

SPECTROPHOTOMETRY. PRINCIPLE AND APPLICATIONS

Third class

Advanced laboratory techniques

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In chemistry, spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength.

Spectrophotometry involves the use of a spectrophotometer, which is a photometer that can measure intensity as a function of the light source wavelength.

A spectrophotometer is commonly used for the measurement of transmittance or reflectance of solutions, transparent or opaque solids, such as polished glass, or gases.

Historically, spectrophotometers use a monochromator containing a diffraction grating to produce the analytical spectrum, but most modern mid-infrared spectrophotometers use a Fourier transform technique to acquire the spectral information (infrared spectroscopy).

UV – VIS AND IR SPECTROSCOPY

Ultraviolet–visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared (NIR)) ranges.

UV/Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied.

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 is called the transmittance, and is usually expressed as a percentage

UV – VIS AND IR SPECTROSCOPY

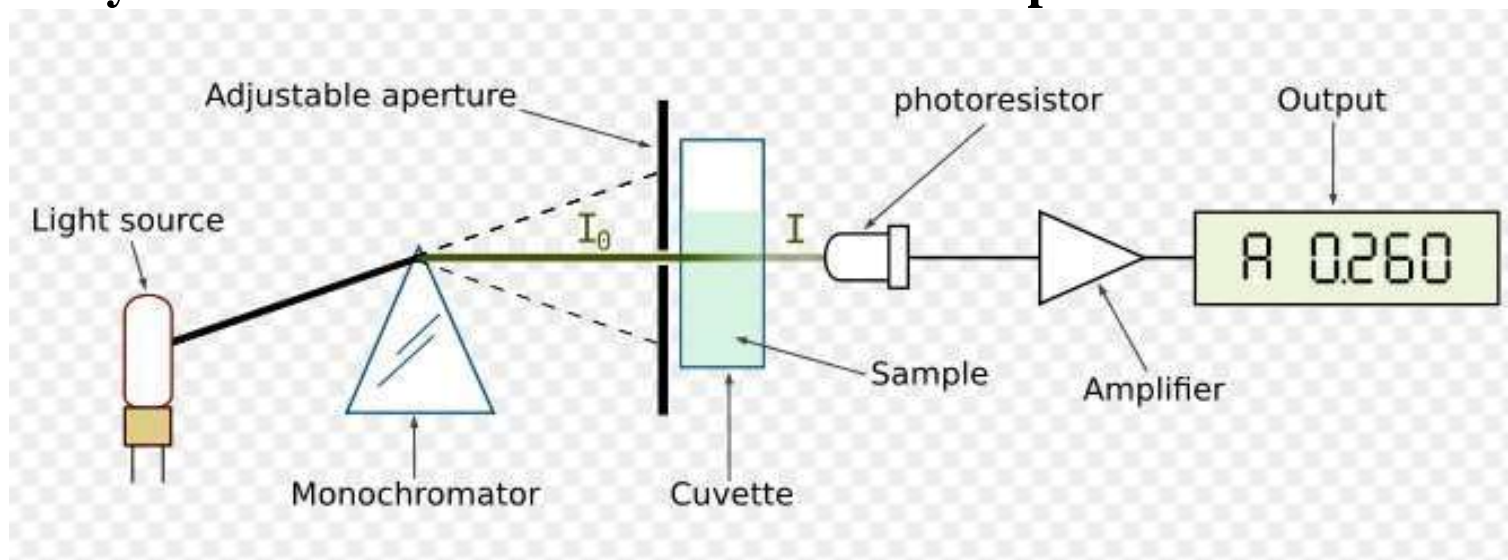
Infrared spectroscopy (IR spectroscopy) is the spectroscopy that deals with the infrared region of the electromagnetic spectrum, that is light with a longer wavelength and lower frequency than visible light. It covers a range of techniques, mostly based on absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify and study chemicals. A common laboratory instrument that uses this technique is a Fourier transform infrared (FTIR) spectrometer.

The infrared spectrum of a sample is recorded by passing a beam of infrared light through the sample. When the frequency of the IR is the same as the vibrational frequency of a bond, absorption occurs.

APPLICATIONS

The use of spectrophotometers spans various scientific fields, such as physics, materials science, chemistry, biochemistry, and molecular biology.

