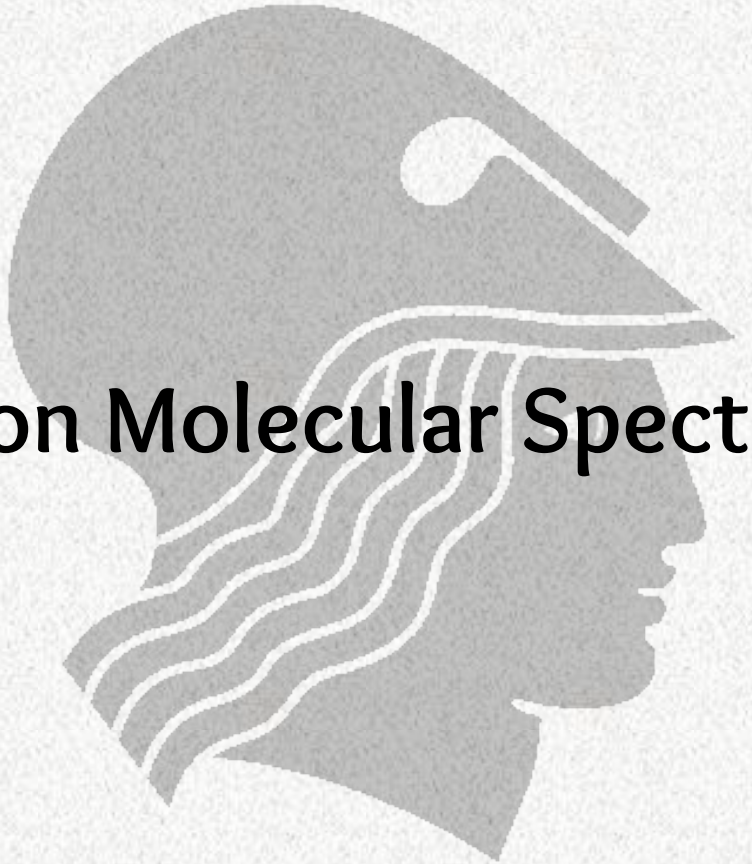


Basics on Molecular Spectroscopy

University of Lincoln
presentation

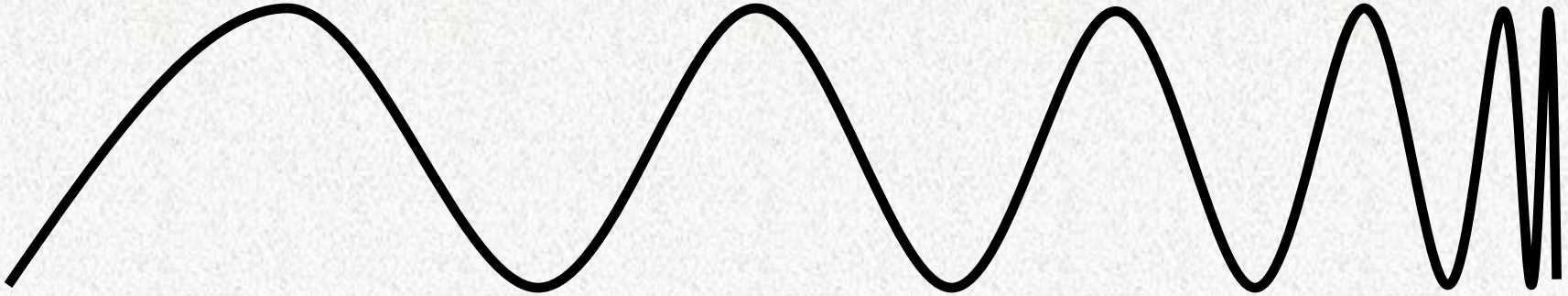
SPECTROSCOPY

- **Interaction of Radiation with a sample**
- **The study of molecular or atomic structure of a substance by observation of its interaction with electromagnetic radiation**
- QUANTITATIVELY - For determining the amount of material in a sample
- QUALITATIVELY – For identifying the chemical structure of a sample

THE ELECTROMAGNETIC SPECTRUM

- Most of us are aware of many different ways of transmitting energy and these phenomena come together in one physical entity called the ELECTROMAGNETIC SPECTRUM
- The difference between these *sources of radiation* is the amount of *energy* they radiate.
- The radiation from these and other sources covers a range of energies

