Spectrophotometer Encyclopedia Article

Spectrophotometer

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Spectrophotometer

A spectrophotometer is an optical device that can determine the concentration of a compound or particles in a solution or suspension.

Light of a pre-selected wavelength is shone through a chamber that houses the sample. The sample particles, **bacteria** for example, will absorb some of the light. The amount of light that is absorbed increases with increasing numbers of bacteria in a predictable way. The relationship between absorbance and the number of absorbing sample molecules is expressed mathematically as the Beer-Lambert Law.

The absorbance of light can also be described as the optical density of the sample solution or suspension.

The percent of light that has been absorbed can be determined and, by comparing this absorption to a graph of the absorption of known numbers of bacteria, the concentration of bacteria in the suspension can be computed. In a microbiology laboratory, such measurements are routinely used in **bacterial growth** studies, to determine the number of bacteria growing in a **culture** at certain times based on the absorbance of the suspension. A standard curve can be constructed that relates the various measured optical densities to the resulting number of living bacteria, as determined by the number of bacteria from a defined portion of the suspensions that grows on **agar** medium.

Some spectrophotometers are equipped with a single measuring chamber. For these so-called single-beam instruments, the absorbance of a sample is taken, followed by the absorbance of a control. Typically, a bacterial control is uninoculated growth medium, so the absorbance should be zero. In typical growth curve studies, the bacterial culture can be grown in a special flask called a side-arm flask. The side arm is a test tube that can be inserted directly into a spectrophotometer.

Double-beam spectrophotometers are also available and are the norm now in research microbiology laboratories. In these instruments the light beam is split into two beams by means of mirrors. One light path goes through the sample chamber and the other light beam passes through what is referred to as the reference cell or chamber. The ration of the absorbance between the two chambers is computed and is used to determine sample concentration.

Depending on the spectrophotometer, absorbance can be taken at a single wavelength, or scanned through a spectrum of wavelengths. The latter can be a useful means of identifying components of the sample, based on their preferential absorption of certain wavelengths of light.