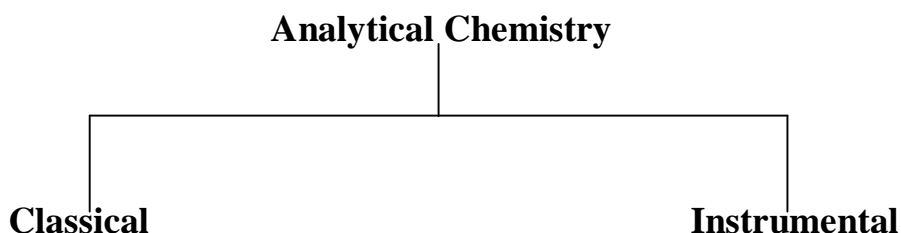


Instrumental Analysis



Introduction:

Analytical Chemistry is the science, which deals with methods for determining the chemical composition of samples of matter (elements or compounds).

Classical methods

Involve separating the components in a sample by precipitation, extraction or distillation.

In **qualitative classical methods**, the separated components treated with reagents can yield products recognized by their colors, boiling points, melting points, solubility's in a series of solvents, odors, optical activities or their refractive index.

In **quantitative classical methods**, the amounts of components are determined by gravimetric or titration methods.

In **gravimetric analysis**, the mass of components is determined.

In **titrimetric analysis**, the volume or mass of a standard reagent, required to react completely with sample components is measured.

Instrumental methods

In the 19th century, chemists began to exploit phenomenon other than those used for classical methods for solving analytical problems. Thus, measurement of physical properties of analysts such as- conductivity, electrode potential, light absorption or emission, mass-to-charge ratio and fluorescence, began to be used

for quantitative analysis of a variety of inorganic, organic and biochemical analysis. Furthermore, highly efficient chromatographic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior to their qualitative and quantitative determination. These newer methods are called **Instrumental methods of analysis**.

The most characteristic properties that are used for instrumental analysis are listed in table (1)

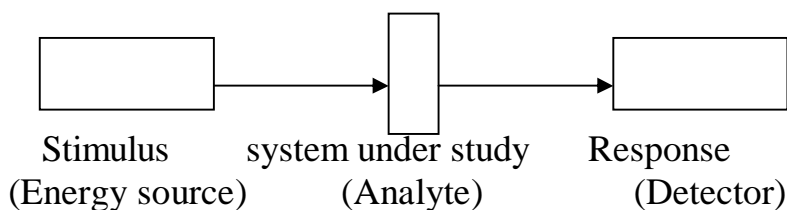
Table (1) Chemical and Physical properties Employed in Instrumental Methods

Characteristic property	Instrumental methods
Interaction with radiation	Spectroscopy methods (UV visible, IR, x-ray and NMR spectroscopy, -- etc.)
Electrical	Potentiometry, conductometry, - -etc.
Mass-to-charge ratio	Gravimetry, mass spectrometry, - -etc
Rate of reaction	Kinetic methods
Thermal characteristics	Thermal gravimetry TG, Differential thermal analysis DTA, differential scanning calorimetry DSC, - -etc.
Radioactivity	Activation and isotope analysis methods

In addition, there is a group of instrumental methods used for separation and resolution of closely related compounds called chromatography. The methods listed in table (1) are used following chromatographic separations.

Instruments for analysis

Instruments for chemical analysis comprise basic components:-



- (a) The stimulus (energy source), usually in the form of electromagnetic, electrical or mechanical energy
- (b) System under study (analyte)
- (c) Response (detector) that converts changes in properties of the analyte to a number that is proportional to the relevant chemical or physical absorption by the analyst.

The detection system converts information to electrical signals by photodiodes, photomultipliers, - etc. Then the electrical signal converts information to numeric or graphic output of a photographic plate, recording paper, or computers. The information appears as the blackening of a photographic plate, a tracing on a recorder or computer output. Most modern analytical instruments contain computers. The intensity of light is determined before and after its interaction with the sample, and the ratios of these intensities (I/I_0) provides a measure of the analyst concentration.

Calibration of instrumental methods

All types of analytical methods require calibration, i.e. relating the measured analytical signal to the concentration of the analyst. To use this technique, several standards containing exactly known concentrations of the analyte are introduced into the instrument and the instrumental response is recorded.

Ordinarily, this response is corrected for the instrumental output obtained with a blank. Ideally, the blank contains all of the components of the original sample except for the analyte. Plotting resulting data gives a graph of corrected instrument response versus analyte concentration.

External standard method

In figure (1), linear plots are obtained. Non linear plots are due to matrix effects, interferences, instrumental drift, - - etc.

Usually an equation ($y=mx+c$)

is developed for the calibration curve,

so that sample concentration can be computed directly.

Where, m =slope of the curve, c = background count rate

The success of the calibration curve method is dependent on: (a) how accurately the analyte concentrations of the standards are and (b) how closely the matrix of the standards resemble that of samples to be analyzed.

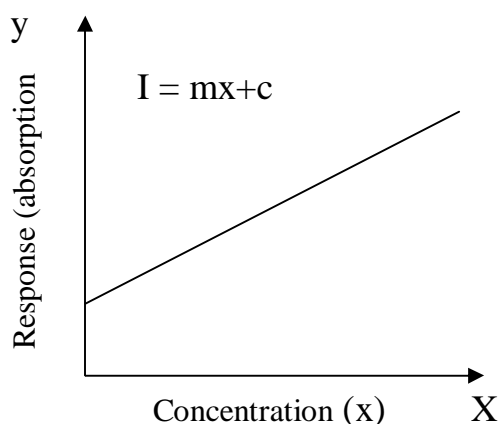


Figure (1)

Internal standard method

Matrix effects lead to interference errors. To minimize these affects, the internal standard method can minimize several types of both random and systematic errors.

The internal standard is a substance added in a constant amount to all samples, blanks and calibration standards in an analysis.

Calibration involves plotting the ratio of the analyte signal to the internal standard signal (I/I_o) as a function

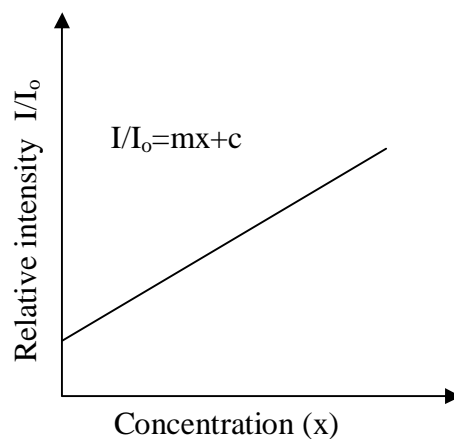


Figure (2)

of the analyst concentration of the standards.

This ratio then used to obtain the analyst concentration from the calibration curve shown in figure (2).

Thus if the analyte and internal standard signals are influenced in the same way, compensation for several types of matrix interferences, random and systematic errors may occur.

The internal standard should be absent from the sample matrix, so that the only source of the standard is the added amount. For example, Lithium is a good internal standard for the determination of sodium (Na) and potassium (K) in blood serum, because the chemical behavior of Li is similar to both analysts, but it does not occur naturally in blood.

Standard addition method (spiking method)

When a suitable standard is not available, it may be possible to use the element to be determined as its own internal standard. This is sometimes more convenient than the use of a different element.

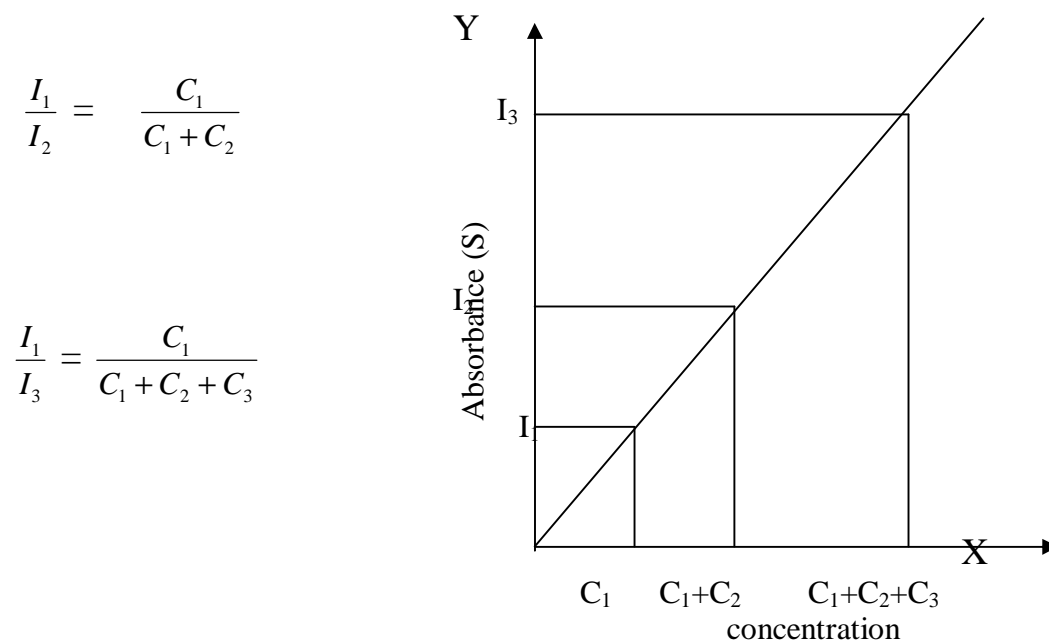


Figure (3) principle of spiking procedure A small quantity (C_2) of the element to be analyzed is added to the sample, and the ratio of the count rates before and after addition are used to estimate the initial concentration (C_1).

