# *Instrumental Analysis*

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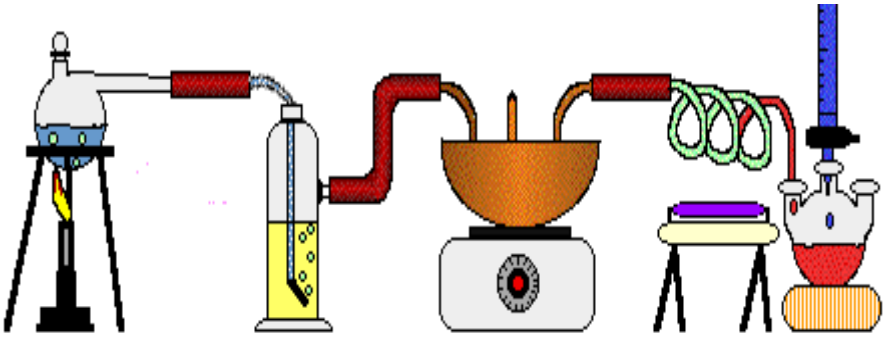

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# المصادر:

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# Introduction

Analytical chemistry deals with methods determining the chemical composition of sample of matter**. Qualitative methods** yields information about the identify of atomic or molecular species or the functional groups in the sample **.quantitative methods** ,in contrast , provides numerical information as to the relative amount of one or more of these components.

## Classification of analytical methods

Analytical methods are often classified as a being either **classical** or **instrumental classical methods**, sometimes called **wet-chemical methods** preceded instrumental methods by century or more.

## Classical methods

In the early years of chemistry, most analysis were carried out by separating the components of interest (the analytes) by precipitation, extraction or distillation .for **qualitative analysis**, the separated components were then treated with reagents that yielded products that could be recognized by their colors, their boiling or melting points, their solubilities in a series of solvents, their odors, their optical activates or their refracts indexes for **quantitative analysis**, the amount of analyte was determined by **gravimetric** or by **volumetric** measurement.

**In gravimetric measurements** the mass of analyte or some compounds produced from the analyte was determined .**in volumetric,** also called titrimetric, procedures, the volume or mass of standerd reagents required to react completely with analyte was measured .

These classical methods for separating and determinatinganalytes are still used in many laboratories .the extent of their general application is, however ,decreasing with the passage of time and with the advent of instrumental methods to supplant them.

## Instrumental methods

Early in the twentieth century, scientists to exploit phenomena other than those used of classical methods for solving analytical problem .thus, measurements of such analyte physical properties as conductivity, electrode potential, light absorption or emission, mass-to-charge ratio, and fluorescence began to be used for quantitative analysis furthermore ,highly efficient chromatographic and electrophoresis techniques began to replace distillation ,extraction and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination these newer methods for separating and determination chemical species are known instrumental methods of analysis.

# Type of instrumental methods

Chemical and physical characteristics are very useful for qualitative or quantitative analysis .the table1 lists most of characteristics properties that are currently used for instrumental analysis.

Table 1: phesical and chemical properties used in instrumental methods

| **Characteristics properties** | **Instrumental methods** |
|---|---|
| Emission of radiation | Emission spectroscopy ,fluorescence, phosphorescence |
| Absorption of radiation | Spectrophotometry and photometry …… |
| Scattering of radiation | Turbidimetry ….. |
| Refraction of radiation | Reflractometry |
| Diffraction of radiation | X-ray and electron diffraction methods |
| Rotation of radiation | Polarimetry, optical rotary… |
| Electrical potential | Potiantiometry |
| Electrical charge | Coulometry |
| Electrical current | Amperometry ,polarography |
| Electrical resistance | Conductometry |
| Mass | Gravimetry |
| Mass-to-charge ratio | Mass spectroscopy |
| Rate of reaction | Kinetic methods |
| Thermal characteristics | Thermal gravimetry ,differential thermal analysis |
| Radioactivity | Activation and isotope dilution methods |

# Error Analysis and propagation

**This section shows how to calculate and propagate experimental errors and discusses the effects of uncertainty on empirical findings.**

**Uncertainty Analysis**

Uncertainty analysis is a mathematical tool for analyzing the imprecision and inaccuracies introduced into an experiment during measurement. Such an analysis is necessary for several reasons:

- You need to know how well you can trust your results.
- You should know if your experimental results agree with any previously measured or theoretical values. Knowing the inaccuracies associated with your results allows you to make more meaning full comparisons. Is the difference between your result and the theoretical value significant or is it just a result of experimental (i.e., measurement) uncertainty?
- If you have measured something in two different ways, you need to know if the difference between the two values is larger or smaller than the measurement uncertainty. Is one method more accurate than another?
- You should know where the greatest uncertainties exist in an experiment so that the procedure may be improved.

**Precision and Accuracy**

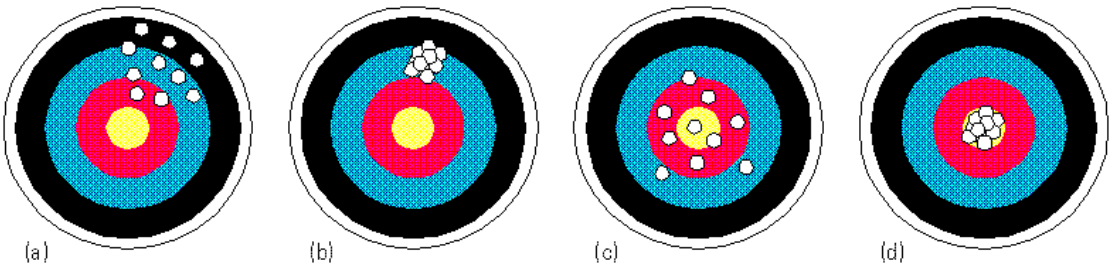
Two terms are widely used in discussions of the reliability of data: **precision and accuracy**.

**Precision** describes the reproducibility of results, that is, the agreement between numerical values for two or more measurements that have been made under identical conditions. In general the precision of a given analytical **method is obtained by simply repeating the measurement**. To describe the precision of a set of replicate data three statistics are widely used: **standard deviation, variance and coefficient of variation**.

**Accuracy** is related to the **CORRECTNESS** of an experimental result. Accuracy is a relative term, which finally depends on the magnitude of the amount measured. Accuracy is expressed in terms of either **absolute error or relative error**.

**N.I:Accuracy refers to how close a measurement or result is to being correct. Precision refers to the repeatability of a measurement. A precise measurement or result is not necessarily accurate.**

**Precision and Accuracy are demonstrate in the following figures:**




| Precision – Accuracy – | Precision + Accuracy – | Precision – Accuracy + | Precision + Accuracy + |
|---|---|---|---|

## Random (indeterminate) and Systematic (determinate) Errors

We will be concerned with two types of errors: random (indeterminate) and systematic (determinate) errors.

**Random Errors**

There is some random error associated with any measurement you make. Random errors come primarily from the inherent limits of the accuracy of an instrument, a piece of glassware, or other experimental apparatus. When you measure the length of an object with a millimeter ruler; you can clearly distinguish between 3 mm and 4 mm, but the gradations are too closely spaced for you to very clearly distinguish between 3.5 and 3.6 mm.

The limitation on the **accuracy** of your measurement comes from the ruler itself. **These errors are random or indeterminate errors because they do not occur in any particular direction**; they are present in every measurement. In the case of the ruler, if the actual length of the object were 3.7 mm, you might, in 6 different measurements, read:

| | | |
|---|---|---|
| 3.7 | 3.5 | 4.0 |
| 3.2 | 4.2 | 3.4 |

The errors are just as likely to be on the low side of the accepted value as they are to be on the high side. **Random error is an inherent part of any measurement, so there is no way to avoid it**. We can reduce the effect of random error on our results by repeating the experiment several times. What you must learn from this section is how random errors affect your experimental results.

## Systematic Errors

Systematic errors are distinguished from random errors largely by being unidirectional and of a consistent degree. A systematic error causes consistently high or consistently low results. Systematic errors in your results can be attributed to the following three sources: **instrumental errors, personal errors and errors introduced by the method of experimentation.** Specifics about possible sources of error and how to correct these errors follows.

### 1. Instrumental Errors

### a. Typical sources of instrumental errors include:

- ❖ Laboratory thermometers sometimes read a degree high or a degree low on every measurement. This is a very common systematic error.
- ❖ Reagents which have decomposed, and whose concentrations have therefore changed, can cause consistently low results in titrations.
- ❖ Balances may be defective, consistently indicating a mass too high or too low.
- ❖ Dirty glassware causes errors by delivering less than the calibrated volume of solution, or by adding impurities to the reagent.

### b. Ways to correct or compensate for these errors:

- Thermometers can be calibrated by measuring the melting points of a number of compounds whose melting points are sharp and precisely known. If the true melting points can be plotted on a graph against the readings obtained with your thermometer, a calibration curve can be constructed. Every time you take a thermometer reading, you can determine an accurate temperature using the calibration curve. Due to lack of time we will not do this in our laboratory.
- Reagents known to decompose can be prepared and stored under special conditions to minimize their deterioration. They can also be **standardized** against reagents of known concentration. Standardization will be done for several reagents in this course.
- Balances, like thermometers, can be calibrated as well**.** There is no way to compensate for a **defective balance** except by being observant. If a balance appears to behave strangely or gives you a 'suspicious' reading, use another balance and report the defective balance to your instructor.
- Glassware must be thoroughly cleaned before each use, and rinsed with the solution with which you will be working.

**2. Personal Errors**

An almost universal source of error is prejudice. Most of us have the natural tendency to estimate scale readings in a direction that improves the precision in a set of results or causes the results to fall closer to a preconceived notion of the accepted value for the measurement.

Personal errors may also be the result of so-called operator defects or blunders. Blunders include adding the wrong reagent or the wrong amount, dropping your sample, or failing to record a pertinent piece of data. Blunders can also account for random errors in your experiment. If you pay careful attention to the procedures and keep a complete record of your observations, you need never compromise your experiments with a major blunder.

**3. Method Errors**

**Examples of systematic errors caused by incorrect method**

- ❖ Running a reaction at too high a temperature may decompose the products, causing a low yield.
- ❖ If the end of a reaction is signaled by an indicator color change, indicators that change color before or after the end of the reaction (endpoint) will cause erroneous results.

**Differentiating between Systematic and Random Error**

You need to be able to differentiate between systematic and random errors and the effects they have on results. Do not confuse a set of random errors that all occur in one direction with systematic error. For example, say you are recording the temperatures of five different solutions whose actual temperatures are shown in the chart below:

| Solution | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Temperature (˚C) | 5 | 10 | 15 | 20 | 25 |

Let us consider what occurs if a systematic error is present. For instance, say the thermometer used consistently reads two degrees too high. The results would then look like this:

| Solution | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Temperature (˚C) | 7 | 12 | 17 | 22 | 27 |

Now consider a case of random error: because you cannot see the level of the alcohol very well, you misread the thermometer every time. You then obtain the following results:

| Solution | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Temperature (˚C) | 6 | 13 | 18 | 21 | 27 |

In both cases, the erroneous results are **higher** than the actual values, but in the systematic error case, the results are consistent
- they are always two degrees higher than the actual value. In the random error case, the results vary, sometimes one degree higher than actual, sometimes three degrees higher than actual. Just because a set of errors is unidirectional does not mean that it is systematic.
**Random error is unpredictable and affects precision, while systematicerror is more regular and affects accuracy.**

## Calculating and Reporting Errors

The calculation of uncertainty in results begins with an estimate of the absolute uncertainty in each measurement. For the instruments you will use, the absolute uncertainties, determined by calculating the standard deviations of successive measurements, are as follow

| Instrument | Absolute Uncertainty |
|---|---|
| 50mL Buret | ± 0.05 mL |
| 250mL Volumetric Flask | ± 0.1 mL |
| 100mL Volumetric Flask | ± 0.08 mL |
| 10mL Graduated Pipette | ± 0.1 mL |
| 1mL Graduated Pipette | ± 0.02 mL |
| Mettler Balance | ± 0.001 g |
| 260°C/360°C Thermometer | ± 0.5 °C |
| 50°C Thermometer | ± 0.05 °C |
| 100mL Graduated Cylinder | ± 0.4 mL |
| 10mL Graduated Cylinder | ± 0.1 mL |
| LKB Visible Spectrophotometer | ± 0.004 (0-1 Absorbance scale)<br>± 0.04 (0-2 Absorbance scale)<br>± 0.4 (%T scale) |
| stopwatch<br>millimeter ruler | ± 0.05 s<br>± 0.5 mm |
| pH meter | ± 0.01 pH units |

For instruments other than the ones mentioned, intelligent estimates of uncertainty can be made. Linearly scaled instruments generally allow measurements as accurate as one-fifth of a gradation. A 50-ml graduated cylinder, for example, has gradations of one ml and an uncertainty of 1 ml x 0.2 = ±0.2 ml. In such instruments, the gradations are spaced far enough apart to allow clear distinctions between one-fourth of the gradation or one-half of the gradation.
For instruments with closely spaced gradations (such as a millimeter ruler), such distinctions are not possible. The uncertainty in such

instruments is taken as half the gradation. For a millimeter ruler, this is 0.5 x 1 mm = 0.5 mm

**Statistical analysis**

How to choose an analytical method? How good is measurement?
How reproducible? - Precision
How close to true value? - Accuracy/Bias
How small a difference can be measured? - Sensitivity
What range of amounts? - Dynamic Range
How much interference? – Selectivity

**mean**. **It is calculated by adding together the numerical values of all measurements and dividing this sum by the number of measurements.**

$$\text{mean} = \bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

**Deviation**: How much each measurement differs from the mean is an important number and is called the **deviation.** A deviation is associated with each measurement, and if a given deviation is large compared to others in a series of identical measurements,

$$\text{deviation} = (x_i - \bar{x})$$

**Precision** - Indeterminate or random errors
Absolute standard deviation:

$$s = \sqrt{\frac{\sum_{i-0}^{i=N} (x_i - \bar{x})^2}{N-1}}$$

***Standard deviation,*** : A useful measure of the precision of the average of a series of *n* measurements **(xi)** of the same quantity;

Relative standard deviation: RSD = s\x
relative % standard deviation = RSDx100

$$\%E_{rel} = \frac{d}{u} \times 100$$

**Accuracy** - Determinate errors (operator, method, instrumental)
'***Absolute error***' is the difference between the ***experimental value***and the ***truevalue***.

***Example :*** An analyst determines a value of 70.55% cineole in a fresh sample of Eucalyptus Oil that actually contains 70.25%, the absolute error is given by :
**70.55 – 70.25 = 0.30%**
The error thus obtained is invariably stated with regard to the actual size of the measured quantity *i.e.*, either in percent (%) or in parts per thousand (ppt). Therefore, the relative error is given

$$\frac{0.30}{70.25} \times 100 = 0.42\% \quad \text{or} \quad \frac{0.30}{70.25} \times 1000 = 4.2 \text{ ppt}$$

**Example 1.1**

The following numerical results were obtained in a given laboratory experiment: 0.09376, 0.09358, 0.09385, and 0.09369. Calculate the relative parts per thousand standard deviation.

***Solution 1.1***

We must calculate both the mean and the standard deviation in order to use Equations (1.3) and (1.5).

First, the mean, m:

$$m = \frac{0.09376 + 0.09358 + 0.09385 + 0.09369}{4} = 0.09372$$

Next, the deviations:

$$d_1 = |0.09372 - 0.9376| = 0.00004$$
$$d_2 = |0.09372 - 0.09358| = 0.00014$$
$$d_3 = |0.09372 - 0.09385| = 0.00013$$
$$d_4 = |0.09372 - 0.09369| = 0.00003$$

Then, the standard deviation:

$$s = \sqrt{\frac{(0.00004)^2 + (0.00014)^2 + (0.00013)^2 + (0.00003)^2}{(4-1)}}$$

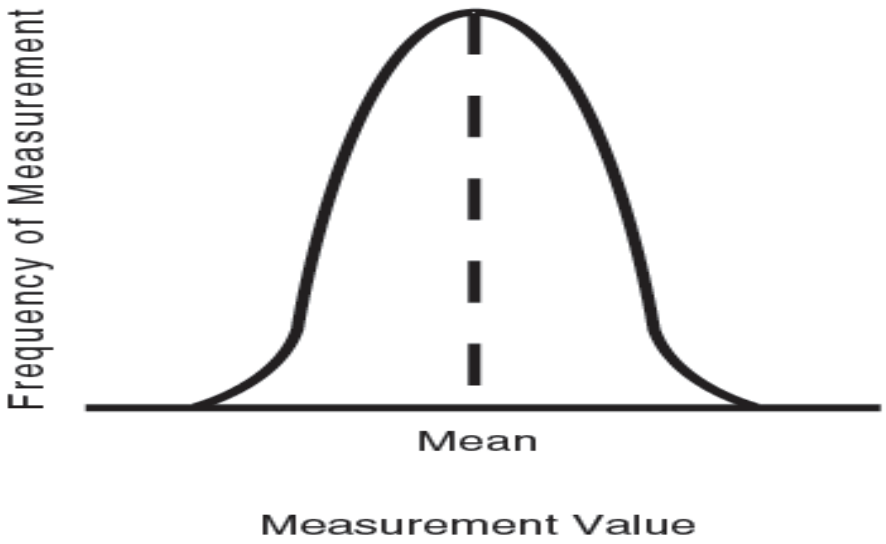
$$= 1.14 \times 10^{-4} = 1.1 \times 10^{-4}$$

Finally, to get the relative parts per thousand standard deviation:

$$RSD = \frac{s}{m} \times 1000 = \frac{1.14 \times 10^{-4}}{0.09372} \times 1000 = 1.2$$

## 7.3 Normal Distribution

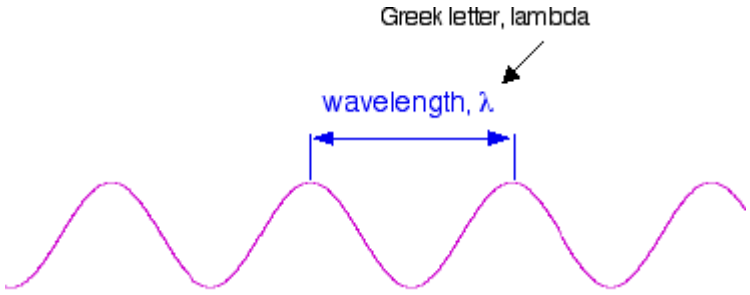
For an infinite data set. A plot of frequency of occurrence *vs* the measurement value yields a smooth bell-shaped curve. It is referred to as bell-shaped because there is equal drop-off on both sides of a peak value, resulting in a shape that resembles a bell. The peak value corresponds to m , the population mean. This curve is called the **normal distribution curve** because it represents a normal distribution of values for any infinitely repeated measurement.



# ELECTROMAGNETIC RADIATION

**Wavelength, frequency and the speed of light**

If you draw a beam of light in the form of a wave (without worrying too much about what exactly is causing the wave!), **the distance between two crests is called the *wavelength* of the light. (It could equally well be the distance between two troughs or any other two identical positions on the wave.)**

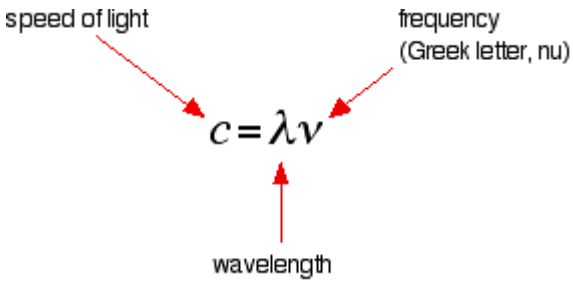



You have to picture these wave crests as moving from left to right. If you counted the **number of crests passing a particular point per second**, you have the ***frequency*** of the light. It is measured in what used to be called "cycles per second", but is now called ***Hertz***, Hz. Cycles per second and Hertz mean *exactly* the same thing.

Orange light, for example, has a frequency of about 5 x $10^{14}$ Hz (often quoted as 5 x $10^{8}$ MHz - megahertz). That means that 5 x $10^{14}$ wave peaks pass a given point every second.

Light has a constant speed through a given substance. For example, it always travels at a speed of approximately 3 x $10^{8}$metres per second in a vacuum. This is actually the speed that all electromagnetic radiation travels - not just visible light.

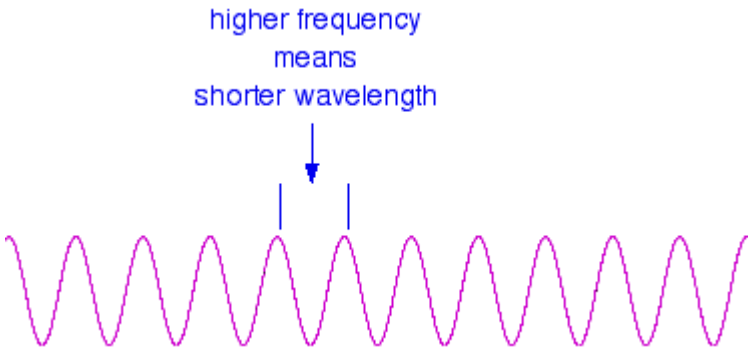
There is a simple relationship between the wavelength and frequency of a particular colour of light and the speed of light:




. . . and you can rearrange this to work out the wavelength from a given frequency and vice versa:

$$\lambda = \frac{c}{\nu} \qquad \nu = \frac{c}{\lambda}$$

**These relationships mean that if you increase the frequency, you must decrease the wavelength.**




Compare this diagram with the similar one above.

and, of course, the opposite is true. **If the wavelength is longer, the frequency is lower**.

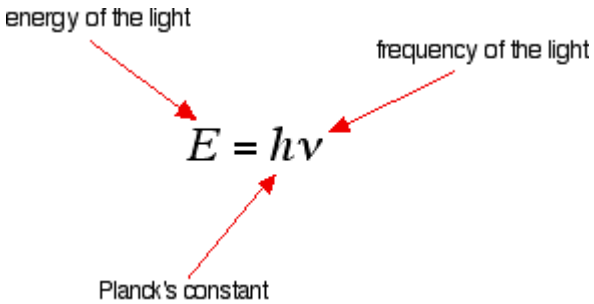
It is really important that you feel comfortable with the relationship between frequency and wavelength. If you are given two figures for the wavelengths of two different colours of light, you need to have an immediate feel for which one has the higher frequency.

For example, if you were told that a particular colour of red light had a wavelength of 650 nm, and a green had a wavelength of 540 nm, it is important for you to know which has the higher frequency. (It's the green - a shorter wavelength means a higher frequency. Don't go on until that feels right!)

## Note: nm = nanometre = $10^{-9}$metre

### The frequency of light and its energy

Each particular frequency of light has a particular energy associated with it, given by another simple equation:




**You can see that the higher the frequency, the higher the energy of the light.**

Light which has wavelengths of around 380 - 435 nm is seen as a sequence of violet colours. Various red colours have wavelengths around 625 - 740 nm. **Which has the highest energy?**

The light with the highest energy will be the one with the highest frequency - that will be the one with the smallest wavelength. In other words, violet light at the 380 nm end of its range.

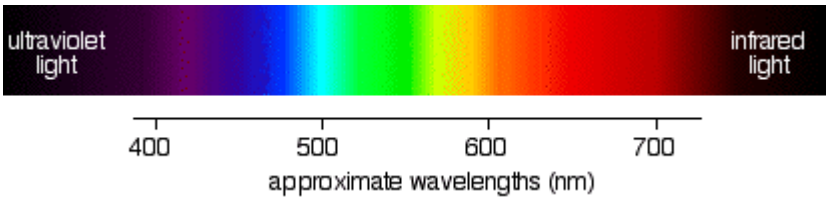
**Wave number:** The number of waves in one centimeter .and it is reversible of wavelength. It is measured by $cm^{-1}$.

$V = 1/\lambda$

## The Electromagnetic Spectrum

### Visible light

The diagram shows an approximation to the spectrum of visible light.




The main colour regions of the spectrum are approximately:

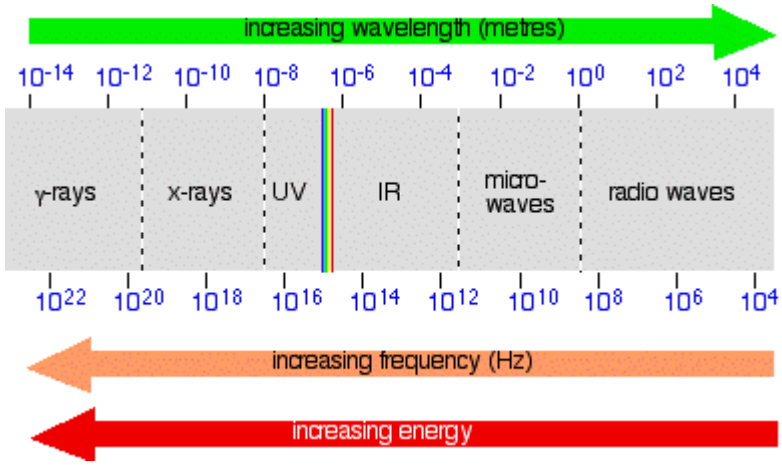
| colour region | wavelength (nm) |
|---|---|
| Violet | 380 - 435 |
| Blue | 435 - 500 |
| Cyan | 500 - 520 |
| Green | 520 - 565 |
| Yellow | 565 - 590 |
| Orange | 590 - 625 |
| Red | 625 - 740 |

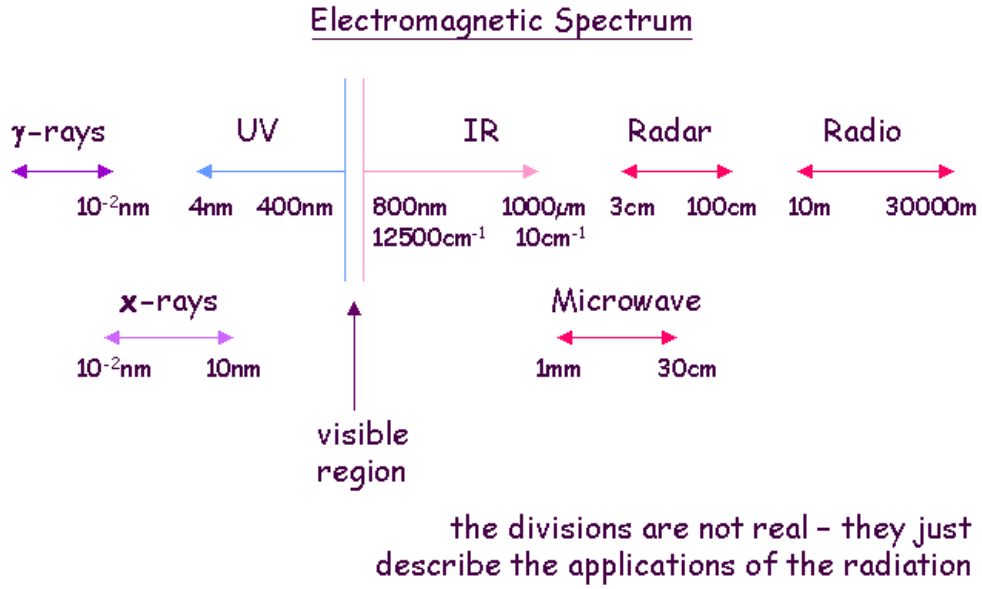
**Don't assume that there is some clear cut-off point between all these colours. In reality, the colours just merge seamlessly into one another - much more seamlessly than in my diagram!**

**Placing the visible spectrum in the whole electromagnetic spectrum**

The electromagnetic spectrum doesn't stop with the colours you can see. It **is perfectly possible to have wavelengths longer than violet light or shorter than red light.**

On the spectrum further up the page, I have shown the ultra-violet and the infra-red, but this can be extended even further into x-rays and radio waves, amongst others. The diagram shows the approximate positions of some of these on the spectrum.



| Wavelength Range (meters) | Photon energy (kJ/mol) | Spectroscopic region | Spectroscopic technique |
|---|---|---|---|
| $10^{-13}$ | $10^{9}$ | γ-radiation | Mössbauer |
| $10^{-10}$ | $10^{6}$ | X-radiation | X-ray diffraction and X-ray scattering |
| $10^{-7}$ | 1200 | Far ultraviolet | Far UV spectroscopy |
| $3 \times 10^{-7}$ (300 nm) | 430 | Near ultraviolet | UV/Vis spectroscopy |
| $4 \times 10^{-7}$ to $7 \times 10^{-7}$ | 300 to 170 | Visible | UV/Vis spectroscopy |
| $10^{-6}$ to $10^{-4}$ | 120 to 1.2 | Infrared | IR spectroscopy |
| 0.01 | 0.01 | Microwave | Electron paramagnetic resonance |
| 0.1 | 0.001 | Radio | Nuclear magnetic resonance |

**Also be aware that the energy associated with the various kinds of radiation increases as the frequency increases**

The spectrum of electromagnetic radiation as a whole spans the range of wavelengths λ from $10^{10}$ m (infrasound) down to $10^{-14}$ m (cosmic radiation). Ultraviolet (UV) and visible (VIS) radiation comprise only a small portion of wavelengths from about $10^{-6}$ to $10^{-7}$ m (see Fig. 1). For all types of electromagnetic radiation, the associated energy (E) is calculated using Planck's constant:

$$E = h \cdot \nu \qquad 1$$

*E* Energy

*h* Planck's constant ($9.626.10^{-34}$ J $s^{-1}$)

ν Frequency in $s^{-1}$

The frequency of the radiation is calculated according to the following equation:

$$\nu = \frac{c}{\lambda} \qquad 2$$

*c* Velocity of light ($3.10^{8}$ m s–1)

λ Wavelength in nm

In UV/VIS spectroscopy, wavelengths are usually expressed in nanometers (nm = $10^{-9}$ m).The presumably best-known example is the yellow colour of the sodium flame, which is due to the 589.5 and 589.0 nm double line of wavelengths. Equations (1) and (2) also readily show that radiation energy increases with shorter wavelengths. Hence, UV radiation contains more energy than does visible light.

Electromagnetic radiation can be generated with a wide range of wavelengths. Different wavelengths have differing energies, and interact with molecules in different ways.

The energy in a mole of 400 nm photons is calculated by:

$$E = h\nu = \frac{hc}{\lambda} = \left( \frac{\left(6.626x10^{-34} J \quad \text{sec}\right)\left(2.9979x10^{8} \frac{m}{\text{sec}}\right)\left(6.022x10^{23} \frac{photons}{mol}\right)}{400x10^{-9} m} \right)$$

The table below gives the range of wavelengths and energies of photons in the electromagnetic spectrum. High-energy photons contain enough energy to break covalent bonds, although they only break bonds under certain conditions. Low energy photons contain too little energy to disrupt covalent interactions, and are limited to contributing energy to molecules, usually without altering the molecular structure.

| Wavelength Range (meters) | Photon energy (kJ/mol) | Spectroscopic region | Spectroscopic technique |
|---|---|---|---|
| $10^{-13}$ | $10^{9}$ | γ-radiation | Mössbauer |
| $10^{-10}$ | $10^{6}$ | X-radiation | X-ray diffraction and X-ray scattering |
| $10^{-7}$ | 1200 | Far ultraviolet | Far UV spectroscopy |
| $3 \times 10^{-7}$ (300 nm) | 430 | Near ultraviolet | UV/Vis spectroscopy |
| $4 \times 10^{-7}$ to $7 \times 10^{-7}$ | 300 to 170 | Visible | UV/Vis spectroscopy |
| $10^{-6}$ to $10^{-4}$ | 120 to 1.2 | Infrared | IR spectroscopy |
| 0.01 | 0.01 | Microwave | Electron paramagnetic resonance |
| 0.1 | 0.001 | Radio | Nuclear magnetic resonance |

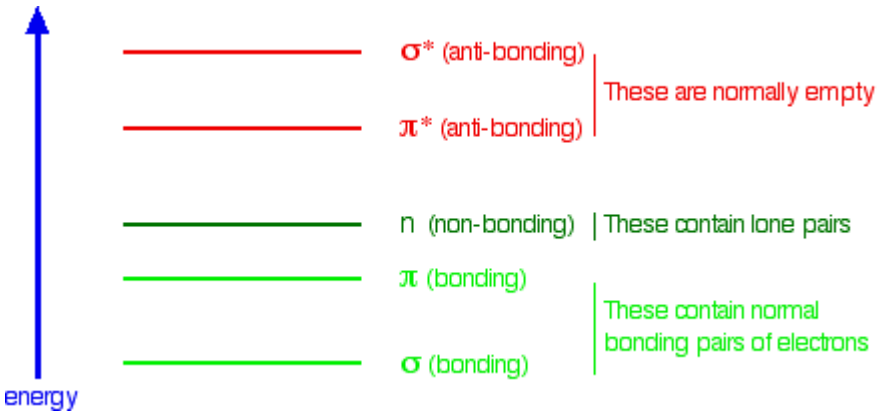
# UV-VISIBLE ABSORPTION SPECTRA

This page explains what happens when organic compounds absorb UV or visible light, and why the wavelength of light absorbed varies from compound to compound.

