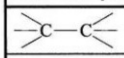
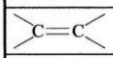
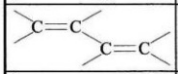
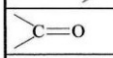
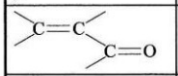
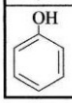
UV SPECTROSCOPY

Chromophore	Wavelength / nm
	150
	150
	190
	210-230
	195 (280 weak)
	210-250
	254
	270

Chromophore = portion of a molecule that absorbs light

Conjugated structure = 2+ adjacent C=C double bonds
More pi-electrons = more conjugated double bonds
↳ compound will absorb more light and have a longer wavelength and chromophore

Analytical purposes

When a beam of radiation is passed through a sample,
- some radiation is **absorbed** by the sample
- some radiation is **reflected** or **scattered**
- some radiation **passes** straight through

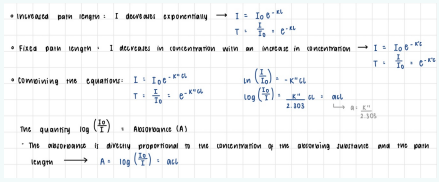
For analytical purposes, we are interested in the **amount ABSORBED** by the sample and so we want to eliminate reflection and scattering.

This is done by:
- taking I0 as the intensity of the light passing through a cell when filled with a **blank solution** (everything except substance being measured)
taking I as the intensity passing through the cell when filled with the **sample**

Calculating transmittance

$$Percentage\ transmittance = \frac{I}{I_o} \times 100$$

Equations



Beer-Lambert Law

Beer-Lambert law= dependence of absorption on concentration and path length

A=acl

a = absorption coefficient
Represents the absorbance of a solution of unit concentration when measured in a cell of unit path length

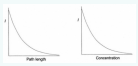
Quantitative analysis

Two methods are used to convert the measured absorbance into a concentration:
1. Calibration curve
2. Using the Beer-Lambert law with a given 'a' value

Calibration curve	5 or more standards are prepared from a sample of pure material to be determined. Their absorbance is measured and their values are used to construct a calibration curve. The sample absorbance is measured and its concentration is read off the curve. **A pure analyte sample must be available**
Beer-Lambert law	Use of **A=acl** will be needed with additional steps to work out the dilution factor

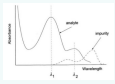
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Relationship between path length and concentration



The value of T (or I) depends on the cell's path length, concentration of absorbing substances and the nature of the substance.
- Increased path length = decreased intensity → light passes MORE through solution and interacts MORE with molecule

Wavelength selection

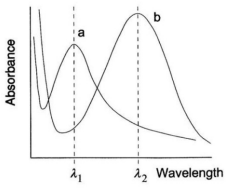


The wavelength for the assay should be chosen so that only the substance of interest absorbs, with no impurities

Analysis of mixtures

If we assume that the components of the mixture do not react with one another, then the absorbance at some wavelength = the sum of absorbance for each component

Mixture of 2 substances



At wavelength λ_1,

$$A(\lambda_1) = \varepsilon_a(\lambda_1)c_a l + \varepsilon_b(\lambda_1)c_b l$$

At wavelength λ_2,

$$A(\lambda_2) = \varepsilon_a(\lambda_2)c_a l + \varepsilon_b(\lambda_2)c_b l$$

For a mixture of 2 substances with different chromophores, each will have different powers of light absorption at some wavelength(s) in the spectrum.
If measurements are made on the mixture at 1 and 2, then a pair of simultaneous equations can be set up to find the unknown concentrations.

FLUORESCENCE SPECTROSCOPY

What is fluorescence?

> When molecules absorb UV-visible radiation, they absorbed energy is converted into kinetic energy due to collisions.
> A few excited molecules get rid of excess energy by emitting the absorbed energy as light = **FLUORESCENCE**

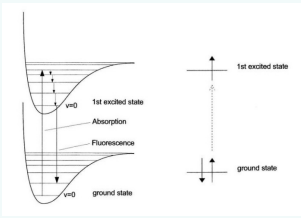
Why use fluorescence?