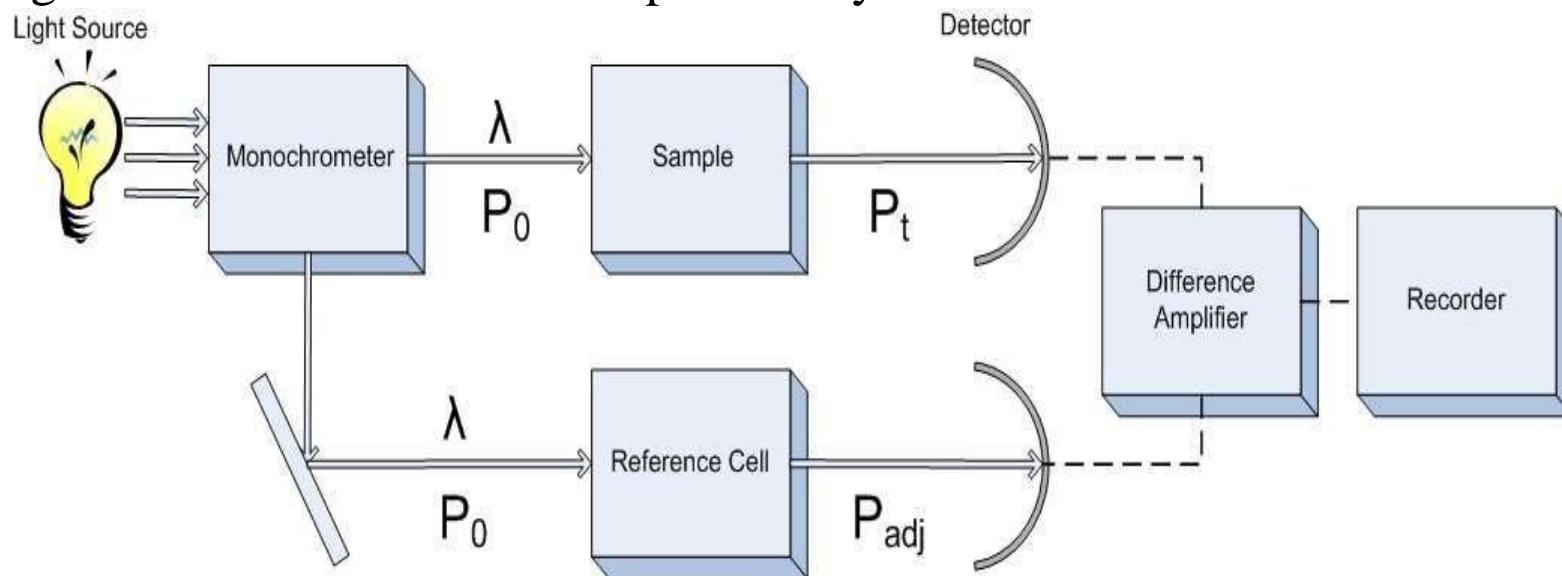
There are two major classes of devices: single beam and double beam. A double beam spectrophotometer compares the light intensity between two light paths, one path containing a reference sample and the other the test sample. A single-beam spectrophotometer measures the relative light intensity of the beam before and after a test sample is inserted.