If the concentration / count rate is linear, the net count rate from an element is proportional to its concentration, and addition of more of the same element should cause the count rate to increase proportionally. Figure (3) illustrates the concept and it can be seen that net count rate (I_1) is proportional to the concentration (C_1). By addition of (C_2) of the analyzed element to give a new concentration of (C_1+C_2) the net count rate increases to (I_2), and with more additions ($C_1+C_2+C_3$) the net count rate increases to I_3 and so on.

This method is useful for the determination of single elements in very complex matrices, and where suitable standards are not available.

Statistical Terms

In order to prove mathematically the reliability of any analysis method, it is important to define some statistical terms:

True result

The true result is that concentration which actually exists in a representative sample (i.e.) the right concentration. This may never be known absolutely, but in standard samples, a chosen concentration sometimes called **accepted reference level** defined as the **most agreed value**, is often used as the true result.

Accuracy

The closeness of the experimental result, or mean of a number of results, to the true value.

Mean (average, \bar{X})

The arithmetic sum of a set of results, divided by their number

$$\bar{X} = \frac{1}{n} \sum_{i=1}^{i=n} X_n$$

Precision (relative or systematic error)

The closeness among replicate results obtained under any set of conditions.

An increase in precision implies a decrease in its absolute numerical value.

The mathematical expression for precision includes:

Standard deviation

The root mean square of a set of results from their arithmetic mean

$$S = \sqrt{\frac{\sum (X - \bar{X})^2}{n - 1}}$$

Variance

The square of the standard deviation

$$V = S^2$$

Relative standard deviation

The standard deviation expressed as the percentage of the mean.

$$RSD = \frac{S}{\bar{X}} \times 100$$

Systematic error

The difference between the true result and the experimental result.

Calibration with blank samples can correct for it

The accuracy of a result is therefore dependent both upon the precision of the measurement and on the systematic error.

Therefore, **a method or measurement may be precise without being accurate.**

Sensitivity

The sensitivity of an analytical method or instrument defined, as the ratio of the change in response (R) to the change in the quantity or concentration (C) that is measured.

$$S = \Delta R / \Delta C$$

The sensitivity is dependent on type of instrument and experimental conditions.

A parameter often used to express sensitivity is the limit of detection

Detection limit

The minimum concentration or mass of analyte that can be detected at a known confidence level.

Detection limits differ widely for several analytical methods, and from one element or compound to another.

Optical Methods

A major class of analytical methods is based on the interaction of electromagnetic radiation with matter. The most widely used spectroscopic methods are based on electromagnetic radiation, which is a type of energy that takes several forms. The most readily recognizable radiations are light and radiant heat.

Note that spectroscopic methods that employ not only visible but also gamma rays and x-rays as well as ultraviolet, infrared, microwave and radiofrequency radiation are often called *optical methods* despite the fact that the human eye is sensitive to neither of the later types of radiations. This might be due for the similarities of the ways of interaction of these types of radiation with matter.

Nature of electromagnetic radiation

Any wave is essentially just a way of shifting energy from one place to another. Relatively small local movements in the environment transfer the energy. The energy of radiation travels because of local fluctuation changes in electrical and magnetic fields- hence **electromagnetic radiation**

In contrast to other wave phenomenon, such as sound, electromagnetic radiation requires no supporting medium for its transmission and thus passes readily through a vacuum.

As indicated in figure (a) and as the name implies, an electromagnetic wave has an electric component and a magnetic component. The two components oscillate in plane perpendicular to each other and perpendicular to the direction of propagation. Only the electric

component is active in ordinary energy transfer interaction with matter. Henceforth, in our discussion of wave behavior, we will consider only the electric component. Figure (b) is a two dimensional representation of the electric component of the ray.

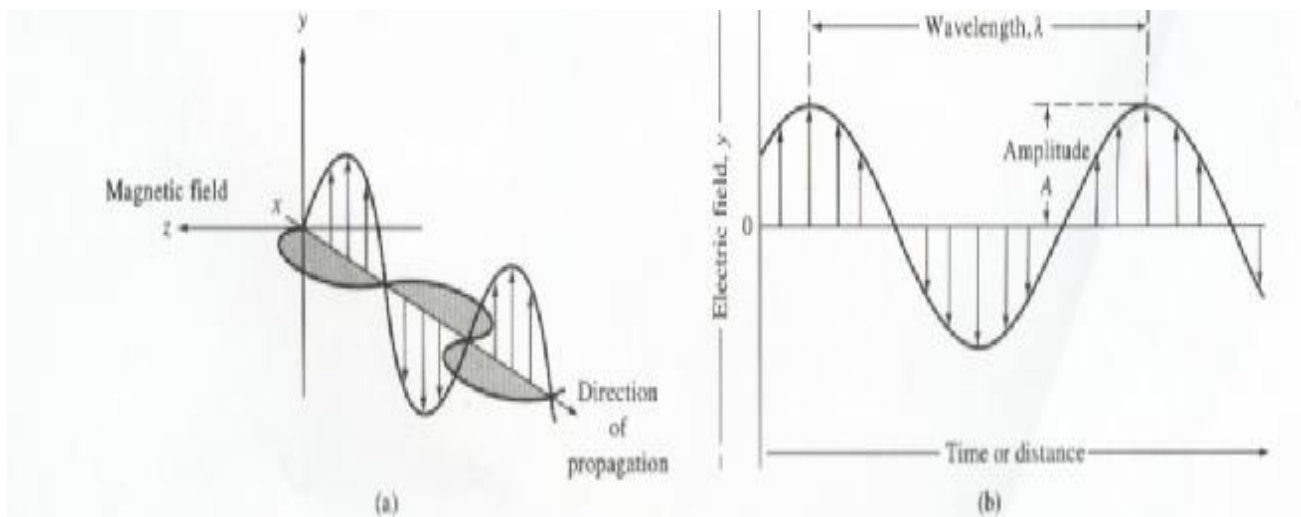
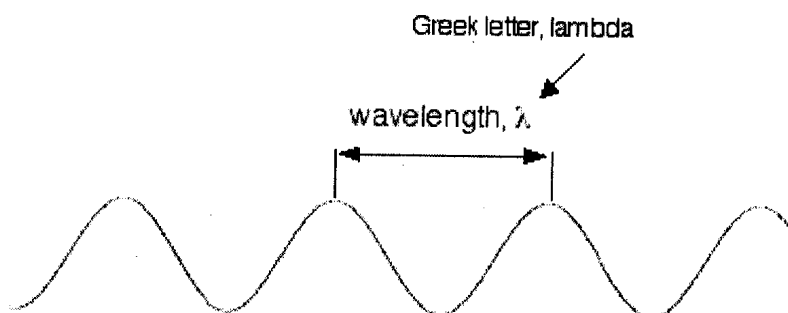


Figure (1) An electromagnetic wave

Radiant energy can be described in terms of a number of parameters:-

Wavelength (λ) is the linear distance between any two equivalent positions on the wave (e.g., successive maxima or minima)

The units of wavelength are the micrometer ($1 \mu\text{m} = 10^{-6} \text{ m}$), usually called micron. The unit widely used in spectroscopy is the angstrom ($1\text{\AA} = 10^{-10} \text{ m}$).



Wave number ($\bar{\nu}$) is the number of waves per unit distance ($\bar{\nu} = \frac{1}{\lambda}$).

The unit most commonly used for wave number is the reciprocal cm (cm^{-1}).

Frequency (ν) is the number of complete wavelength units which pass a fixed point per unit of time.