The Electromagnetic Spectrum



Radio waves
Microwave
Infra-red
Visible
Ultraviolet
X-rays
Gamma rays

Long ← Wavelength → Short

Low → Energy → High

Low → Frequency → High

RADIATION IS TRANSMITTED IN A WAVEFORM

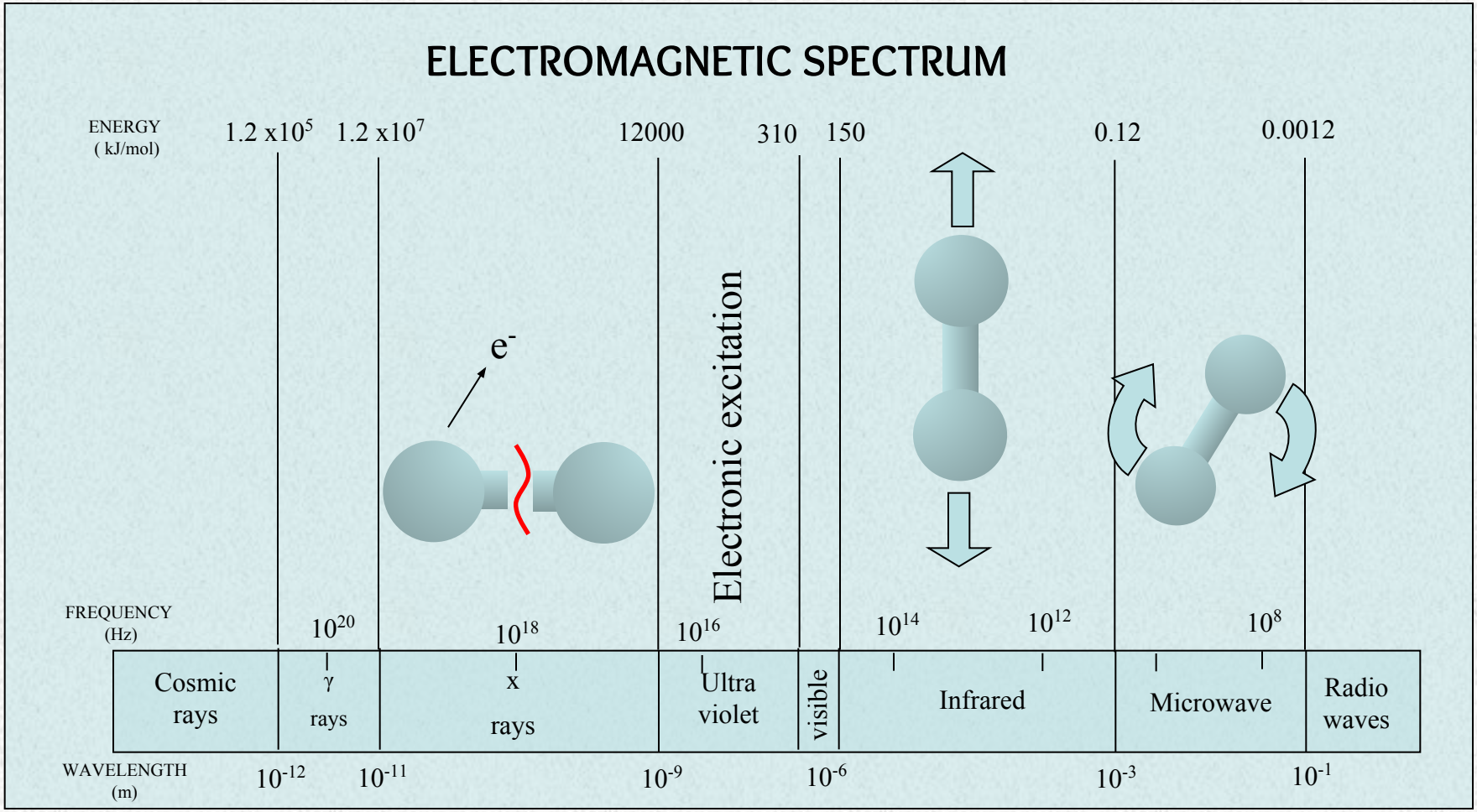
- LOW ENERGY RADIATION has a LONG WAVELENGTH