Single beam spectrophotometer

APPLICATIONS

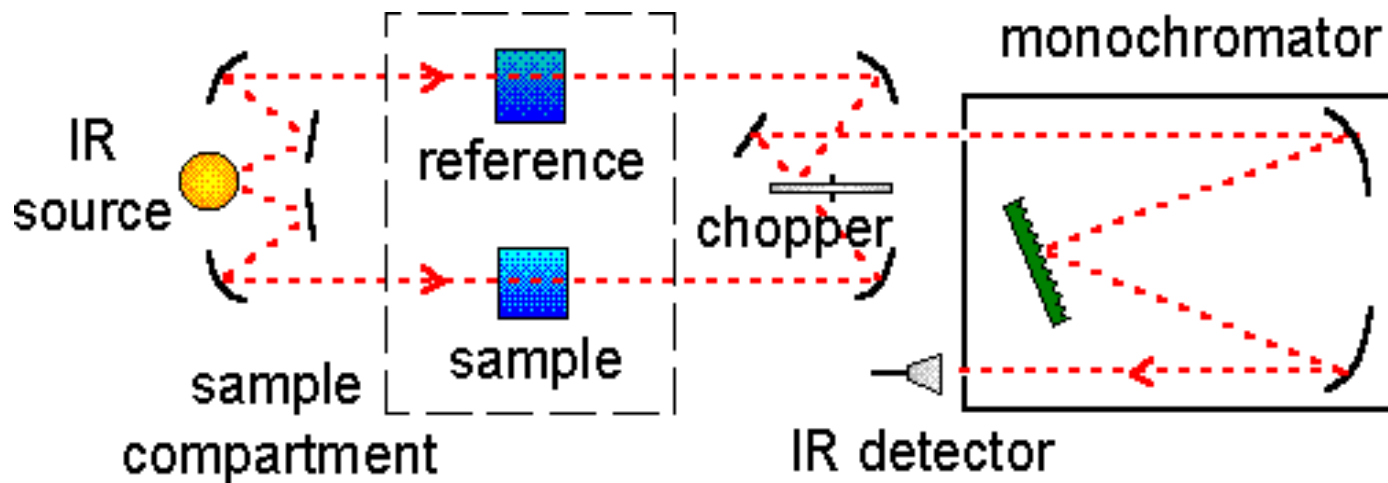
Although comparison measurements from double-beam instruments are easier and more stable, single-beam instruments can have a larger dynamic range and are optically simpler and more compact. Additionally, some specialized instruments, such as spectrophotometers built onto microscopes or telescopes, are single-beam instruments due to practicality.



Double beam spectrometer

APPLICATIONS

Infrared spectroscopy is a simple and reliable technique widely used in both organic and inorganic chemistry, in research and industry. It is used in quality control, dynamic measurement, and monitoring applications such as the long-term unattended measurement of CO₂ concentrations in greenhouses and growth chambers by infrared gas analyzers.



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FTIR spectrometer

ADVANTAGES / DISADVANTAGES

UV - VIS

ADVANTAGES	DISADVANTAGES
<p>A light source shutter controls the amount of light from a specialized lamp that passes through the sample. The shutter is the only moving component of a UV-vis spectrometer. The advantage of this system lies in the simplistic design of the instrument.</p>	<p>However, no single lamp emits all the light wavelengths necessary for analysis. For example, a deuterium lamp emits wavelengths from 180 nm to 370 nm, and a tungsten lamp emits wavelengths from 315 nm to 900 nm. Changing the lamp is a time-consuming process.</p>
<p>Sample analysis using UV-vis is a very quick process compared to other methods of sample detection, such as HPLC. This rapid analysis is achieved only through proper calibration.</p>	<p>UV-vis spectrometers require frequent calibrations to retain the accuracy and precision of the instrument. Choosing what type of material to use as the calibrator requires the knowledge of the type of sample being analyzed.</p>
<p>The UV-vis technique is non-destructive to the sample and has a high sensitivity for detecting organic compounds.</p>	<p>However, stray light can be a problem for UV-Vis spectrometers. This can be caused by the user trying to detect the sample using too wide of a wavelength range or by poor instrument design.</p>

ADVANTAGES / DISADVANTAGES

FT - IR

ADVANTAGES	DISADVANTAGES
<p>The multiplex advantage arises because all of the spectral elements are measured simultaneously. Thus, a spectrum can be obtained very quickly.</p>	<p>FTIR instruments do not measure spectra; they measure interferograms which are difficult to interpret without performing a Fourier transform to produce a spectrum.</p>
<p>The throughput advantage arises because unlike the dispersive spectrometers, FTIR spectrometers have no slits which attenuate the infrared light.</p>	<p>In systems which are source noise limited, the disadvantage arises from the fact that if noise occurs in one part of the radiation from the infrared source, it will spread throughout the spectrum.</p>
<p>The frequency scale of the spectrum is known very accurately.</p>	<p>FTIR instruments have a single beam, and for highly sensitive work experiments can take a long time and changes in infrared absorbing gas concentrations can severely affect the results.</p>

CONCLUSIONS

- The use of spectrophotometry as it results from the presented materials proves to be of real help in regards to analyzing the properties of different materials from the point of view of their chemical properties.
- The large variety of methods used and equipment typology shows the technological development in this field of interest relative to its possibilities in analyzing materials in different domains i.e. medical, industrial, etc.