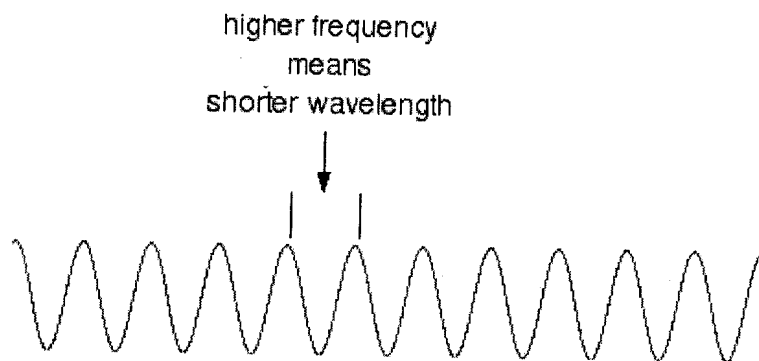
The units of frequency are cycles per second or Hertz (Hz)

Light has a constant speed through a given substance, e.g., it always travels at a speed of approximately 3×10^8 m/sec in a vacuum. This is actually the speed that electromagnetic radiation travels.

The wavelength (λ) and frequency (ν) are related to the velocity of light in vacuum by the expression:

$$\nu = \frac{C}{\lambda} = \bar{\nu} C, \quad \left(\bar{\nu} = \frac{1}{\lambda} \right)$$

These relationship means that if the wavelength is longer, the frequency is lower.



Particle properties

To describe how electromagnetic radiation interacts with matter, consider the beam of radiation as a train of photons. The energy of each photon is proportional to the frequency of radiation given by the relationship:

$$E = h \nu = h \frac{C}{\lambda}$$

Where, E = energy of the photon (ergs)

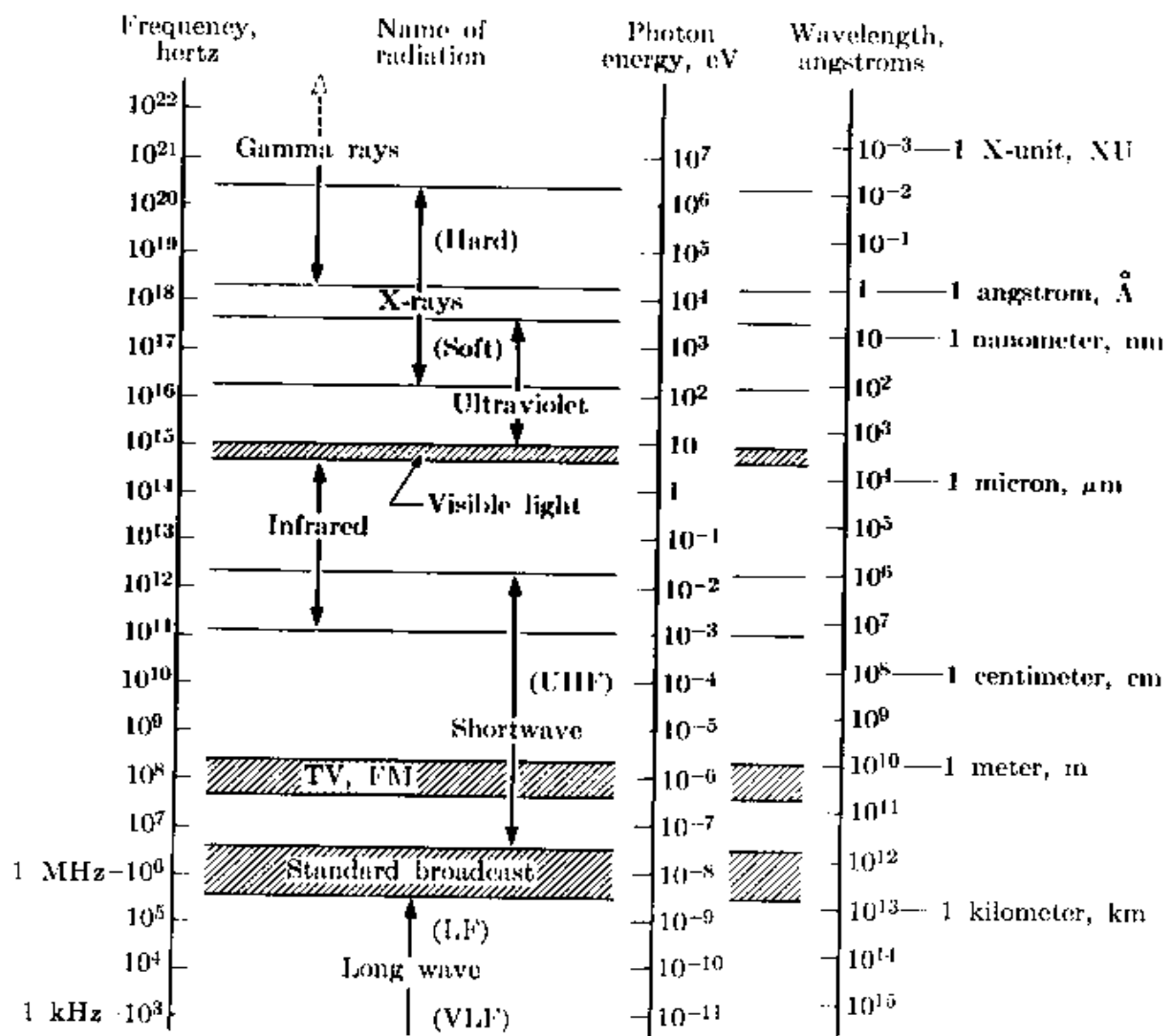
ν = frequency of electromagnetic radiation (Hz)

h = plank's constant = 6.624×10^{-27}

The higher the frequency, the higher the energy of radiation (i.e.) a photon of high frequency (short wavelength) has higher energy content than one of lower frequency (longer wavelength).

The intensity of a beam of radiation is proportional to the number of photons and is independent of the energy of each photon. Since energy per unit time is power, **Intensity is often referred as the radiant power emitted by the source.**

Electromagnetic Spectrum



Figure(2)- The electromagnetic spectrum

The electromagnetic spectrum is composed of a large range of wavelengths and frequencies (energies). It varies from the highly energetic gamma rays to the very low energy radio-waves. The entire range of radiation is commonly referred to as the **electromagnetic spectrum**. The major spectral regions of the spectrum are shown in figure (1) and the divisions are based on the methods required to generate and detect various types of radiations.

Table (1) lists the wavelength ranges for the regions of the spectrum that are important in analytical purposes and, also gives the names of the various spectroscopic methods associated with each and the types of transitions that serves as the basis for the various spectroscopic techniques.

Table (1)

Type spectroscopy	Type of transitions	Wavelength range
Gamma rays	Nuclear	$(10^{-10} - 10^{-14})$ m
X-rays	Inner K-and L-shell electrons	$(10^{-9}) - (6 \times 10^{-12})$ m
Ultraviolet rays	Valence and middle-shell electrons	$(3.8 \times 10^{-7}) - (6 \times 10^{-10})$ m
Visible	Valence electrons	$(7.8 - 3.8) \times 10^{-7}$
Infrared	Molecular vibrations and rotations	$(10^{-3}) - (7.8 \times 10^{-7})$ m
Microwave	Molecular rotations	0.3m-1mm
Radio waves		Few km-0.3 m

Absorption and Emission of Radiation

Electromagnetic radiation can interact with matter in a number of ways.

If the interaction results in the transfer of energy from a beam of radiant energy to the matter, it is called "**absorption**". The reverse process in which a portion of the internal energy of matter converted into radiant energy is called "**emission**". In emission process, species in an excited state can emit photons of characteristic energies by returning to lower energy states or ground states.

Part of the radiation which passes into matter, instead of being absorbed, may be **scattered or reflected** or may be re-emitted at the same wavelength or a different wavelength upon emerging from the sample. Radiation, which is neither absorbed nor scattered, may undergo changes in **orientation** or **polarization** as it passes through the sample.

Absorption of radiation

When radiation passes through a layer of solid, liquid or gas, certain frequencies may be selectively removed by ***absorption***, a process in which electromagnetic energy is transferred to the atoms, ions, or molecules composing the sample. Absorption promotes these particles from their normal room temperature state, or ground state to one or more higher-energy excited states.

According to quantum theory, atoms, molecules, or ions have only a limited number of discrete, energy levels. For absorption of radiation to occur, the energy of the exciting photon must exactly match the energy difference between the ground state and one of the excited states of the absorbing species. Since these energy differences are unique for each species, a study of the frequencies of absorbed radiation provides a means of characterizing the constituents of a sample of matter. A plot of absorbance as a function of wavelength or frequency called the ***absorption spectrum***. The nature of the spectrum is influenced by differences between absorption spectra for atoms and those for molecules.

