

UV-Visible Spectroscopy

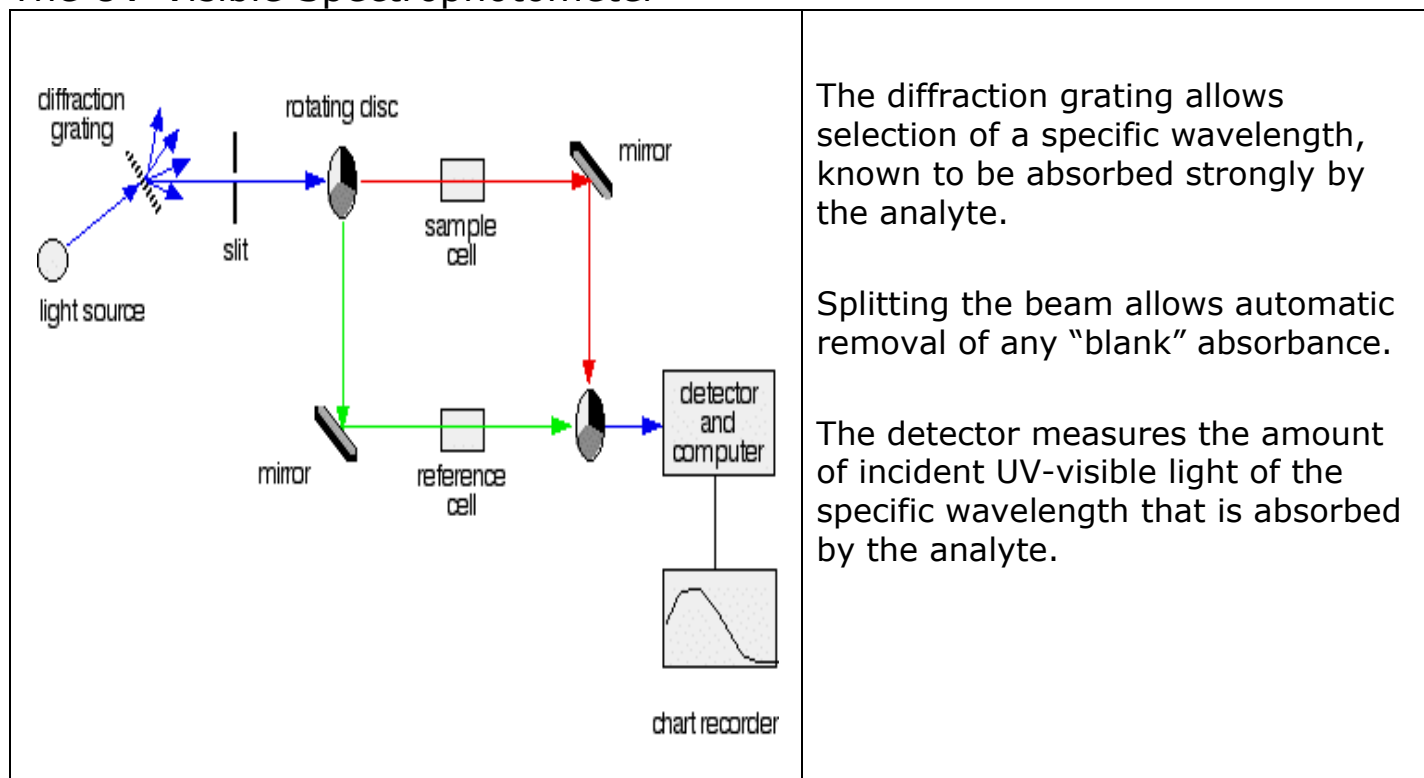
UV-visible spectroscopy is a quantitative analytical method based on the absorption of light of a particular wavelength by the analyte in a sample. The light absorbed by the analyte has an energy that promotes electrons in atoms, molecules or ions into higher energy states.

A colorimeter is a relatively cheap, but useful, instrument that shines filtered light through a coloured analyte [or an analyte that can form a coloured compound], the amount of the filtered light absorbed being dependant on the concentration of the coloured analyte in the sample. For example, a blue solution containing copper sulfate absorbs light in the red end of the spectrum [thus appearing blue]. The absorption of red filtered light passing through the sample could be used to determine the concentration of the copper sulfate in the solution.

A UV-visible spectrophotometer is a far more selective and sensitive [and expensive] instrument than a colourimeter, because the incident light is a specific wavelength and colourless analytes, that absorb in the UV region, can also be analysed.

