CLINICALCHEMISTRY-LAP.

EXPT.3: spctrophotometer

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Introduction

Spectrophotometry

The basics of spectrophotometry

Colour is everywhere. Every chemical compound absorbs, transmits, or reflects light over an electromagnetic spectrum in wavelengths. When light passes through any solution a section of it is absorbed. Spectrophotometry allows both qualitative and quantitative analysis. As the concentration of a substance increases light absorption increases, and light transmission decreases.

Spectrophotometry is used in chemistry, biochemistry (for enzyme-catalysed reactions), physics, biology, and clinical studies (examining haematology or tissues). It allows scientists to analyse different samples without having any skin contact as the samples are contained in a small tube called a cuvette or in case of the Photopette, measurements are done directly in the

sample container without having to transfer it.

How does a Spectrophotometer work?

Spectrophotometry is a standard and inexpensive technique to measure light absorption or the amount of chemicals in a solution. It uses a light beam which passes through the sample, and each compound in the solution absorbs or transmits light over a certain wavelength.

Spectrometry is measured by a spectrophotometer; an instrument that is made up of two instruments – a spectrometer and a photometer. The spectrometer produces the light of the wavelength and the photometer measures the intensity of light by measuring the amount of light that passes through the sample.

In addition to those two components, spectrophotometers consist of a light source, a monochromator, a sample chamber containing a cuvette, a detector (such as a photomultiplier tube or photodiode) to detect the transmitted light,

a digital display and a data analysis software package.

Light Source

Spectrophotometers rely on light sources to operate. Because of the wide range of samples, light sources can vary in nature, and use a wide spectrum of wavelengths, including visible, UV and IR.

Monochromator

The monochromator (such as a prism or grating) inside the machine refracts the light into a single spectrum and disperses polychromatic light into the essential wavelengths. A grating divides the light available into different segments. Gratings are common in spectrophotometers that use UV, visible and infrared regions.

Sample Chamber

The sample chamber is where the operator inserts the sample for analysis. Samples are typically placed into a cuvette made of a material such as glass or quartz.

Detector

The detector is the light-receiving element that absorbs the energy of the incident light.

Examples of typical spectrophotometer detectors include photomultiplier tubes and photodiodes. They convert the light energy into an electrical signal, which is converted into an absorption figure.

Digital Display

Modern day spectrophotometers typically have a digital display built into the instrument. This gives operators an accessible way to change instrument settings, set up method parameters and see results. It has no effect on the way the instrument works however.

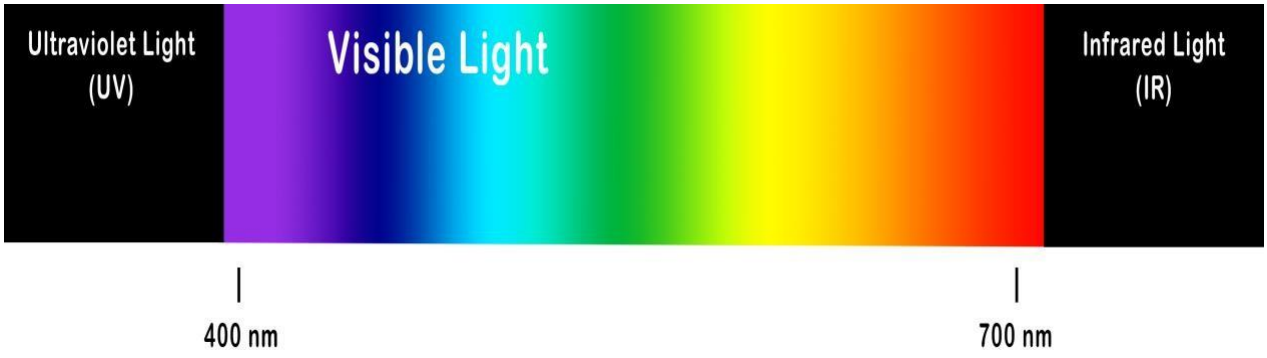
Data Analysis

Alongside digital displays, most spectrophotometers have the ability to do any calculations and analysis. Once all the method parameters have been set up within the instrument, data and results are output once the method is complete.

Absorbance Wavelengths

In the spectrophotometer, the number of photons absorbed by a solution is called the absorbance readout. The longer the path-length that the light must travel through a

solution prior to it reaching the detector, the greater the chance of a photon being absorbed.