Atomic spectra

The passage of electromagnetic ultraviolet radiation through a medium that consists of mono-atomic particles, such as sodium vapor, results in the absorption of few well-defined frequencies as in figure (3a).

Excitation can occur only by an electronic process in which one or more of the electrons of the atom are raised to a higher energy level. For example, all the atoms in sodium vapors are in the ground state under ordinary conditions. Their valence electrons lie in the 3S level.

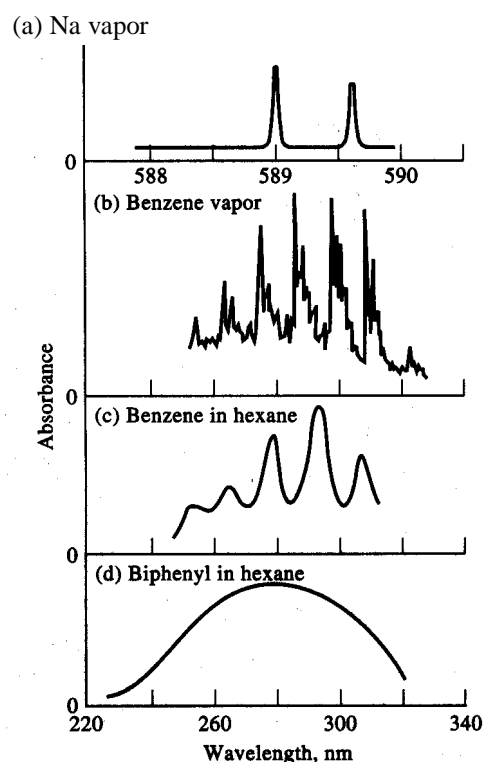


Figure (3)-Some typical UV absorption spectra

If irradiated with a beam of energy, the outer electrons of many of the atoms will absorb photons and accelerate to the 3P levels. The excited electron has a strong tendency to return to its normal 3S state and in so doing emits a photon. This emitted photon possesses a definite amount of energy, dictated by the spacing of the energy levels. As a result, the spectrum of the sodium vapor exhibits sharp absorption peak in the yellow region of the visible spectrum at 589 nm. If the electron is given more energy, it may be raised to some higher level than 3P such as 4P or 5P resulting for an ultraviolet peak at about 285 nm.

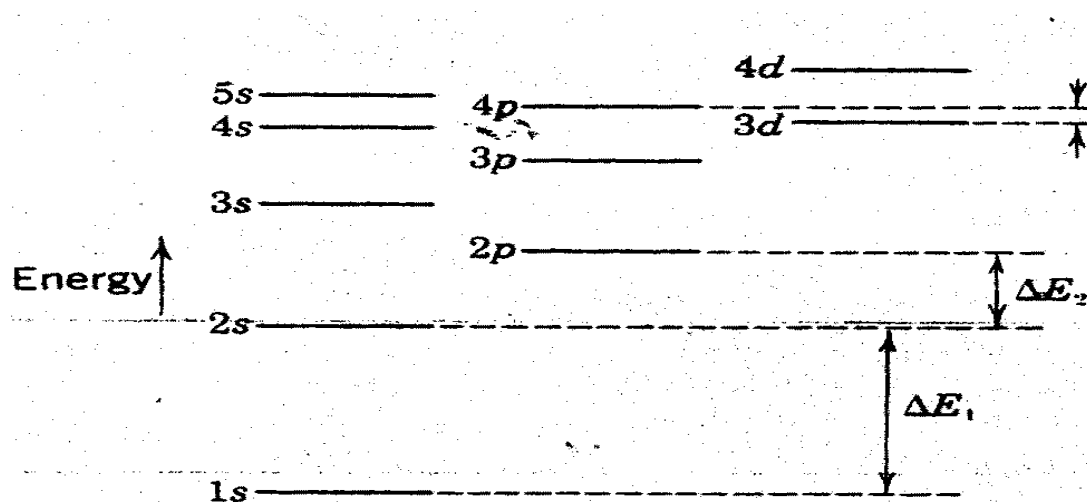


Figure (4)- Energy level diagram for sub-shells in poly electron atom

The vertical lines labeled ΔE_1 , ΔE_2 and so on indicate allowed transitions between energy levels. Such transitions can occur only if photons of exactly the same energy are available; otherwise, no absorption can occur.

With a highly energetic source of excitation, many electrons (not only the outermost) in any element can be excited to varying degrees, and the resulting emitted radiation may contain up to several thousand discrete and reproducible wavelengths mostly in the UV and visible regions.

If even more energy is available for excitation, an inner electron can be torn entirely away from the atom. An electron from some higher level will then drop to fill the vacancy. The radiation emitted will be of much greater energy. This describes the emission of X-rays from atoms subjected to bombardment by a beam of fast moving electrons.

Molecular spectra

The absorption of radiation by a molecule is far more complex than absorption by individual atoms. The total energy state of a molecule includes electronic, vibration and rotational components. That is

$$E = E_{\text{electronic}} + E_{\text{vibrational}} + E_{\text{rotational}}$$

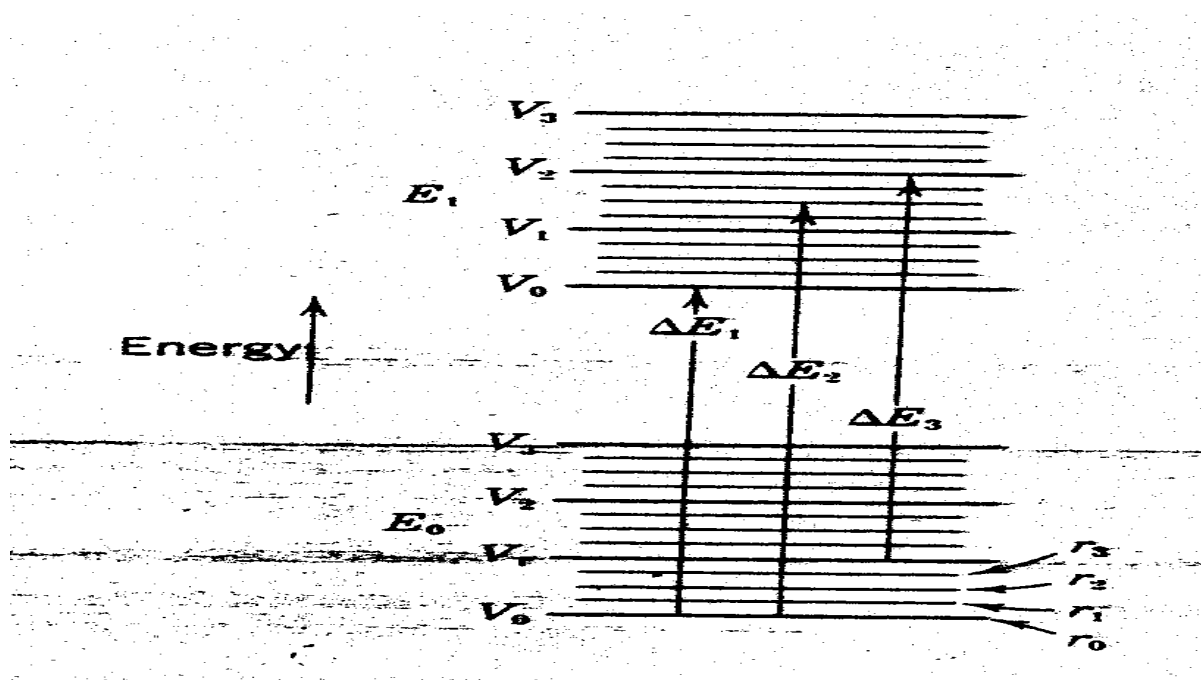


Figure (5) molecular electronic, vibrational and rotational energy levels

For each electronic level, there will be several sublevels corresponding to vibration states; and the later further subdivided into rotational levels.

For example, in figure (5), ΔE_1 , ΔE_2 and ΔE_3 all represent electronic transitions involving the same two electronic levels but different vibration and rotational levels. Each absorption thus correspond to energy transfer from radiation of a given frequency or wavelength.

As can be seen from figure (5), the energy difference between the ground state and an electronically excited state is large relative to the energy differences between vibration levels in a given electronic state.

Transitions within molecular species can be studied by observation of the selective absorption of radiation passed through them or by emission processes. For example, transitions between electronic levels are found in the ultraviolet and visible regions; those between vibration levels but within the same electronic level lie in the **near and mid-IR** and can be observed with **Raman techniques**.