- HIGH ENERGY RADIATION has a SHORT WAVELENGTH

Radiation Energy

- The strength of the radiation energy will interect with the molecules in different ways:
  - High energy sources produce breaking of bonds
    - X-Ray, γ Rays, …
  - Medium energy sources excite electrons
    - UV / VISIBLE Spectroscopy
  - Low energy sources produce vibrations in chemical bonds
    - Infrared Energy
  - Very low energy sources produce rotation of the chemical bonds
    - Microwaves and Radio waves

EFFECT OF ENERGY ON A MOLECULE



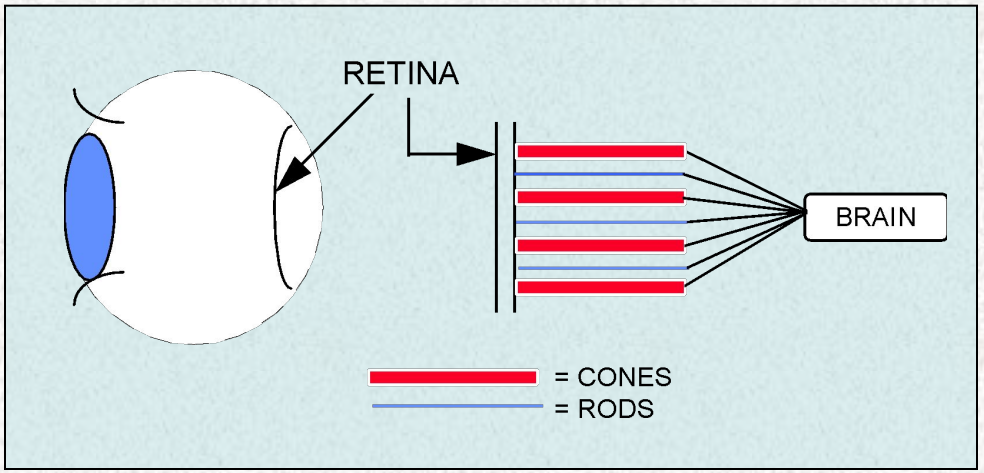
VISIBLE SPECTROSCOPY

WHAT IS COLOUR?

Colour is a sensation which occurs when light enters the eye and focuses on the retina at the back of the eye. The light actually initiates a photochemical reaction in the nerve cells attached to the retina. These transmit impulses to the brain and stimulate our sense of colour

CONES - Give colour and three types which pick up red, blue and green

RODS - Give grey/black and also used for night vision.



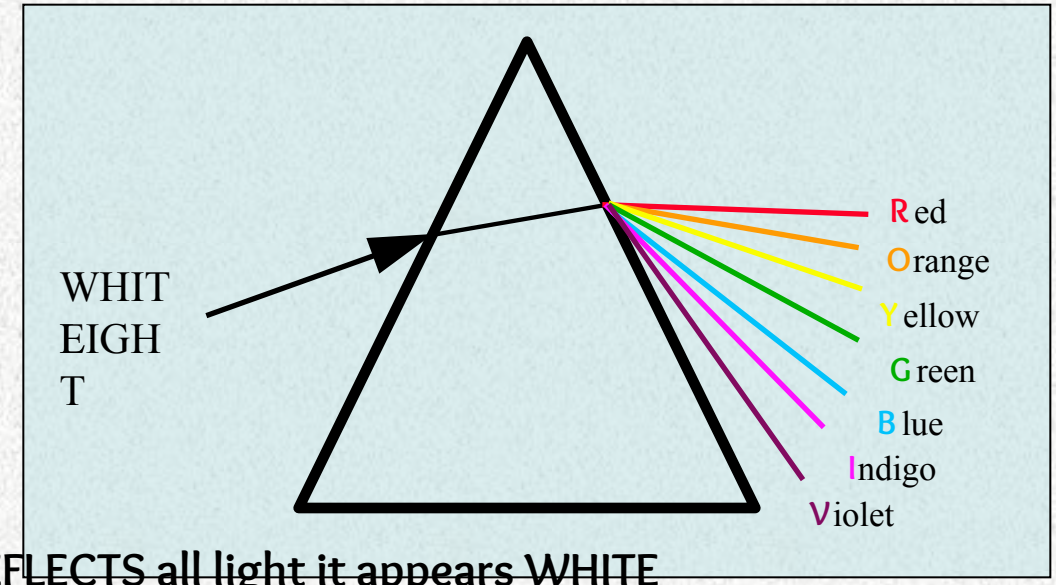
All the colours we actually sense are made up of these three colours together with white and grey and black.