## What happens when light is absorbed by molecules?

### Promotion of electrons

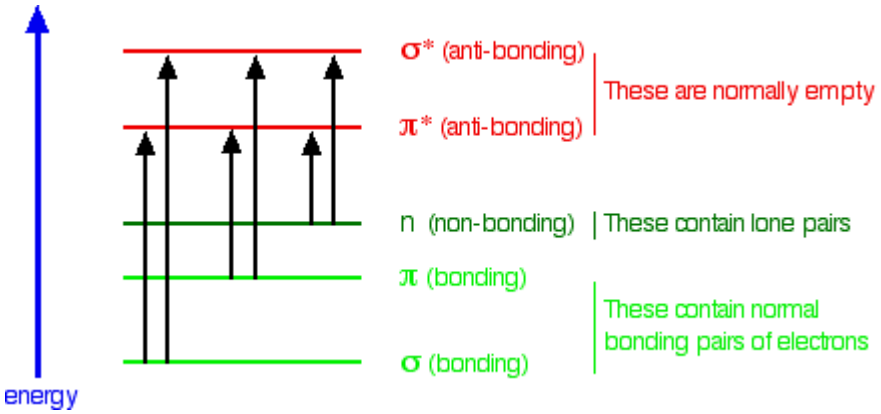
When we were talking about the various sorts of orbitals present in organic compounds on the introductory page you will have come across this diagram showing their relative energies:




Remember that the diagram isn't intended to be to scale - it just shows the relative placing of the different orbitals.

When light passes through the compound, energy from the light is used to promote an electron from a bonding or non-bonding orbital into one of the empty anti-bonding orbitals.

The possible electron jumps that light might cause are:

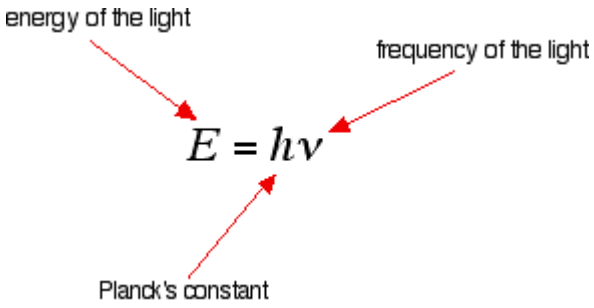



In each possible case, an electron is excited from a full orbital into an empty anti-bonding orbital. Each jump takes energy from the light, and a big jump obviously needs more energy than a small one.

**Each wavelength of light has a particular energy associated with it. If that particular amount of energy is just right for making one of these energy jumps, then that wavelength will be absorbed - its energy will have been used in promoting an electron.**

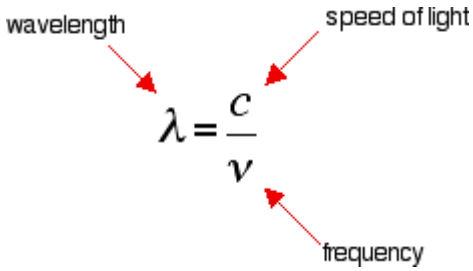
**We need to work out what the relationship is between the energy gap and the wavelength absorbed. Does, for example, a bigger energy gap mean that light of a lower wavelength will be absorbed - or what?**

It is easier to start with the relationship between the *frequency* of light absorbed and its energy:




You can see that if you want a high energy jump, you will have to absorb light of a higher frequency. The greater the frequency, the greater the energy.

That's easy - but unfortunately UV-visible absorption spectra are always given using wavelengths of light rather than frequency. That means that you need to know the relationship between wavelength and frequency.



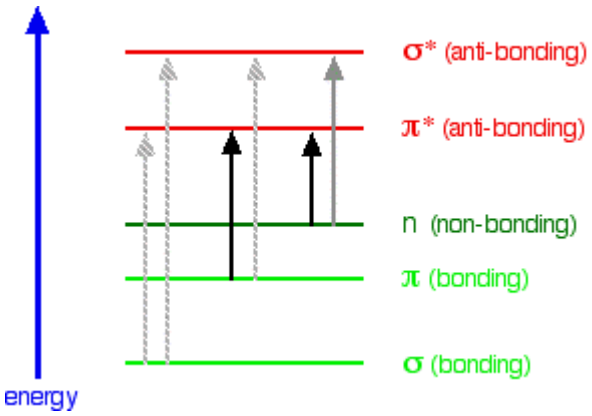

**You can see from this that the higher the frequency is, the lower the wavelength**

**So . . . If you have a bigger energy jump, you will absorb light with a higher frequency - which is the same as saying that you will absorb light with a lower wavelength.**

## Some jumps are more important than others for absorption spectrometry

An absorption spectrometer works in a range from about 200 nm (in the near ultra-violet) to about 800 nm (in the very near infra-red). Only a limited number of the possible electron jumps absorb light in that region.

Look again at the possible jumps. This time, the important jumps are shown in black, and a less important one in grey. The grey dotted arrows show jumps which absorb light outside the region of the spectrum we are working in.




**Remember that bigger jumps need more energy and so absorb light with a shorter wavelength. The jumps shown with grey dotted arrows absorb UV light of wavelength less that 200 nm.**

**The important jumps are:**

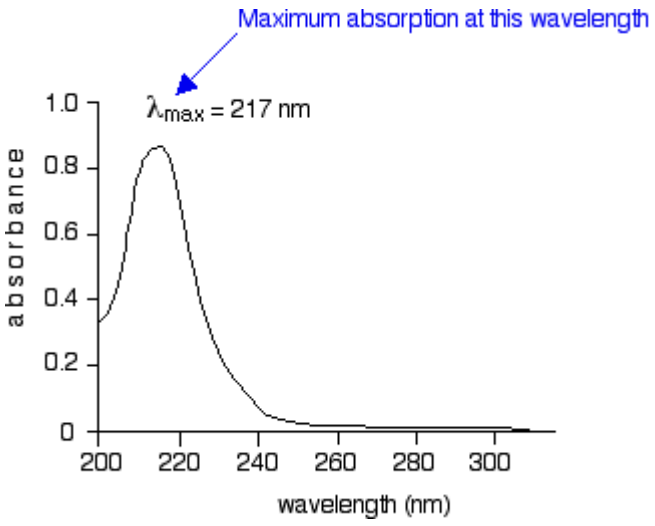
- from pi bonding orbitals to pi anti-bonding orbitals;
- from non-bonding orbitals to pi anti-bonding orbitals;
- from non-bonding orbitals to sigma anti-bonding orbitals.

That means that in order to absorb light in the region from 200 - 800 nm (which is where the spectra are measured), the molecule must contain either pi bonds or atoms with non-bonding orbitals. Remember that a non-bonding orbital is a lone pair on, say, oxygen, nitrogen or a halogen.

Groups in a molecule which absorb light are known as ***chromophores***.

**What does an absorption spectrum look like?**

The diagram below shows a simple UV-visible absorption spectrum for buta-1,3-diene - a molecule we will talk more about later. Absorbance (on the vertical axis) is just a measure of the amount of light absorbed. The higher the value, the more of a particular wavelength is being absorbed.



You will see that absorption peaks at a value of 217 nm. This is in the ultra-violet and so there would be no visible sign of any light being absorbed - buta-1,3-diene is colourless. You read the symbol on the graph as "lambda-max".

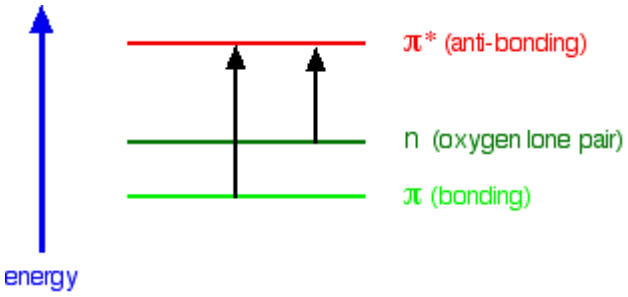
In buta-1,3-diene, $CH_2=CH-CH=CH_2$, there are no non-bonding electrons. That means that the only electron jumps taking place (within the range that the spectrometer can measure) are from pi bonding to pi anti-bonding orbitals.

**A chromophore producing two peaks**

**A chromophore such as the carbon-oxygen double bond in ethanal, for example, obviously has pi electrons as a part of the double bond, but also has lone pairs on the oxygen atom.**

That means that both of the important absorptions from the last energy diagram are possible.

You can get an electron excited from a pi bonding to a pi anti-bonding orbital, or you can get one excited from an oxygen lone pair (a non-bonding orbital) into a pi anti-bonding orbital.




The non-bonding orbital has a higher energy than a pi bonding orbital. That means that the jump from an oxygen lone pair into a pi anti-bonding orbital needs less energy. That means it absorbs light of a lower frequency and therefore a higher wavelength.

Ethanal can therefore absorb light of two different wavelengths:

- the pi bonding to pi anti-bonding absorption peaks at 180 nm;
- the non-bonding to pi anti-bonding absorption peaks at 290 nm.

**Both of these absorptions are in the ultra-violet, but most spectrometers won't pick up the one at 180 nm because they work in the range from 200 - 800 nm.**

The importance of conjugation and delocalisation in what wavelength is absorbed Consider these three molecules:

| $CH_2=CH_2$ | $CH_2=CH-CH=CH_2$ | $CH_2=CH-CH=CH-CH=CH_2$ |
|---|---|---|
| ethene | buta-1,3-diene | hexa-1,3,5-triene |

Ethene contains a simple isolated carbon-carbon double bond, but the other two have conjugated double bonds. In these cases, there is delocalisation of the pi bonding orbitals over the whole molecule.

Now look at the wavelengths of the light which each of these molecules absorbs.

| **molecule** | **wavelength of maximum absorption (nm)** |
|---|---|
| **ethene** | 171 |
| **buta-1,3-diene** | 217 |
| **hexa-1,3,5-triene** | 258 |

**All of the molecules give similar UV-visible absorption spectra - the only difference being that the absorptions move to longer and longer wavelengths as the amount of delocalisation in the molecule increases.**

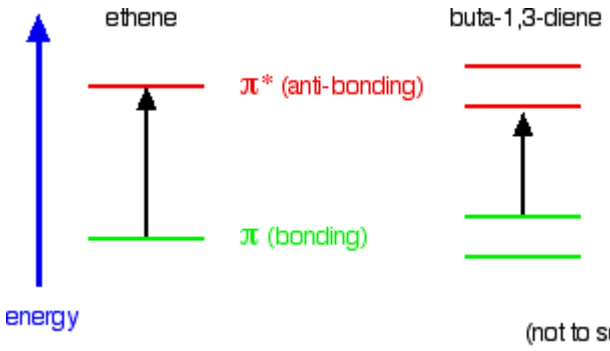
Why is this?

You can actually work out what must be happening

- The maximum absorption is moving to longer wavelengths as the amount of delocalisation increases.
- Therefore maximum absorption is moving to shorter frequencies as the amount of delocalisation increases.
- Therefore absorption needs less energy as the amount of delocalisation increases.
- Therefore there must be less energy gap between the bonding and anti-bonding orbitals as the amount of delocalisation increases.

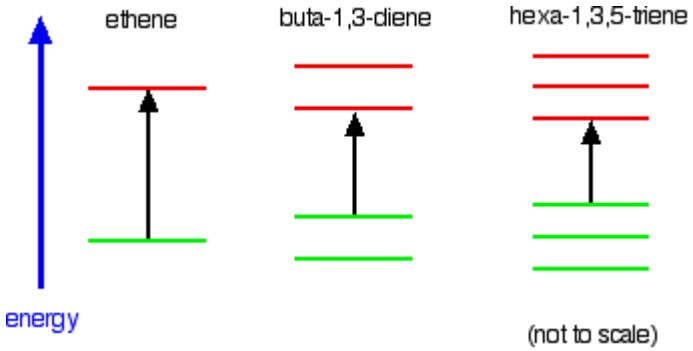
. . . and that's what is happening.

Compare ethene with buta-1,3-diene. In ethene, there is one pi bonding orbital and one pi anti-bonding orbital. In buta-1,3-diene, there are two pi bonding orbitals and two pi anti-bonding orbitals. This is all discussed in detail on the introductory page that you should have read.




The highest occupied molecular orbital is often referred to as the HOMO - in these cases, it is a pi bonding orbital. The lowest unoccupied molecular orbital (the LUMO) is a pi anti-bonding orbital.

**Notice that the gap between these has fallen. It takes less energy to excite an electron in the buta-1,3-diene case than with ethene.In the hexa-1,3,5-triene case, it is less still.**

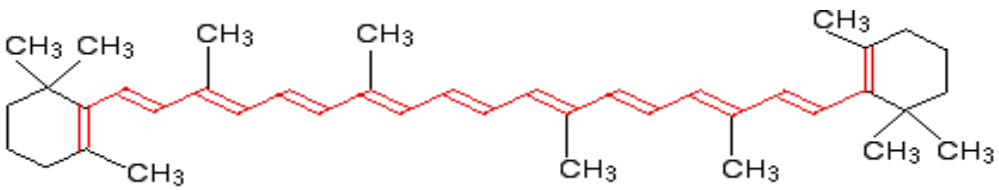



Note: In this case, you will have to work out for yourself why there are 3 bonding and 3 anti-bonding

**If you extend this to compounds with really massive delocalisation, the wavelength absorbed will eventually be high enough to be in the visible region of the spectrum, and the compound will then be seen as coloured. A good example of this is the orange plant pigment, beta-carotene - present in carrots, for example.**

**Why is beta-carotene orange?**

Beta-carotene has the sort of delocalisation that we've just been looking at, but on a much greater scale with 11 carbon-carbon double bonds conjugated together. The diagram shows the structure of beta-carotene with the alternating double and single bonds shown in red.



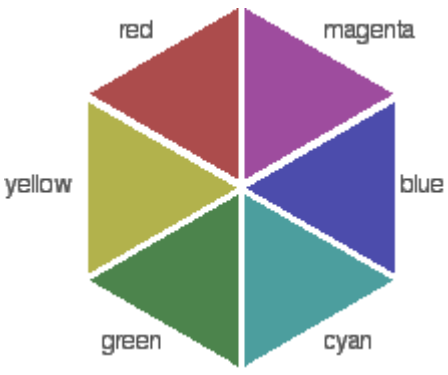
**The more delocalisation there is, the smaller the gap between the highest energy pi bonding orbital and the lowest energy pi anti-bonding orbital. To promote an electron therefore takes less energy in beta-carotene than in the cases we've looked at so far - because the gap between the levels is less.**

**Remember that less energy means a lower frequency of light gets absorbed and that's equivalent to a longer wavelength.**

Beta-carotene absorbs throughout the ultra-violet region into the violet - but particularly strongly in the visible region between about 400 and 500 nm with a peak about 470 nm.

If you have read the page in this section about electromagnetic radiation, you might remember that the wavelengths associated with the various colours are approximately

| colour region | wavelength (nm) |
|---|---|
| violet | 380 - 435 |
| blue | 435 - 500 |
| cyan | 500 - 520 |
| green | 520 - 565 |
| yellow | 565 - 590 |
| orange | 590 - 625 |
| red | 625 - 740 |



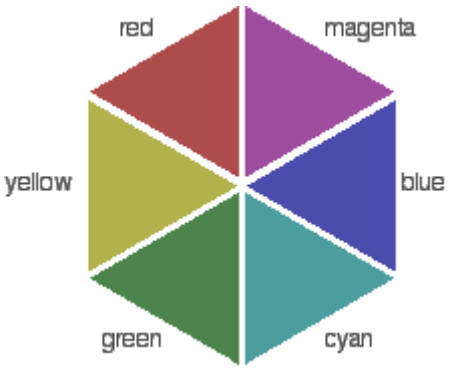

**So if the absorption is strongest in the violet to cyan region, what colour will you actually see?**

Sometimes what you actually see is quite unexpected. Mixing different wavelengths of light doesn't give you the same result as mixing paints or other pigments.

You can, however, sometimes get some estimate of the colour you would see using the idea of ***complementary colours***.

## *Complementary colours*

If you arrange some colours in a circle, you get a "colour wheel". The diagram shows one possible version of this. An internet search will throw up many different versions!




**Colours directly opposite each other on the colour wheel are said to be complementary colours. Blue and yellow are complementary colours; red and cyan are complementary; and so are green and magenta.**

### Mixing together two complementary colours of light will give you white light.

What this all means is that if a particular colour is absorbed from white light, what your eye detects by mixing up all the other wavelengths of light is its complementary colour.

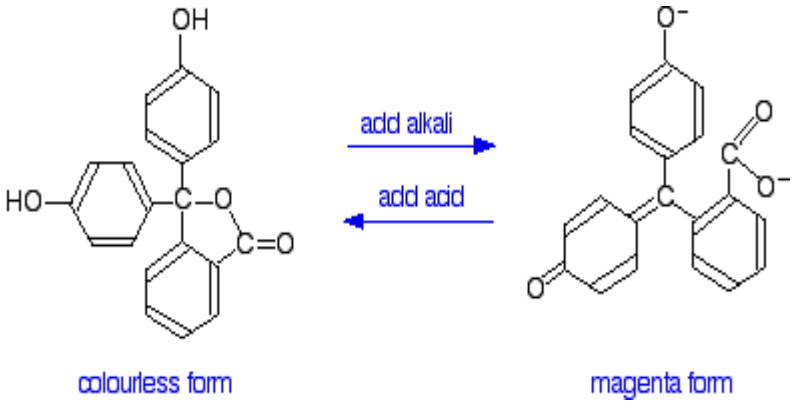
**In the beta-carotene case, the situation is more confused because you are absorbing such a range of wavelengths. However, if you think of the peak absorption running from the blue into the cyan, it would be reasonable to think of the colour you would see as being opposite that where yellow runs into red - in other words, orange.**

## Applying this to the colour changes of two indicators

### Phenolphthalein

You have probably used phenolphthalein as an acid-base indicator, and will know that it is colourless in acidic conditions and magenta (bright pink) in an alkaline solution. How is this colour change related to changes in the molecule?

The structures of the two differently coloured forms are:




Both of these absorb light in the ultra-violet, but the one on the right also absorbs in the visible with a peak at 553 nm.

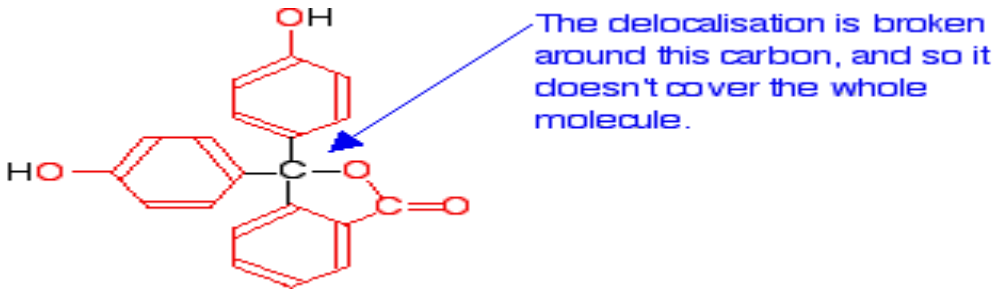
The molecule in acid solution is colourless because our eyes can't detect the fact that some light is being absorbed in the ultra-violet. However, our eyes do detect the absorption at 553 nm produced by the form in alkaline solution.

**553 nm is in the green region of the spectrum. If you look back at the colour wheel, you will find that the complementary colour of green is magenta - and that's the colour you see.**

**So why does the colour change as the structure changes?**

What we have is a shift to absorption at a higher wavelength in alkaline solution. As we've already seen, a shift to higher wavelength is associated with a greater degree of delocalisation.

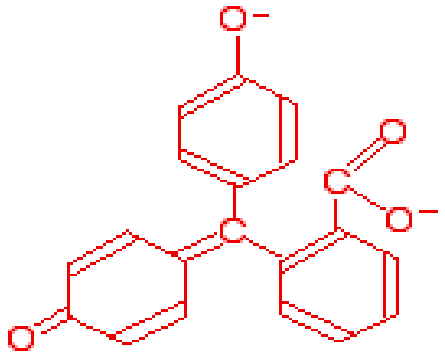
Here is a modified diagram of the structure of the form in acidic solution - the colourless form. The extent of the delocalisation is shown in red.




Notice that there is delocalisation over each of the three rings - extending out over the carbon-oxygen double bond, and to the various oxygen atoms because of their lone pairs.

But the delocalisation doesn't extend over the whole molecule. The carbon atom in the centre with its four single bonds prevents the three delocalised regions interacting with each other.

Now compare that with the magenta form:



The delocalisation covers absolutely everything in the ion formed in alkaline solution.

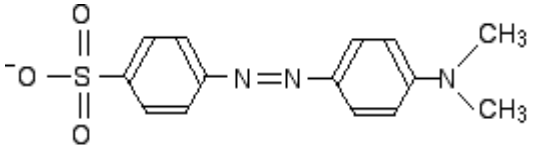
**The rearrangement now lets the delocalisation extend over the entire ion. This greater delocalisation lowers the energy gap between the highest occupied molecular orbital and the lowest unoccupied pi anti-bonding orbital. It needs less energy to make the jump and so a longer wavelength of light is absorbed.**

***Remember: Increasing the amount of delocalisation shifts the absorption peak to a higher wavelength.***

**Methyl orange**

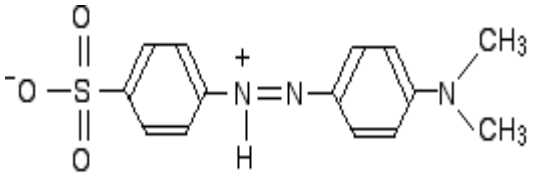
You will know that methyl orange is yellow in alkaline solutions and red in acidic ones.

The structure in alkaline solution is:

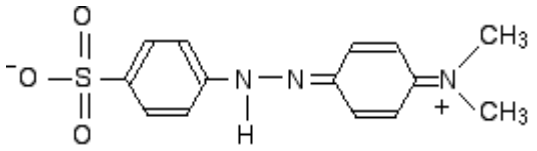


yellow form of methyl orange

In acid solution, a hydrogen ion is (perhaps unexpectedly) picked up on one of the nitrogens in the nitrogen-nitrogen double bond.



This now gets a lot more complicated! The positive charge on the nitrogen is delocalised (spread around over the structure) - especially out towards the right-hand end of the molecule as we've written it. The normally drawn structure for the red form of methyl orange is . . .



red form of methyl orange

***So which is the more delocalised structure - red or yellow?***

Let's work backwards from the absorption spectra to see if that helps.

**The yellow form has an absorption peak at about 440 nm. That's in the blue region of thespectrum, and the complementary colour of blue is yellow. That's exactly what you would expect.**

**The red form has an absorption peak at about 520 nm. That's at the edge of the cyan region of the spectrum, and the complementary colour of cyan is red.**

**Notice that the change from the yellow form to the red form has produced an increase in the wavelength absorbed. An increase in wavelength suggests an increase in delocalisation.**

***That means that there must be more delocalisation in the red form than in the yellow one.***

# UV-Vis Luminescence Spectroscopy

## Introduction

Luminescence is the emission of light by a substance. It occurs when an electron returns to the electronic ground state from an excited state and loses it's excess energy as a photon.

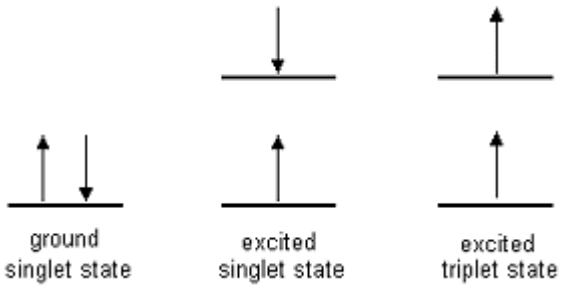
Luminescence spectroscopy is a collective name given to *three* related spectroscopic techniques. They are;

- Molecular fluorescence spectroscopy
- Molecular phosphorescence spectroscopy
- Chemiluminescence spectroscopy

## Fluorescence and phosphorescence (photoluminescence)

The electronic states of most organic molecules can be divided into *singlet* states and *triplet* states;

**Singlet state:** All electrons in the molecule are spin-paired
**Triplet state:** One set of electron spins is unpaired




### Fluorescence

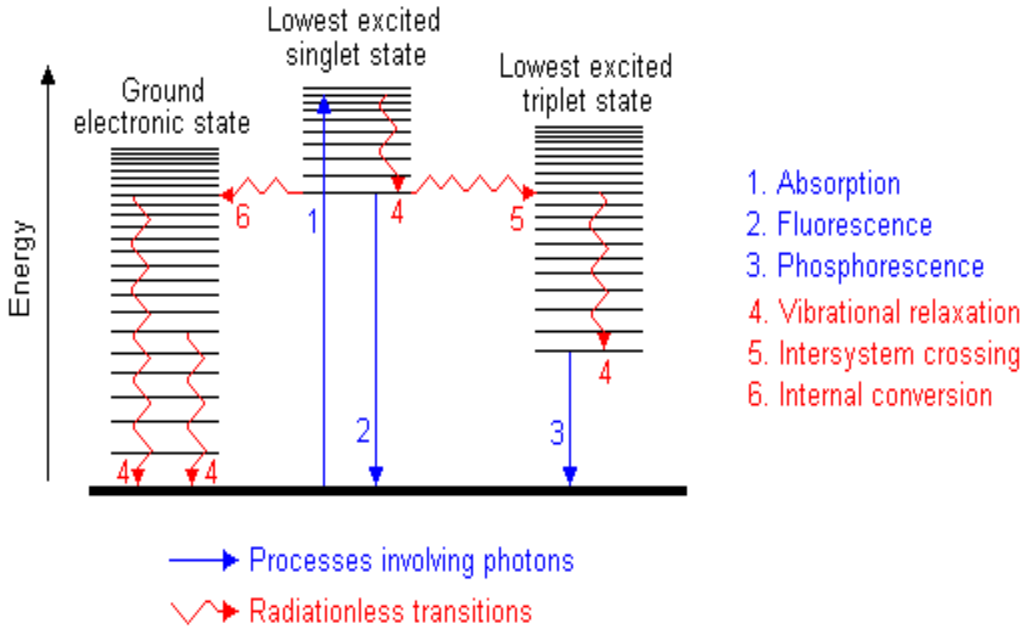
**Absorption of UV radiation by a molecule excites it from a vibrational level in the electronic ground state to one of the many vibrational levels in the electronic excited state**. This excited state is usually the first excited *singlet* state.

A molecule in a high vibrational level of the excited state will quickly fall to the lowest vibrational level of this state by losing energy to other molecules through collision. The molecule will also partition the excess energy to other possible modes of vibration and rotation.

**Fluorescence occurs when the molecule returns to the *electronic* ground state, from the excited singlet state, by emission of a photon.**

If a molecule which absorbs UV radiation does not fluoresce it means that it must have lost its energy some other way. These processes are called***radiationless transfer of energy***. Have a look at this diagram:

**Possible physical process following absorption of a photon by a molecule**




**Intra-molecular redistribution of energy between possible electronic and vibrational states**

**The molecule returns to the electronic ground state.The excess energy is converted to vibrational energy (*internal conversion*),** and so the molecule is placed in an extremely high vibrational level of the electronic ground state. This excess vibrational energy is lost by collision with other molecules (***vibrational relaxation***).

**A combination of intra- and inter-molecular energy redistribution**

**The spin of an excited electron can be reversed, leaving the molecule in an excited *triplet* state; this is called *intersystem crossing***. The triplet state is of a **lower electronic energy** than the excited singlet state.

The probability of this happening is increased if the vibrational levels of these two states overlap. For example, the lowest singlet vibrational level can overlap one of the higher vibrational levels of the triplet state.

A molecule in a high vibrational level of the excited triplet state can lose energy in collision with solvent molecules, leaving it at the lowest vibrational level of the triplet state

**Phosphorescence**

A molecule in the excited triplet state may not always use intersystem crossing to return to the ground state. It could lose energy by emission of a photon. A triplet/singlet transition is much less probable than a singlet/singlet transition. The lifetime of the excited triplet state can be up to 10 seconds, in comparison with $10^{-5}$ s to $10^{-8}$ s average lifetime of an excited singlet state. Emission from triplet/singlet transitions can continue after initial irradiation. Internal conversion and other

radiationless transfers of energy compete so successfully with phosphorescence that it is usually seen only at low temperatures or in highly viscous media.

### Chemiluminescence

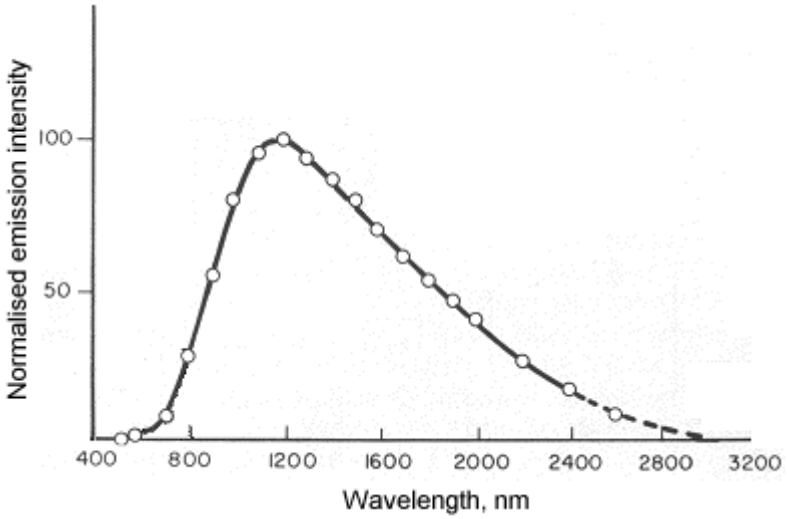
Chemiluminescence occurs when a chemical reaction produces an electronically excited species which emits a photon in order to reach the ground state. These sort of reactions can be encountered in biological systems; the effect is then known as *bioluminescence*. The number of chemical reactions which produce chemiluminescence is small. However, some of the compounds which do react to produce this phenomenon are environmentally significant.

A good example of chemiluminescence is the determination of nitric oxide:

$NO + O_3 \rightarrow NO_2^* + O_2$

$NO_2^* \rightarrow NO_2 + h\nu$ ($\lambda$ = 600 - 2800 nm)

The following graph shows the spectral distribution of radiation emitted by the above reaction:




# Spectral analysis methods

Spectral analysis methods are divided into two parts

A - Absorption spectrometry methods

B - Emission spectroscopy methods

A- Absorption spectrometry methods:

The method of absorption spectrometry :means absorb part of the electromagnetic radiation by the materials in the atomic or molecular state, This part of the absorbed radiation called absorption, it is direct proportion with the concentration .

The absorption spectrometry methods divided into five sections:

**1 -** The molecular absorption method of ultraviolet spectrum:
This method relies on molecules in the solution absorb part of UV radiation incident it.
**2 -** The molecular absorption method of visible spectrum:
This method relies on molecules in the solution absorb part of the visible radiation incident it.
**3 -** The molecular absorption method of infrared radiation:
This method relies on molecules in the solution absorb part of infrared incident it . This method has been used in the analysis of qualitative and synthetic organic and in organic compounds
**4 -** The nuclear magnetic resonance (NMR): This method relies on the nuclei particles absorb the part to incident by the radio-ray. This method has been used in the analysis of qualitative and synthetic organic compounds also
**5 -** method of atomic absorption spectrometer (AAS):
This method relies on the atoms of material absorb in the gas-state ultraviolet or visible incident it. This method has been used in quantitative analysis of metals.

**II / Emission spectroscopy methods:**

Emission spectroscopy depend of excitation atoms or molecules by radiation or thermal or electric energy and then measuring the intensity of emitted radiation from the atoms or molecules after re-entry to the ground state.
Divided methods of atomic emission spectroscopy into five sections:
**1 –** The flame atomic emission spectroscopy: This method depend to excite the atoms of the material that found in the gas state by heat of flame and then measuring the intensity of radiation that emitted from the atoms
**2 –**The atomic emission electric method: This method depend to excite the atoms of the material in the gas state by electric energy and then measuring the intensity of radiation that emitted from the atoms .
**3 -** The luminescence molecular spectrum: This method to excite the molecules in solution by beam of visible or ultraviolet radiation and then measure the intensity emissions of these molecules.
**4 -** The luminescence atomic spectrum: This method to excite the atoms in the gas state by beam visible or ultraviolet radiation and then measure the intensity of emissions of these atoms.
**5 -** The luminescence atomic X-ray: This method to excite the atoms in the gas state by a package of X-ray and then measuring the intensity of X-rays emitted from the atoms.

# Instrumentation

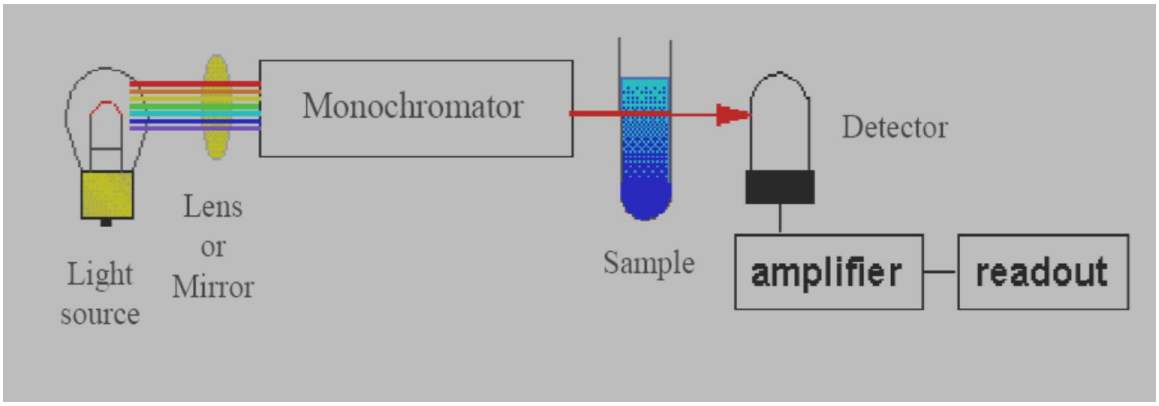
The minimum requirements of an instrument to study absorption spectra (a spectrophotometer) are shown below

1. A source of radiation of appropriate wavelengths.

2. A means of isolating light of a single wavelength and getting it to the sample compartment - monochromator and optical geometry.

3. A means of introducing the test sample into the light beam - sample handling.

4. a means of detecting and measuring the light intensity.