In summary, an atom or molecule accept energy only in quanta to cause an excitation from one energy level to another.

Mathematical Theory (Beer's Lambert's Law)

If a beam of white light passes through a glass container (cuvet) filled with liquid, the emergent radiation is always less powerful than the entering. The loss is due in part to "reflections" at the surfaces and to scattering by any suspended particles present. But in the absence of such particles, it is primarily accounted for "absorption" of the radiant energy by the liquid

Figure (6) depicts a beam of parallel radiation before and after it has passed through a medium that has a thickness of (b) cm and a concentration of (c) of an absorbing species. As a consequence of interaction between the photons and absorbing atoms

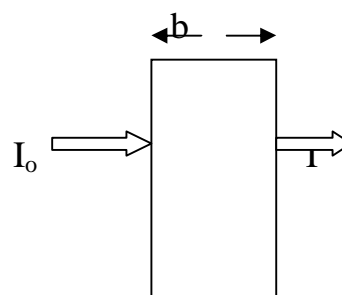


Figure (6) Absorbing solution of concentration (c)

or molecules, the power of the beam is attenuated from I_0 to I .

The transmittance T of the medium is then the fraction of incident radiation transmitted by the medium:

$$T = P / P_0$$

Transmittance is often expressed as a percentage or

$$\%T = I/I_0 \times 100\%$$

The **Absorbance** (A) of a medium is defined by the equation:

$$A = \log I_0/I = -\log T$$

Note that the ratio in contrast to transmittance, the absorbance of a medium increases as attenuation of the beam becomes greater.

Beer's Law

For monochromatic radiation, absorbance is directly proportional to the path length (b) through the medium and the concentration (c) of the absorbing species,

$$A (\text{absorbance}) = \log I_0/I = a b c$$

Where (a) is the proportionality constant called the **absorptivity**, units ($L.g^{-1}.cm^{-1}$).

When the concentration is expressed in moles per liter, the absorptivity is called the **molar absorptivity** and is given the symbol (ϵ)

$$A = \epsilon b c \text{ (units of } \epsilon = L.mole^{-1}.cm^{-1})$$

Note:- Absorptivity (ϵ) is a property of a substance (intensive property), whereas absorbance (A) is a property of a particular sample (extensive property) and will therefore vary with the concentration and length of light path through the container.

Emission of radiation

The interaction of electromagnetic radiation with matter is a reversible phenomenon. Let us examine the events, which might follow the absorption of

radiation raising the energy of a molecule from its ground state, S_0 , to an excited vibration level of an excited singlet state, S_2 , (Arrow A).

The molecule may lose the acquired energy through one of several alternate pathways:

(a) The process might be reversed immediately in which the emitted radiation is identical in frequency to the radiation employed for excitation (Arrow E). This process called **Resonance Fluorescence**.

(b) The excitation energy is more likely that it is converted to kinetic energy by collisions with other molecules and fall to the lowest vibrational level of the S_2 state, resulting in a minute increase in the temperature of the system. A process called **Vibrational Relaxation**, (Arrow R).

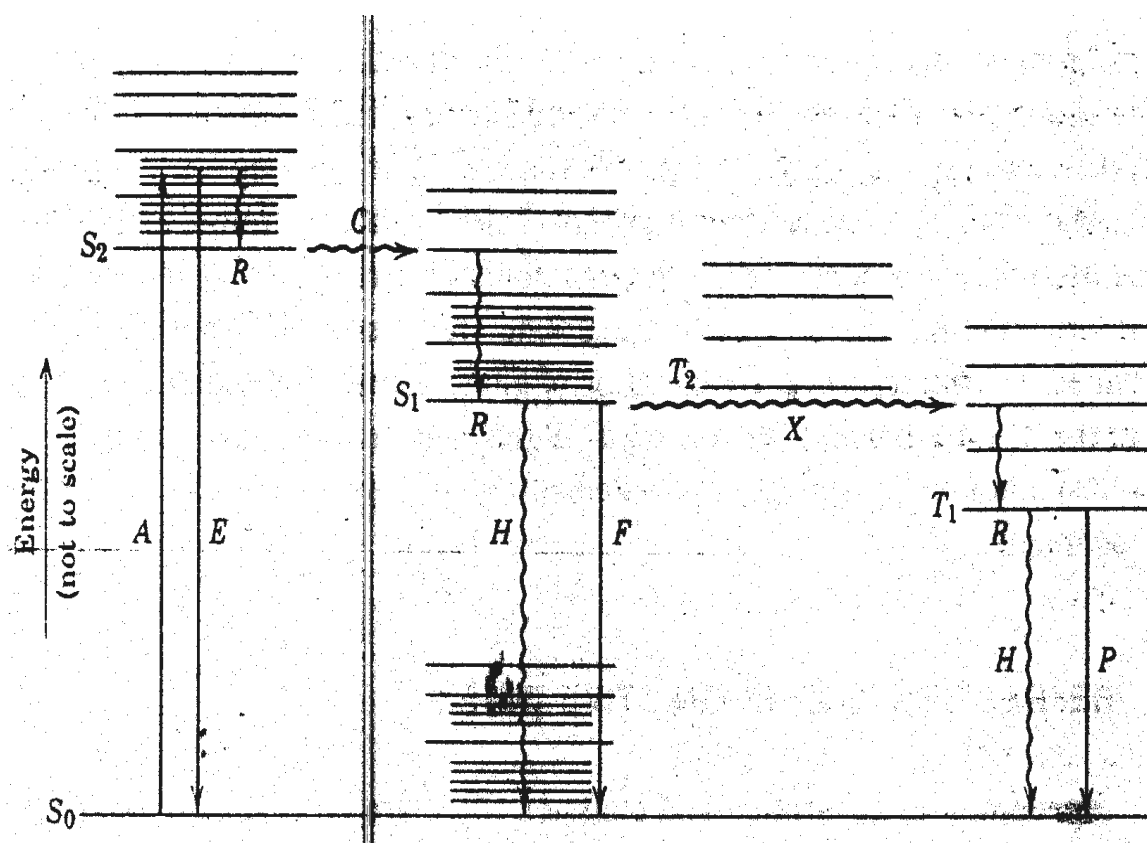


Figure (7) Energy level diagram of singlet and triplet states of a molecule showing luminescence phenomena

(c) A transition from the lowest S_2 state to the next lower singlet state S_1 is highly favored, and is called **Internal Conversion**, (Arrow C). The molecule then rapidly loses energy through additional collisions until it reaches the lowest level of the lowest singlet state S_1 . The molecule may return directly from the S_1 level to the ground state by emitting a photon, termed **normal fluorescence** (Arrow F). The frequency of normal fluorescence will be lower than the resonance fluorescence.

Many organic and some inorganic compounds fluorescence in the visible region when they are irradiated with ultraviolet light.

Another possibility that the molecule may return to the ground state by further collisions, dissipating the energy as non radiated heat (Arrow H).

A third possibility is that the molecule can shift from the singlet state to the corresponding triplet state ($S_1 \rightarrow T_1$), a phenomenon called **Intersystem Crossing** (Arrow X). This crossing involves unpairing of the two electrons, leaving the molecule in an excited vibrational level.

The lifetime of the T_1 state is relatively long (> 10 sec) and its energy is lower than the S_1 state, therefore a triplet molecule is more likely to lose energy through collisions.

However, some substances do return from the triplet state to the ground state via photon emission (Arrow P), a process called **Phosphorescence**. The duration of phosphorescence depends on the lifetime of the T_1 state and may last as long as 10 sec. Only a few type of molecules exhibit phosphorescence.

Refraction of radiation

When radiation passes from one medium to another, it is partially reflected and partially transmitted. When radiation passes at an angle through the interface between two transparent media that have different intensities, an abrupt change in direction or refraction of the beam is observed because of a difference in velocity of the radiation in the two media.

The index of refraction (n) of an optical medium is defined as the ratio of the velocity of a particular frequency in vacuum (c) to that in a medium (c_m).

$$n = c / c_m = v_{\text{vacuum}} / v_{\text{medium}} \quad \text{--- (1)}$$

The index of refraction of air is so close to unity ($n_{\text{air}} = 1.00027$), that for ordinary purposes:

$$n = v_{\text{air}} / v_{\text{medium}} \quad \text{--- (2)}$$

When the beam passes from a less dense to a more dense environment, as in figure (8), the bending is towards the normal to the interface. Bending away from the normal occurs when the beam passes from a more dense to a less dense medium.