Fluorescence is more selective than absorption of energy
↳ The chances of finding 2 substances which absorb **and emit** at the same wavelength is **LESS** than finding 2 substances that absorb at the same wavelength

Not all substances fluoresce
↳ Advantage: it makes fluorimetry more selective for compounds that fluoresce
Disadvantage: not all compounds fluoresce

It's more selective than absorption spectrophotometry

Process of fluorescence



- The emitted radiation is of lower energy than the absorbed radiation
- Molecules in the ground state absorb light and are excited to a vibrational level in the 1st excited state
- Excess vibration is then lost by collisions with other molecules until they are in the V=0 level of the 1st excited state
- The molecules get rid of the remaining energy by emitting it as radiation and dropping to a vibrational level in the ground state

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Types of fluorescence

1. Chemi--lumine-scence	When the product molecules are left in an excited state, light is emitted when the molecules return to the ground state
2. Bio-lu-min-escence	Biochemical reactions that produce light

Relationship between structure and fluorescence

e.g. Fluorescein → fluorescent and rigid structure (prevents loss of energy)
e.g. Phenolphthalein → non-fluorescent and non-rigid structure (can twist which converts its absorbed energy into rotational and vibrational energy)

Fluorescence enhancers and inhibitors

Fluorescence enhancers	Fluorescence inhibitors
OH, OCH3, NH2, NHR, NR2 ↳ Increase the no. of delocalised electrons	COOH, NO2, NO, F, Cl, Br, I

Factors affecting fluorescence intensity

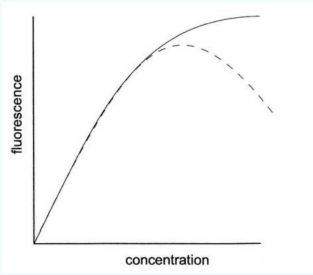
$$I = I_0\, 10^{-\varepsilon c l}$$

$$F \propto (I_o - I)$$

More energy absorbed = more energy emitted
Intensity of fluorescence is PROPORTIONAL to amount of light absorbed

Relationship between I0 and I given by the Beer-Lambert law
- Intensity of emitted radiation is directly proportional to conc
- Fluorescence is directly proportional to intensity of excitation state
↳ brighter lamp = greater fluorescence
- Fluorescence depends on molar absorption coefficient (E)
↳ stronger absorbance = greater fluorescence

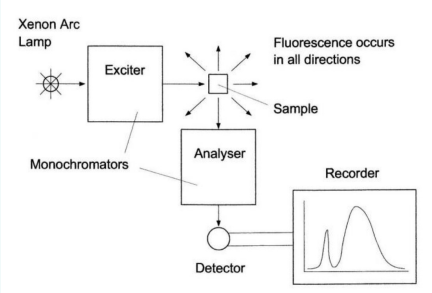
Fluorescence intensity vs. concentration



At high concentrations, the fluorescence emission becomes concentrated near the **sides of the sample cell** to which the exciting radiation enters.
As the exciting radiation passes through the sample, its intensity falls and the fluorescence produced also decreases.

Under these conditions, the equation for fluorescence intensity no longer holds (as Ecl is no longer small

Experimental measurement of fluorescence



1. Light source
- As intense as possible at wavelength of max absorption
- Continuous source
- Wavelength region of 200-500nm

2. Excitor and detector monochromator
- Used to select excitation/emission wavelengths
- Low diminishing and high light gathering powers
- Filters can be used but are less versatile

3. Sample cell
- Usually silica
- 1x1cm
- 4 clear, optically worked surfaces

4. Detector
- Must be sensitive as possible (varies)
- Photomultiplier tube

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Factors affecting fluorescence measurements	
1. Lower limit of detection	Lowest concentration of substance that can be determined
2. Fluorescent impurities	Usually from the reagents or from the cell Ensure the cell is clean and is always placed in the same way (as the 4 sides have different background intensities)
3. Photodecomposition	The sample can undergo decomposition due to the high intensity of the exciting radiation samples, and standards should NOT be exposed to the radiation longer than necessary
4. Quenching	Substance fluorescence is affected more by its environment then the absorption of the sample **Quenching = when fluorescence is decreased by the presence of another sample**

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