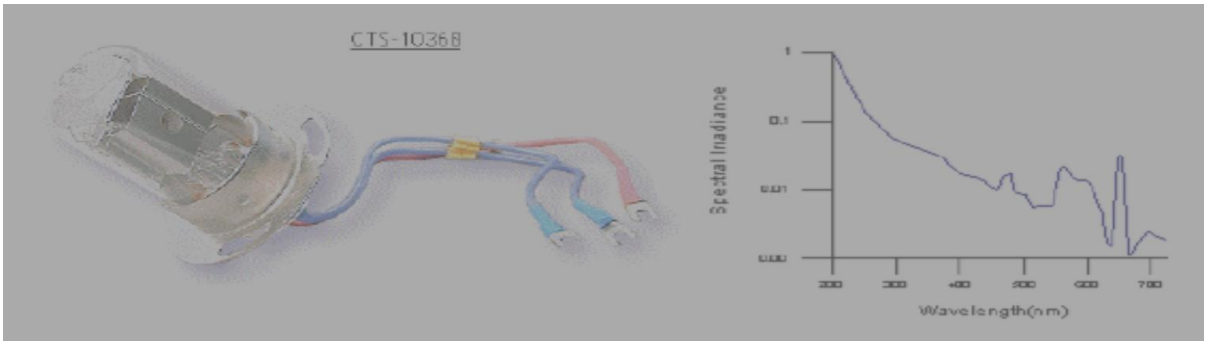

## (I) Sources of Radiation

**The source must provide a beam of radiation that is**

- Stable with sufficient energy for easy detection and measurements.
- Continuous i.e., made up of all wavelengths within the spectral region to be used

**Common sources for spectrophotometry**

| **Source** | **λ Region, nm** | **Type of spectroscopy** |
|---|---|---|
| $H_2$ and $D_2$ lamps | 160-380 | UV molecular absorption |
| Tungsten lamp | 380-800 | Visible molecular absorption |

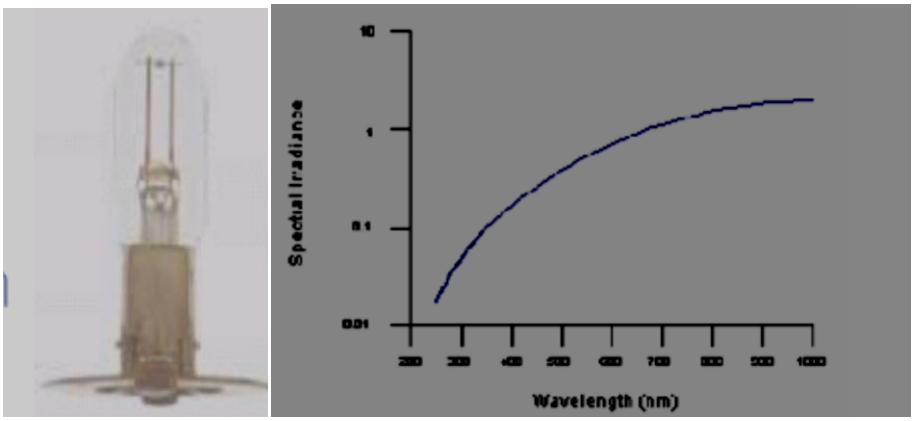



## Deuterium Arc Lamp

- UV Region
- WavelengthRange :190~420nm

## Tungsten Lamp

- WavelengthRange : Part of the UV and the whole of the Visible range (350 ~ 2,500nm)




## _ Xenon Lamp

- WavelengthRange : 190~800nm

Continuous Spectrum from UV to IR •

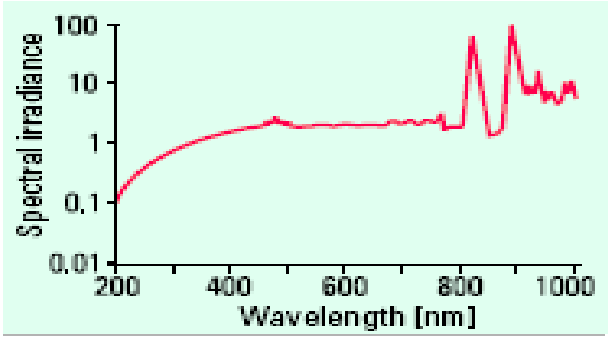



**Figure 18**
**Intensity spectrum of the xenon lamp**

# Properties of continuous source

Range – Quality Results throughout the λ Range
High Optical output
More Energy Better
Exceptional Flash Stability Consistent Quality Precision
Low Heat Radiation •
No Temperature effect on Assay
Long Life •
No need to change Lamp required
No required Warm Up.

## (2) Monochromators (Dispersing system)

- Instrument that can isolate a selected narrow band of wavelengths within a wide spectral range.

or

- It converts polychromatic light to monochromatic light.

## Three types of monochromators are :

(1) Filters (2) Prisms (3) Gratings

## [1] Filters

***Absorption filters (Colored filters)***

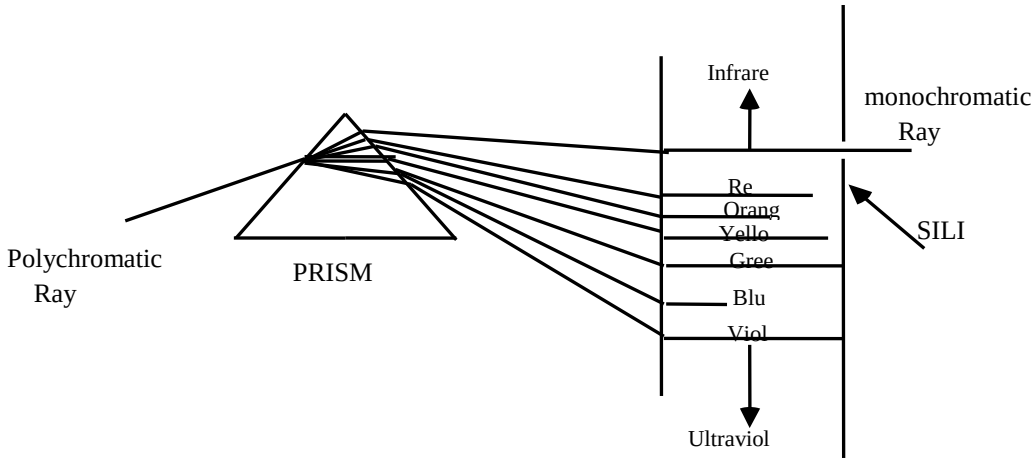
- They absorb radiation of unwanted wavelengths and transmit a limited desired wavelength ranges of spectrum.
- They are widely used for band selection in the visible region.
- The most common type consists of_ colored (tinted) glass.

The transmitted light from the filter is the complementary color of the sample, which is needed to be absorbed.

**[2] Prisms**: - They can be used to disperse UV, visible, and IR radiation.

- They act by refraction of light.

- The material used for prism construction depending on the spectralwavelength region.
- Glass prisms are used in visible region, while quartz prisms are used in UV/visible region



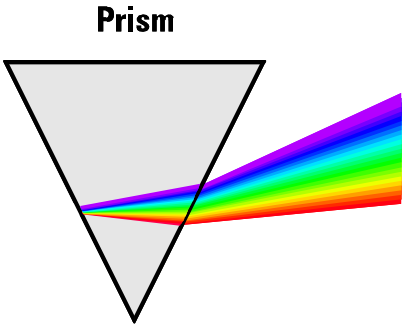

Polychromatic Ray ⟶ Monochromatic Ray

- Glass prisms cannot be used in UV region, since glass absorbs UV

radiation.

- Dispersion power of glass prisms is greater than that of quartz prisms for visible region.

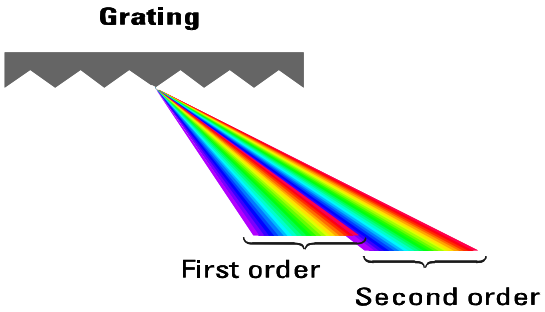
_So, glass prisms are preferred over quartz prisms for visible region.



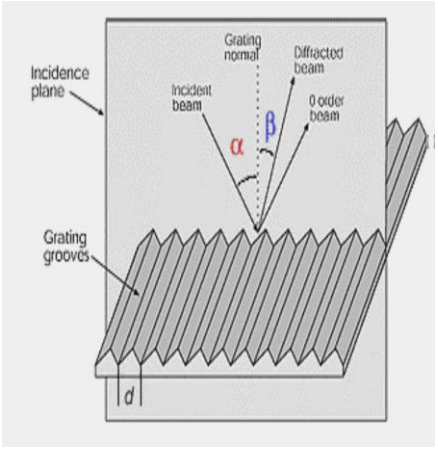

- **Non-linear dispersion**
- **Temperature sensitive**

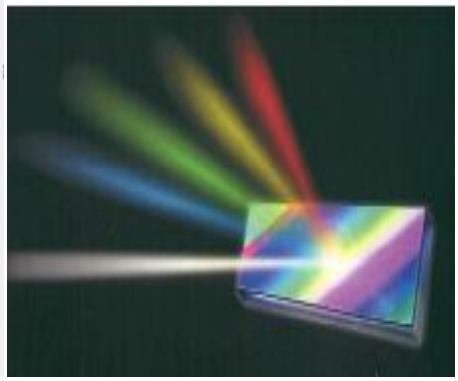
**[3] Gratings :** Act by diffraction and interference of light.

- They consist of a large number of parallel grooves or lines ruled very close to each other on a highly polished surface (e.g. aluminum or aluminized glass).
- For UV/visible region, the grating contains from *** to 600 grooves/ mm.
- Through diffraction and interference, the grating disperses the light beam into almost single λ light




**-Linear Dispersion**
**- Different orders**



- Resolution power _ number of grooves in grating.
- Resolution power of gratings > prisms > filters

* As the bandwidth __ Resolution

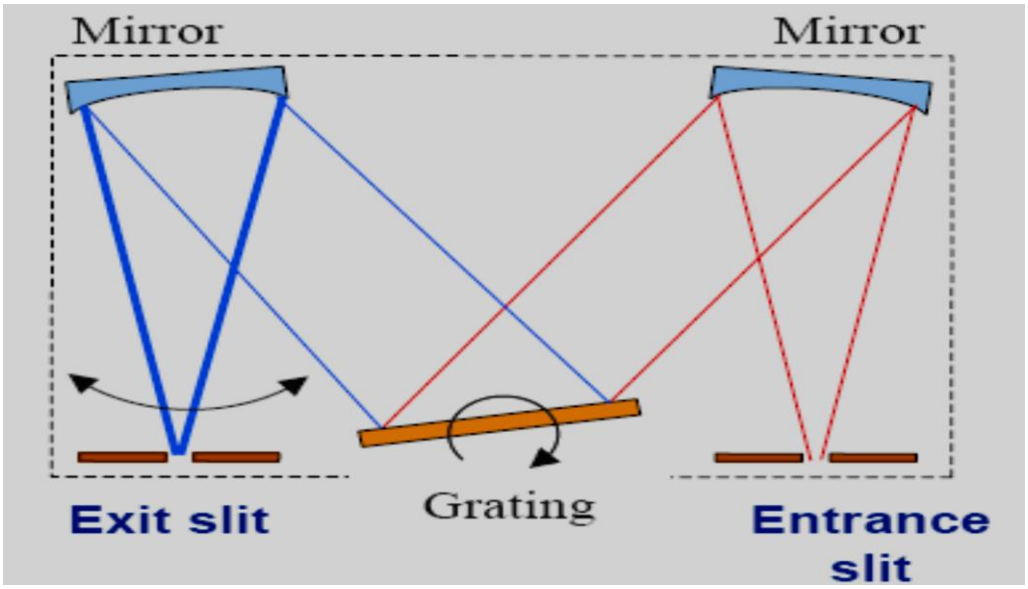
## A monochromator consists of three elements

**1- Entrance slit :** allows light from the source to strike the dispersing system.

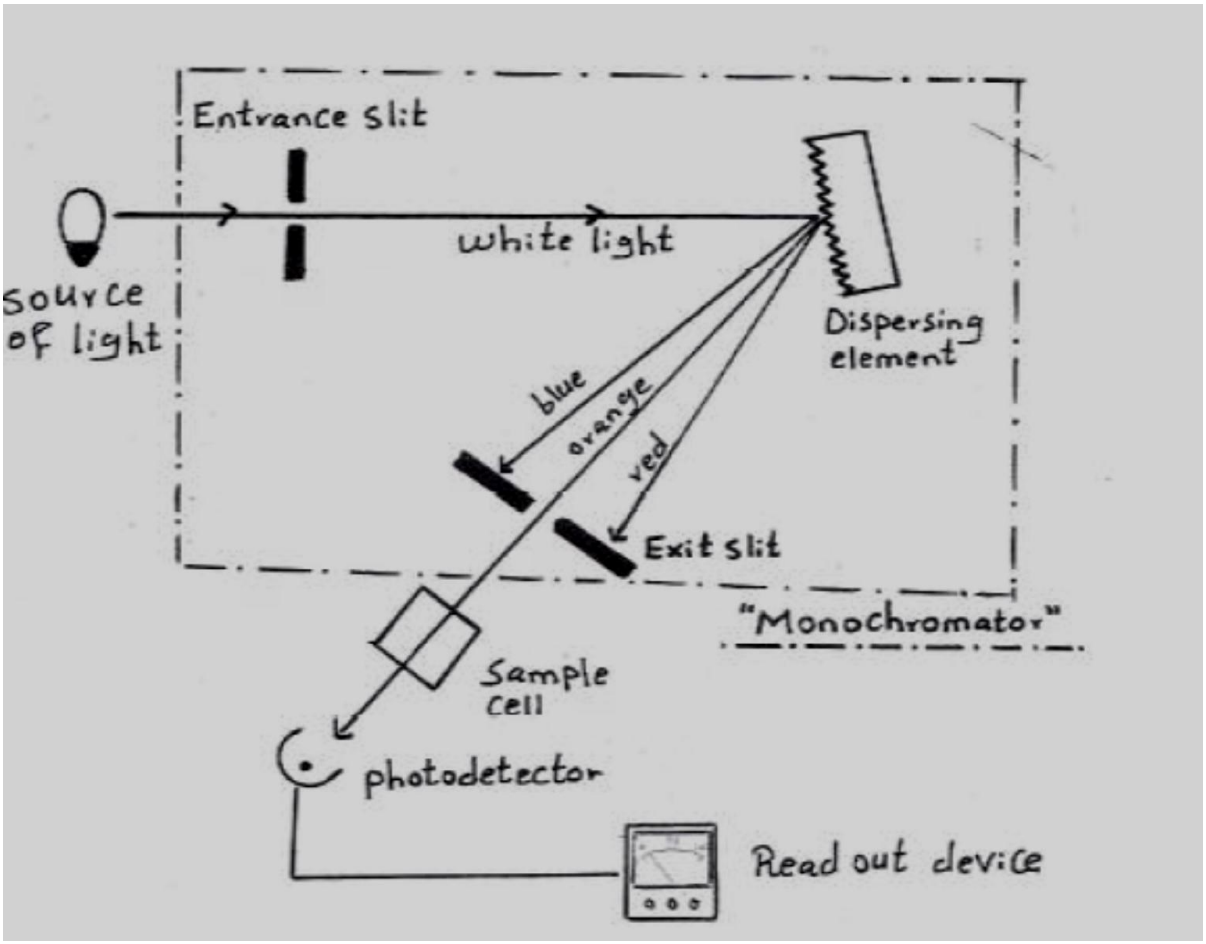
**2- Dispersing element :** Filter or prism or Grating breaks the white light into its component wavelengths (colors)

**3- Exit slit :** allows passage of monochromatic light to the sample compartment.

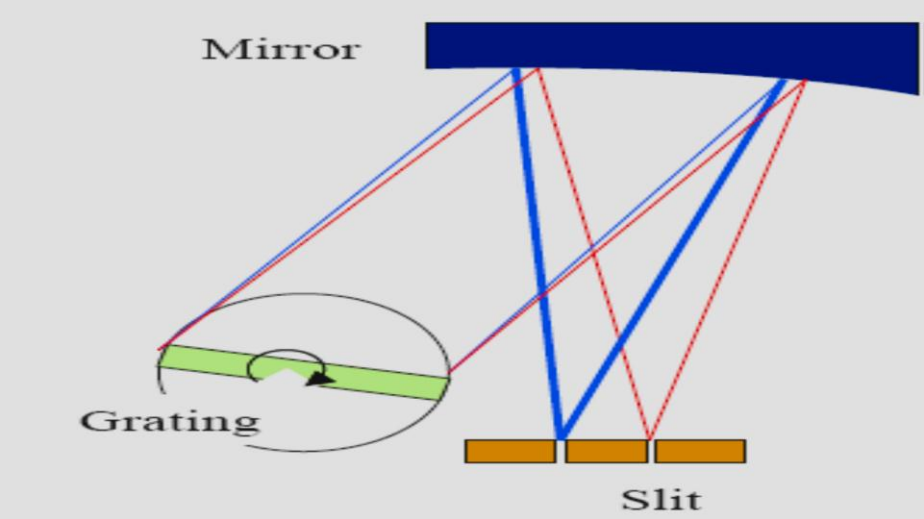
Associated optics are used to control light intensity such as collimating lenses and mirrors for proper alignment of the beam




**Monochromator**




**Spectrophotometer**

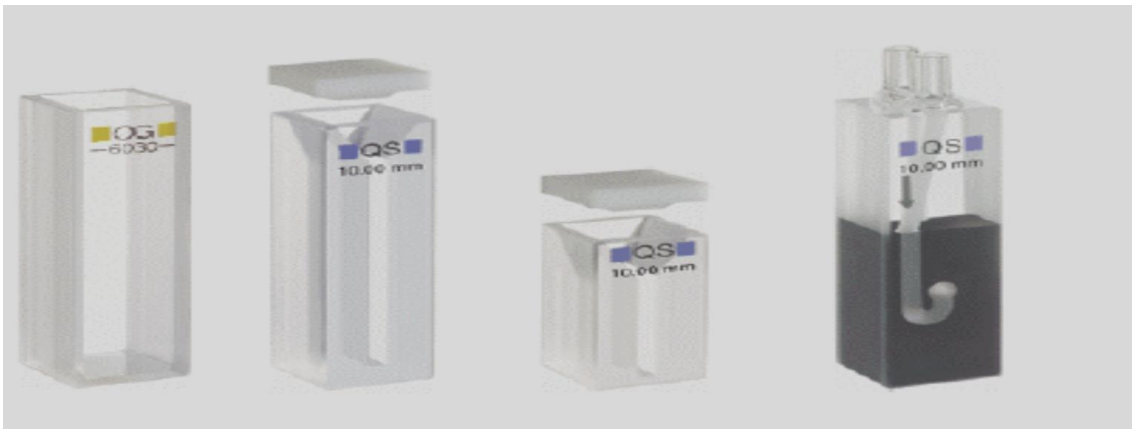



MonochromatorLittrow

## (3) Sample Compartment :

- Sample containers, usually called cells or cuvettes.
- They are rectangular with 1 cm, 2 cm, 4 cm or 5 cm pathlength.
- Two faces f the cell are transparent and the other two are opaque.
- It is made of glass for visible region and quartz or fused silica for UV region

- The most common cell pathlength for studies in the UV/visible region is 1 cm.
- Shorter cells are used for blood analysis where small volume of sample is required.



**(4) Detectors :** they convert optical energy to electrical energy.

**Properties of detectors**

1-Signal that produced from the detector directly proportional to the energy radiation that stroked him.

2 - That have a high sensitivity to detect low levels of energy radiation .

3 - To be a response to a wide range of wavelengths

4 – To be high stability and fast response time

5 - Electric signal can easily be amplified

6 - Noise level is relatively low

Several types of photon detectors are available, including :

## [i] Photo cell (photovoltaic cell) or Barrier-Layer cells

- When radiation falls upon the semiconductor surface.
- The electrons then migrate toward the metallic film.
- The result is an electric current that is proportional to the intensity of incident light

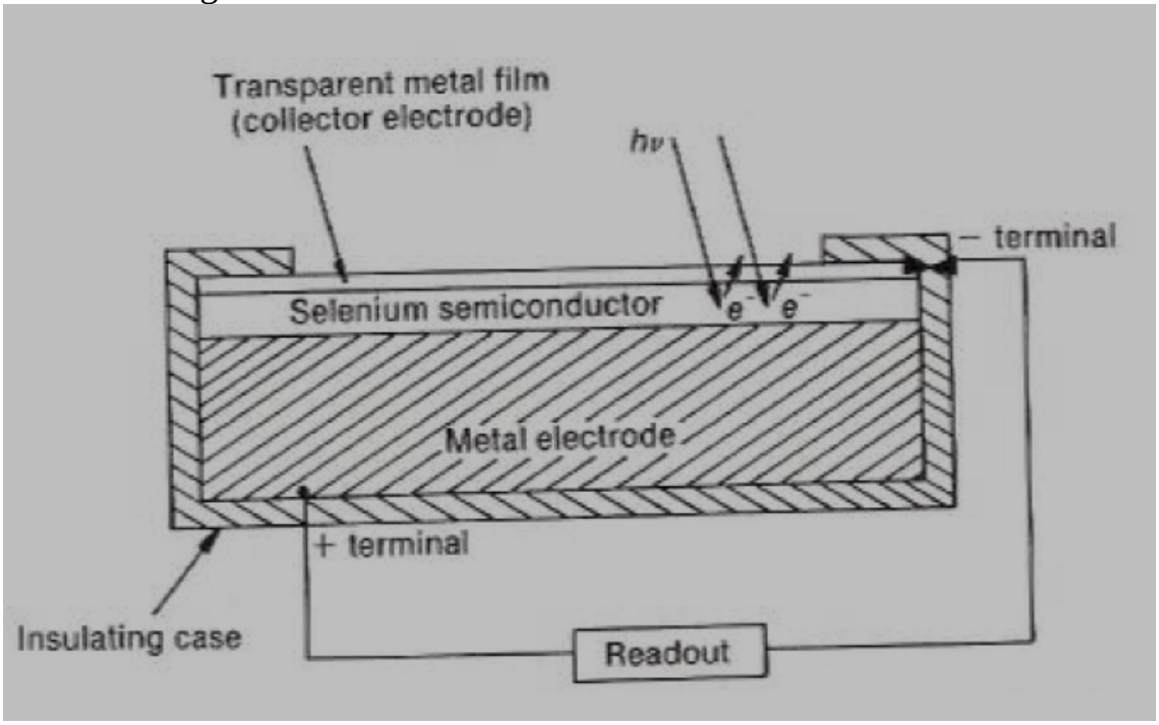
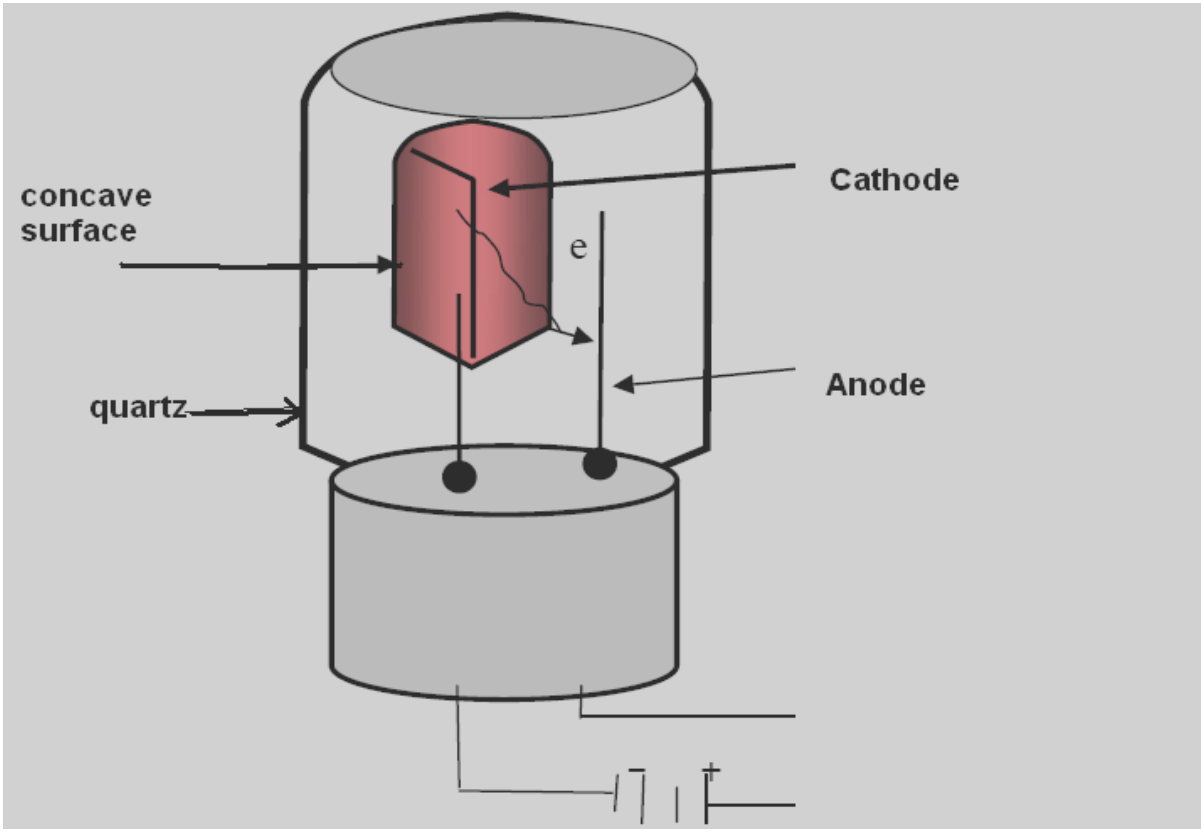



**Photo cell**

## [ii] Phototube :

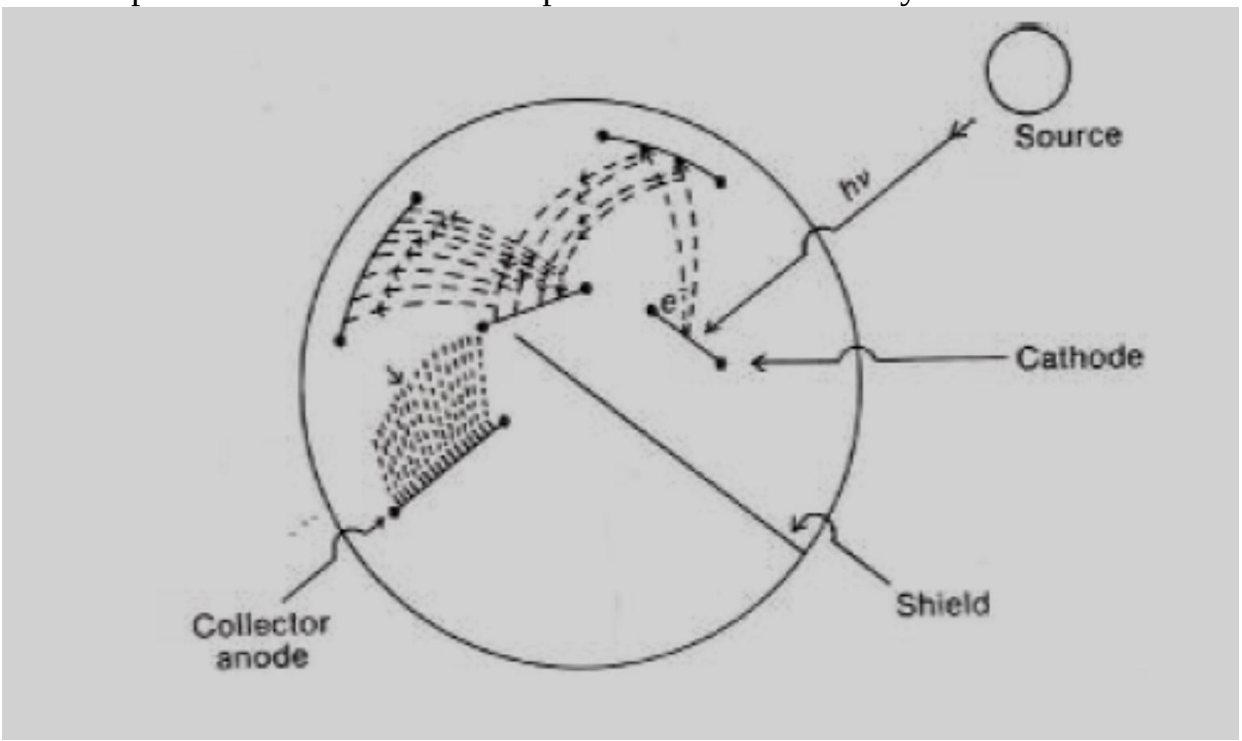
- Consists of a semicylinderical photocathode and a wire anode sealed inside an evacuated transparent glass or quartz envelope.
- The concave surface of the cathode supports a layer of photoemissive material such as an alkali metal or metal oxide.
- When light falls on the photoemissive layer, electrons are emitted from the surface.
- By applying a potential across the electrodes, the emitted photoelectrons are attracted to the +ve changed wire anode to give a photocurrent that is proportional to the intensity of the radiation.

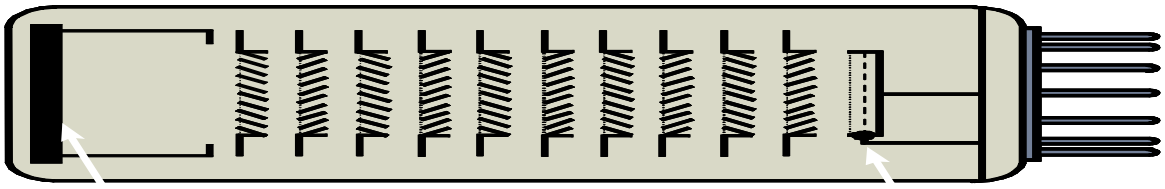



# Phototube

## [iii] Photomultiplier tube (PMT)

- The PMT is similar in construction to the phototube, but it is more sensitive (Magnification of current produced).
- The photocathode is similar to that of the phototube with electrons being emitted upon exposure to radiation.
- In place of a single wire anode, however, the PMT has a series of electrodes called dynodes as shown in fig. below.
- The electrons emitted from the cathode are accelerated toward the first dynode that is maintained 90-100 V positive the cathode.
- Each accelerated photoelectron that strikes the dynode surface produces several electrons, called secondary electrons that are then accelerate to dynode 2, which is held 90-100 V more positive than dynode 1.
- By the time this process has been repeated at each of the dynodes (9 times), 105 to 107 electrons have been produced for each incident photon.
- This cascade of electrons is finally collected at the anode to provide a current that is amplified and electronically measured.

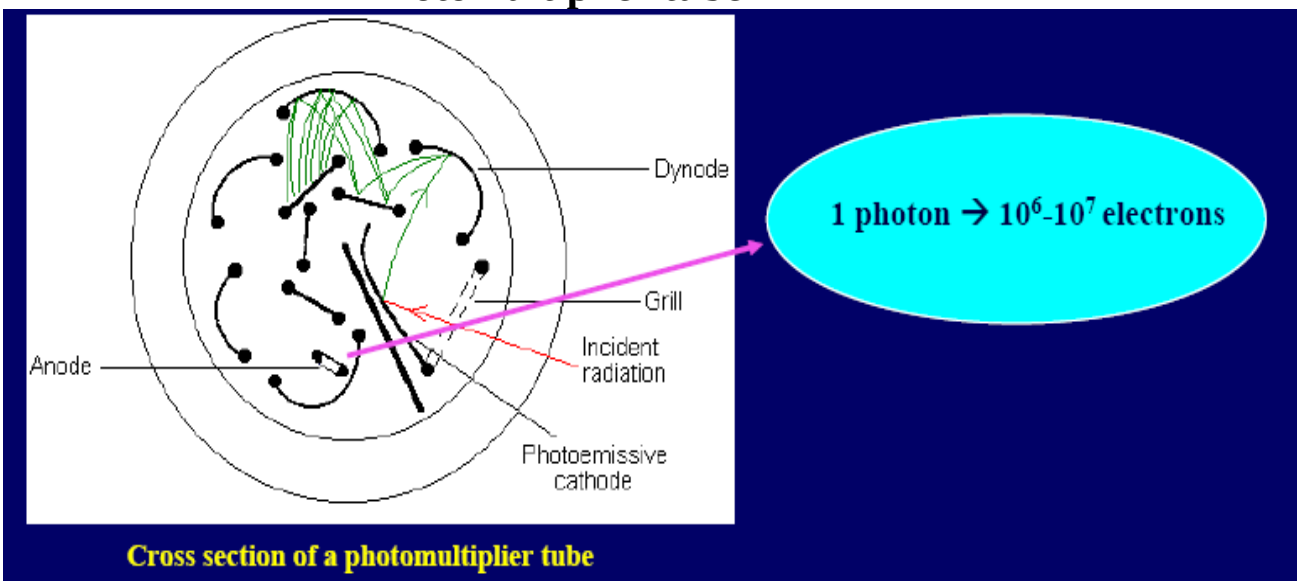


Cathode

**Anode**

## Photomultiplier tube




- a commonly used detector in UV-Visible spectroscopy
- consists of photo emissive cathode(a cathode which emits electrons when struck by photons of radiation), a number of dynodes(which emit several electrons for each electron striking them) and an anode1

## UV/visible photometers and Spectrophotometers

The optical components of spectrometer can be combined in various ways to produce two types of instruments for absorbance measurements.