Snell's law gives the extent of refraction:

$$\sin \Theta_1 / \sin \Theta_2 = n_2 / n_1 = v_1 / v_2 \quad \text{--- (3)}$$

Since n_1 (air) ≈ 1

rearrangement of eq.(3):-

$$n_2 = \sin \Theta_1 / \sin \Theta_2 \quad \text{(Snell's law) --- (4)}$$

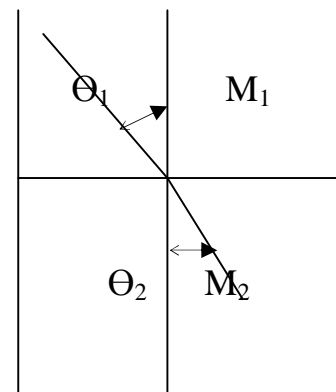
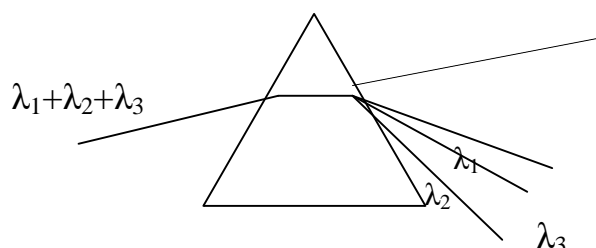


Figure (8) Refraction of light in passing from a less dense medium M_1 into a more dense medium M_2 where its velocity is lower.

The variation of refractive index of a substance with wavelength (or frequency) is called the **dispersion**. This statement is extremely important because it indicates that **light of different frequencies is refracted at different angles**.

The refractive index is an important property of transparent materials. Its variation with wavelength is responsible for chromatic aberration in lenses, and the characteristic dispersion of radiation by prisms both are of significant value in the design of optical instruments.

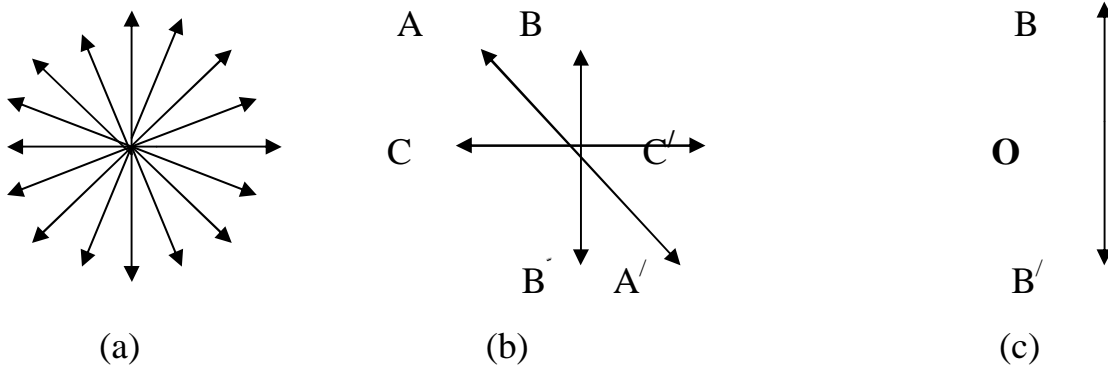


Dispersion of polychromatic radiation by 60°

Polarization and optical activity

Ordinary radiation consists of a bundle of electromagnetic waves in which the vibrations are equally distributed among a huge number of planes centered along the path of the beam.

Figure 9(a) shows a cross section of such a ray which is proceeding in a direction perpendicular to the plane of the paper.



If the beam is passed through a polarizer, each separate wave in the bundle, for example, that vibrating along the vector AOA' ,figure 9(b), is resolved into its orthogonal components BOB' and COC' in the direction of the X and Y axes characteristic of the polarizer.

The polarizing material has the property of absorbing one of these component vibrations (say COC') and passing the other (BOB'). Thus, the emerging beam will consist of vibrations in one plane only, figure 9(c), and is said to be **plane polarized**.

Plane polarized electromagnetic radiation is produced by certain radiant energy sources. For example, the radio waves emanating from an antenna, and microwaves produced by a klaystron tube are both plane-polarized

Polarized ultraviolet and visible radiation produced by passage of radiation through media that selectively absorb, reflect or refract radiation that vibrates in only one plane is illustrated in figure (10).

Radiation from a lamp rendered parallel by a lens, passes through a polarizer (A) which has its axis oriented vertically. The analyzer (B) also with a vertical

axis, has no further effect on the beam, but (C) with its axis horizontal, cuts the radiation into zero. If (C) is rotated on its own plane, the power of the transmitted radiation will vary as the sine of the angle. Two polarizer placed in series are said to be "crossed" if their axes are mutually perpendicular.

A beam of radiation may possess any degree of plane polarization from zero (complete symmetry) to 100 percent (complete polarization).

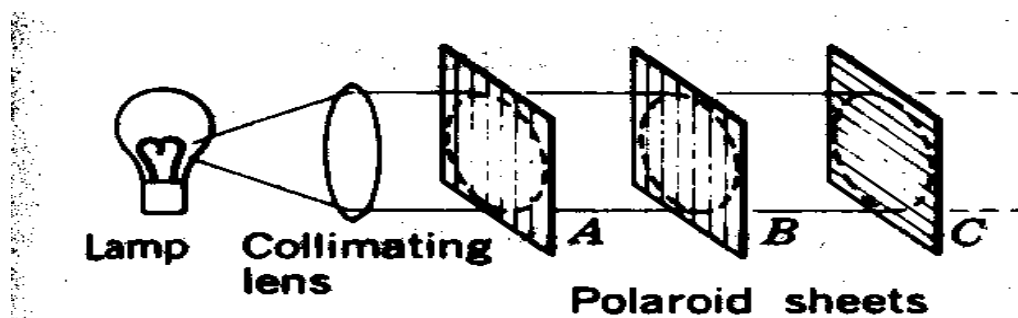


Figure (10):- Plane polarization of radiation

Polarization is important in chemistry because of the ability of some crystals and liquids to rotate the plane of polarized radiation passed through them. This is the property known as "**optical activity**"

Instruments for Spectroscopy

Introduction

The instruments that are used to study the absorption or emission of electromagnetic radiation as a function of wavelength are called ‘**spectrometers**’ or **spectrophotometers**’.

The essential components of a spectrophotometer include:

- 1- A stable source of radiant energy
- 2- A system of lenses, mirrors, and slits which define, collimate (make parallel) and focus the beam.
- 3- Monochromators to resolve the radiation into component wavelengths or bands of wavelength.
- 4- A transparent container to hold the sample.
- 5- Radiation detector
- 6- Readout system (meter, recorder or computer).

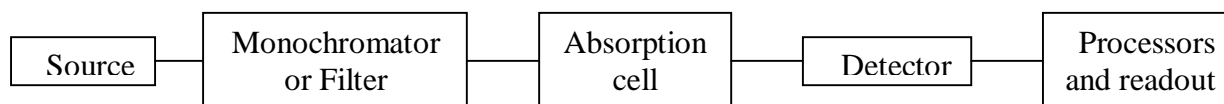


Figure (1) Block diagram of a spectrophotometer

Commercial instruments may be very complex, but all spectrophotometers represent variations of the simple diagram in figure (1).

Sources of radiant energy

Sources of radiant energy consist of materials that are excited by a high voltage electric discharge or by electrical heating. As the materials return to lower energy states, they emit photons of characteristic energies corresponding to ΔE , the energy difference between the excited and lower quantum states.

Sources of Ultraviolet Radiation

The hydrogen lamp and deuterium lamp are the most common sources of UV radiation. They consist of a pair of electrodes which are enclosed in a glass tube provided with a quartz window and filled with hydrogen or deuterium gas at low pressure. When a stabilized high voltage is applied to the electrodes, an electron discharge occurs which excites other electrons in the gas molecules to high energy states. As the electrons return to their ground states they emit radiation in the region roughly between 180 and 350 nm.

Sources of Visible Radiation

A tungsten (W) filament lamp is the most satisfactory and inexpensive source of visible radiation. The filament is heated by a d-c power supply, or by a storage battery. The tungsten filament emits continuous radiation in the region between 350 and 2500 nm.

Sources of Infrared Radiation

The Globar and Ernst glower are the primary sources of infrared radiation. The Globar is a silicon carbide (SiC) rod heated to approximately 1200 °C. It emits continuous radiation in the (1-40) μm region.

Wavelength Selectors

These are devices which resolve wide band polychromatic radiation from the source into narrow bands or, even better, monochromatic radiation. There are two types of resolving devices **filters** and **mono-chromators**.

Filters prepared from special materials allow transmission of only limited wavelength regions while absorbing most of the radiation of other wavelengths.

Monochromators resolve poly-chromatic into its individual wavelengths and isolate these wavelengths into very narrow bands.

