmittance

I_0 = the intensity of the incident light beam

I = the intensity of the transmitted light beam

Although absorbance does not have true units, it is quite often reported in "Absorbance Units" or AU. Accordingly, optical density is measured in ODU, which are equivalent to AU cm⁻¹. The higher the optical density, the lower the transmittance. Optical density times 10 is equal to a transmission loss rate expressed in decibels per cm, e.g., an optical density of 0.3 corresponds to a transmission loss of 3 dB per cm.

Beer-Lambert law

The method is most often used in a quantitative way to determine concentrations of an absorbing species in solution, using the Beer-Lambert law:

$$A = -\log_{10}(I/I_0) = \epsilon \cdot c \cdot L, \quad (3)$$

where A is the measured absorbance, I_0 is the intensity of the incident light at a given wavelength, I is the transmitted intensity, L the pathlength through the sample, and c the concentration of the absorbing species. For each species and wavelength, ϵ is a constant known as the molar absorptivity or extinction coefficient. This constant is a fundamental molecular property in a given solvent, at a particular temperature and pressure, and has units of $1 / M * cm$ or often $AU / M * cm$. The absorbance and extinction ϵ are sometimes defined in terms of the natural logarithm instead of the base-10 logarithm.

The Beer-Lambert Law is useful for characterizing many compounds but does not hold as a universal relationship for the concentration and absorption of all substances.

Ultraviolet-visible spectrophotometer

The instrument used in ultraviolet-visible spectroscopy is called a UV/VIS spectrophotometer. It measures the intensity of light passing through a sample (I), and compares it to the intensity of light before it passes through the sample (I_0). The ratio I / I_0 is called the *transmittance*, and is usually expressed as a percentage (%T). The absorbance, A , is based on the transmittance:

$$A = - \log(\%T) \quad (4)$$

The basic parts of a spectrophotometer are a light source (often an incandescent bulb for the visible wavelengths, or a deuterium arc lamp in the ultraviolet), a holder for the sample, a diffraction grating or monochromator to separate the different wavelengths of light, and a detector. The detector is typically a photodiode or a CCD. Photodiodes are used with monochromators, which filter the light so that only light of a single wavelength reaches the detector. Diffraction gratings are used with CCDs, which collects light of different wavelengths on different pixels.

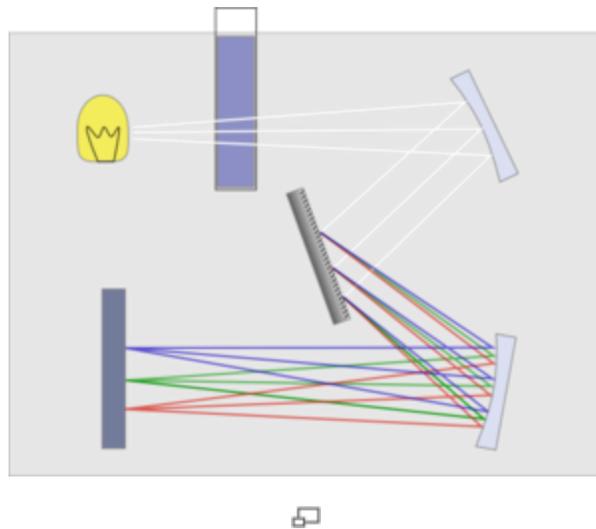


Diagram of a single-beam UV/vis spectrophotometer.

A spectrophotometer can be either *single beam* or *double beam*. In a single beam instrument, all of the light passes through the sample cell. I_0 must be measured by removing the sample. This was the earliest design, but is still in common use in both teaching and industrial labs.

Samples for UV/Vis spectrophotometry are most often liquids, although the absorbance of gases and even of solids can also be measured. Samples are typically placed in a transparent cell, known as a cuvette. Cuvettes are typically rectangular in shape, commonly with an internal width of 1 cm. (This width becomes the path length, L , in the Beer-Lambert law.) Test tubes can also be used as cuvettes in some instruments. The best cuvettes are made of high quality quartz, although glass or plastic cuvettes are common. (Glass and most plastics absorb in the UV, which limits their usefulness to visible wavelengths.)

Instrument Measurement range

Any real measuring instrument has a limited range over which it can accurately measure absorbance. An instrument must be calibrated and checked against known standards if the readings are to be trusted. Many instruments will become non-linear (fail to follow the

Beer-Lambert law) starting at approximately 2 AU (~1% Transmission). It is also difficult to accurately measure very small absorbances that are very close to zero absorbance. The theoretical best accuracy for most instruments is in the range near 1 AU. When possible, the path length or concentration should be adjusted to achieve readings near this range. It is extremely difficult to achieve a large instrument range, between 0 and 6 AU.

Ultraviolet-visible spectrum

An ultraviolet-visible spectrum is essentially a graph of light absorbance versus wavelength in a range of ultraviolet or visible regions. Such a spectrum can often be produced directly by a more sophisticated spectrophotometer, or the data can be collected one wavelength at a time by simpler instruments. Wavelength is often represented by the symbol λ . Similarly, for a given substance, a standard graph of the extinction coefficient (ϵ) vs. wavelength (λ) may be made or used if one is already available. Such a standard graph would be effectively "concentration-corrected" and thus independent of concentration. For the given substance, the wavelength at which maximum absorbance in the spectrum occurs is called λ_{max} , pronounced "Lambda-max".

Applications in Gold Nanoparticles

UV/Vis spectroscopy is routinely used in the quantitative determination of gold nanoparticle solutions.

The Beer-Lambert law states that the absorbance of a solution is directly proportional to the solution's concentration. Thus UV/VIS spectroscopy can be used to determine the concentration of a solution. It is necessary to know how quickly the absorbance changes with concentration. This can be taken from references (tables of molar extinction coefficients), or more accurately, determined from a calibration curve.

We use UV/VIS spectroscopy for characterization of our gold nanoparticles. With it we can determine size, concentration, binding events, and of course, optical absorption characteristics.