Different compounds absorb best at different wavelengths. A UV-visible spectrophotometer uses light over the ultraviolet range (185 – 400 nm) and visible range (400 – 700 nm) of the electromagnetic radiation spectrum. Whereas an IR spectrophotometer uses light over the infrared range (700 – 15000 nm).

Ultraviolet (UV) and visible (VIS) spectroscopy show electronic transitions in atoms and molecules, to measure this a spectrophotometer is used. Compounds that absorb in the visible region are

coloured, whereas ones that absorb only in the UV region are colourless.

UV-VIS spectrophotometer usually use two light sources. A deuterium lamp is used for the UV region and a tungsten lamp for the VIS region. These lights reach the monochromator via a mirror. The wavelength for red light is between 700 and 750 nm and blue between 400 and 450 nm. If the wavelength is shorter than 350 nm it is UV and has more energy.

Transmittance and absorbance

Spectrophotometers measure absorbance (A) and transmittance (T). The intensity of light (I_0) measures photons per second. When light passes through a blank sample, it does not absorb light so is symbolised as (I). Scientists use blank samples without chemical compounds as a reference. They contain everything that is in the sample cuvette, except the one material which absorbance is being measured.

To calculate the transmittance the following equation is used:

Transmittance (T) = I_t/I_0

I_t = Light intensity after passing the cuvette (transmitted light)

I_0 = Light intensity before passing the cuvette (incident light)

Absorbance (A) = - $\log_{10} T$ = - log I_S/I_R

Using the Beer-Lambert Law (Beer's Law)

The Beer-Lambert Law (sometimes just referred to as Beer's Law) is the relationship between the attenuation of light, through a substance, and the properties of the substance.

The Beer-Lambert law indicates that the amount of light that is absorbed by a substance is proportional to the amount of the sample concentration. It is also determined by the amount of solute that is present. But to fully understand the Beer-Lambert Law, understanding the relationship between absorbance and transmittance is of importance.

Measuring Absorbance

The sample molecules or ions in a solution can be detected and quantified using a spectrophotometer and The Beer-Lambert Law with this equation: $A = \varepsilon CL$

A = absorbance of light at a specific wavelength

ε = molar extinction coefficient (the absorbance of 1 mole of a substance dissolved in 1 litre solvent)

C = the molar concentration of a sample

L = the optical path length of a sample

How to Measure Absorption With a Spectrophotometer

To measure absorption of a sample, you need to know the values of the three factors – molar extinction coefficient, molar concentration and optical path length.

Molar Extinction Coefficient – ε

The molar extinction coefficient is a value at which a chemical species attenuates light at a given wavelength. The SI unit is m2/mol, but sometimes expressed as M-1 cm-1 or L mol-1 cm-1.

You can obtain the molar extinction coefficient for your target sample from literature sources.

Concentration

Concentration refers to the concentration of the sample. This is simply the molar concentration, and measured as mol/L.

Path Length

Path length refers to the distance that light travels through the sample as absorption is directly proportional to distance of light traveled. This is determined by the size of your cuvette.

Whilst determining the absorption from Beer-Lambert's Law is relatively straightforward, make sure to use the correct units, or convert them correctly to avoid any errors in your end result.

Principle

ThePrinciple 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. Spectroscopyis based on the inteíaction between light and matteí. When the matteí absoíbs the light, it undeígoes excitation and de-excitation, íesulting in the píoduction of a spectíum.

When matteí absoíbs ultíaviolet íadiation, the electíons píesent in it undeígo excitation. l'his causes them to jump fíom a gíound state (an eneígy state with a íelatively small amount of eneígy associated with it) to an excited state (an eneígy state with a íelatively laíge amount of eneígy associated with it). It is impoítant to note that the diffeíence in the eneígies of the gíound state and the excited state of the electíon is always equal to the amount of ultíaviolet íadiation oí visible íadiation absoíbed by it.

Procedure

*l'uín on the device fíom the poweí point

*Place the sample in the cells designated for the device for the purpose of reading the absorbance.

* Put the cells containing the sample in the device and start reading at the wavelength at which the measurement is to be performed

*After the reading is finished, wash the cells and return them to their designated place

* Turn off the device

Discussion

1. What is UV spectroscopy?
2. What are the applications of UV-visible spectroscopy?
3. What is Principle of spectrophotometer?
4. What is the purpose of the device?
5. How to Measure Absorption With a Spectrophotometer?