**(a)Photometer :**

- Includes a filter for wavelength selection

and a photodetector.

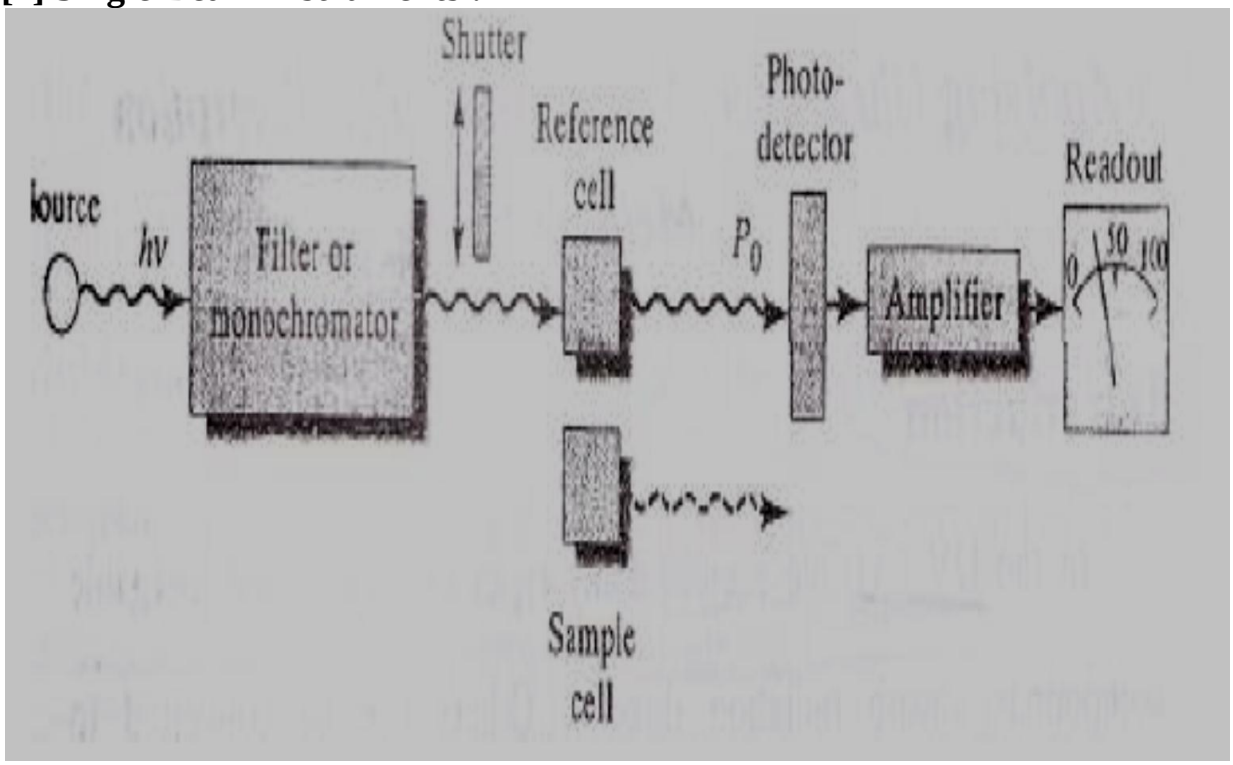
- Often used for the visible region.

**(b)Spectrophotometer :**

- Includes a monochromator (grating or prism) for _ selection and aphotodetector.
- Used for UV/visible and near IR regions.

Both (a & b) can be obtained in single-and double-beam varieties.

**[1] Single-Beam Instruments :**




- Radiation from the filter or monochromator passes through either the reference cell or the sample cell before striking the photodetector.
- Single-beam instruments have the advantages of simplicity of

instrumentation, low cost, and ease of maintenance.

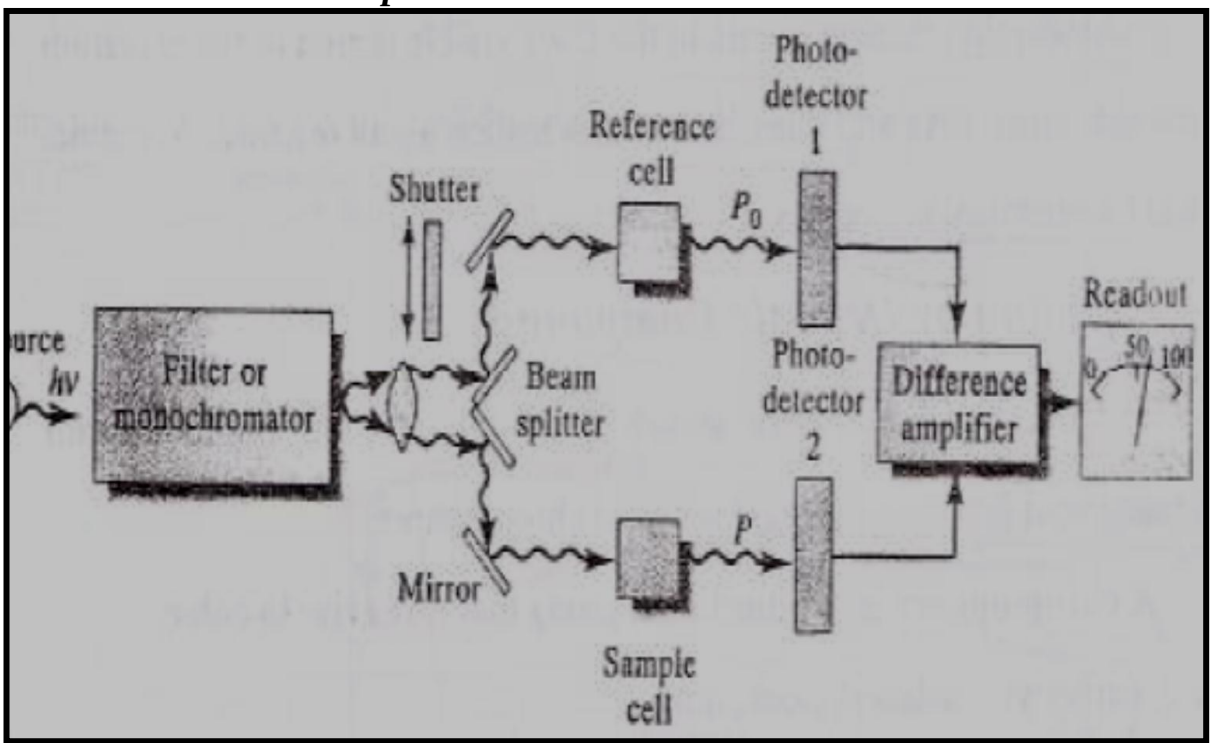
## The 0% T and 100% T adjustment :

The pointer of the meter is first zeroed with the sample cell empty so that the shutter blocks the beam and no radiation reaches the detector.
This process is called the 0% T adjustment.
The cell containing the blank (often the solvent) is then inserted into the cell holder, and the pointer is brought to the 100% T mark. This process is called the 100% T adjustment (zero absorbance).

**N.B. : The 0% T and 100% T adjustments should be made immediately before each transmittance or absorbance measurement.**

**[2] Double-Beam Instruments :**

***Double-beam-in-space :***




- Radiation from the filter or monochromator is split into beams that simultaneously pass through the reference and sample cells before striking two matched photodetectors.
- The two outputs are amplified and the logarithm of their ratio (log Io/It) is obtained electronically and then displayed on the output device.

## SUMMARY:

**In single-beam UV-Visible absorption spectroscopy, obtaining a spectrum requires manually measuring the transmittance of the sample and solvent at each wavelength.**

**The double-beam design greatly simplifies this process by measuring the transmittance of the sample and solvent simultaneously.**

**The detection electronics can then manipulate the measurements to give the absorbance.**

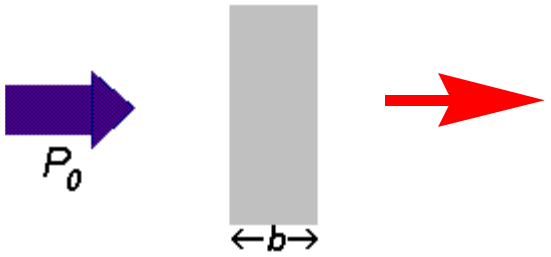
**(5) Recorder or display :**

Consisting of electronic hardware which may be digital or acomputer.
Electric signal produced in detector is fed to a sensitive
Galvanometer, its seal graduated in absorbance or a transmittance

# Beer's Law

## Introduction

Many compounds absorb ultraviolet (UV) or visible (Vis.) light. The diagram below shows a beam of monochromatic radiation of radiant power $P_0$, directed at a sample solution. Absorption takes place and the beam of radiation leaving the sample has radiant power $P$.




The amount of radiation absorbed may be measured in a number of ways:

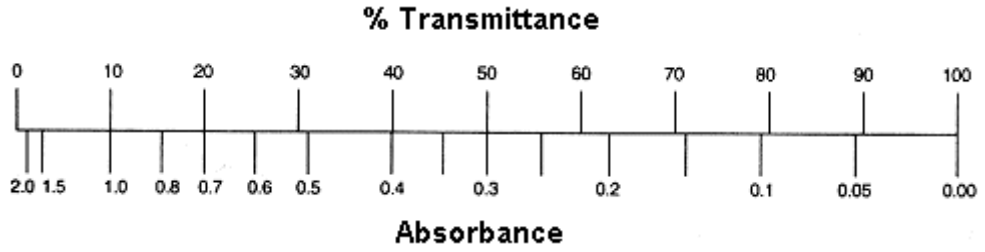
**Transmittance**, $T = P / P_0$
**% Transmittance**, $\%T = 100\ T$

**Absorbance**,

$A = \log_{10} P_0 / P$
$A = \log_{10} 1 / T$
$A = \log_{10} 100 / \%T$
$A = 2 - \log_{10} \%T$

The last equation, $A = 2 - log_{10}\ \%T$ , is worth remembering because it allows you to easily calculate absorbance from percentage transmittance data.

The relationship between absorbance and transmittance is illustrated in the following diagram:




So, if all the light passes through a solution *without* any absorption, then absorbance is zero, and percent transmittance is 100%. If all the light is absorbed, then percent transmittance is zero, and absorption is infinite.

**Example:** The wavelength (λ) of Maximum Absorption is known for different compounds. For example, the coloured compound formed for analysis of Phosphate (molybdenum blue) has maximum light absorption at (λ) = 640 nm. Conversely, a minimum amount of light is *transmitted* through the compound at(λ) = 640 nm. This is shown schematically in Figure below:

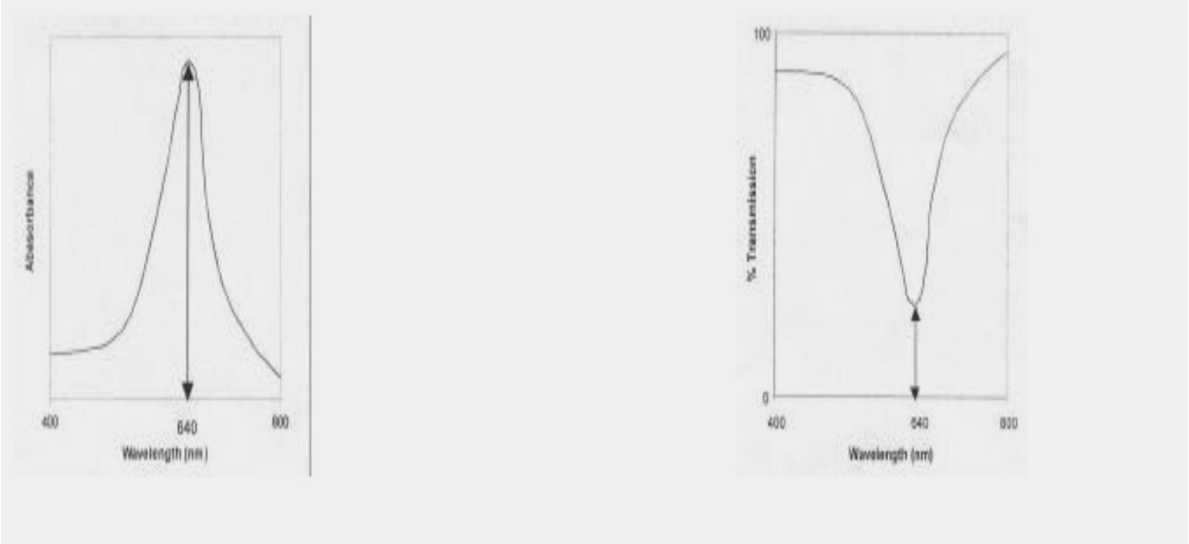



**Figure: Light Absorption and Transmission by Phosphate-molybdenum blue compound. Schematic diagram showing maximum light absorption (and minimum light transmission) at (λ)= 640 nm.**

**The Beer-Lambert Law**

Now let us look at the Beer-Lambert law and explore it's significance. This is important because people who use the law often don't understand it - even though the equation representing the law is so straightforward:

**A=εbc**

Where A is absorbance (no units, since $A = log_{10}P_0/ P$ )
**ε** is the molar absorbtivity with units of L $mol^{-1}$ $cm^{-1}$
**b** is the path length of the sample - that is, the path length of the cuvette in which the sample is contained. We will express this measurement in centimeters.
**c** is the concentration of the compound in solution, expressed in mol $L^{-1}$

The reason why we prefer to express the law with this equation is because absorbance is directly proportional to the other parameters, as long as the law is obeyed. We are not going to deal with deviations from the law.

**When the The Beer-Lambert Law**

**A=εbc**

The values of **molar absorpitivity (ε)**that has unit ( L $mol^{-1}$ $cm^{-1}$)does not rely on concentration not on the beam along the corridor and not on the intensity of incident light and it is a significant of the molecule ,while Absorption(A) status significant of the sample measured depends on the concentration and a corridor along the beam and the beam intensity and type of incident and the type of solvent

**When the The Beer-Lambert Law**

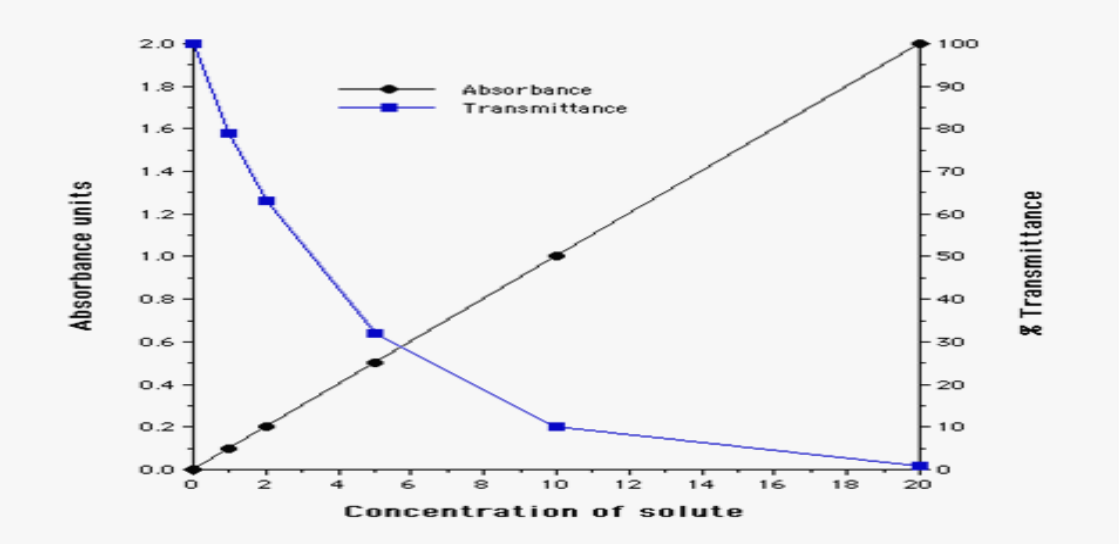
**A=abc**

**Specific absorpitivitycoeffiesiont(a)** that has unit ( L $gm^{-1}$ $cm^{-1}$)Factor is a natural character that is dependent on the wavelength of radiation incident on the sample and the sample type that does not depend on the concentration and use of the material did not know the molecular weight

**Example**: Potassium dichromate $K_2Cr_2O_7$showin medium base at the highest absorption wavelength 372 nm, the base of solution containing $3X10^{-5}$M that has transmittance 71.6% of the intensity of incident light at 372nm when placed in the cell that has length 1 cm.

1 – calculate the absorption of this solution?
2 - What molar absorpitivity? for potassium dichromate at 372nm ?
3 – what the percentage of transmittance(T%) when the absorption cell has length 3 cm?

**The relationship between absorbance , transmittance and concentration**

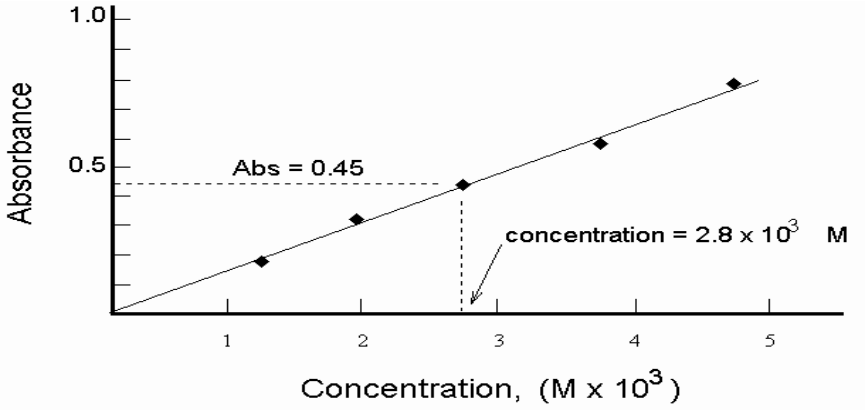



## UV/visible: Applications

### Using UV-absorption spectra to find concentrations

**A=εbc**

This equation and relationship between absorbance and concentration can be used to create a Beer's Law Plot. By plotting the known concentrations of several samples of a particular chemical on the x-axis and the corresponding maximum absorbance on the y-axis, a Beer's Law Plot is obtained.




The Plot can then be used to determine an unknown concentration of the same chemical. At dilute concentrations,

many chemical solutions obey Beer's Law, resulting in a straight line plot with the general equation for a straight line **y = mx + b**.

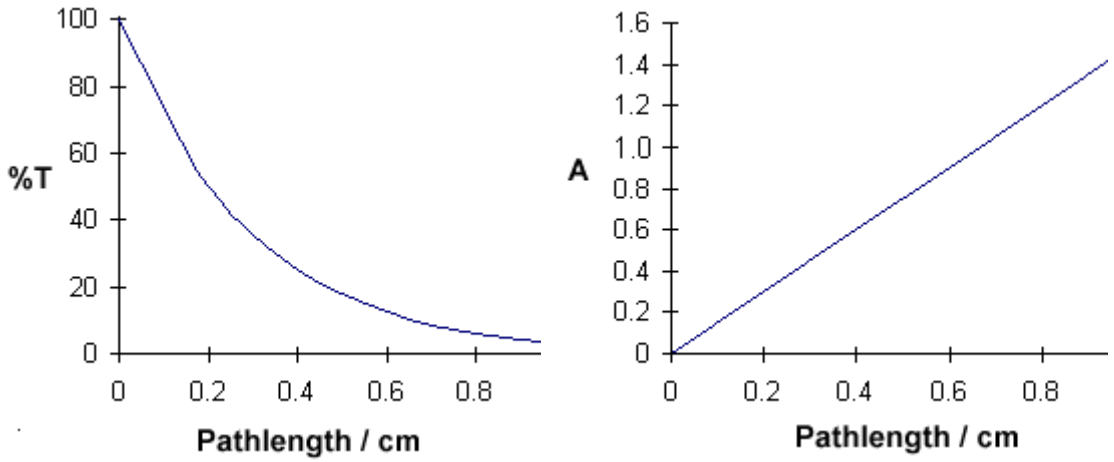
**Question :** Why do we prefer to express the Beer-Lambert law using absorbance as a measure of the absorption rather than %T ?

**Answer :** To begin, let's think about the equations...

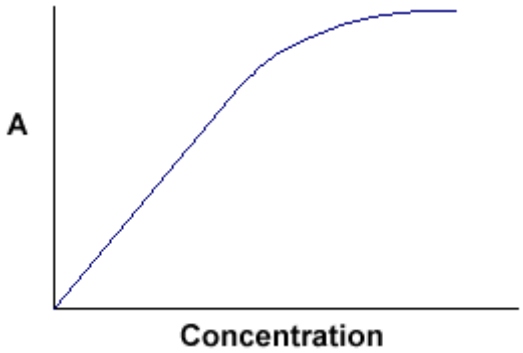
**$A=\varepsilon bc$**

**$\%T = 100\ P/P_0 = e^{-\varepsilon bc}$**

| Path length / cm | 0 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 |
|---|---|---|---|---|---|---|
| %T | 100 | 50 | 25 | 12.5 | 6.25 | 3.125 |
| Absorbance | 0 | 0.3 | 0.6 | 0.9 | 1.2 | 1.5 |




**A = εbc** tells us that absorbance depends on the total quantity of the absorbing compound in the light path through the cuvette. If we plot absorbance against concentration, we get a straight line passing through the origin (0,0).




Note that the Law is not obeyed at high concentrations. This deviation from the Law is not dealt with here.

The linear relationship between concentration and absorbance is both simple and straight forward, which is why we prefer to express the Beer-Lambert law using absorbance as a measure of the absorption rather than %T.

**Question:** What is the significance of the molar absorbtivity, **ε** ?

**Answer:** To begin we will rearrange the equation A = **ε**bc :

**ε** = A / bc

In words, this relationship can be stated as " **ε** is a measure of the amount of light absorbed per unit concentration".

Molar absorbtivity is a constant for a particular substance, so if the concentration of the solution is halved so is the absorbance, which is exactly what you would expect.

Let us take a compound with a very high value of molar absorbtivity, say 100,000 L $mol^{-1}$ $cm^{-1}$, which is in a solution in a 1 cm pathlength cuvette and gives an absorbance of 1.

**ε** = 1 / 1 x c

100,000 = 1 / 1 c

Therefore, c = 1 / 100,000 = 1 $x10^{-5}$mol $L^{-1}$

Now let us take a compound with a very low value of **ε**, say 20 L $mol^{-1}$ $cm^{-1}$ which is in solution in a 1 cm pathlength cuvette and gives an absorbance of 1.

**ε** = 1 / 1x c

Therefore, c = 1 / 20 = 0.05 mol $L^{-1}$

The answer is now obvious - a compound with a high molar absorbtivity is very effective at absorbing light (of the appropriate wavelength), **and hence low concentrations of a compound with a high molar absorbtivity can be easily detected.**

**Question:** What is the molar absorbtivity of $Cu^{2+}$ ions in an aqueous solution of $CuSO_4$? It is either 20 or 100,000 L $mol^{-1}$ $cm^{-1}$

**Answer:** I am guessing that you think the higher value is correct, because copper sulphate solutions you have seen are usually a beautiful bright blue colour. However, the actual molar absorbtivity value is 20 L $mol^{-1}$ $cm^{-1}$! The bright blue colour is seen because the concentration of the solution is very high.

β-carotene is an organic compound found in vegetables and is responsible for the colour of carrots. It is found at exceedingly low concentrations. You may not be surprised to learn that the molar absorbtivity of β - carotene is 100,000 L $mol^{-1}$ $cm^{-1}$.

## Using UV-absorption spectra to help identify organic compounds

If you have worked through the rest of this section, you will know that the wavelength of maximum absorption (lambda-max) depends on the presence of particular chromophores (light-absorbing groups) in a molecule.

For example, on another page you came across the fact that a simple carbon-carbon double bond (for example in ethene) has a maximum

absorption at 171 nm. The two conjugated double bonds in buta-1,3-diene have a maximum absorption at a longer wavelength of 217 nm.

# Spectroscopy – Multimolecules Effect

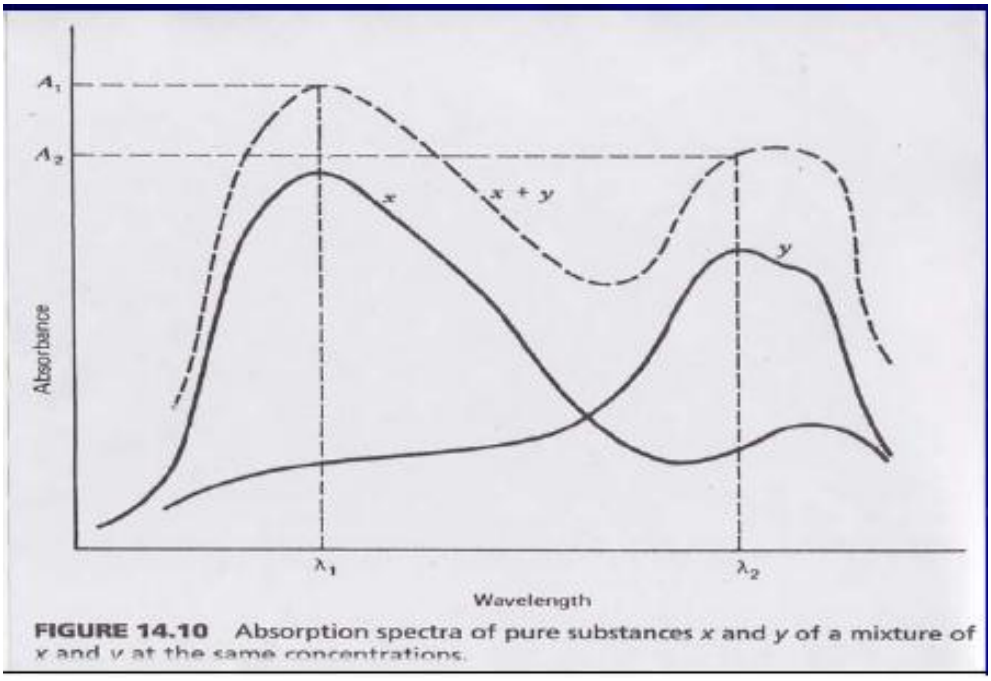



**FIGURE 14.10** Absorption spectra of pure substances *x* and *y* of a mixture of *x* and *y* at the same concentrations.

# Spectroscopy - Mixture

**Multi-component mixture:**

$$A_T = A_1 + A_2 + A_3 + A_n$$

$$A_T = \varepsilon_1 bc_1 + \varepsilon_2 bc_2 + \varepsilon_3 bc_3 + \varepsilon_n bc_n$$

**Where: 1,2,3,….,n – refer to absorbingComponents**

**Example:**absorption were measured every three solutions alone are Z, Y, and the third solution is a combination of both (Y + Z) measure concentration of the Z, Y in mix by use cell that have length one centimeter by use information listed below:

| SOLUTION | $A_{475}$ | $A_{670}$ |
|---|---|---|
| 0.001M(Y) | 0.9 | 0.2 |
| 0.01M(Z) | 0.15 | 0.65 |
| MIXED (Y+Z) | 1.65 | 1.65 |

## Effect of solvent, concentration, pH, and temperature

A number of factors, including the solvent used as well as the concentration, pH, and temperature of the sample, can effect the position and intensity of absorption bands of molecules. These parameters should be controlled to ensure maximum precision and when comparing spectra measured under different conditions.

**The polarity** of a solvent can modify the electronic environment of the absorbing chromophore. In general, the magnitude of the shift can be correlated with solvent polarity.

Thus, for example, the absorption maximum of acetone can vary from 259 to 279 nm, depending on the solvent used. For comparative analysis, a single solvent should be used for all measurements.

**Concentration** normally affects only the intensity of bands.At higher concentrations, however, molecular interactions (for example, dimerization) may cause changes in the shape and position of the absorbance band.

These changes in turn affect the linearity of the concentration versus absorbance relationship and may lead to inaccurate quantitative results.

**The effects of pH** on absorbance spectra can be very large and result primarily from the shifting of equilibrium between two different forms.

For example, pH indicators visually change color at different pH values. If the spectrum of the sample under study is found to be effected by pH, a buffer should be used to control this parameter.

**Note**, however, that most buffers themselves exhibit significant absorbance, which may effect the wavelength range over which measurements can be performed.

**Temperature** also can effect UV-visible measurements. Simple expansion of the solvent, especially for some organic solvents, may be

sufficient to change the apparent absorbance and thereby the accuracy of quantitative results.

In addition, temperature may affect equilibrium, which can be either chemical or physical. A good example of a physical equilibrium is the denaturation of nucleic acids as temperature is increased, which changes absorptivity.

## Spectroscopy – Beer's Law Limitations

**Deviations from the direct proportionality (b=const)**

**1-Instrumental Deviations 2-Chemical Deviations**

## Chemical Deviations

**_ Deviations in absorptivity coefficients**

**- at high concentrations (>0.01M) due to electrostatic interactions**

**Between molecules in close proximity**

**_ Scattering of light, due to particulates in the sample**

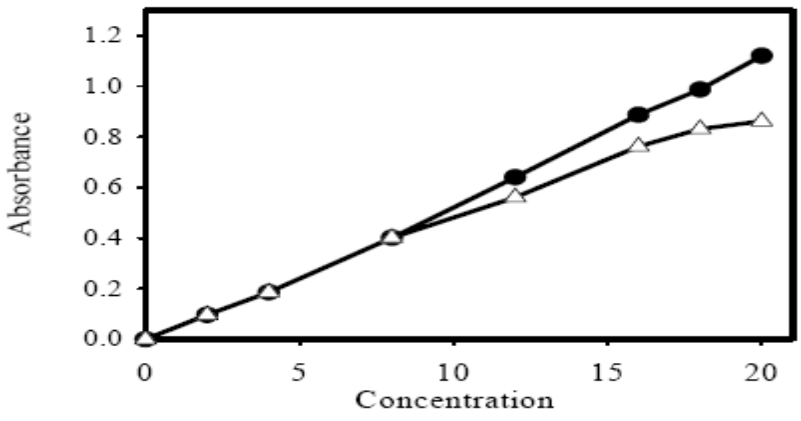
**_ Fluorescence or Phosphorescence of the sample**

**_ Changes in refractive index at high analysis concentration**

**_ Shifts in chemical equilibria as a function of concentration**

**_ Stray light**

## Spectroscopy - Deviations



## Example of chemical interference

Such as interaction the solvent with the soluble substance change of the composition of soluble substance example, chemical reason for the apparent deviation of the change is the color of the solution, for example in the case $K_2Cr_2O_7$solution changes color from orange to yellow at dilute with water

$$K_2Cr_2O_7 + H_2O \underset{\text{Base}}{\overset{\text{Acid}}{\rightleftharpoons}} K_2CrO_4 + 2H^+$$

Where the use of acid medium produced $K_2CrO_4$ acid and the use of the base medium $\mathbf{K_2Cr_2O_7}$ for stop interaction control of the pH solution

# Instrumental Deviations

- **monochromatic radiation – quality of monochromator and control of bandwidth and slit**
- **Instrumental noise – accuracy of measurement of transmittance –quality of detector**

### Spectrophotometric Titrations

In volumetric analyses, the color changes that signify the end point of a titration are most often detected through visual inspection. This process is inherently subjective and can be a source of error. The use of a spectrophotometer for endpoint detection introduces objectivity into the analysis and lends itself to automation

## Properties of Photometric Titration

1 - reduce interferences of the other elements

2 - use of a large number of measurements in determining the concentration of component or one element

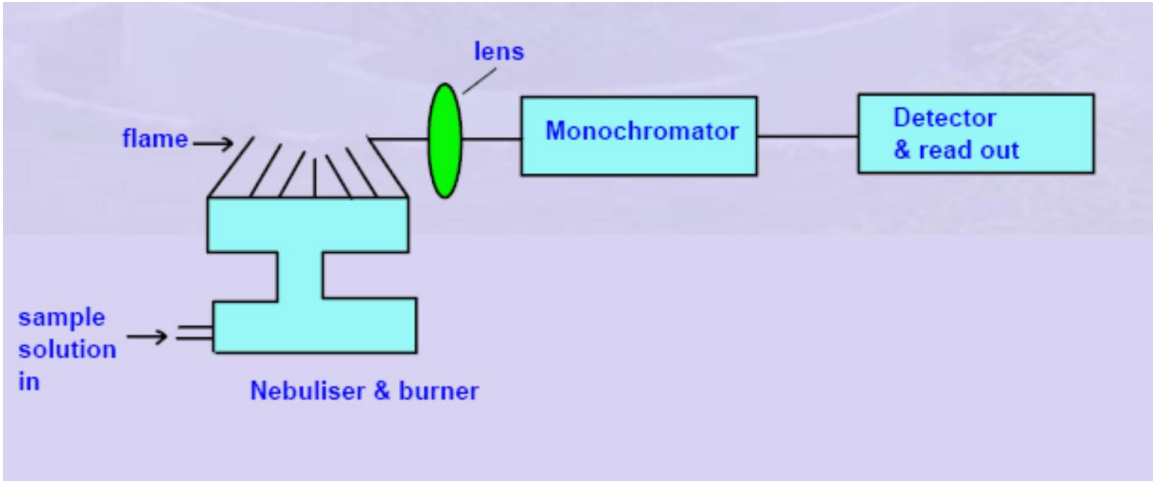
3 - in this type of titration not be necessary fixed high dissociation of the composite as in other types of titration

4 - could be used it to for titration that were color titration is difficult especially when titrated weak acids and bases, or cannot be titration

5 - the results more accuracy and precision from the results of a routine titration

## Flame photometry:

This technique is used in water analysis for determining the concentration of alkali and alkali metal ions such as sodium, potassium and calcium. The following diagram (fig. Below) shows the basic components of a flame photometer.




