Spectrophotometry

Light is an electromagnetic wave that can interact with materials in different ways. Such interactions:

- Reflection
- Scattering
- Absorption
- Transmission

**PRINCIPLE OF ABSORPTION**

The orbital electrons (of atoms that make up a molecule) are on discrete energy levels of the ground state (vibrational and rotational levels) according to the thermal equilibrium with their environment. If such amount of energy ($E = hf$) is transmitted to an electron on the ground state that is sufficient for the excitation to a certain excited level, then the electron will be in an excited state. This process is called absorption or absorption of light. Because all atomic and molecular system are characterized by their electron distribution (energy-term structure), the absorption is a physical parameter the value which is suitable for the characterization of the examined sample.

**THE PHENOMENON OF ABSORPTION**

When light propagates in materials, it can also lose its intensity through absorption.
The decrease in light intensity in dilute solutions can be described by an exponential function that is called the Lambert-Beer law:

\[ I = I_0 e^{-\varepsilon(\lambda)cx} \]

where \( I \) is the intensity of the light leaving the solution, \( I_0 \) the intensity of the light entering the solution, \( \varepsilon(\lambda) \) is the absorption (or extinction) coefficient at a given wavelength, \( c \) is the concentration, and \( x \) is the thickness of the sample.

From the definition of absorption it is expected to be easily measurable, and also be additive. Therefore, the negative logarithm of the quotient of the outgoing and incident light defined as absorbance, absorbance or optical density (OD):

\[ A = OD = \log \frac{I_0}{I} = \varepsilon(\lambda)cx \]

The concentration of a solution with a known value of \( \varepsilon(\lambda) \) can be determined with ease if we know the layer thickness (x) and the measured absorbance (A) of the solution:

\[ c = \frac{A}{\varepsilon(\lambda)x} \]

Concentration measurement is the most commonly used field of absorption photometry.

**WAVELENGTH DEPENDENCE OF ABSORPTION**

Light interacts with the material in a wavelength-dependent manner, and thus the extent of light absorption will also be a function of wavelength. The wavelengths (energy doses, quantums) that match the electronic system of the substance are going to be absorbed and the non-matching wavelengths will be transmitted (passed through). In this sense, absorption is a wavelength-dependent physical quantity.

Tryptophan, tyrosine and phenylalanine amino acids have typical spectra in the UV range. These amino acids are present in the vast majority of proteins, so most of the proteins have an absorption maximum around 280 nm. The proteins have a decent absorption peak even at around 220 nm made by the
peptide bonds. Nucleic acids have an absorption maximum at around 260 nm due to pyrimidine and purine bases. (http://elte.prompt.hu)

TRANSMISSION

Transmission is the quotient of the intensity of transmitted and incident light:

\[ T = \frac{I}{I_0} \]

Its value is characteristic of the tested material. It is not an additive physical parameter.

MEASUREMENT OF ABSORPTION

By detecting the wavelength dependence of absorbance an absorption spectrum is obtained. The spectrum contains valuable information about the electronic structure of the investigated molecular system, but due to the wide bands, it is not suitable for qualitative identification alone.

Example: Absorption spectrum of curcumin.

The absorption spectrum of curcumin can be seen at different concentrations. The absorbance values at \( \lambda = 425 \) nm represented as a function of concentration provide linear function (small inset figure).
RECORDING THE ABSORPTION SPECTRUM

The absorption spectrum can be recorded with a spectrophotometer. The instrument’s main components are:

- Light source (F): illuminates in the wavelength range of 200-800 nm
- Monochromator (M): the appropriate wavelength range can be selected
- Sample holder (S - sample solution, R - reference solution)
- Photodetector (D): measures light intensity (using a photoelectron multiplier)
- Electronic unit (E)
- Computer (C)
- Mirrors (T)

![Spectrophotometer Diagram]

In some applications, additional units such as lenses, optical filters and optical slits may also be required.

The arrangement of a spectrophotometer can be single-beam or double-beam, depending on the geometry of the measurement. The absorption properties of a reference sample must be measured as well in both embodiments.

Only one cuvette can be placed in a single-beam spectrophotometer at a time, so the intensity of the light transmitted by the reference and the sample can be measured one after the other.

Two cuvettes can be placed in a double-beam spectrophotometer simultaneously, so the intensity of the light transmitted by the reference and the sample can be measured at the same time.

APPLICATIONS OF ABSORPTION

Spectrophotometry is one of the most commonly used analytical methods in biochemistry. The method is definitely suitable for fast, simple, routine measurements of small amounts of materials.

If the absorption maximum is in the visible range of the spectrum, the material is coloured. The number of analyzations that can be performed in this range is very large.

UV range analyses are also widespread as many colourless materials have intense absorption band in this range (190-320 nm).