VISIBLE SPECTROSCOPY

COMPOSITION OF WHITE LIGHT

- Sunlight is white light and covers a wavelength range of 380-750nm. A simple physics experiment shows that white light is actually a composition of a range of colours i.e., light of different energies and hence wavelengths.

When white light falls on an object the colour detected by the eye will depend upon the ABSORPTION/REFLECTION properties of the material in the object;

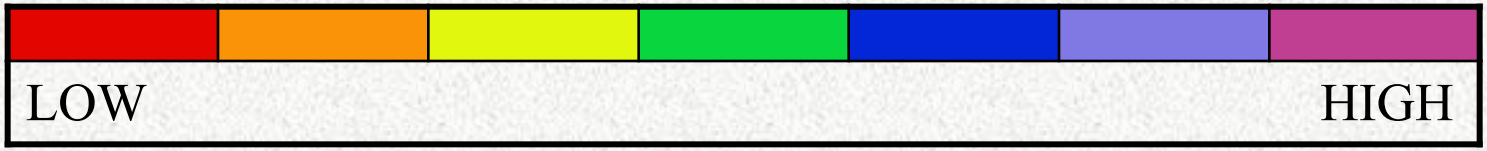


- If the material completely REFLECTS all light it appears WHITE
- If the material absorbs a constant fraction of the light across the spectrum it appears GREY.
- If the material completely ABSORBS all the light it appears BLACK