**Flame photometer**

The liquid sample to be analysed is sprayed under controlled conditions into the flame where the water evaporates, leaving behind the inorganic salts as minute particles. These salts decompose into constituent atoms or radicals and may become vapor raised. The vapours containing the metal atoms are excited by thermal energy of the flame and this causes electrons of the metallic atoms to be raised to higher energy levels. When these excited electrons fall back to their original positions, they give off discrete amounts of radiant energy. The emitted radiation is passed through the monochromator where the desired region isolated. A photocell and an amplifier are then used to measure the intensity of isolated radiation. Normally for alkali metals a propane-compressed air mixture is used as a fuel.

A linear concentration range (for sodium and potassium 1 to 10 mg $l^{-1}$ and for calcium 10-50 mg $l^{-1}$) is within the range expected for environmental water samples. The method is simple and sample preparation is not needed. However care has to be taken that the calibration of the instrument and analytical measurements are performed quickly after each other

# Infra-Red Absorption Spectroscopy

## *Theoretical Principles*

### Introduction

The term "infra red" covers the range of the electromagnetic spectrum between 0.78 and 1000 μm. In the context of infrared spectroscopy, wavelength is measured in "wavenumbers", which have the unit's $cm^{-1}$.

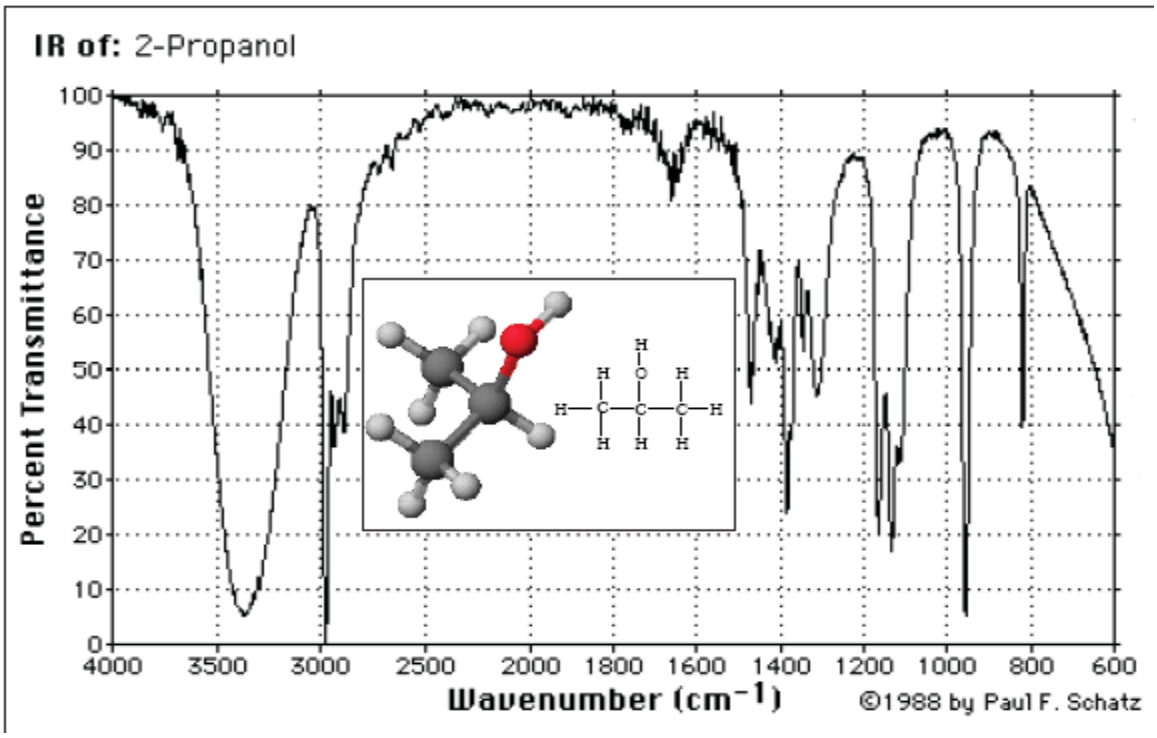
Wave number = 1 / wavelength in centimeters

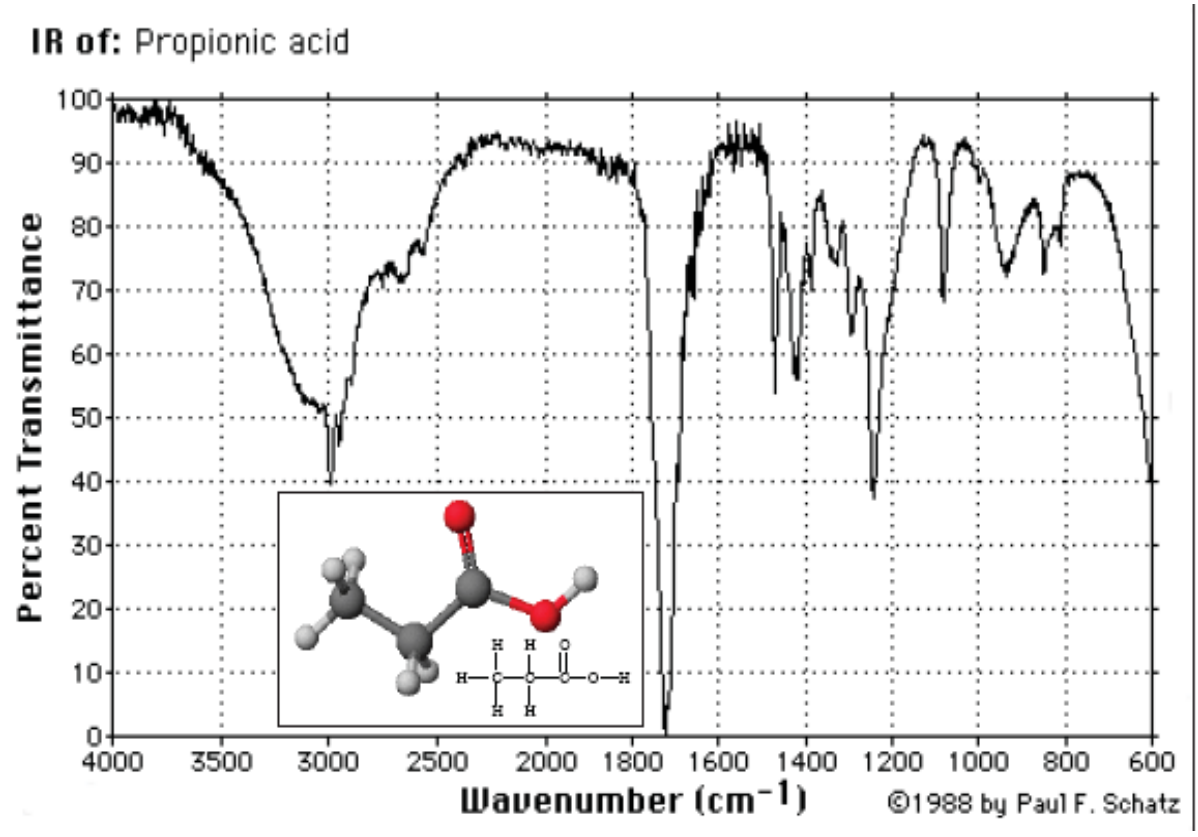
It is useful to divide the infrared region into three sections; ***near***, ***mid*** and***far***infrared;the most useful I.R. region lies between 4000 - 670$cm^{-1}$.

| **Region** | **Wavelength range** (μm) | **Wave number range** ($cm^{-1}$) |
|---|---|---|
| **Near** | **0.78 - 2.5** | **12800 - 4000** |
| **Middle** | **2.5 - 50** | **4000 - 200** |
| **Far** | **50 -1000** | **200 - 10** |

Infrared spectroscopy is widely used by chemists and scientists, as **it is a well-developed technique to identify chemical compounds or monitor changes occurring in the course of a chemical reaction.**

For example, the spectra of two related molecules, 1-propanol (an alcohol), and propanoic acid (a carboxylic acid) are given on next page. Their spectra are considerably different in the 3000-3500 and 1600-1800 wave number regions, and there are less prominent differences in the region less than 1500 wave numbers. In this experiment you will learn how to tell what important features a molecule has by looking at its infrared spectrum.




**The infrared spectra of two common organic molecules, 2-propanol and propanoicacid.**

## Theory of infra red absorption

IR radiation does not have enough energy to induce electronic transitions as seen with UV. Absorption of IR is restricted to compounds with small energy differences in the possible vibration and rotational states.

For a molecule to absorb IR, the vibrations or rotations within a molecule must cause a net change in the dipole moment of the molecule. The alternating electrical field of the radiation (remember that electromagnetic radiation consists of an oscillating electrical field and an oscillating magnetic field, perpendicular to each other) interacts with fluctuations in the dipole moment of the molecule. If the frequency of the radiation matches the irrational frequency of the molecule then radiation will be absorbed, causing a change in the amplitude of molecular vibration.

**N.I: The energy of infrared radiation is sufficient to change the vibrational energy states of a molecule. If the dipole moment of a molecule changes as it vibrates, infrared radiation can interact with the molecule. When the frequency of the radiation matches the frequency of a particular vibration, energy is transferred to the molecule, increasing the amplitude of the vibration.**

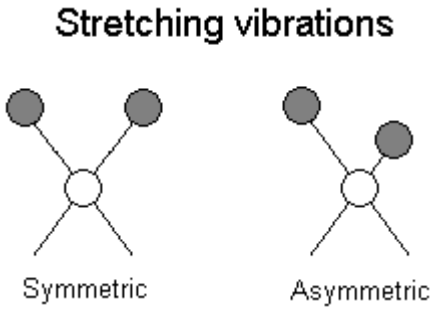
**Molecular rotations**

Rotational transitions are of little use to the spectroscopist. Rotational levels are quantized, and absorption of IR by gases yields line spectra. However, in liquids or solids, these lines broaden into a continuum due to molecular collisions and other interactions.

## Molecular vibrations

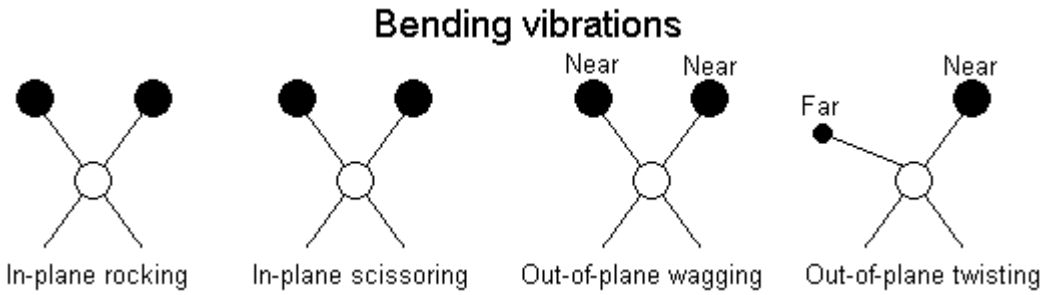
The positions of atoms in molecules are not fixed; they are subject to a number of different vibrations. Vibrations fall into the two main categories of ***stretching*** and ***bending*****.**

**Stretching:** Change in inter-atomic distance along bond axis




**Bending:** Change in angle between two bonds. There are four types of bend:

- Rocking
- Scissoring
- Wagging
- Twisting



## Vibrational coupling

In addition to the vibrations mentioned above, interaction between vibrations can occur (*coupling*) if the vibrating bonds are joined to a single, central atom. Vibrational coupling is influenced by a number of factors;

- Strong coupling of stretching vibrations occurs when there is a common atom between the two vibrating bonds
- Coupling of bending vibrations occurs when there is a common bond between vibrating groups
- Coupling between a stretching vibration and a bending vibration occurs if the stretching bond is one side of an angle varied by bending vibration
- Coupling is greatest when the coupled groups have approximately equal energies
- No coupling is seen between groups separated by two or more bonds

# *Instrumentation*

## Introduction

In this look at instrumentation for IR spectroscopy, we will be limiting our attention to instrumentation concerned with spectroscopy in the middle region (4000 - 200$cm^{-1}$). It is absorption in this region, which gives structural information about a compound.

# Instrumental components

## Sources

an inert solid is electrically heated to a temperature in the range 1500-2000 K. The heated material will then emit infrared radiation.

***The Nernst glower*** is a cylinder (1-2 mm diameter, approximately 20 mm long) of rare earth oxides. Platinum wires are sealed to the ends, and a current passed through the cylinder. The Nernst glower can reach temperatures of 2200 K.

***The Globar source*** is a silicon carbide rod (5mm diameter, 50mm long), which is electrically heated to about 1500 K. Water cooling of the electrical contacts is needed to prevent arcing. The spectral output is comparable with the Nernst glower, except at short wavelengths (less than 5 μm) where it's output becomes larger.

***The incandescent wire source***is a tightly wound coil of **nichrome wire,** electrically heated to 1100 K. It produces a lower intensity of radiation than the Nernst or Globar sources, but has a longer working life.

## Detectors

There are three catagories of detector;

- Thermal
- Pyroelectric
- Photoconducting

***Thermocouples*** consist of a pair of junctions of different metals; for example, two pieces of bismuth fused to either end of a piece of

antimony. The potential difference (voltage) between the junctions changes according to the difference in temperature between the junctions

***Pyroelectric detectors*** are made from a single crystalline wafer of a pyroelectric material, such as triglycerinesulphate. The properties of a pyroelectric material are such that when an electric field is applied across it, electric polarisation occurs (this happens in any dielectric material). In a pyroelectric material, when the field is removed, the polarisation persists. The degree of polarisation is temperature dependant. So, by sandwiching the pyroelectric material between two electrodes, a temperature dependant capacitor is made. The heating effect of incident IR radiation causes a change in the capacitance of the material. Pyroelectric detectors have a fast response time. They are used in most Fourier transform IR instruments.

Photoelectric detectors such as the mercury cadmium **telluride** detector comprise a film of semiconducting material deposited on a glass surface, sealed in an evacuated envelope. Absorption of IR promotes nonconducting valence electrons to a higher, conducting, state. The electrical resistance of the semiconductor decreases. These detectors have better response characteristics than pyroelectric detectors and are used in FT-IR instruments - particularly in GC - FT-IR.

## What is FT-IR?

FT-IR stands for Fourier Transform Infrared, the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). **The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample**. Like a fingerprint no

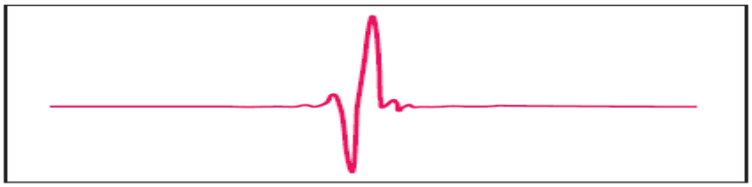
two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis.

**Fourier Transform Infrared (FT-IR)** spectrometry was developed in order to overcome the limitations encountered with dispersive instruments.

The main difficulty was the slow scanning process. A method for measuring all of the infrared frequencies **simultaneously**, rather than individually, was needed.

A solution was developed which employed a very simple optical device called an **interferometer**. The interferometer produces a unique type of signal, which has all of the infrared frequencies "encoded" into it.

The signal can be measured very quickly, usually on the order of **one second** or so. Thus, the time element per sample is reduced to a matter of a few seconds rather than several minutes.



Most interferometers employ a **beamsplitter, which** takes the incoming infrared beam and divides it into two optical beams. One beam reflects off of a flat mirror, which is fixed in place.

The other beam reflects off of a flat mirror which is on amechanism which allows this mirror to move a very short distance (typically a few millimeters) away from the beamsplitter.

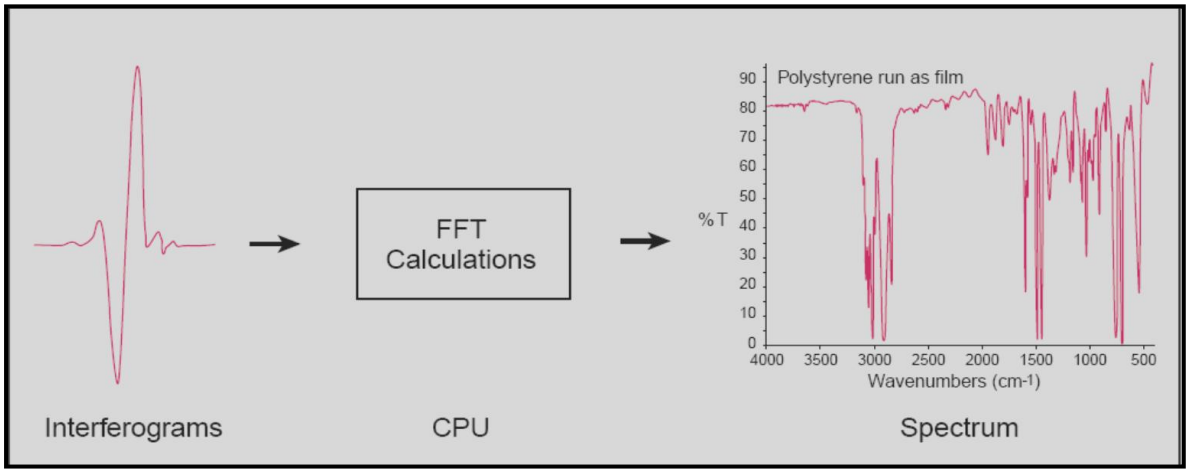
The two beams reflect off of their respective mirrors and are recombined when they meet back at the beamsplitter.

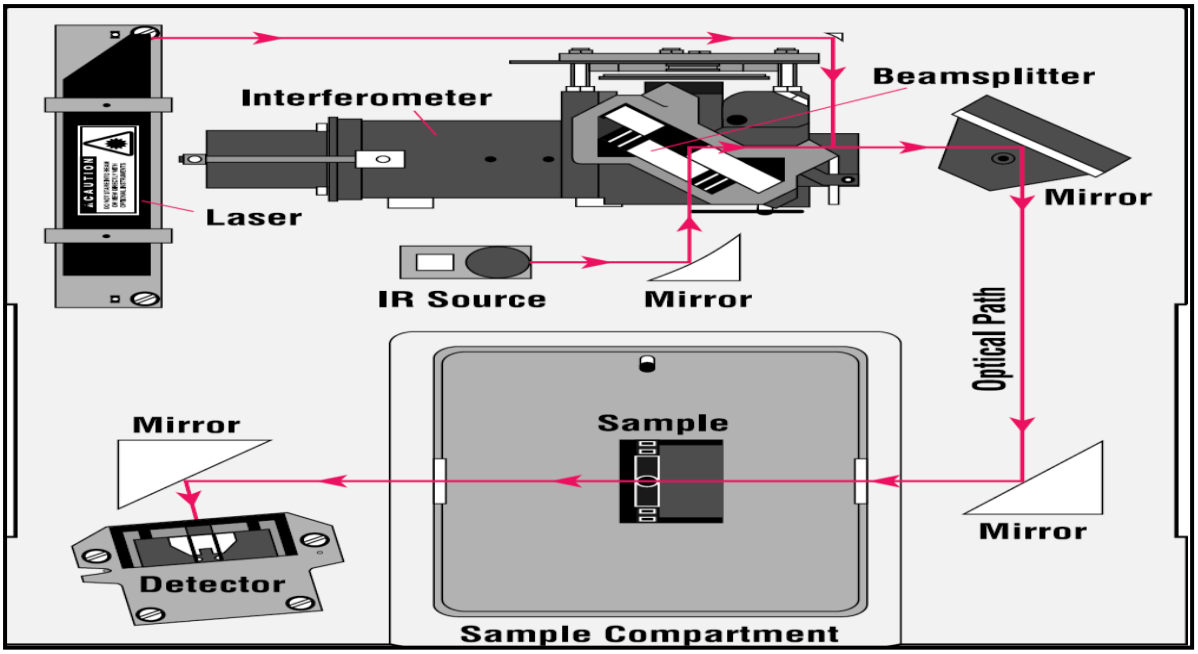
Because the path that one beam travels is a fixed length and the other is constantly changing as its mirror moves, the signal which exits the interferometer is the result of these two beams "interfering" with each

other. The resulting signal is called an **interferogram, which** has the unique property that every data point (a function of the moving mirror position), which makes up the signal has information about every infrared frequency, which comes from the source.

This means that as the interferogram is measured, all frequencies are being measured **simultaneously**. Thus, the use of the interferometer results in extremely fast measurements.

Because the analyst requires a **frequency spectrum** (a plot of the intensity at each individual frequency) in order to make identification, the measured interferogram signal cannot be interpreted directly. A means of "decoding" the individual frequencies is required. This can be accomplished via a well-known mathematical technique called the **Fourier transformation**. This transformation is performed by the computer, which then presents the user with the desired spectral information for analysis.





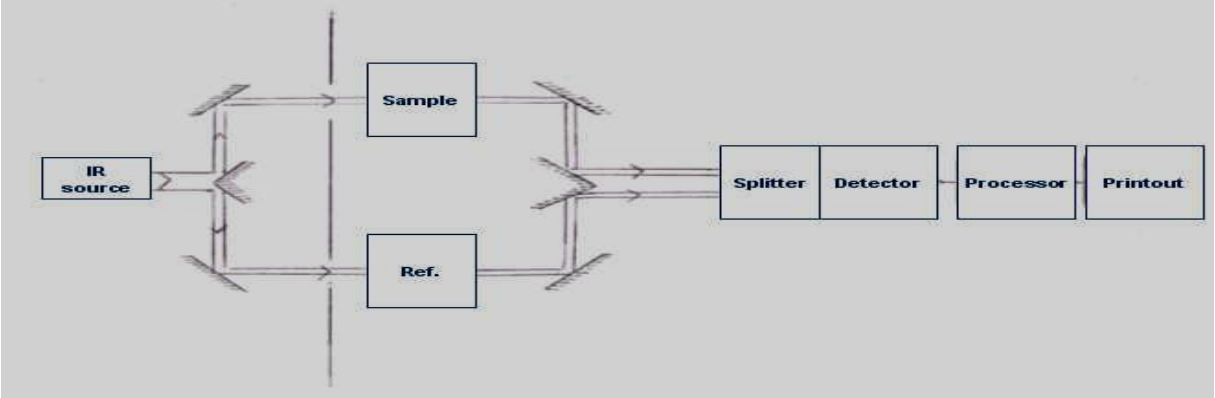




Figure : Diagram of an FTIR instrument

***So, what information can FT-IR provide?***

- It can identify unknown materials
- It can determine the quality or consistency of a sample
- It can determine the amount of components in a mixture

## *The Sample Analysis Process*

The normal instrumental process is as follows:

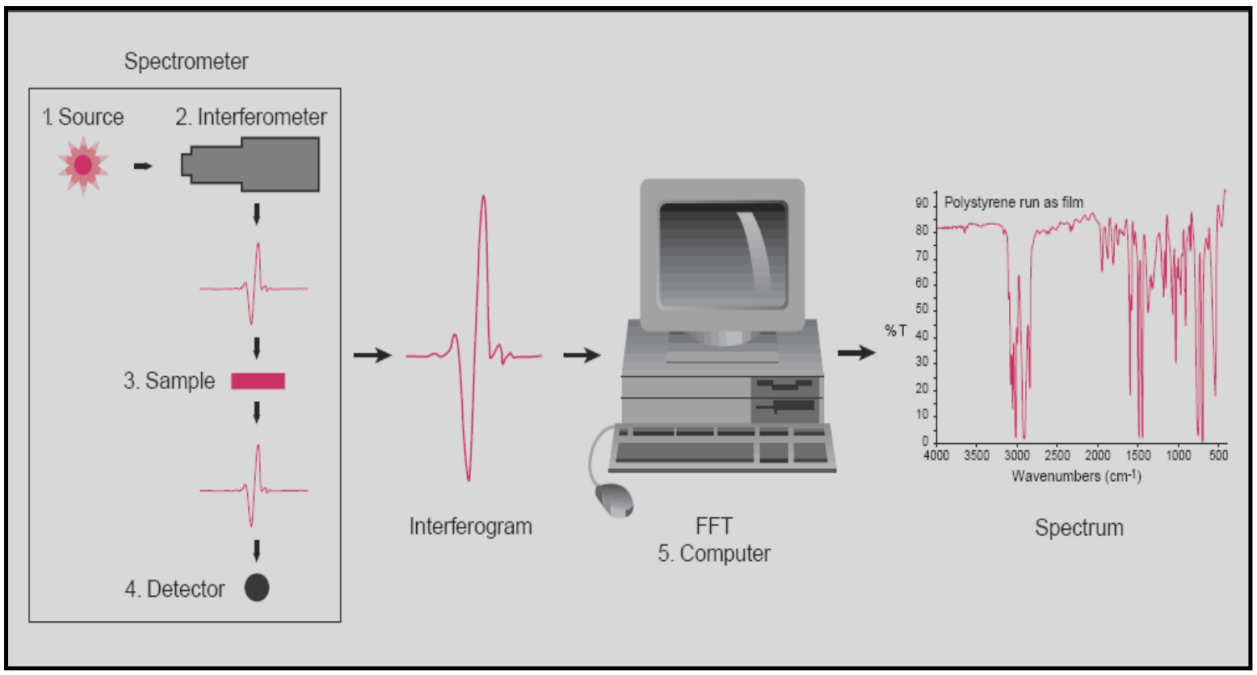
**1. The Source:** Infrared energy is emitted from a glowing blackbody source. This beam passes through an aperture, which controls the amount of energy presented to the sample (and, ultimately, to the detector).

**2. The Interferometer:** The beam enters the interferometer where the "spectral encoding" takes place. The resulting interferogram signal then exits the interferometer.

**3. The Sample:** The beam enters the sample compartment where it is transmitted through or reflected off of the surface of the sample, depending on the type of analysis being accomplished. This is where specific frequencies of energy, which are uniquely characteristic of the sample, are absorbed.

**4. The Detector:** The beam finally passes to the detector for final measurement. The detectors used are specially designed to measure the special interferogram signal.

**5. The Computer:** The measured signal is digitized and sent to the computer where the Fourier transformation takes place. The final infrared spectrum is then presented to the user for interpretation and any further manipulation




## Advantages of Fourier transform IR over dispersive IR

- Improved frequency resolution
- Improved frequency reproducibility (older dispersive instruments must be recalibrated for each session of use)
- Higher energy throughput
- Faster operation
- Computer based (allowing storage of spectra and facilities for processing spectra)
- Easily adapted for remote use (such as diverting the beam to pass through an external cell and detector, as in GC - FT-IR)

**Because there needs to be a relative scale for the absorption intensity, a background spectrum must also be measured. This is normally a measurement with no sample in the beam. This can be compared to the measurement with the sample in the beam to determine the "percent transmittance." This technique results in a spectrum, which has all of the instrumental characteristics, removed. Thus, all**

**spectral features, which are present, are strictly due to the sample. A single background measurement can be used for many sample measurements because this spectrum is characteristic of the instrument itself.**

**Sample Preparation**

Samples for FTIR can be prepared in a number of ways. For **liquid samples**, the easiest is to place one drop of sample between two plates of sodium chloride (salt). Salt is transparent to infrared light. The drop forms a thin film between the plates. **Solid samples** can be milled with potassium bromide (KBr) to form a very fine powder. This powder is then compressed into a thin pellet, which can be analyzed. KBr is also transparent in the IR. Alternatively, solid samples can be dissolved in a solvent such as methylene chloride, and the solution placed onto a single salt plate. The solvent is then evaporated off, leaving a thin film of the original material on the plate. This is called cast film, and is frequently used for polymer identification.

## Calculation of frequency

The frequency of vibrations is obtained from Hooke's law,

$$\nu = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}}$$

where ν is the vibrational frequency (in wave numbers, $cm^{-1}$), μ" is the reduced mass of atoms in the bond (in grams), c is the velocity of light ($2.998 \times 10^{-10} cm \cdot s^{-1}$), and k is the force constant of the bond (in $dyne \cdot cm^{-1}$). Let us look at each part of the equation:

***a) Frequency:***

Recall that the energy of electromagnetic radiation is proportional to frequency

(E = h***v***) and that frequency is given by

**Frequency = velocity of light (cm/sec) / wavelength (cm)**

Because the velocity of light is a constant, we recognize that the frequency is simply proportional to the reciprocal of the wavelength.In infrared spectroscopy a typical wavelength for a vibration is 4.0 x $10^{-4}$cm. Taking the reciprocal, we have 2500 $cm^{-1}$. Chemists say that this vibration has a frequency of 2500 "wavenumbers." The infrared spectra youwill see will have energies in the range of 4000 $cm^{-1}$ to 500 $cm^{-1.}$

***b) Reduced mass****, ":*

The reduced mass is the quotient of the product of the masses of the two bonded atoms divided by their sum.

**Reduced mass == $m_1 \bullet m_2/m_1 + m_2$**

The Relation of Atomic Masses and Vibrational Frequency

| Bond | Energy ($cm^{-1}$) |
|---|---|
| H—F | 4100 |
| H—Cl | 3000 |
| H—Br | 2650 |
| H—I | 2300 |

**The important point is that the energy involved in a vibration is inversely related to the masses of the atoms involved.That is, the heavier the atoms involved, the lower the energy. (You will see the effects of this in this experiment.) In the margin is a table showing that as the masses of atoms involved in a bond increase, the energy (given in wave numbers) declines.**

***c) Force constant, k:***

The force constant, which can be thought of as a measure of bond "stiffness," can be correlated with such bond properties as bond order and bond strength. Because the frequency is related directly to the square root of the force constant, we know that the frequency of bond vibrations

should decrease as bonds decrease in strength. This trend is illustrated by the series of carbon-nitrogen bond frequencies given in the table in the margin. The force constant, and thus the vibrational frequency, is also related to the electronegativity difference between the atoms of the bond. As the electronegativity difference increases, the bond strength increases. This means the force constant should increase, and so the vibrational frequency should also increase. This is seen in the series of bonds having an H atom attached to C, N, O, and F.

The Relation of Bond Order and Vibrational Frequency

| Bond | Energy ($cm^{-1}$) |
|---|---|
| C≡N | 2150 |
| C=N | 1650 |
| C-N | 1100 |

The Relation Between the Electronegativity of the Bonded Atoms and Vibrational Frequency

| Bond | Energy ($cm^{-1}$) |
|---|---|
| H—C | 2861.6 |
| H—N | 3300 |
| H—O | 3735.2 |
| H—F | 4183.5 |

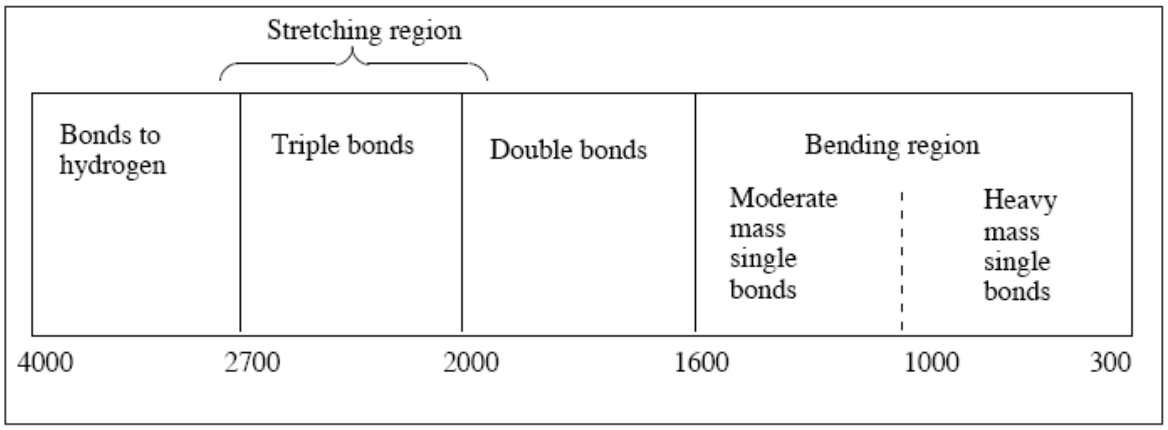



**Figure 3.** Characteristic wavelength regions (in wavenumbers, $cm^{-1}$) for different vibrations.

## Characteristic Patterns in Infrared Spectra

Just as each person has a unique set of fingerprints, each chemical compound has a unique infrared spectrum. If you have available an enormous library of reference spectra, and a powerful computer, in principle you could match the spectrum of an unknown compound with the library and uniquely identify the compound. This is in fact done,the Chemistry Department has an infrared system with this capability.

But what if you have a spectrum of a completely new compound, or onethat is not in your database? It turns out that different parts of a molecule,its***functional groups***, give rise to infrared vibrations in reasonably well defined regions of the infrared region. For example, as *Figure 3* shows, stretching vibrations of bonds to H usually occur at higher energies, triple bonds have higher energy stretching vibrations than those of double bonds, whereas bending vibrations occur at lower energies. Extensive experience has led to the compilation of correlation tables. For example, chemists know that the stretching motions of bonds commonly found in organic compounds occur in the ranges given in the table below.

**Chemistry 112 Laboratory: Infrared Spectroscopy–An Introduction Page 97,Revised: December 2005**

| Bond | Frequency ($cm^{-1}$) | Shape and Intensity |
|---|---|---|
| O—H | 3650-2500 | Often broad, strong |
| N—H | 3400-3200 | Sharp, variable |
| —C—H | 2960-2850 | Sharp, medium |
| =C—H | 3100-3000 | Sharp, medium |
| ≡C—H | 3300 | Sharp, medium |
| C=C | 1680-1620 | Sharp, variable |
| C≡C | 2230-2100 | Sharp, variable |
| C=O | 1800-1630 | Sharp, strong, |
| C—O | 1300-1000 (variable) | Sharp, strong |
| C—N | 1360-1020 | |

Much more extensive correlation tables of infrared bands can be found in books on infrared spectroscopy and instrumental analysis. Chemists in "crime" laboratories use infrared spectroscopy to identify chemical compounds implicated in crimes.