The components of a monochromator include: (1) an entrance slit which admits polychromatic radiation from the source; (2) a collimating device either a lens or a mirror; (3) a dispersing device, either a prism or grating which resolve the radiation into small bands of wavelengths emerging at different angles; (4) A focusing lens or mirror; (5) An exit slit.

Sample Containers

The cells or cuvettes that hold the samples must be made of material that is transparent to radiation in the spectral region of interest. Quartz or fused silica is required for work in the ultraviolet region (< 350 nm). Plastic containers have also found application in the visible region. Crystalline sodium chloride (NaCl) is the most common substance employed for cell windows in the infrared region.

Radiation Detectors

Any detector absorbs the energy of the radiation and converts this energy to a measurable quantity such as the darkening of a photographic plate, electric current or thermal changes. Any detector must generate a signal which is quantitatively related to the radiant power striking it. The noise of a detector refers to the **background signal** or **dark current** generated when no radiant power from the sample reaches the detector.

Phototubes

Ultraviolet and visible radiation, possess enough energy to cause photoejection of electrons when they strike surfaces which have been treated with specific type of compounds. Their absorption may also cause bound, non-conducting electrons to move into conducting bands in certain semiconductors. Both processes generate an electric current which is directly proportional to the radiant power of the absorbed radiation.

Photomultiplier tube

If the ejected electron is accelerated by an electric field, it acquires more energy; and if it strikes another electron-active surface, it may transfer some of its energy, ejecting several more electrons. These electrons may in turn be accelerated to another surface and produce even more electrons, and so on.

A cross section of this device is shown in figure (). Each succeeding electron active plate, or dynode, is at higher electrical potential and thus acts as an amplification stage for the original photon. After nine stages of amplification, the original photon has been amplified by a factor of approximately 10^6 .

Photoconductivity detectors

These are semiconductors whose resistance decreases when they absorb radiation in the region (0.75 to 3 μm). The application of photoconductors is important in Fourier Transform Infrared instrumentation FTIR.

Absorption of radiation by semiconductor materials promotes some of their bound electrons into an energy state in which they are free to conduct electricity. The resulting change in conductivity can then be measured.

Thermal detectors

In thermal detectors, the radiation absorbed is converted to thermal energy (heat) and a corresponding temperature change is noted. These are various types of rapid response thermometers such as thermocouples, resistance thermometers (bolometer), gas thermometers, and pyroelectric transducers.

Processors and readout

The electronic signal generated by any radiation detector must be translated into a form that can be interpreted. This process is typically accomplished with amplifiers, ammeters, potentiometers and potentiometer recorders.

Amplifiers

The amplifier takes an “input” signal from the circuit of the sensing component and, through a series of electronic operations, produces an “output” signal which is many times larger than the “input”. The amplification factor (ratio of output to input) is called “**the gain**” of the amplifier.

Readout devices

Several types of readout devices are found in modern instruments. Some of these devices include the digital meters, the scale of potentiometers, cathode ray tubes and computers.

The instrument is calibrated so that there are 100 units on the meter from ($I_t = 0$) to ($I = I_0$) and these units are linear with respect to I_t . When an absorbing sample is substituted for the “blank”, the detector response will show between

0 and 100 units on the meter

Types of UV-visible instruments

(a) Single beam instruments

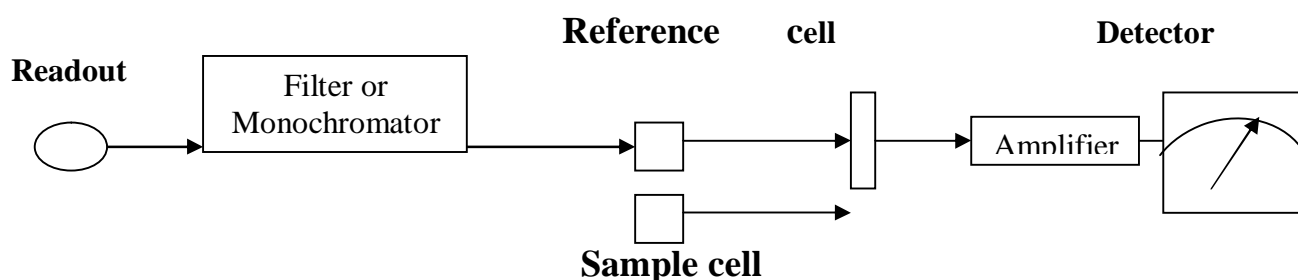


Figure (1) Single beam instrument

It consists of the radiation source, a filter or monochromator for wavelength selection, matched cells that can be imposed alternately in the radiation beam, the photodetector, an amplifier and readout device.

A single beam instrument require a stabilized voltage supply to avoid errors resulting from changes in the beam intensity during the time required to make the 100% T adjustment and determine %T for the analyte.

(b) Double beam instruments

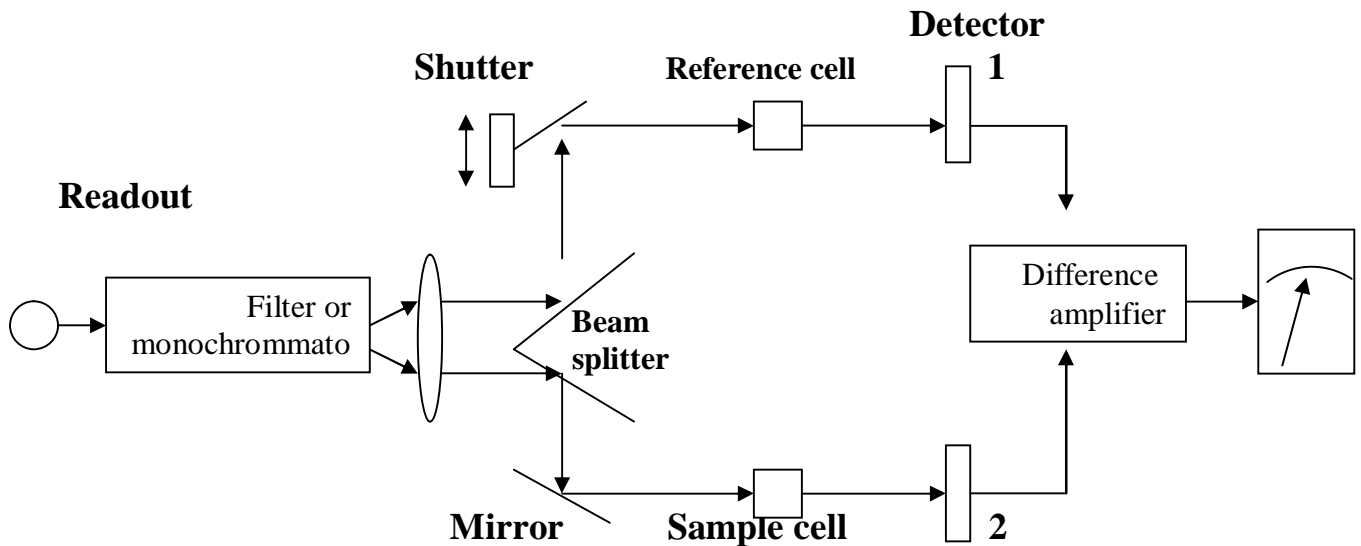
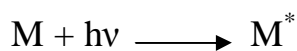


Figure (2) Double beam instrument

Two beams are formed in space by a V-shaped mirror called a beam splitter. One beam passes through a reference solution to a detector, and the second simultaneously traverses the sample to a second, matched detector. The two outputs are amplified, and their ratio (or the log of their ratio) is determined electronically and displayed by the readout device.

Application of UV/visible Molecular Absorption Spectroscopy

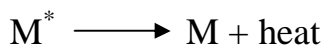
The absorption of ultraviolet or visible radiation by an atomic or molecular species M can be considered to be a two step process, the first of which involves electronic excitation as shown by the equation:



Where, M^* is an excited species

The lifetime of the excited species ($10^{-8} - 10^{-9}$ S) is terminated by any of several excited species, these involve:

- (a) Conversion of excitation energy to heat



- (b) Decomposition of M^* to form new species, such a process is called ***photochemical reaction***.

- (c) Re-emission of fluorescence or phosphorescence.

The absorption of ultraviolet or visible radiation generally results from excitation of bonding electrons; as a consequence, the wavelengths of absorption peaks can be correlated with the types of the bonds in the species under study.

The electronic transitions in the UV/visible region involve: (1) π , σ , and n electrons,

(2) d and f electrons, and (3) charge transfer electrons.