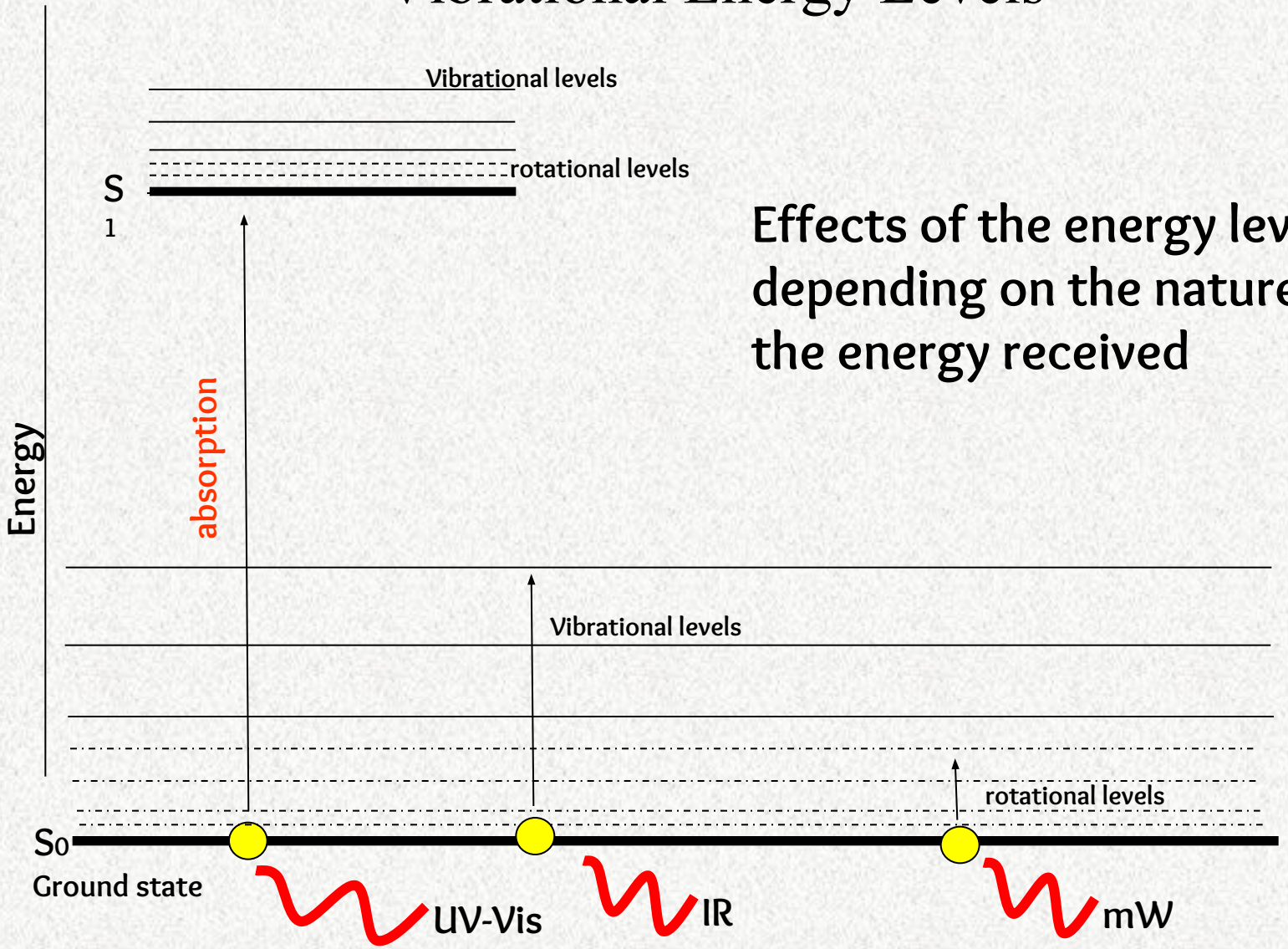
VISIBLE SPECTROSCOPY

When a sample only absorbs light of a single wavelength the eye sees COMPLEMENTARY colours.

Wavelength Range Absorbed	Colour Absorbed	Colour Seen By Eye
380 - 430	Violet	Yellow - Green
430 - 480	Blue	Yellow
480 - 490	Green - Blue	Orange
490 - 500	Blue - Green	Red
500 - 560	Green	Purple
560 - 580	Yellow - Green	Violet
580 - 590	Yellow	Blue
590 - 610	Orange	Green - Blue
610 - 750	Red	Blue - Green



Vibrational Energy Levels



Effects of the energy levels depending on the nature of the energy received

UV / VISIBLE SPECTROSCOPY

UV Radiation – Wavelength range 220 - 380nm

VISIBLE Radiation – Wavelength range 380 - 780nm

Substances can absorb varying amounts of UV and/or Visible radiation at particular wavelengths – Coloured compounds absorb energy in both UV and visible region of the electromagnetic spectrum.

Substances can be liquids or solids and measurements are made with instruments called SPECTROPHOTOMETERS or SPECTROMETERS.

Modern instruments can be coupled to microscopes which allow solid samples and very small samples of solids and liquids to be analysed both qualitatively and quantitatively.

UV / VISIBLE SPECTROSCOPY - THEORY

- If a particular wavelength of UV or Visible radiation can be isolated from the source and passed through a sample which can ABSORB some of the radiation then the TRANSMITTED light intensity (I t) will less than the INCIDENT light intensity (I o).

- The amount of light transmitted with respect to the incident light is called TRANSMITTANCE (T) ie.,

$$T = \frac{I_t}{I_o}$$

- Sample can absorb all or none of the incident light and therefore
- transmittance often quoted as a percentage eg.,

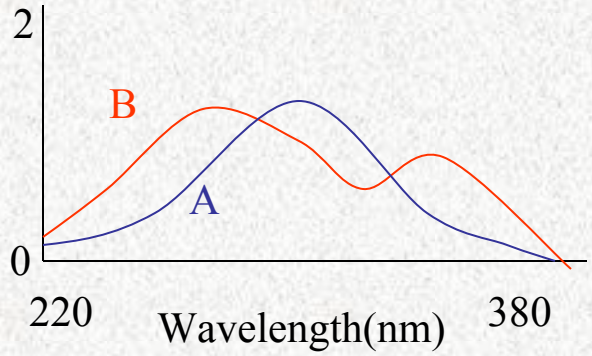
$$\% \ T = \frac{I_t}{I_o} \times 100$$

UV / VISIBLE SPECTROSCOPY - THEORY

ABSORBANCE $A = -\log_{10} T$

$$A = -\log_{10}\left(\frac{I_t}{I_o}\right)$$

$$A = \log_{10}\left(\frac{I_o}{I_t}\right)$$



For of %T = 0 and 100 the corresponding absorbance values will be 0 and 2 respectively

By plotting Absorbance vs wavelength an ABSORBANCE SPECTRUM is generated. The absorbance spectra for the same compounds A and B are shown.

With the advantage that absorbance measurements are usually linear with Concentration, absorbance spectra are now used

THE LAWS OF SPECTROPHOTOMETRY

There are two very important basic laws and a third one which is a combination of the two.

LAMBERTS LAW – ABSORBANCE (A) proportional to the PATHLENGTH (l) of the absorbing medium.

