

Principle of Spectrophotometry

The principle of spectrophotometry is based on the measurement of the absorption or transmission of light by a substance as a function of its wavelength. It is a widely used technique in analytical chemistry and biochemistry to quantify the concentration of a substance in a sample.

Spectrophotometry involves the use of a spectrophotometer, which is an instrument that emits light of various wavelengths and measures the intensity of light that passes through a sample or is absorbed by it. The basic principle is governed by the Beer-Lambert Law, which states that the absorbance of a sample is directly proportional to its concentration and the path length of the light through the sample. The process typically involves the following steps:

Selection of an appropriate wavelength: The spectrophotometer allows the user to select a specific wavelength of light for the analysis. Different substances absorb light at different wavelengths, so choosing the appropriate wavelength is crucial for accurate measurements.

Preparation of a blank: A blank solution, which is a solvent or a solution without the substance of interest, is used as a reference. It is used to calibrate the instrument and compensate for any background absorption or scattering.

Measurement of the sample: The sample solution containing the substance to be analyzed is placed in a cuvette, which is then inserted into the spectrophotometer. The spectrophotometer emits light of the selected wavelength, and the intensity of light transmitted through or absorbed by the sample is measured.

Calculation of absorbance or transmittance: The spectrophotometer provides a readout of either the transmitted light intensity or the absorbed light intensity. Absorbance (A) is calculated as the logarithm of the ratio of incident light to transmitted light, while transmittance (T) is the ratio of transmitted light to incident light.

Determination of concentration: The absorbance or transmittance values obtained from the sample are compared to a calibration curve or standard curve generated from known concentrations of the substance. By comparing the measured absorbance or transmittance value to the curve, the concentration of the substance in the sample can be determined.

Spectrophotometry is widely used in various applications, including quantitative analysis of chemical compounds, determination of the concentration of biomolecules like DNA, RNA, and proteins, and monitoring enzyme reactions, among others.