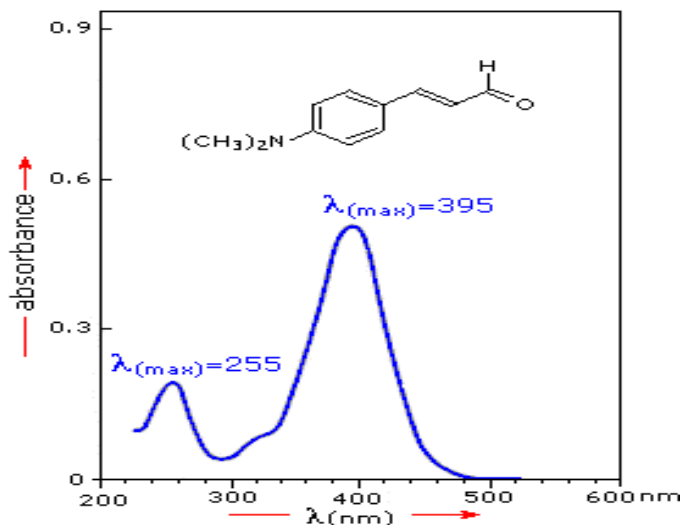
Any absorbance of the solution by substances other than the analyte is detected by measuring the absorbance of a reagent *blank* [having everything that's in the sample solution, except the analyte]. A double beam UV-visible spectrophotometer is able to automatically remove the absorbance of the reagent blank.

The UV-Visible Spectrophotometer



UV-Visible Spectroscopic Analysis

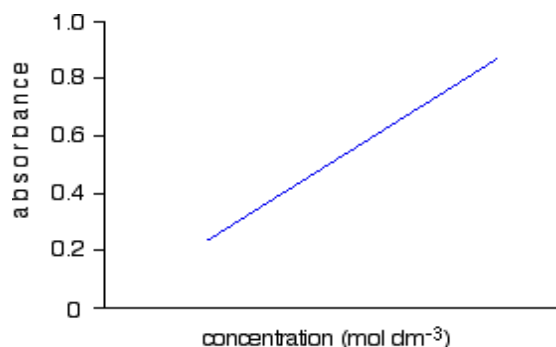
To choose a suitable wavelength for UV-visible spectroscopy, a spectrum of the pure analyte is obtained and a wavelength that is absorbed strongly by it [but not by other substances in the sample] is chosen.



In this case the analyte, C₁₁H₁₃NO, absorbs strongly at 395 nm and, to a lesser extent, 255 nm in the UV region of the EM spectrum. As there is insignificant absorption at 395 nm by other substances in the sample, this wavelength is chosen for analysis.

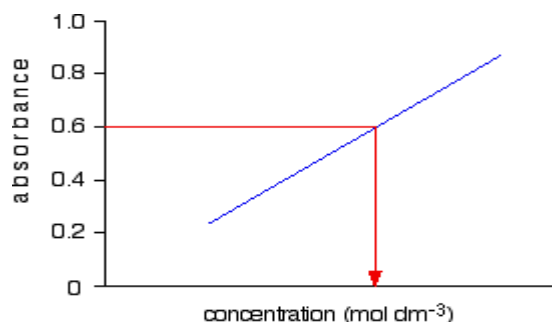
The absorbance at 395 nm for a range of standard concentrations of the pure analyte is then measured and a calibration curve established. When the absorbance of the sample is measured, reference to the calibration curve will establish the concentration of the analyte in the sample.

**Absorbance [@395 nm] of
C₁₁H₁₃NO standards**



Analyte Calibration Curve

**Absorbance [@395 nm] of the
sample solution = 0.600**



The concentration of analyte in the sample is read off the calibration curve.

Example of UV-visible Spectroscopy

Determination of the phosphate content of detergent

The quantitative analysis of phosphate in detergent can be performed by UV-visible spectroscopy. The phosphate present in a prepared solution of the powdered detergent is reacted with ammonium molybdate to produce the molybdenum blue complex, which is known to absorb strongly at a wavelength of 715 nm.

A set of phosphate standards and a reagent blank are prepared and reacted in exactly the same way as the sample. The absorbance of the sample solution and set of standards at 715 nm, corrected for the blank, is then determined using a double beam UV-visible spectrophotometer.

UV-visible Procedure:

- sample preparation: 1.250 g of detergent dissolved in distilled water and diluted to 500.0 mL with distilled water.
- aqueous solutions of AR grade sodium dihydrogen orthophosphate were prepared, containing 5.0, 10.0, 15.0, 25.0 and 40.0 mg/L PO_4^{3-} .
- sample and standards reacted with acidified ammonium molybdate reagent to produce the molybdenum blue complex.
- absorbance @ 715 nm determined by a LUV 1000E double beam UV-visible spectrophotometer

UV-visible Results:

solution analysed	absorbance @ 715 nm
5.0 mg/L PO_4^{3-} (aq) standard	0.061
10 mg/L PO_4^{3-} (aq) standard	0.124
15 mg/L PO_4^{3-} (aq) standard	0.185
25 mg/L PO_4^{3-} (aq) standard	0.310
40 mg/L PO_4^{3-} (aq) standard	0.495
diluted detergent sample	0.238

Analysing the Results:

- a calibration graph of absorbance vs. mg/L phosphate is constructed
- the phosphate content [mg/L] of the diluted detergent sample is taken from the calibration graph
- the phosphate content of the detergent in %[w/w] is calculated from the mg/L concentration and the dilution factor