BEERS LAW - ABSORBANCE (A) proportional to the CONCENTRATION (c) of the sample.

BEER- LAMBERT LAW - ABSORBANCE (A) proportional to c x l

$$A \propto cl$$

$$A = Ecl$$ (A is a ratio and therefore has no units)

The constant E is called the MOLAR EXTINCTION COEFFICIENT

Link to "Beer-Lambert law" video

UV / VISIBLE SPECTROSCOPY - THEORY

UNITS OF THE MOLAR EXTINCTION COEFFICIENT

- CONCENTRATION (c) - Moles litre^{-1}
- PATHLENGTH (l) - cm

$A = Ecl$ Hence $E = \frac{A}{c\,l}$

$E = \frac{1}{\text{mole litre-1} \times \text{cm}}$

$E = \text{mole-1 litre} \times \text{cm}^{-1}$

But 1 litre = 1000cm3

$E = 1000 \text{ mole -1 cm3} \times \text{cm}^{-1}$

Hence Units of E = 1000 cm2 mole $^{-1}$

UV / VISIBLE SPECTROSCOPY - THEORY

IMPORTANCE OF THE BEER LAMBERT LAW

$A = Ecl$ but if E and l are constant

ABSORBANCE $\propto$ CONCENTRATION and should be linear relationship

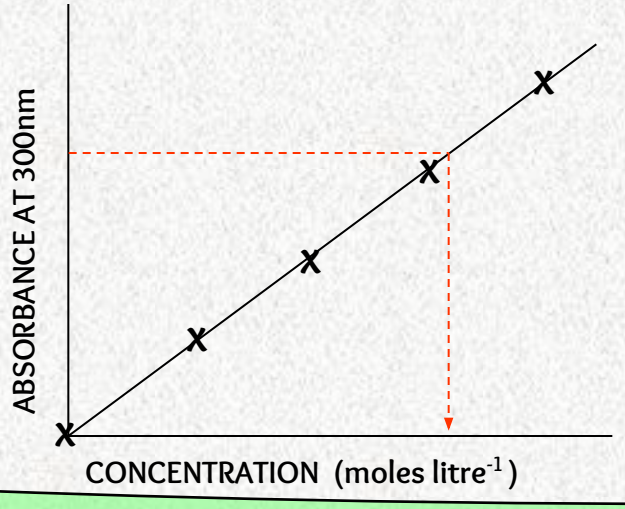
Prepare standards of the analyte to be quantified at known concentrations and measure absorbance at a specified wavelength.

Prepare calibration curve.

From measuring absorbance of sample

Concentration of analyte in sample can be obtained from the calibration curve

E can be obtained from the slope of the calibration curve for a given wavelength (λ)



UV / VISIBLE SPECTROSCOPY - THEORY

RULES FOR QUANTITATIVE ANALYSES

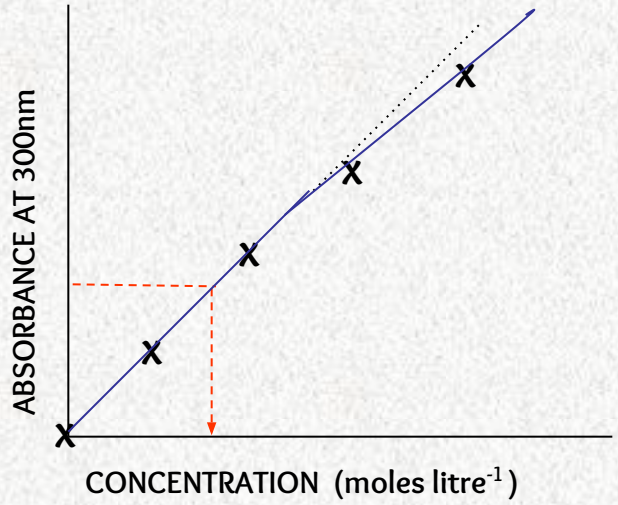
At high concentrations the calibration curve may deviate from linearity – Always ensure your concentration of the sample falls within the linear range – if necessary dilute sample