## Identifying Compounds

The various classes of organic compounds. Each is characterized by a ***functional group***, an atom or group of atoms within the molecule that give the compound its characteristic chemistry.

the different classes of compounds have characteristic vibrations that are unique. Therefore, a chemist skilled in "reading" an infrared spectrum can often decide what type of compound has given rise to the spectrum. Furthermore, if reference spectra are available in a catalog, or in a database searchable by computer, the exact identity of the compound can be determined. This is widely used by research chemists, chemists responsible for quality control in pharmaceutical production, for example, and by forensic chemists.

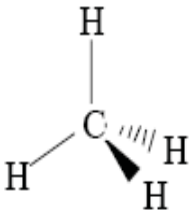
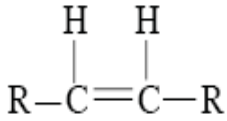
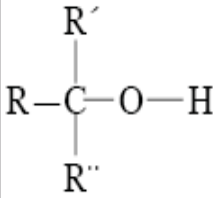
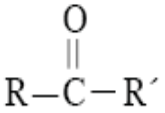
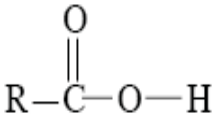
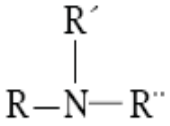
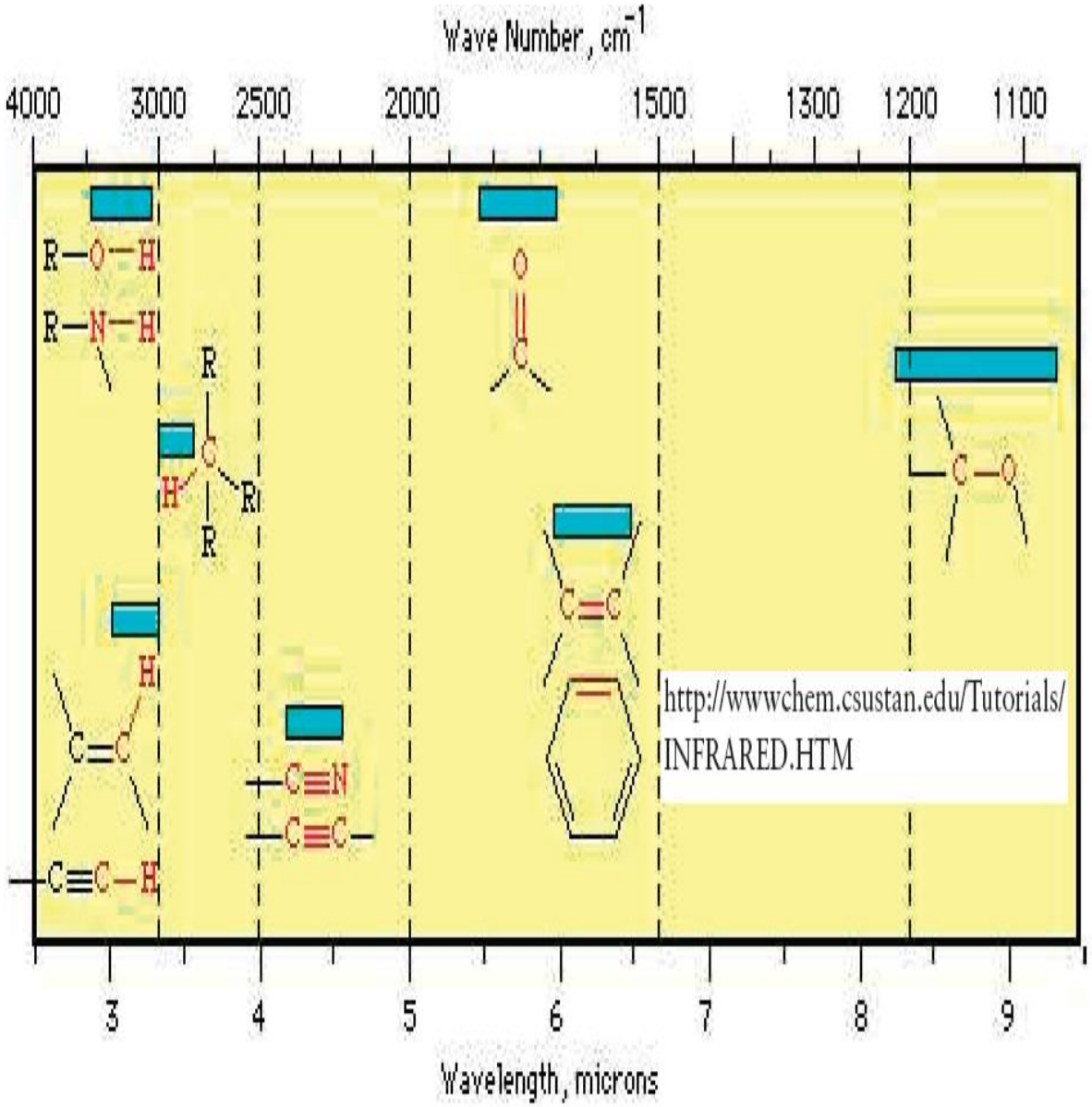
| Schematic Structure | Class of Compound | Characteristic Vibrations |
|---|---|---|
|  | Alkane, $C_nH_{2n+2}$ | Only C–H vibrations |
|  | Alkene | C=C double bond stretch |
|  | Alcohol | O—H stretch |
|  | Aldehyde (when R´ = H)<br>Ketone (when R´ = organic group) | C=O stretch |
|  | Carboxylic acid | C=O and O–H vibrations |
|  | Amine | N–H stretch |

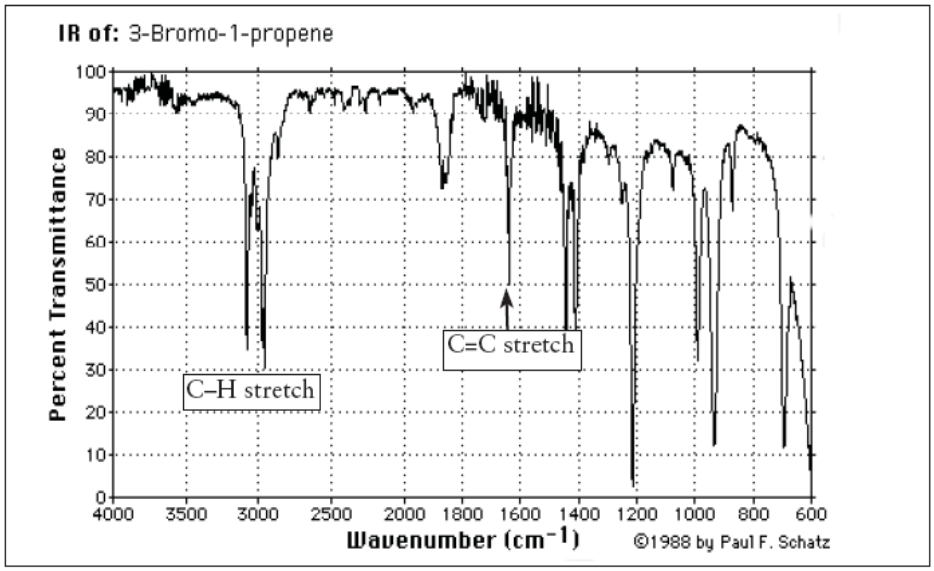
**Figure 4** Some classes of organic compounds, their functional groups, and characteristic vibrations of those groups.

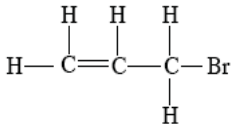
CORRELATION CHART: wavelength regions for vibrations of some common groups.



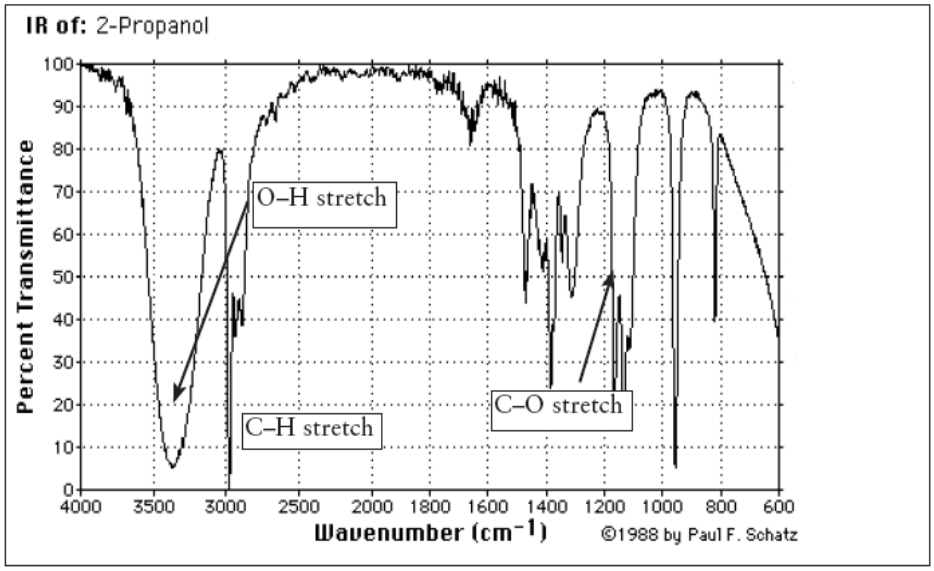
# Representative Infrared Spectra

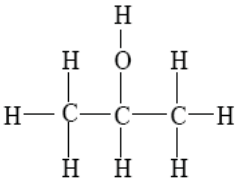
*3-Bromo-1-propene*



This molecule has a carbon-carbon double bond that has a sharp band at about 1650 $cm^{-1}$. The sharp bands at about 2950-3100 are C–H stretches. The band at about 3100 $cm^{-1}$ is due to the C–H bonds around the C=C double bond, whereas the band at 2950 $cm^{-1}$ is C–H bonds of the $–CH_2Br$ group.

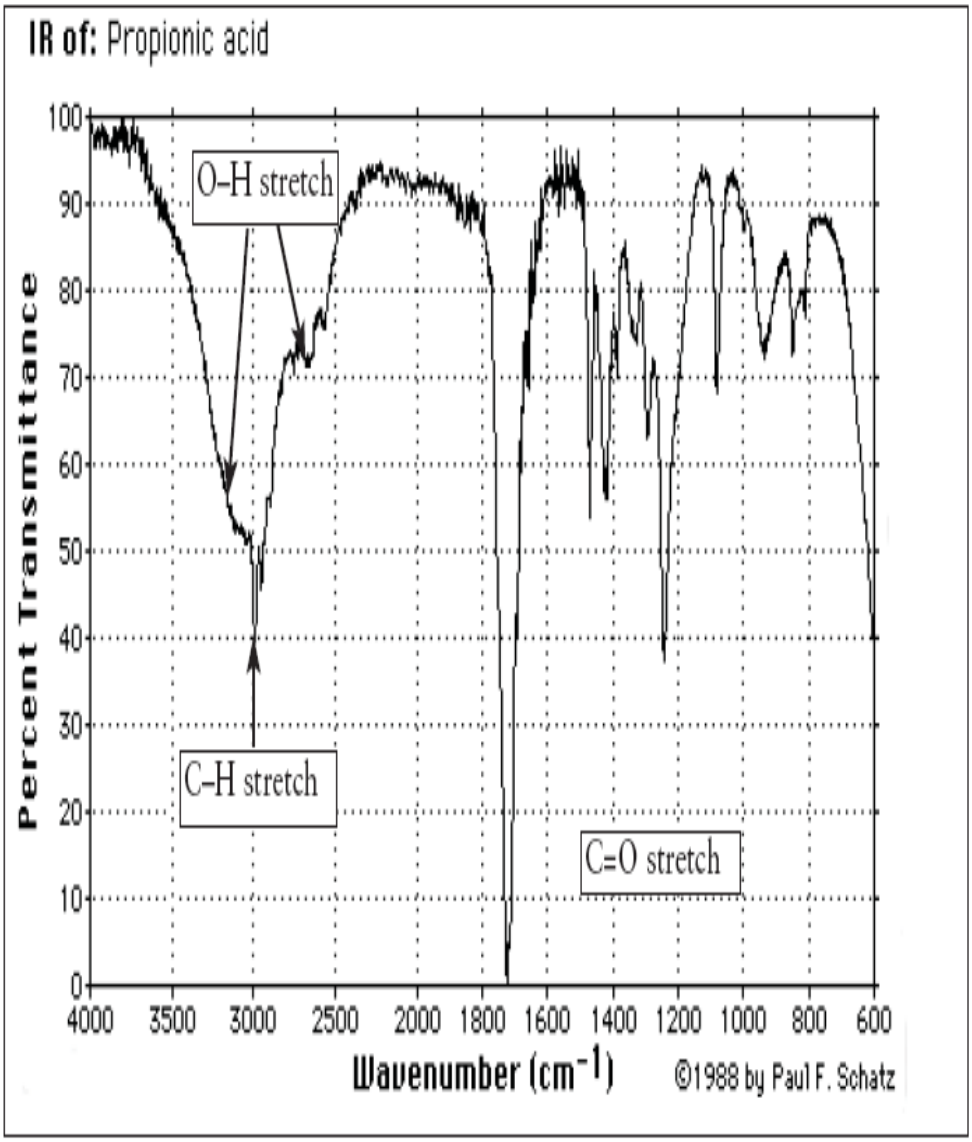


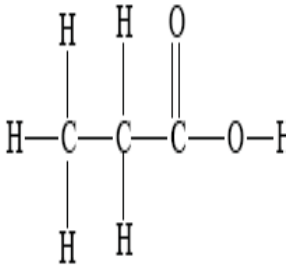



*2-Propanol*

This alcohol has an obvious, broad and strong band at about 3400 $cm^{-1}$. This is characteristic of an O–H stretching vibration. The C–H stretch is again at about 3000 $cm^{-1}$. Finally, you now see a C–O stretch in the 1100-1200 $cm^{-1}$ region.

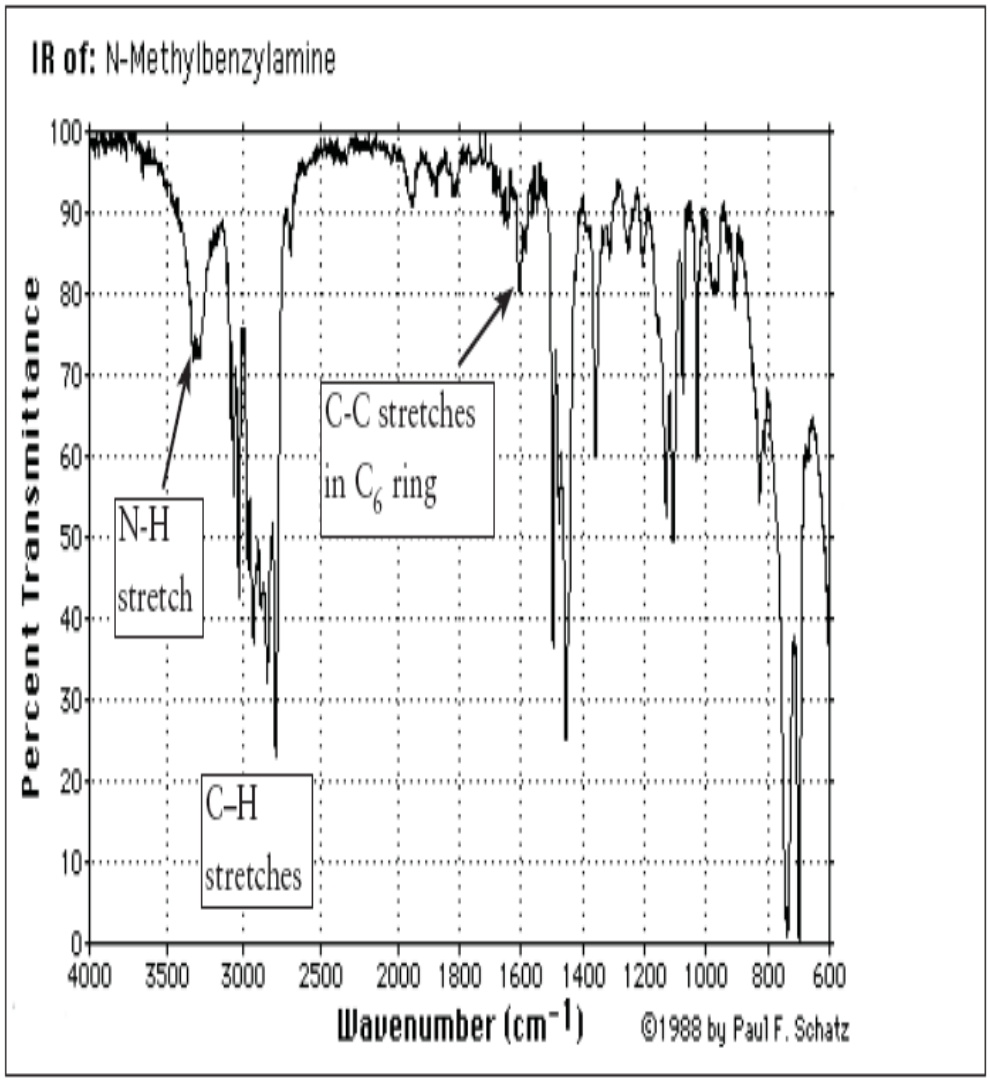
*Propanoic Acid*

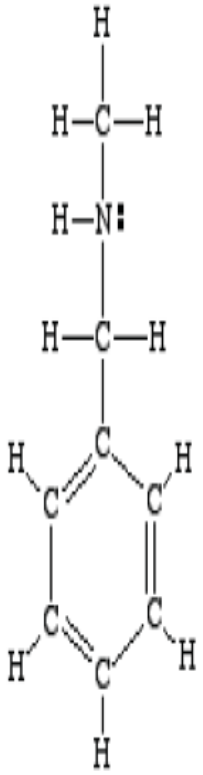


An organic acid is characterized by the $-CO_2H$ group (the carboxylic acid group). This group has a very clear infrared fingerprint. Here you see the strong, broad O–H stretching band (overlapping the C–H stretch) and the sharp, strong C=O band.

*N-Methylbenzylamine*



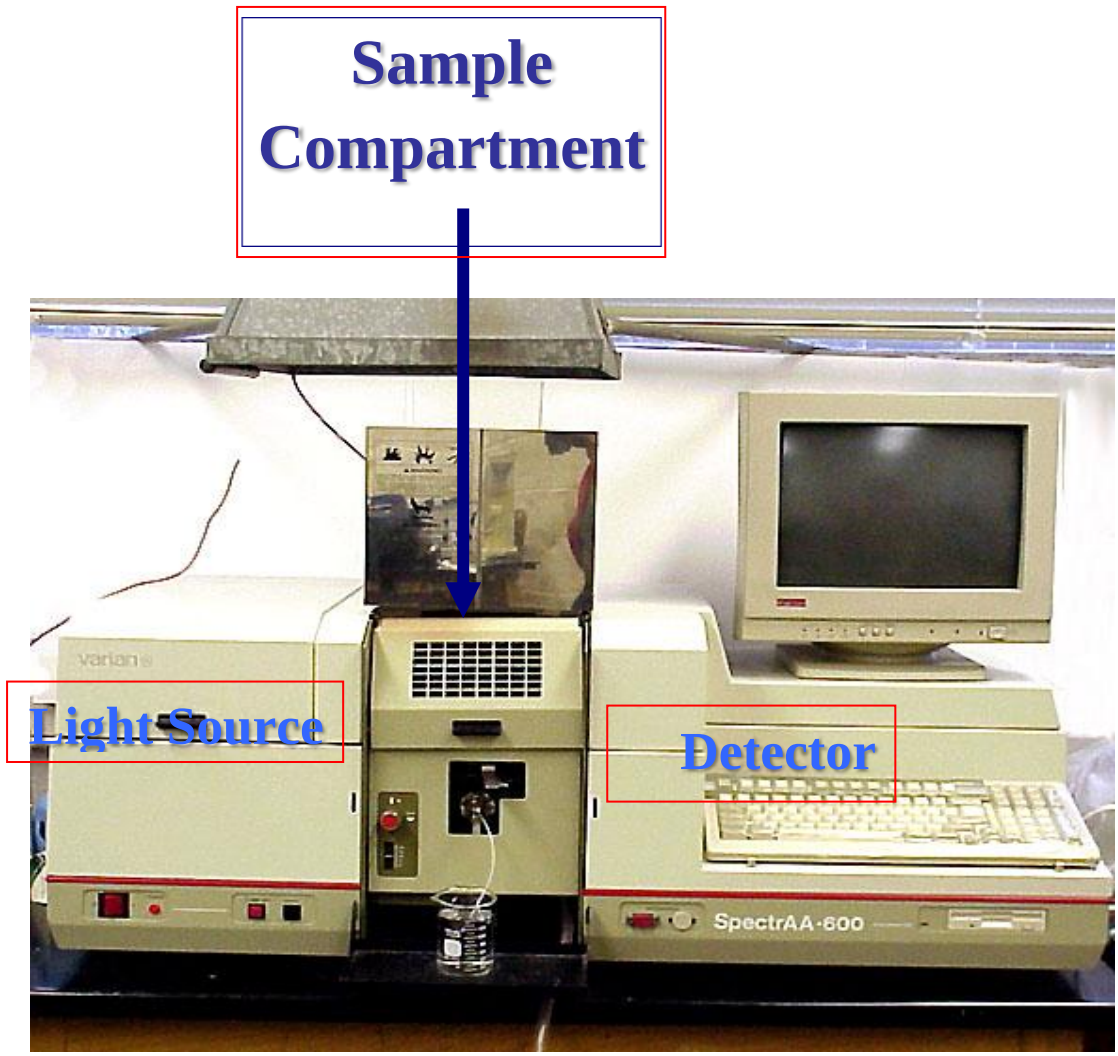



This molecule is classified by organic chemists as an "amine." In this case, there is an H atom attached to the N, and this has a characteristic medium intensity band at about 3400 $cm^{-1}$ (see page 5). In addition, there is a group of bands around 3000 $cm^{-1}$ that arise from C–H stretching vibrations. Finally, we again see a weakish set of bands at about 1600 $cm^{-1}$ that arise from the stretching of the C–C bonds in the $C_6$ benzene ring. (See the spectrum of acetophenone on the preceding page.)

# Atomic absorption spectroscopy

Atomic absorption spectrophotometry is commonly used in many analytical laboratories for determination of trace elements in water samples and in acid digests of sediment or biological tissues. Itis one of the important instrumentaltechniques for both quantitative and qualitative analysis of metallic and nonmetallic elements in inorganic or organic materials.

Atomic absorption spectroscopy can be used to analyze the concentration of over 62 different metals in a solution.An atomic absorption spectrophotometer consists of a light source, a sample compartment and a detector.



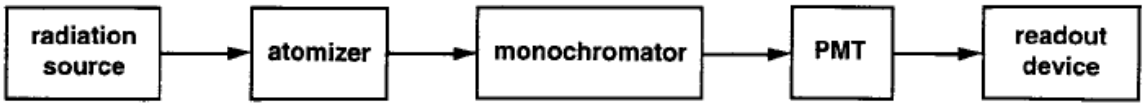



Fig. The essential components of an atomic absorption spectrophotometer.

## EXCITATION SOURCES

### A. Hollow-Cathode Lamps

The line source of radiation most frequently employed in AAS is the hollow-cathode lamp (HCL)—see Figure below for a schematic rendering. Commercially, hollow cathode lamps are available as both a single element and multi-element sources. A potential of 350V to 500V (producing a current of 1 to 50 mA) is placed across the cathode and anode terminals.

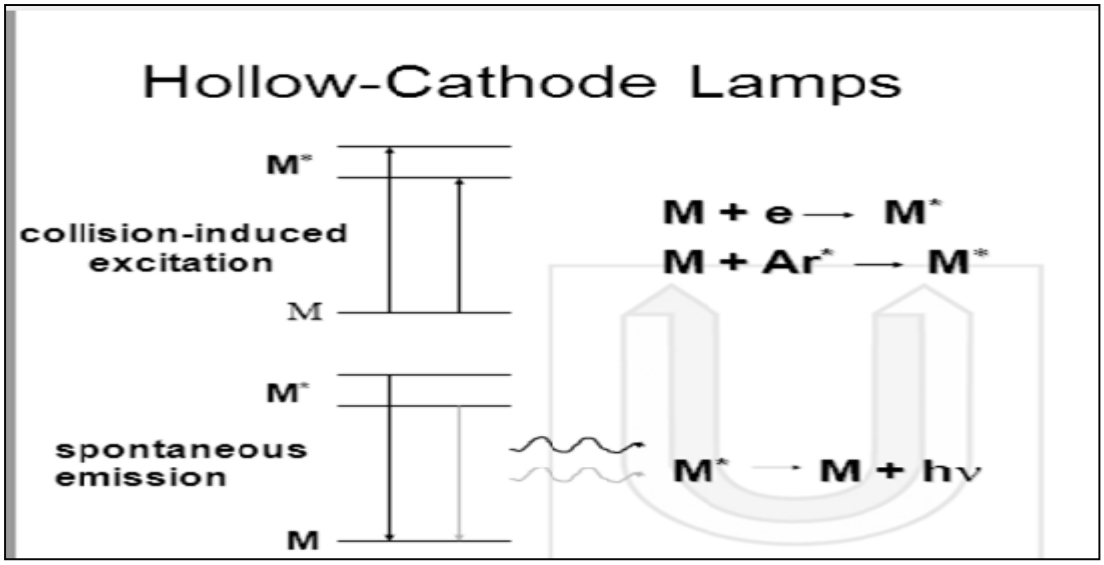
The element selectivity of the HCL is achieved by fabricating the cathode from, or coating the interior of the cathode with, the element of interest. The anode and cathode are sealed in a Pyrex tube containing a quartz window at the opposite end for transmission capability throughout the ultraviolet spectral region.

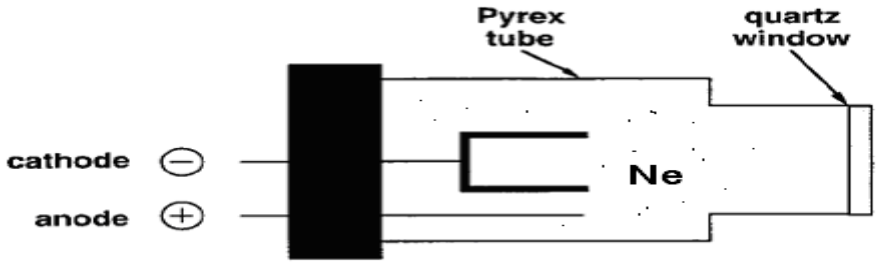
The interior of the tube is evacuated and filled with an inert gas, such as neon. Under the applied potential, the neon atoms become ionized at the anode and are accelerated toward the cathode. The kinetic energy of the accelerated neon ions is imparted to the material of the cathode upon impact, causing metal atoms to be sputtered off the cathode surface into the gas phase. The sputtered metal atoms undergo collisions with other neon ions, producing excited state metal atoms. Upon relaxation to the ground state, the metal atoms emit radiation at their characteristic wavelengths.

$$Ne \longrightarrow Ne^{+} + e^{-}$$

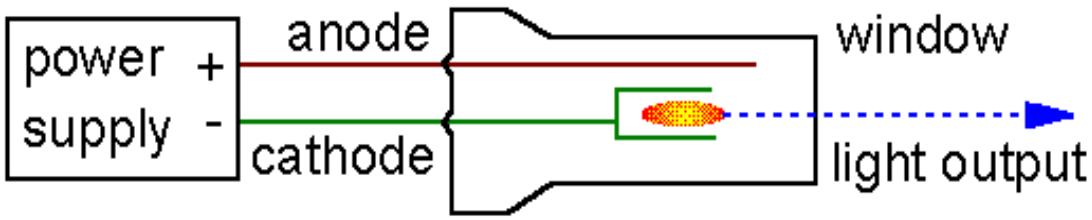
$$Ne^{+} + M \longrightarrow M^{*} + 2e^{-}$$

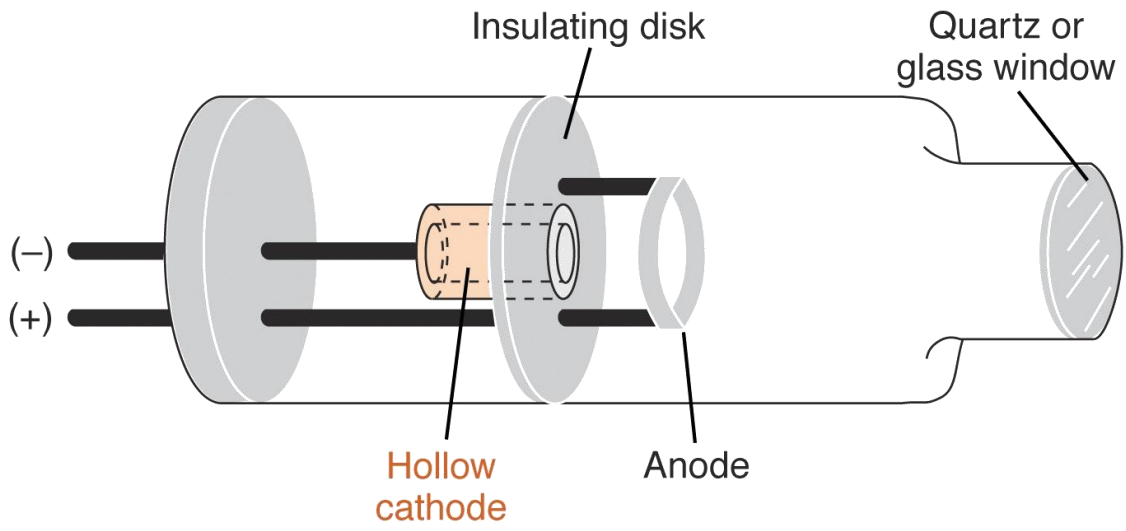
$$M^{*} \longrightarrow M + h\nu$$




Schematic of a hollow-cathode lamp.







**A hollow cathode lamp for Aluminum (Al)**

**The intensity of the atomic line radiation produced depends on the number of sputtered metal atoms, which depends on the kinetic energy of the neon ions,which, in turn, is dictated by the lamp current.**

**Consequently, a higher lamp current produces a greater spectral line intensity from the source, which in turn provides a greater analytical sensitivity. However, at high lamp currents, an excess of metal atoms is sputtered from the cathode surface. The resulting dense cloud of metal atoms absorbs radiation emitted from near by excited-state metal atoms.** When **self-absorption** of this type occurs within the HCL,

the level of radiation available for absorption by the free atoms from the sample, produced via atomization, is reduced and sensitivity is lost.

This process of **self-absorption** is called "**self-reversal**" and dictates the maximum operating current of the HCL and the peak of spectrum will be dent.

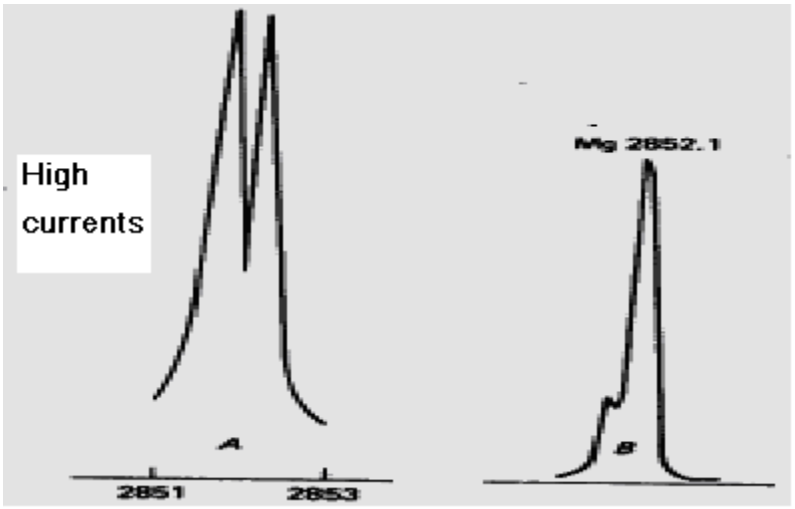



**FIGURE :Curve *A* illustrates the self reversal that occurs with high current of the HCL**

The multi-element HCL **operates** under the same principles governing the single element HCL, except that the cathode is fabricated from pressed alloys or powders of several elements, instead from just a single element.

For a **cost** comparable to that of the single-element HCL, the multi-element HCL provides simultaneous detection of several metals. Despite the economic advantages, the multi-element HCL typically has a shorter lifetime than the single-element HCL, primarily because the metal atoms of the cathode have different degrees of volatility.

The sensitivities of the inaccessible metal atoms will decrease over time and ultimately shorten the effective lifetime of the HCL.

The lamp is housed inside the lamp compartment of the instrument.

The atomic spectra of most elements originate from the transition of electrons from the ground state to the excited state, giving rise to what are commonly called **resonance lines**.

The diagrams in Figure below are transitions – selected lines for sodium and potassium. Some elements in the periodic table contain very complicated electronic structures and display several resonance lines close together. The widths of most atomic lines are extremely small $10^{-6}$ nm, and when broadened in various ways the width never exceeds $10^{-2}$ nm. Fortunately, the modern optics available on the latest instruments can isolate lower bandwidths.