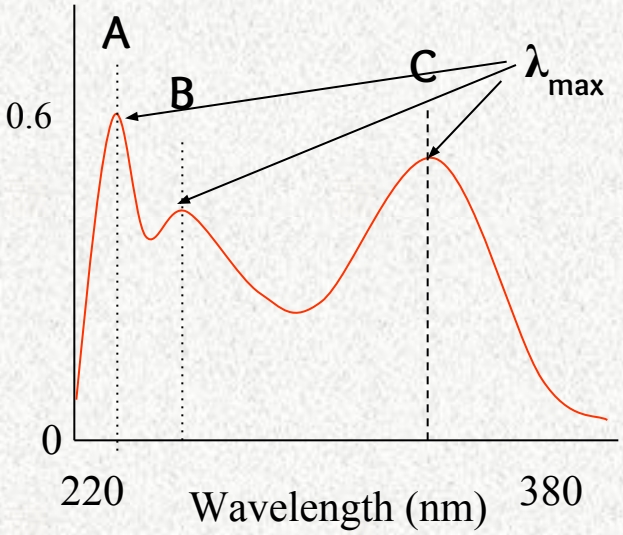
Absorbance not to exceed 1 to reduce error*

CHOOSE CORRECT WAVELENGTH
An analyte may give more than one absorbance maxima (λ_{max}) value.

Many compounds absorb at 220-230nm hence do not use A

Need to choose wavelength more specific to compound (SELECTIVITY) and if more than one select one with highest absorbance as this gives less error – hence use C





Let's play a bit !

A couple of things to take into account…

The intensity of incident light from the light source is always 110.0 photons/sec

You have to calculate Transmittance $T = I_t / I_o$ and Absorbance $A = -\text{Log } T$ by yourself and supply the website with the values you obtain

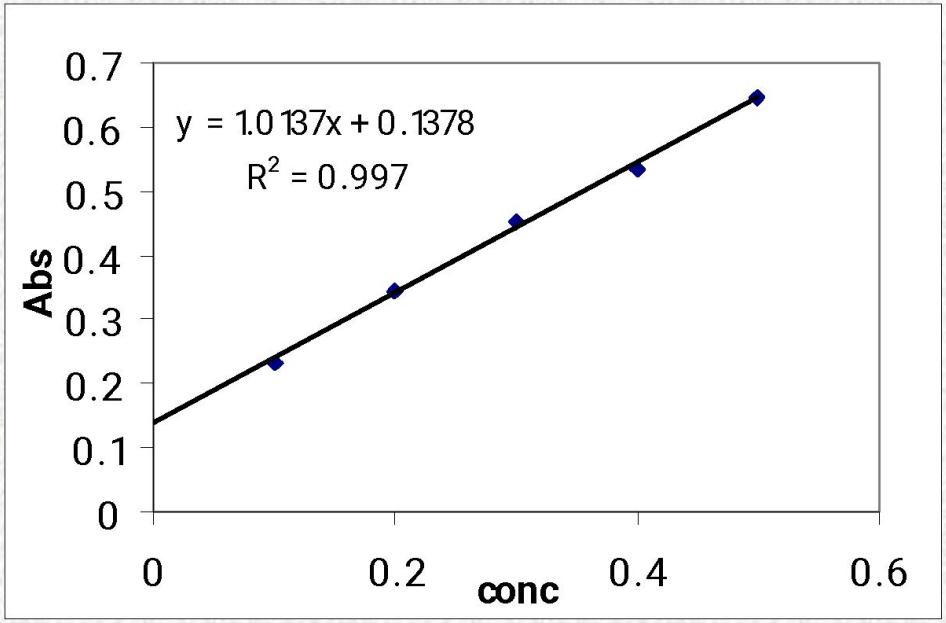
Now you can play with the virtual spectrophotometer changing the path length, concentration, calculate the Molar Absorptivity (or Molar Extinction Coefficient) And run a calibration curve….

Enjoy!!

Example of calculations for photometry

Given the following set of data for a compound C:
Can you give the least square equation better fitting the curve?
(Conc=X, Abs=Y)

Conc (M)	Abs
0.1	0.2322
0.2	0.3456
0.3	0.4532
0.4	0.5331
0.5	0.6453



Is the fitting of the curve to the equation acceptable? How can you tell?

What is the concentration of C when we obtain an Absorbance of 0.3321?

The concentration is: Abs= 1.0137 * Conc + 0.1378

Abs= 0.3321 – Abs blank= 0.3321- 0.13800 = 0.1941

$$\text{Conc} = \frac{\text{Abs} - 0.1378}{1.0137} = \frac{0.1941 - 0.1378}{1.0137} = 0.055\ \text{M}$$

Acknowledgements

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