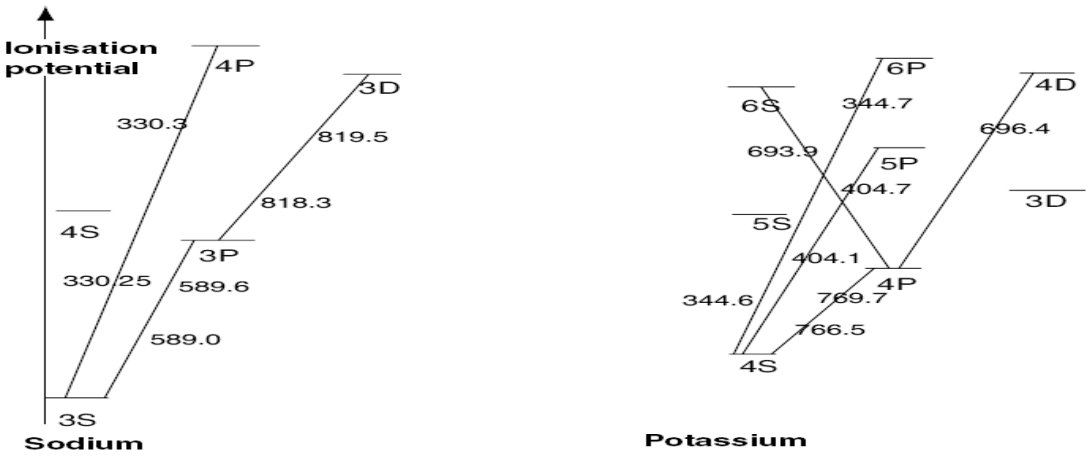



Figure : Energy levels and wave number (nm) diagram for sodium and potassium

AAS is relatively free from elemental **interferences** because it **determines elements using absorption by specific lamps for the metal under test. However, it is prone to background interferences caused by:**

(a) absorbing molecular species of the sample, e.g. some absorptions by other species in the sample may occur at the same wavelength as the element under test;

(b) absorbing atomic species from other elements in the sample, e.g. phosphorus in the presence of calcium;

(c) particles from high salt concentration in the atom cell can cause light scattering.

**B. Electrodeless Discharge Lamps**

The electrodeless discharge lamp (EDL) is another atomic line source used in AAS. The EDL is also available in a multi-element format. The EDL is fabricated by sealing a small amount of the metal or metal iodide of interest in a quartz tube filled with an inert gas, usually argon, at reduced pressures. The EDL is placed in a microwave, radio-frequency field to ionize the inert gas. The metal or metal iodide species collides with the free electrons from the ionized inert gas, producing excited-state metal atoms. In a process analogous to that described for the HCL, these excited-state metal atoms emit the characteristic atomic lines of the metal as they relax to the ground state.

In comparison to the HCL, the EDL produces an atomic line source of greater intensity, resulting in enhanced analytical sensitivities. However, the EDL requires a separate power supply that increases the instrumental cost of the AAS.

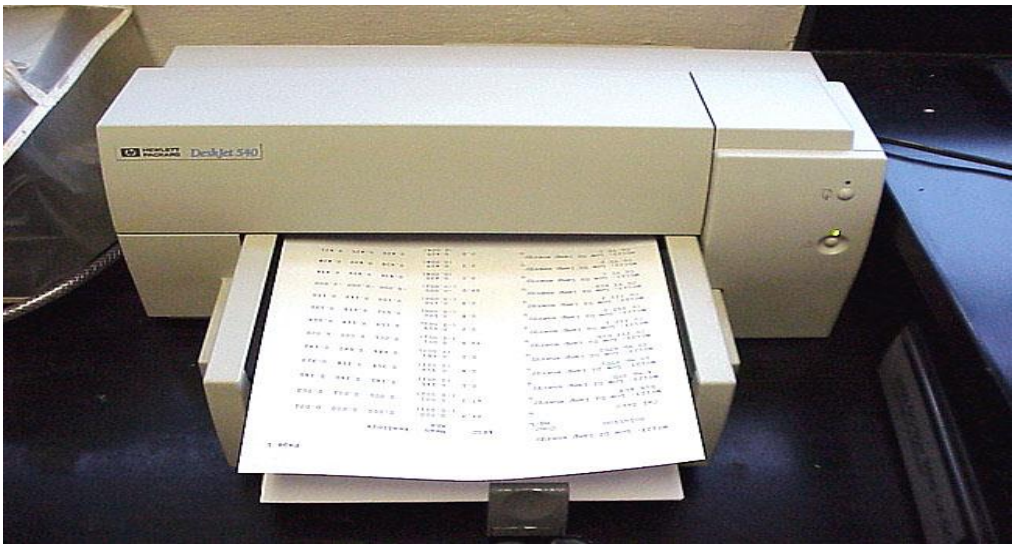
## C. Continuum Sources

For purely economic reasons, the use of a continuum source in AAS is attractive because it eliminates the need to purchase multiple HCL sources. However, in practice the bandpass of a typical monochromator (0.1 to 0.2 nm) is much greater than the atomic line-width of the absorbing atoms (0.002 nm). Consequently, a large fraction of the radiation produced by the source falling on the detector is unabsorbed. The small amount of radiation that is actually absorbed results in a low analytical sensitivity. Continuum sources utilizing scanning Echelle grating monochromators have largely overcome the band pass difficulty, but overall they still provide lower sensitivity in comparison to the narrow lines produced by the HCL. When using a continuum source, the high-intensity xenon arc lamp is preferred over the deuterium continuum source because of the higher analytical sensitivity obtainable.

**The sample compartment** is really the flame since it is in the flame that the atoms absorb radiation from the source.

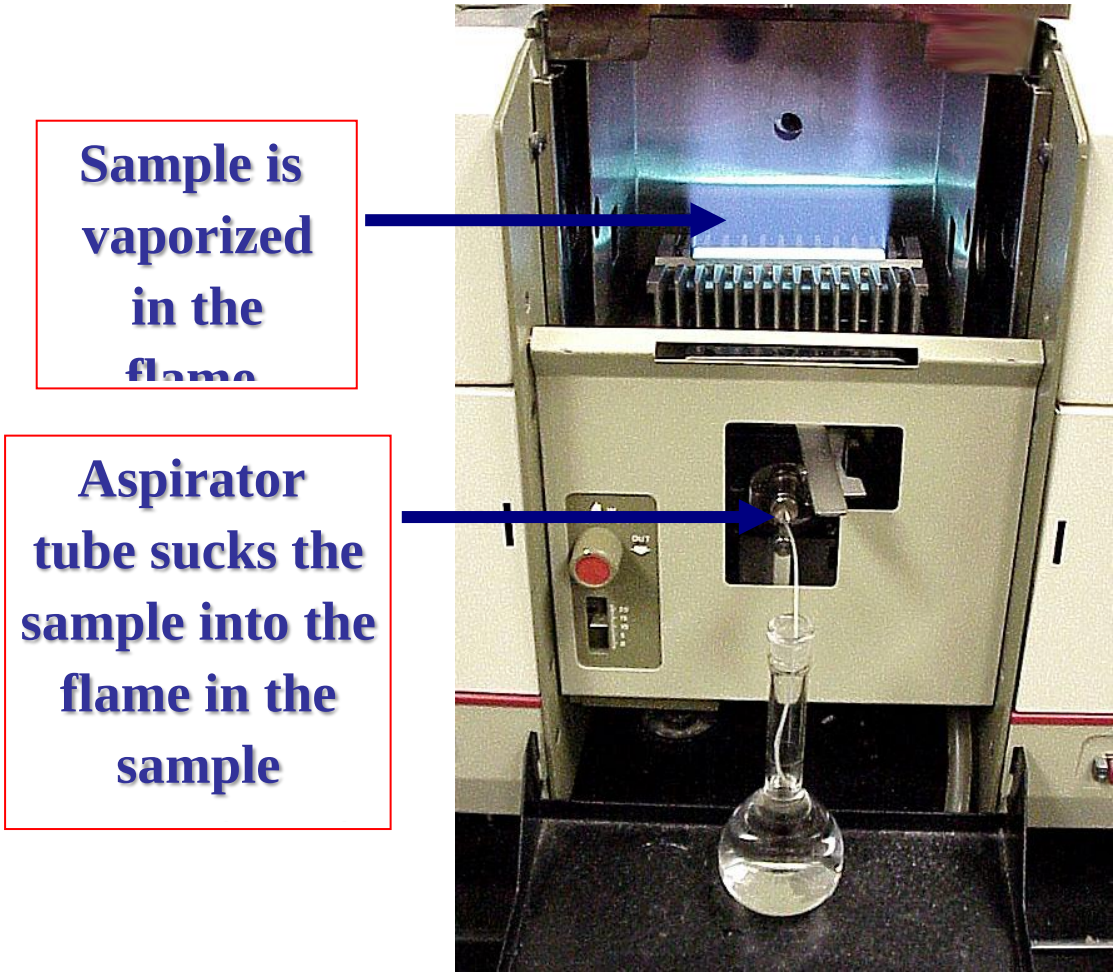
The signal from the detector is transferred to the computer, and the output registers on the monitor in a manner specified by the user.

**Hardcopy of the data is usually then sent to the printer.**




One of the most common means of introducing the sample into the flame is by preparing a solution of the sample in a suitable solvent, frequently water.

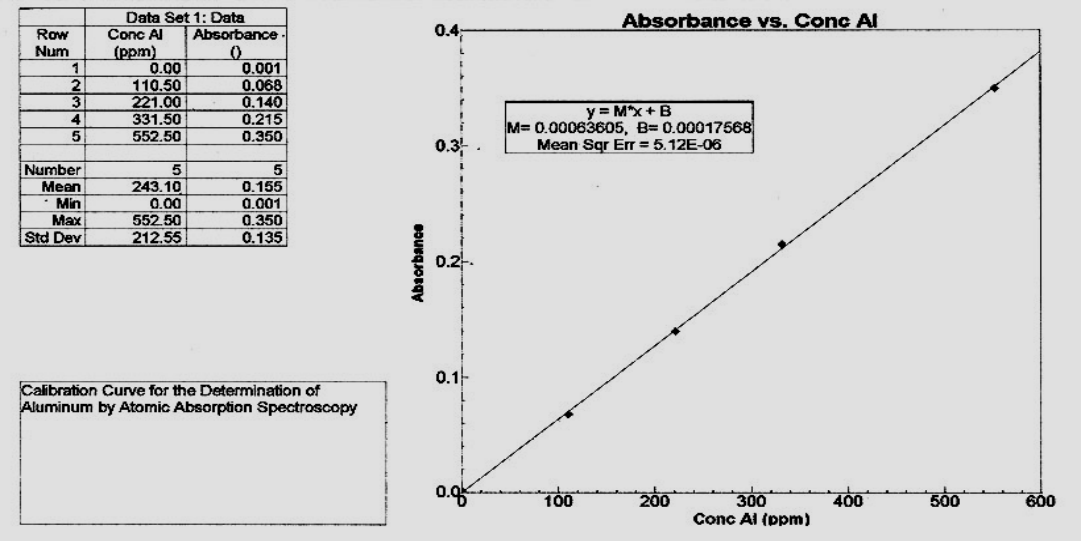
The flame gases flowing into the burner create a suction that pulls the liquid into the small tube from the sample container. This liquid is transferred to the flame where the ions are atomized. These atoms absorb light from the source.




The readings specified by the user are displayed on the screen for each sample measured.

Quantitative analysis can be achieved by measuring the absorbance of a series of solutions of known concentration.

A calibration curve and the equation for the line can be used to determine an unknown concentration based on its absorbance.



| Row Num | Data Set 1: Data Conc Al (ppm) | Absorbance () |
|---|---|---|
| 1 | 0.00 | 0.001 |
| 2 | 110.50 | 0.068 |
| 3 | 221.00 | 0.140 |
| 4 | 331.50 | 0.215 |
| 5 | 552.50 | 0.350 |
| | | |
| Number | 5 | 5 |
| Mean | 243.10 | 0.155 |
| Min | 0.00 | 0.001 |
| Max | 552.50 | 0.350 |
| Std Dev | 212.55 | 0.135 |



**Rf.Written by Bette Kreuz,Produced by Ruth Dusenbery,University of Michigan-Dearborn2000**

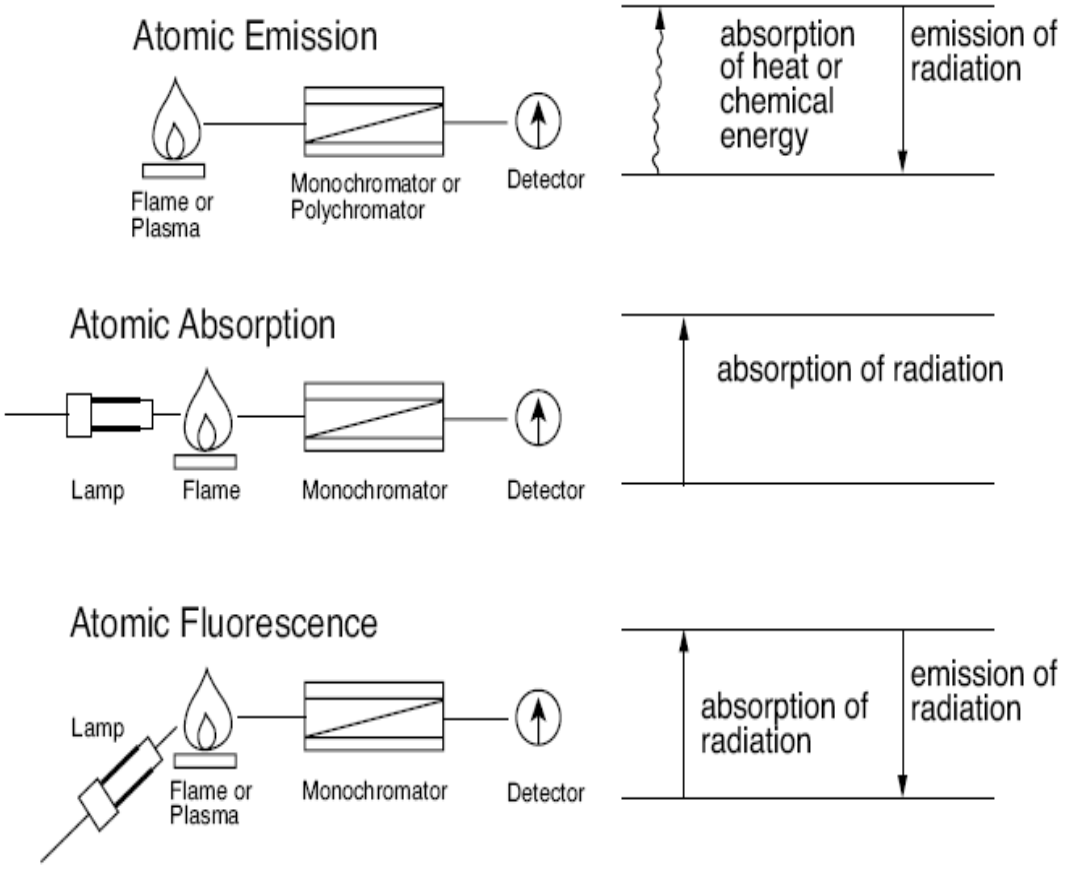




Figure 1.5 Three types of atomic spectroscopy techniques shown diagrammatically. (Reproduced by kind permission: copyright &1999–2008, all rights reserved, PerkinElmer,Inc.)

Atomic emission spectroscopy is applied to the measurement of light emitted by thermal energy caused by the thermal source from the chemical species present. Examples of emission, absorption and fluorescence spectroscopy can be shown schematically, as in Figure above.

The colour of light obtained from excited atoms stems from the chromophore of the valence shell electrons emitting light as electromagnetic radiation. Photons are absorbed during the promotion of an electron between waves' mechanically allowed (i.e. quantised) energy levels. The ultraviolet (165–400 nm) and visible regions (400– 800 nm) of the electromagnetic spectrum are the regions most commonly used for analytical atomic spectroscopy. Wavelengths from 700 nm upwards are in the infrared region and are inapplicable to atomic spectroscopy. The 165–700 nm region in the electromagnetic spectrum is generally referred to as 'light' although, technically, **all electromagnetic radiation can be considered as light. Known wavelengths for elements can vary from 1 for Li to 5700 lines for Fe. Some lines are more sensitive than others and this fact is in itself useful because low levels would need very sensitive lines while less sensitive lines can be useful for higher concentrations of elements in samples.**

# Summary: Applications of Atomic Spectroscopy

Flame atomic absorption and flame emission techniques were developed before inductively coupled plasma emission spectroscopy and are still used extensively for analysis of a wide variety of samples on a routine and non-routine basis. They are very useful techniques for elemental analysis of selected sample matrices; however, the lower temperature ($\approx 2800C^0$) of these techniques limits their sensitivity to a range of important samples. The development of plasma sources (1970s) capable of achieving temperatures of $9000C^0$ has revived the use of emission instrumentations enabling improved sensitivity and multi-elemental analysis at major, minor and trace levels. The use of plasma as an excitation source has an added analytical advantage in its ability to use a wider linear dynamic range allowing little or no dilution and reducing chemical and physical interferences. The higher excitation temperature of plasmas compared with flames results in a more efficient atom excitation which leads to increased sensitivity especially for refractory elements such as B, P, W, Nb, Zr and U. The plasma source geometry and dynamics mean fewer sample atoms in the plasma and temperature profiles result in minimal line reversal and matrix interferences.

Both flame and plasma sources are sensitive and selective techniques measuring as little as $10^{-16}$ g of analyte solution in complex mixtures. However, understanding the techniques and the type of sample being analysed helps in deciding which technique is suited for a particular application.

Elements occur in natural and synthetic compounds at various levels and since the beginning of the development of atomic spectroscopy analytical instruments, more information about toxicity, benefits, etc., became known. Modern atomic spectroscopy instrumentation can determine from % levels to trace levels (ppm) and sub-trace low levels (sub-ppb) with a

high degree of accuracy and precision. At whatever concentration, knowing the concentration of these elements plays a very important role in understanding more about products in terms of health issues, benefits, shelf life, stabilities, etc. Table below is a summary of elements of great importance that need to be monitored in order that any changes can be interpreted as part of behaviour that could be good or bad.

Table :List of some elements of importance requiring quantification using atomic spectroscopy techniques

| **Element** | **Area of importance** |
|---|---|
| **Mg, P, S, K, Si, V, Cr, Fe, Co, Ni Co, Zn, As, Sc, Mo, Sn and I** | **Biochemistry and medicine** |
| **Almost all elements in the periodic table** | **Environmental science** |
| **Contamination and wear metals** | **Crude and virgin oil, and petroleum** |
| **e.g. Fe, Ni, Co, Mn, Cr, Mo, W, Na, K** | **Industries** |
| **Radioactive elements used for industrial and medicinal purposes** | **e.g. $U^{238}$, $U^{235}$ $Cs^{132}$, $Pu^{239}$, B, Si, Cd, etc.** |
| **Si, Al, As, Fe, Cu, Mo, Hg, Cr, Se, BaSr, Sb, Au Bi, Nb, Zr and Pb** | **, Electronics and semiconductor industries** |
| **Almost all earth and clay containing elements** | **Geological research** |
| **Cu, Pb, Al, P, S, Ca, Mg, Si, Hg, Cr, Co, etc.** | **Works of art, paint mixtures, etc.** |
| **Si, B, Cd, Pb, Fr, Cs, U Hg, As, Sn, Sr, Ni, Ge, Ga, etc.** | **Ceramic industries** |
| **All elements** | **Forensic support** |

## Interferences

Many metals can be determined by direct aspiration of sample into an air-acetylene flame .So called "chemical" interference occurs when the flame is not hot enough to dissociate the molecules or when the dissociated atoms are oxidized to a compound that will not dissociate further at the flame temperature. Such interferences can sometimes be overcome by adding specific elements or compounds to the sample solution. Dissociation of the molecules of silicon, aluminum, barium, beryllium and vanadium requires a hotter flame, and nitrous oxide-acetylene is used. Molecular absorption and light scattering caused by solid particles in the flame can cause high absorption values and consequently positive errors. Background correction techniques can be used to obtain correct values.

### VI. INTERFERENCES

### A. Chemical Interferences

Of all the interferences, chemical interferences are predominantly responsible for removing metal atoms from their ground state. Chemical interferences arise during the vaporization processes in atomization. Radical species are produced that can react with the metal compound undergoing vaporization to produce metal hydroxides, metal hydrides, or metal oxide species. In flame AAS, these species cannot be dissociated at cooler flame temperatures. This fact effectively reduces the number of free metal atoms produced in the flame, resulting in a loss in sensitivity. One method to reduce the effects of chemical interferences is to adjust the flame-gas stoichiometry. A fuel-rich flame produces a reducing environment, with correspondingly fewer of the reactive oxide species. An alternate approach is to use a hotter flame, such as the nitrous oxide-acetylene flame, to vaporize and dissociate any refractory metal oxides. A

third commonly employed technique is to use a releasing agent that can compete with the metal-oxide formation equilibrium. An example often cited is the addition of lanthanum when analyzing for calcium in the presence of high concentrations of phosphate ions.

**In this example**, the lanthanum competes with the calcium for complex formation with the phosphate ions in the flame. The lanthanum phosphate complex is more stable than the calcium phosphate complex and predominates, resulting in a greater number of free calcium atoms in the flame providing a higher sensitivity for the calcium. Another useful scavenging agent is ethylenediaminetetraaceticacid (EDTA). EDTA forms stable complexes with most metals that can be vaporized in a flame. Furthermore, the carbon atoms of EDTA surrounding the metal effectively produce a reducing environment during the dissociation step.

**B. Ionization Interferences**

To some extent, all metallic atoms produced at the temperatures of the flame undergo ionization. This can certainly be minimized by using cooler flames such as the acetylene-air flame and by adjusting the flame-gas stoichiometry. Another method is to chemically alter the ionization equilibrium within the flame. The ionization of a metal written as an equilibrium expressionis:

$$M^0 \rightarrow M^{n+} + ne^-$$

Therefore, elements that provide electrons to the flame will shift this equilibrium to the left. Such a species is called a “radiation buffer” or an “ionization buffer”. Radiation buffers are species that are easily ionized, such as cesium.To ensure similar atomization efficiencies for

quantitation, the buffer is typically added in excess to both standards and unknowns.

**C. Matrix Interferences**

A matrix interference involves the bulk physical properties of the sample to be analyzed. In practice, first a blank solvent is atomized and the instrument zeroed so that the measured voltage at the photo-multiplier tube (PMT) corresponds to 100% transmittance. Solutions containing the analyte of interest are subsequently atomized, and their atomic absorbances are measured.

The Beer-Lambert law is used to relate the unknown analyte concentration to the concentration of standard solutions. To ensure that the Beer- Lambert law is followed, atomization efficiencies must be matched between the calibration curve and the unknown sample. In flame AAS, matrix interferences will affect nebulization efficiencies during the atomization stage, primarily because nebulization efficiencies are affected by the physical properties of viscosity, surface tension, and density. For quantitative results using the Beer-Lambert law, these properties must be matched in both standard and unknown solutions. Instead of taking the time to matrix-match standards and unknown solutions, if the unknown is sufficiently concentrated, it can be diluted to approximately match the physical properties of the standard solutions, which are typically dilute.

The most common way to overcome a matrix interference is to use the method of standard additions (Figure 6). This method effectively creates a calibration curve by using incremental additions of a standard solution to an unknown. The incremental additions are of a minuscule volume so as not to alter the bulk physical properties of the unknown, effectively providing a matched matrix. The plot obtained is extrapolated to the x-axis intercept.

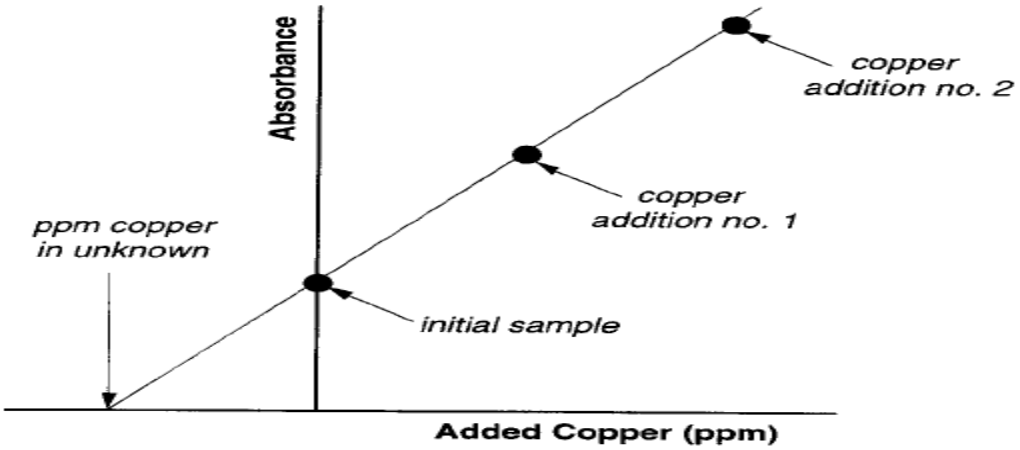



FIG. 6. An example of the method of standard additions for the analysis ofcopper concentration in an unknown.

The absolute value of the x-axis intercept gives the analyte concentration in the unknown sample. When using the method of standard additions, care must be taken to ensure that one is operating in the linear region of the calibration curve. In AAS, the Beer-Lambert typically holds at low concentrations, and the calibration plot often exhibits nonlinearity at higher analyte concentrations.

This is caused by stray source radiation that reaches the detector and to flame-diffusion effects, both of which can severely reduce the accuracy of the measurement and the results.

**D. Spectral Interferences**

Spectral interferences in AAS, although rare, do occasionally occur and can be corrected by manipulation of instrumental variables. Spectral interferences arise when radiation of a wavelength different from that of the wavelength of the element of interest falls on the detector. An example of direct overlap of spectra produced by atomic species in the unknown occurs when sodium and magnesium are both present. When analyzing for dilute amounts of magnesium in a sample with a high concentration of sodium, the 285.28 nm sodium line will overlap the

285.21 nm magnesium line. Therefore, in the flame, sodium atoms will absorb radiation from the magnesium HCL, resulting in a falsely high magnesium concentration detected at the PMT. As most monochromators do not have a resolution sufficient to separate the two closely spaced lines, an alternative wavelength for magnesium quantitation must be selected in such cases. Another similar spectral interference occurs when using a multi-element HCL. In this instance, lines from other elements in the HCL occur near, or overlap, the line of the analytical element of interest.

This is the case when using the nickel 232.0 nm line in a multi-element HCL containing iron, chromium, and manganese. To overcome the problem, either the slit can be narrowed to permit transmission of only the nickel wavelength, or a single-element HCL can be used. Other spectral interferences include emission interferences and non-atomic absorption. These have been addressed in the section on background correction.

## VII. CONTINUING RESEARCH

Although the major innovations in AAS appear to have been accomplished, research still continues in many laboratories to improve atomization efficiencies. Despite the fact that flame AAS instruments are the most commonly used type of instrument, new electrothermal atomizers are being developed to increase sensitivity levels of AAS. Modern computer control has reduced much of the operator error in use of both flame and electrothermal atomizers, and if the precision of electrothermal AAS can be increased to rival flame AAS, the technique could become more widely accepted. AAS isfinding increasing use as an element-selective detector in liquid chromatography, gas chromatography, and even supercritical fluid chromatography. The element selectivity of AAS has secured the place of AAS in diverse

analytical laboratories, just as the inefficiency of the atomization process will surely stimulate novel sensitivity breakthroughs in the future.

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## Inductively Coupled Plasma

Inductively Coupled Plasma (ICP) is an analytical technique used for the detection of trace metals in environmental samples. The primary goal of ICP is to get elements to emit characteristic wavelength specific light which can then measure.

The technology for the ICP method was first employed in the early 1960 with the intention of improving upon crystal growing techniques. Since then, ICP has been refined and used in conjunction with other procedures for quantitative analysis.

An ICP is a very high temperature (7000-8000k) excitation source that efficiently desolvates, vaporizes, excites, and ionizes atoms. Molecular interferences are greatly reduced with this excitation source but are not eliminated completely.

An ICP typically includes the following components:

1- Sample introduction system (nebulizer)

2- ICP torch

3- High frequency generator

4- Transfer optics and spectrometer

5-Computer interface

An ICP requires that the elements which are to be analyzed be in solution. An aqueous solution is preferred over an organic solution, as organic solutions require special manipulation prior to injection into the ICP.

The sample is nebulized and entrained in the flow of plasma support gas, which is typically Ar.

The plasma torch consists of

1-**concentric quartz tubes**

2- the **inner tube containing the sample aerosol**and **Ar support gas**.

3-**the outer tube containing an Ar gas flow to cool the tubes**.

A radiofrequency (RF) generator (typically 1-5 kW @ 27 MHz or 41MHz) produces an oscillating current in an induction coil that wraps around the tubes.

The induction coil creates an oscillating magnetic field. The magnetic field in turn sets up an oscillating current in the ions and electrons of the support gas.

$$Ar \xrightarrow{hv} Ar^{+} + e^{-}$$

$$Ar^{+} + M \longrightarrow M^{*} + 2e^{-}$$

These ions and electrons transfer energy to other atoms in the support gas by collisions to create a very high temperature plasma.

$$M^{*} \longrightarrow M + hv$$

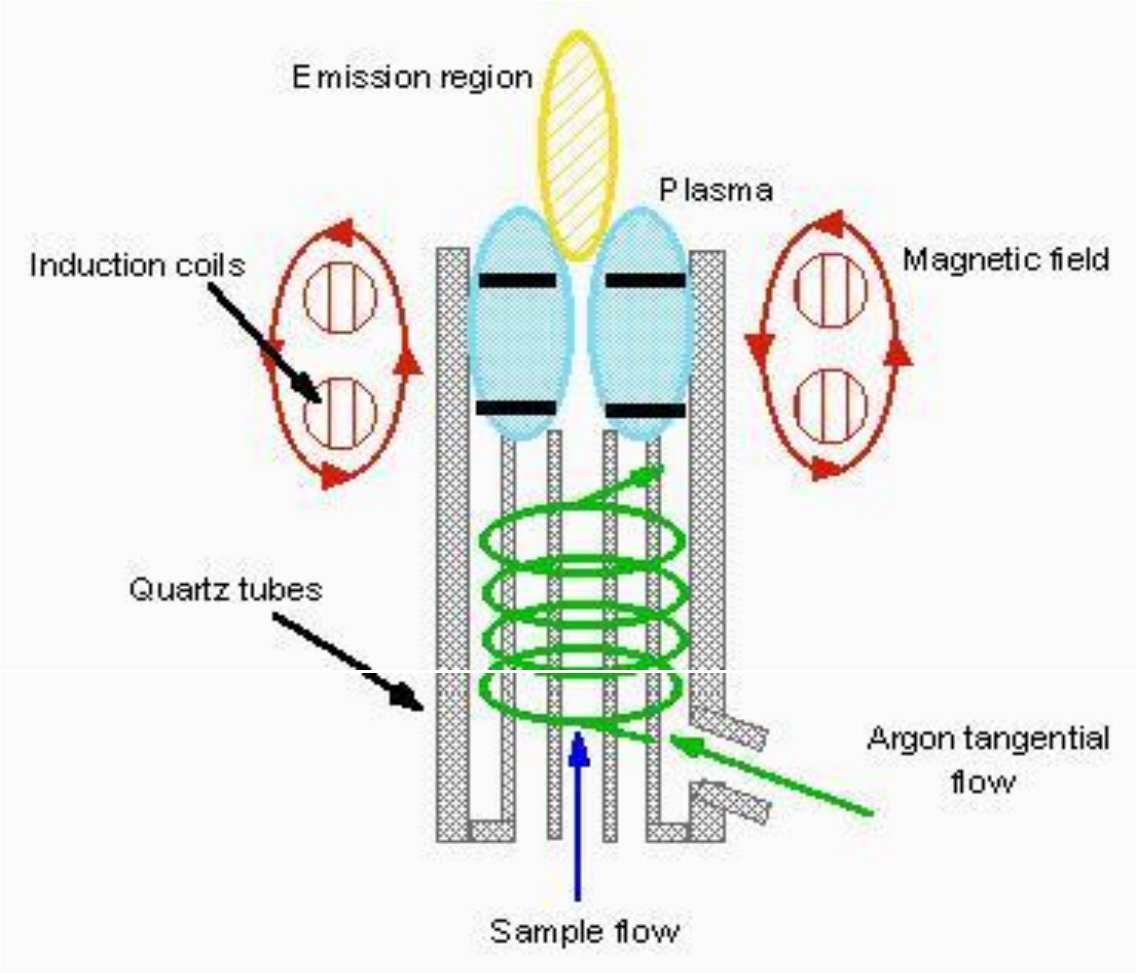



**Figure: Schematic cross-section of an ICP**

The light emitted by the atoms of an element in the ICP must be converted to an electrical signal that can be maturated quantitatively.

The light emitted by the atoms or ions in the ICP is converted to electrical signals by the photomultiplier in the spectrometer.

The intensity of the electron signal is compared to previous measured intensities of known concentration of the element and a concentration is computed.

Each element will have many specific wavelengths in the spectrum which could be used for analysis. Thus, the selection of the best line the analytical application in hand requires considerable experience of ICP wavelengths.

## Advantages and Disadvantages

Advantages of using an ICP include :

1-its ability to identify and quantify all elements with the exception of Argon.

2-since many wavelengths of varied sensitivity are available for determination of any one element, the ICP is suitable for all concentration from ultra-trace levels to major components

3-detection limits are generally low for most elements with a typically range of 1_ 100 g/L.

4-Probably the largest advantage of employing an ICP when performing quantitative analysis is the fact that multielemental analysis can be accomplished, and quite rapidly.

5-A complete multielement analysis can be undertaken in a period as short as 30 seconds, consuming only 0.5 ml of sample solution.

6-Although in theory, all elements except Argon can be determined using and ICP, certain unstable elements require special facilities for handling the radioactive fume of the plasma.

7-Also, an ICP has difficulty handling halogens, special optics for the transmission of the very short wavelengths become necessary.

# Gas chromatography

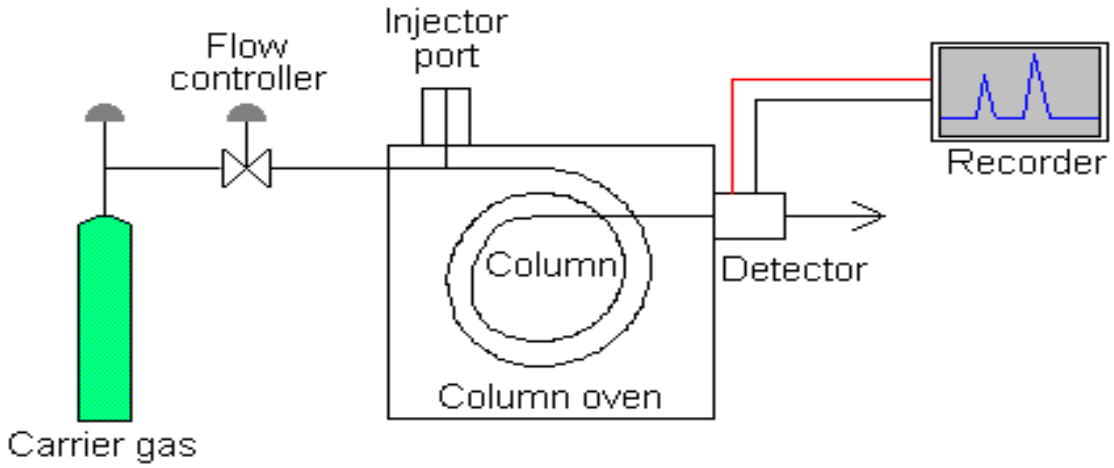
## Introduction

Gas chromatography - specifically gas-liquid chromatography - involves a sample being vaporized and injected onto the head of the chromatographic column.

The sample is transported through the column by the flow of inert, gaseous mobile phase.

The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

Have a look at this schematic diagram of a gas chromatograph:




## Instrumental components

### Carrier gas

The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependant upon the type of detector which is used. The carrier gas system also contains a molecular sieve to remove water and other impurities.

**Sample injection port**

For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapour - slow injection of large samples causes band broadening and loss of resolution.

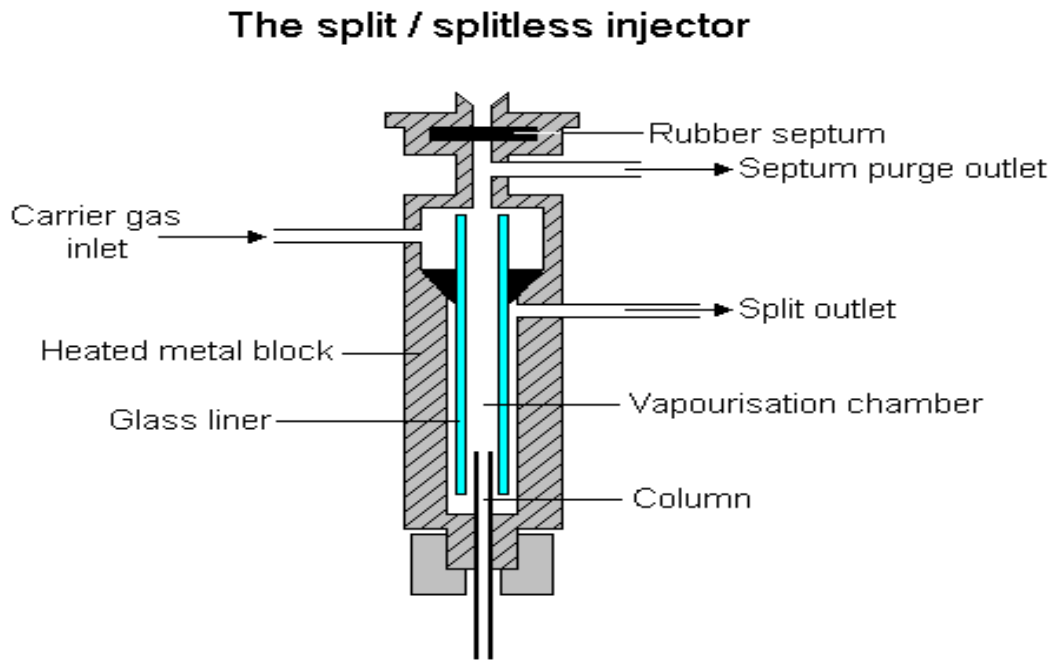
The most common injection method is where a microsyringe is used to inject sample through a rubber septum into a flash vapouriser port at the head of the column.

The temperature of the sample port is usually about 50□C higher than the boiling point of the least volatile component of the sample.

For **packed columns**, sample size ranges from tenths of a microliter up to 20 microliters.

**Capillary columns**, on the other hand, need much less sample, typically around $10^{-3}$µL.

For capillary GC, split/splitless injection is used. Have a look at this diagram of a split/splitless injector;



**The injector can be used in one of two modes; split or splitless. The injector contains a heated chamber containing a glass liner into which the sample is injected through the septum.**

**The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). The sample vapourises to form a mixture of carrier gas, vapourised solvent and vapourised solutes. A proportion of this mixture passes onto the column, but most exits through the split outlet. The septum purge outlet prevents septum bleed components from entering the column.**

## Columns

There are two general types of column, *packed* and *capillary* (also known as *open tubular*).

**Packed columns** contain a finely divided, inert, solid support material (commonly based on ***diatomaceous earth***) coated with liquid stationary phase. Most packed columns are **1.5 - 10m** in length and have an internal diameter of **2 - 4mm.**

**Capillary columns** have an internal diameter of a few tenths of a millimeter. They can be one of two types;

***wall-coated open tubular*** (WCOT) or ***support-coated open tubular*** (SCOT). **Wall-coated columns** consist of a capillary tube whose walls are coated with liquid stationary phase.

In **support-coated columns**, the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. SCOT columns are generally less

efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.

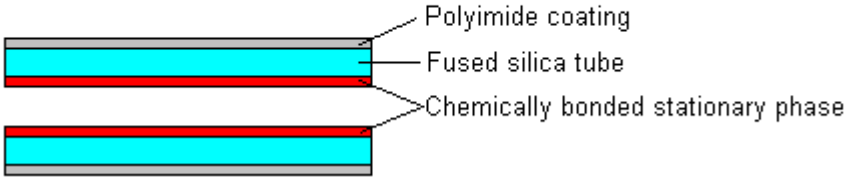
# التراب الدياتومي

Diatomaceous earth:

صخر مساميّ، فاتح اللون، ناعم الحُبَيبات ينشأ عن تراكم جدران الدياتوم diatom السِّليكيّة وتحجُّرها في قاع المحيط. يُستخدم بوصفه مرشِّحاً filter ومادة كاشطة أو حاكّة abrasive، ويُستعان به في صناعة الدهانات والورنيش، والورق، والآجرّ، والخزف، واللدائن(البلاستيك)، والصابون وضروب المنظِّفات. ومما يُذكر أن ألفرد نوبل Nobel استخدم التراب الدياتومي في تجاربه التي أدت إلى إنتاج الديناميت. يكثر وجوده في سواحل الولايات المتحدة الأميركية، والدانمرك، وفرنسا، وألمانيا الغربية، والجزائر

In 1979, a new type of WCOT column was devised - the *Fused Silica Open Tubular* (FSOT) column;

**Cross section of a Fused Silica Open Tubular Column**




These have much thinner walls than the glass capillary columns, and are given strength by the polyimide coating. These columns are flexible and can be wound into coils. They have the advantages of physical strength, flexibility and low reactivity.

**Column temperature**

For precise work, column temperature must be controlled to within tenths of a degree.

The optimum column temperature is dependant upon the boiling point of the sample. As a rule of thumb, a temperature slightly above the average boiling point of the sample results in an elution time of 2 - 30 minutes.

Minimal temperatures give good resolution, but increase elution times. If a sample has a wide boiling range, then temperature programming can be useful. The column temperature is increased (either continuously or in steps) as separation proceeds.

**Detectors**

There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity.

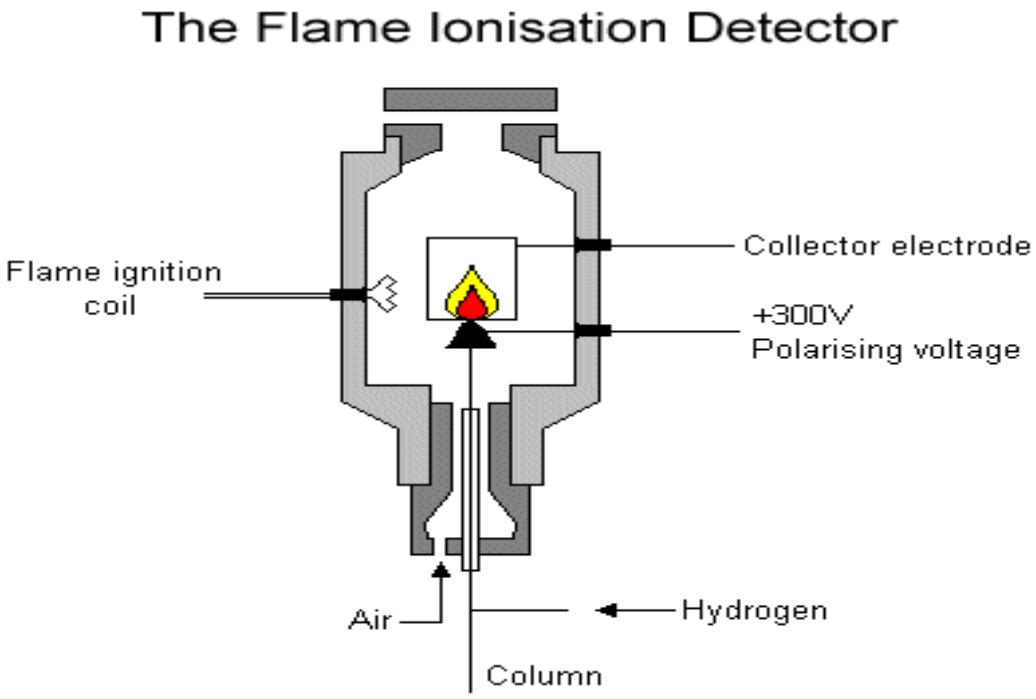
**A *non-selective*** detector responds to all compounds except the carrier gas,

A***selective detector*** responds to a range of compounds with a common physical or chemical property and a *specific detector* responds to a single chemical compound. Detectors can also be grouped into *concentration dependant detectors* and *mass flow dependant detectors*.

The signal from a concentration dependant detector is related to the concentration of solute in the detector, and does not usually destroy the sample Dilution of with make-up gas will lower the detectors response.

**Mass flow**dependant detectors usually destroy the sample, and the signal is related to the rate at which solute molecules enter the detector. The response of a mass flow dependant detector is unaffected by make-up gas. Have a look at this tabular summary of common GC detectors:

| **Detector** | **Type** | **Support gases** | **Selectivity** | **Detectability** | **Dynamic range** |
|---|---|---|---|---|---|
| Flame ionization (FID) | Mass flow | Hydrogen and air | Most organic cpds. | 100 pg | $10^7$ |
| Thermal conductivity (TCD) | Concentration | Reference | Universal | 1 ng | $10^7$ |
| Electron capture (ECD) | Concentration | Make-up | Halides, nitrates, nitriles, peroxides, anhydrides, organometallics | 50 pg | $10^5$ |
| Nitrogen-phosphorus | Mass flow | Hydrogen and air | Nitrogen, phosphorus | 10 pg | $10^6$ |
| Flame photometric (FPD) | Mass flow | Hydrogen and air possibly oxygen | Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium | 100 pg | $10^3$ |
| Photo-ionization (PID) | Concentration | Make-up | Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics | 2 pg | $10^7$ |
| Hall electrolytic conductivity | Mass flow | Hydrogen, oxygen | Halide, nitrogen, nitrosamine, sulphur | | |




The effluent from the column is mixed with hydrogen and air, and ignited. Organic compounds burning in the flame produce ions and electrons which can conduct electricity through the flame.

A large electrical potential is applied at the burner tip, and a collector electrode is located above the flame.

The current resulting from the pyrolysis of any organic compounds is measured. FIDs are mass sensitive rather than concentration sensitive; this gives the advantage that changes in mobile phase flow rate do not affect the detector's response.

**The FID is a useful general detector for the analysis of organic compounds; it has high sensitivity, a large linear response range, and low noise. It is also robust and easy to use, but unfortunately, it destroys the sample**.

# THE MEASUREMENT OF CONDUCTANCE

**A conductance measurement requires a source of electrical power, a cell to contain the solution, and a suitable bridge to measure the resistance of the solution**.

## Power Sources

Use of an alternating current source. There are, however, both upper and lower limits to the frequencies that can be employed; audio oscillators that produce signals of about 1000 Hz are the most satisfactory.

## The cells

Figure below show three common types of cells for the measurement of conductivity. Each contains a pair of electrodes firmly fixed in a constant geometry with respect to one another. The electrodes are ordinarily platinized to increase their effective surface and thus their polarization; growing currents are minimized as a result.

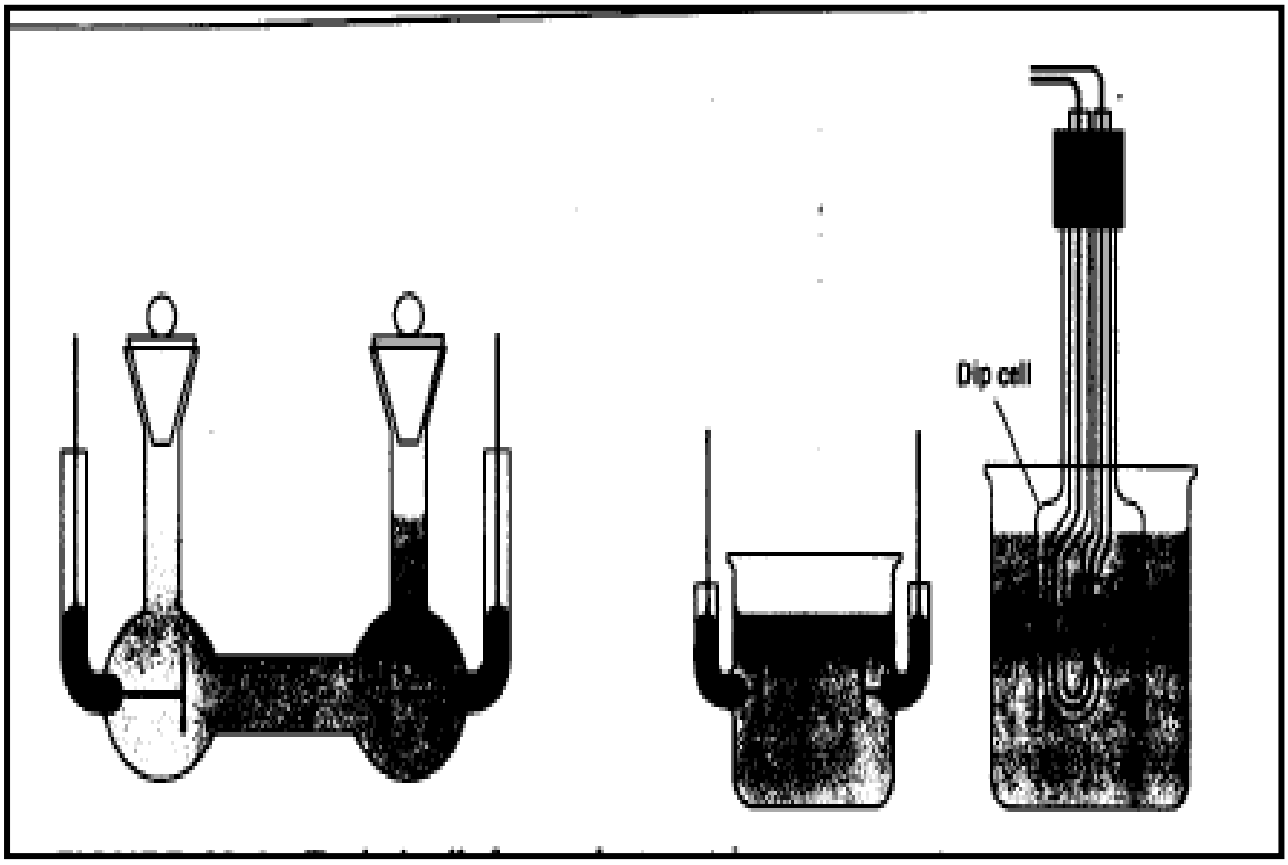


Figure :types of cell conductometric measurements

**CONDUCTOMETRIC TITRATION**

**Conductometric measurement provide convenient means for locating end points in titration .the conductometric data are plotted as a functions of titrant volume.**

## Acid-Base Titration.

**Neutralization titrations are particularly well adapted to the conductometric end point because of the large ionic conductances of hydrogen and hydroxide ions compared with the conductances of the species that replace them in solution.**

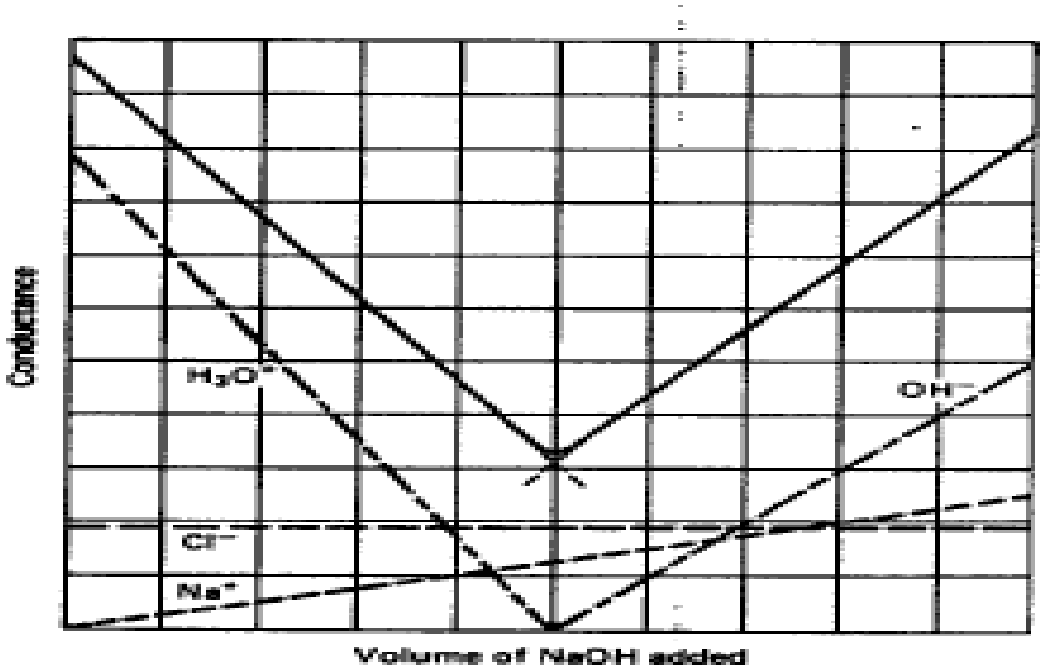
Titration of Strong Acids or Bases. The solid line in Figure below represents a curve(corrected for volume change) obtained when hydrochloric- acid is titrated with sodium hydroxide.

Also plotted are the calculated contributions of the individual ions to the conductance of the solution. During neutralization, hydrogen ions are replaced by an equivalent number of less mobile sodium ions; the conductance changes to lower values as a result of this substitution.

**At the equivalence point, the concentrations of hydrogen and hydroxide ions are at a minimum and the solution exhibits its lowest conductance**.

A reversal of slope occurs past the end point as the sodium ion and hydroxide ion concentrations increase.

**With the exception of the immediate equivalence-point region**, an excellent linearity exists between conductance and the volume of base added.




**kinds of conductometric titration**

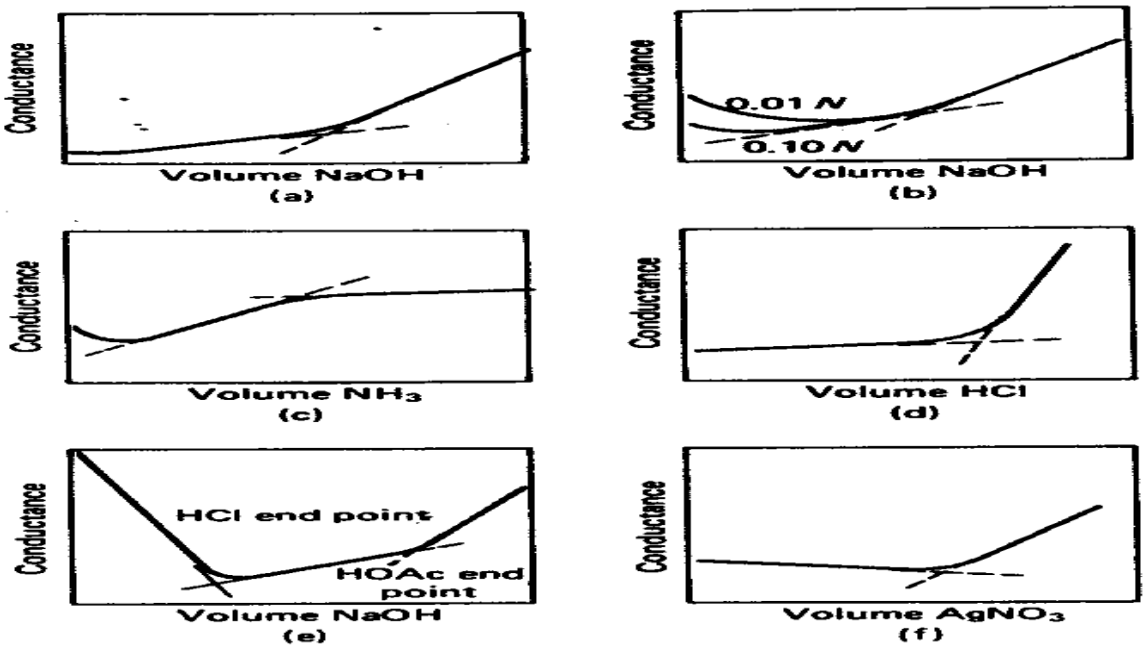



**FIGURE 22-3** Typical conductometric titration curves. Titration of (a) a very weak acid ($K_a \approx 10^{-10}$) with sodium hydroxide; (b) a weak acid ($K_a \approx 10^{-5}$) with sodium hydroxide (Note that for 0.01 *N* solutions, conductance × 10 is plotted); (c) a weak acid ($K_a \approx 10^{-5}$) with aqueous ammonia; (d) the salt of a weak acid; (e) a mixture of hydrochloric and acetic acids with sodium hydroxide; and (f) chloride ion with silver nitrate.

# 1.1 Definitions of Thermal Analysis

**Old Definition**

THERMAL ANALYSIS (TA) refers to a group of techniques in which a property of a sample is monitored against time or temperature while the temperature of the sample, in a specified atmosphere, is programmed.

**Newer definition**

THERMAL ANALYSIS (TA) means the analysis of a change in a property of a sample, which is related to an imposed temperature alteration.

Arguments in favour of the newer definition include:

1 "Analysis" means more than "monitoring". Analysis implies not only the recording of the data but also any subsequent interpretation.

2 It is usually the case that a change in the property rather than the property itself which is studied.

3 We can only really control the temperature of the environment of the sample, rather than the sample itself.

4 The "specified atmosphere" should really be an experimental parameter rather than part of the definition.

## 1.2 Properties Studied by Thermal Analysis

There are a wide range of properties that may be studied, and each has an associated technique which can be used:

specific heat ,thermal expansion, weight, viscosity,…….

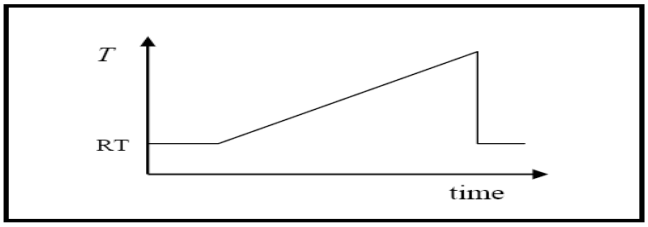
phase changes and lattice parameter

commercial instrumentation. And Available in this analysis are:**DSC, DTA, TGA, DTA+TGA, TMA**, DTMA, Dielectric TA, Rheometry, dilatometry, EGA, XRD

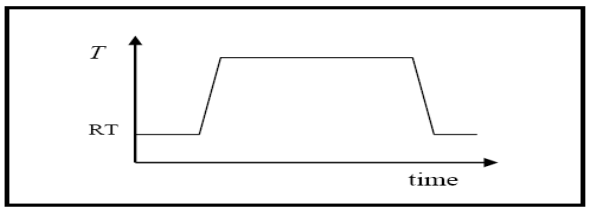
+ heating

## 1.3 Common Types of Experimental Scans

1. Temperature scan




2. Isothermal heat treatment




The isothermal heat treatment is usually best suited to kinetic studies.

The temperature scan is generally more useful to examine thermodynamic properties such as phase transformations.

**1.4 Why Use Thermal Analysis Techniques?**

a) to measure a property of interest, such as specific heat, latent heat or heat of reaction

b) to fingerprint a particular material or substance

c) to assess the high-temperature stability of materials

d) to detect phase transformations

e) fundamental studies of reaction kinetics

This course mainly concentrates on the purely thermal techniques, DTA and DSC.

**2. Differential Thermal Analysis**

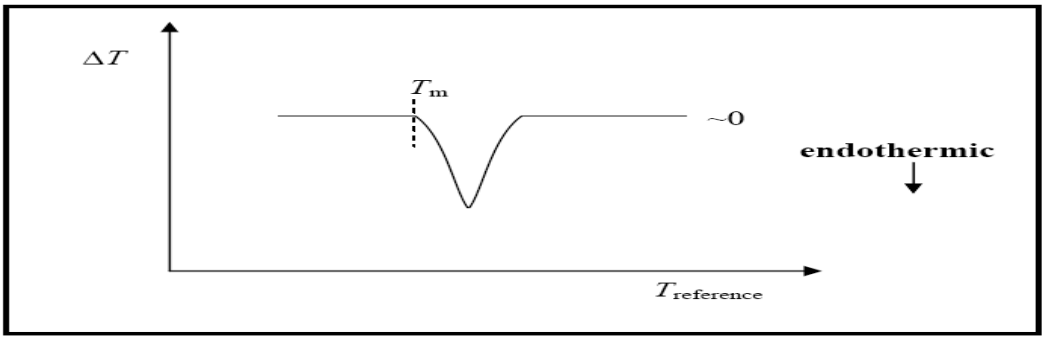
**2.1 DTA Apparatus**

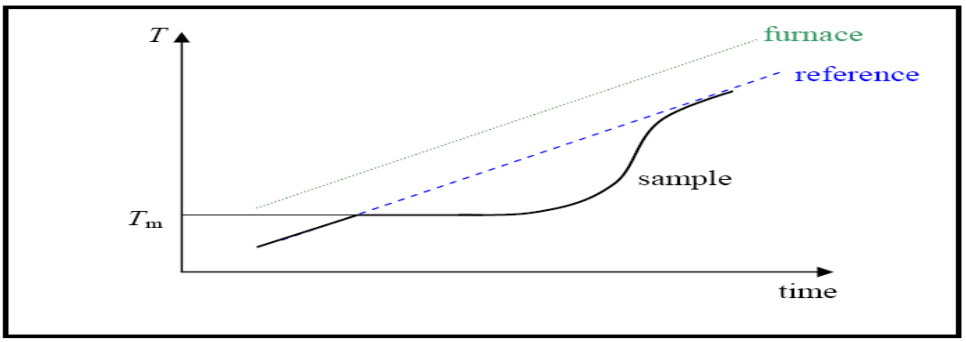
This is an older technique which is simple to perform and understand.

The sample of interest (S) and an inert reference (R) are placed in the same furnace and hence are subject to the same heat treatment. The thermocouples are usually placed directly into the samples and the difference in temperature is monitored.

## 2.2 DTA Scans

Temperature scan for a sample (a pure metal) melting




Note that the melting point (*T*m) is characterised by the onset of a temperature difference between the sample and reference. The position of the peak has no significance and is only affected by factors such as sample size, latent heat of melting and thermal conductivity.

The convention when plotting $\Delta T$ is that energy evolved by a transition or reaction is positive. Hence for the endothermic process shown above, where heat is absorbed by the sample when it melts, the temperature change is negative.

## DTA:

In more modern DTA instruments, the thermocouples are usually placed in the container below the sample. This reduces the effect of sample properties on the peak area, enabling a slightly more quantitative approach.

## 3. Differential Scanning Calorimetry

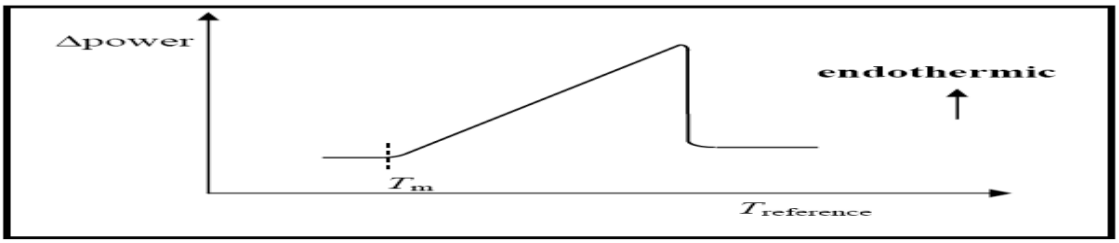
### 3.1 DSC Apparatus

There are two methods of carrying out DSC. ***Power-compensated DSC***is the method usually discussed in textbooks, though most practical instruments are based on ***heat-flux DSC***. However, in practice these usually give equivalent experimental results.

#### *3.1.1 Power-compensated DSC (Patented by Perkin-Elmer)*

The sample and reference are in separate furnaces, each with a heater coil and a thermocouple. The aim is to maintain both at the same temperature, even during a thermal event in the sample. The difference in power supplied to the two furnaces to maintain zero temperature differential between the sample and the reference is measured.

If we do a temperature scan, the melting peak should be a better-defined shape than for DTA:




The temperature difference between sample and reference is converted to a differential thermal power, Δ***q***(or Δpower), which is supplied to the heaters to maintain the temperature of the sample and reference at the programmed value.

The sample and reference are both within the same furnace and are connected by a low-resistance heat-flow path. If any difference in temperature develops, heat flows in proportion to that temperature difference.

# Differential Scanning Calorimetry

## Introduction

**Differential scanning calorimetry (DSC) is a technique for measuring the energy necessary to establish a nearly zero temperature di®erence between a substance and an inert reference material, as the two specimens are subjected to identical temperature regimes in an environment heated or cooled at a controlled rate.**

**There are two types of DSC systems in common use (Fig. 1). In power{compensation DSC the temperatures of the sample and reference are controlled independently using separate, identical furnaces. The temperatures of the sample and reference are made identical by varying the power input to the two furnaces; the energy required to do this is a measure of the enthalpy or heat capacity changes in the sample relative to the reference.**

**In heat{°ux DSC, the sample and reference are connected by a low{resistance heat{°ow path (a metal disc). The assembly is enclosed in a single furnace. Enthalpy or heat capacity changes in the sample cause a di®erence in its temperature relative to the reference; the resulting heat °ow is small compared with that in di®erential thermal analysis (DTA) because the sample and reference are in good thermal contact. The temperature di®erence is recorded and related to enthalpy change in the sample using calibration experiments.**

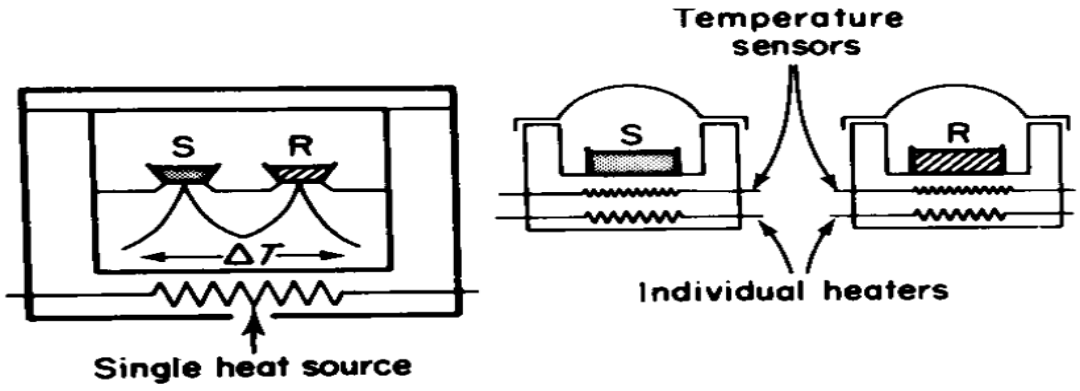


Fig. 1: (a) Heat °ux DSC; (b) power{compensation DSC Heat{°ux DSC

This section is based largely on a description of the Dupont DSC system by Baxter and Greer.

The system is a subtle modi¯cation of DTA, di®ering only by the fact that the sample andreference crucibles are linked by good heat{°ow path. The sample and reference are enclosed in the same furnace. The di®erence in energy required to maintain them at a nearly identical temperature is provided by the heat changes in the sample. Any excess energy is conducted between the sample and reference through the connecting metallic disc, a feature absent in DTA. As in modern DTA equipment, the thermocouples are not embedded in either of the specimens; the small temperature di®erence that may develop between the sample and the inert reference (usually an empty sample pan and lid) is proportional to the heat °ow between the two. The fact that the temperature di®erence is small is important to ensure that both containers are exposed to essentially the same temperature programme.