Types of absorbing electrons

The molecular orbitals associated with single bonds in organic molecules are designated as *sigma* (σ) orbitals, and the corresponding electrons are σ electrons.

As shown in figure (3), the distribution of charge density of a sigma orbital is rotationally symmetric around the axis of the bond.

The double bond in an organic molecule contains two types of molecular orbitals:

a sigma (σ) orbital corresponding to one pair of the bonding electrons and a pi (π) molecular orbital associated with the other pair.

Pi (π) orbitals are formed by the parallel overlap of atomic p-orbitals.

Also shown in figure (3) are the charge density distributions for antibonding sigma and pi orbitals; these orbitals are designated by σ^* and π^* .

In addition to σ and π electrons, many organic compounds contain nonbonding electrons. These unshared electrons are designated by the symbol (n)

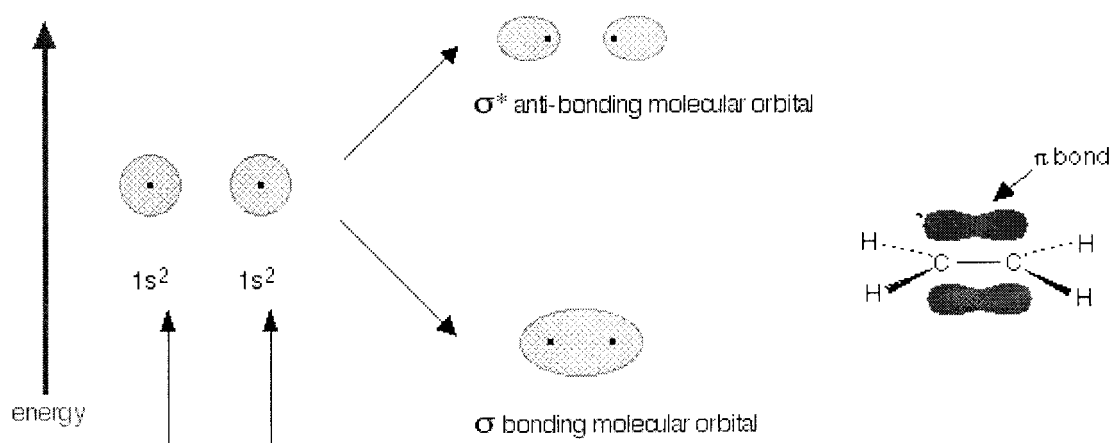


Figure (3) Electron distribution in σ and π molecular orbitals

Electronic transitions among certain of the energy levels can be brought about by the absorption of radiation. Four types of transitions are possible: $\sigma \rightarrow \sigma^*$, $n \rightarrow \sigma^*$, $n \rightarrow \pi^*$, and $\pi \rightarrow \pi^*$.

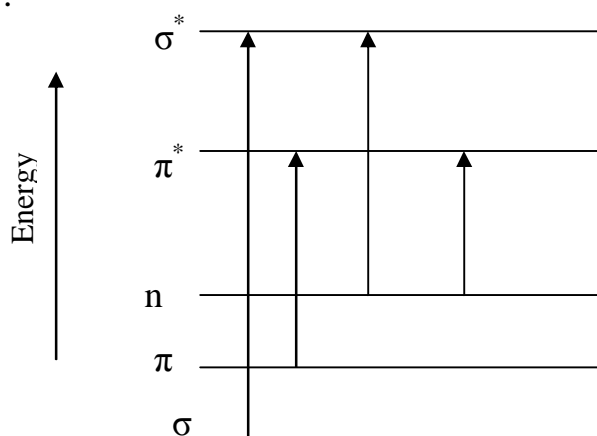


Figure (4) Electronic molecular energy levels

The energy required to induce $\sigma \longrightarrow \sigma^*$ transition is large (figure-4) corresponding to radiation frequencies in the vacuum ultraviolet region. Example: methane contain only single C—H bonds and can thus undergo only $\sigma \longrightarrow \sigma^*$ transitions, exhibits an absorption maximum at 125 nm. Ethane has an absorption peak at 135 nm which arise from the same type of transition in addition to the C-C bond.

Absorption spectrometer works in a range from about 200 nm (in the near ultraviolet) to about 800 nm (in the very near infrared). Therefore, only a limited number of electron transitions absorb light in that region and the important transitions are:

- From pi bonding orbitals to pi anti-bonding orbitals; $\pi \longrightarrow \pi^*$
- From non-bonding orbitals to pi anti-bonding orbitals; $n \longrightarrow \pi^*$
- From non-bonding orbitals to sigma anti-bonding orbitals; $n \longrightarrow \sigma^*$

That means that in order to absorb light in the region from (200-800)nm, the molecule must contain either pi bonds or atoms with non-bonding orbitals.

Non-bonding orbital is a lone pair on, say, oxygen, nitrogen or a halogen and groups in a molecule that absorb light are known as **chromophores**.

The UV/visible spectrum

Figure (5) shows a simple UV-visible spectrum.

Absorbance (on the vertical axis) is a measure of the amount of light absorbed. The higher the value, the more of a particular wavelength is being absorbed.

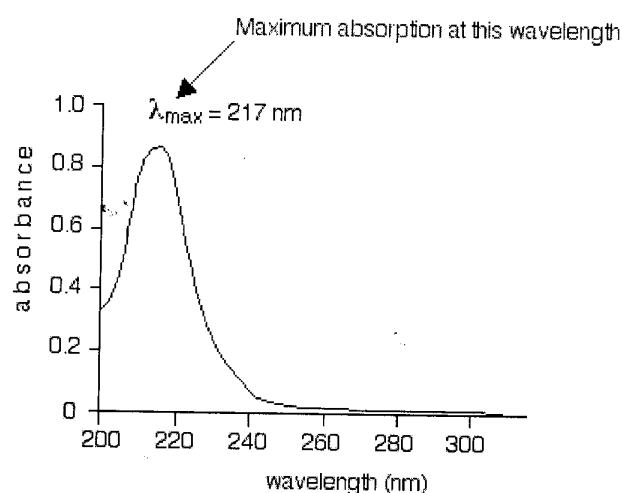


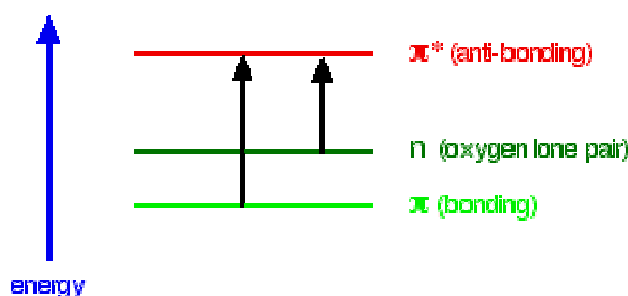
Figure (5) Typical absorption spectra for buta-1,3-diene ($\text{CH}_2=\text{CH}-\text{CH}=\text{CH}_2$)

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.

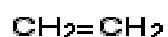
Ethanal can therefore absorb light of two different wavelengths:

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

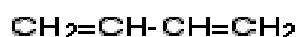
Both of these absorptions are in the ultra-violet, but most spectrometers won't pick up the one at 180 nm because they work in the range from 200 - 800 nm.

The importance of conjugation and delocalisation in what wavelength is absorbed

Consider these three molecules:



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.

- 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.

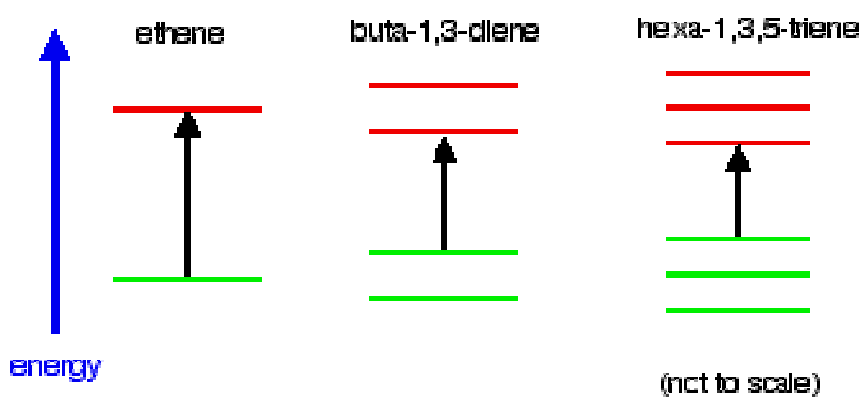
Compare ethene with buta-1,3-diene. In ethene, there is one pi bonding orbital and one pi anti-bonding orbital. In buta-1,3-diene, there are two pi bonding orbitals and two pi anti-bonding orbitals.



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.



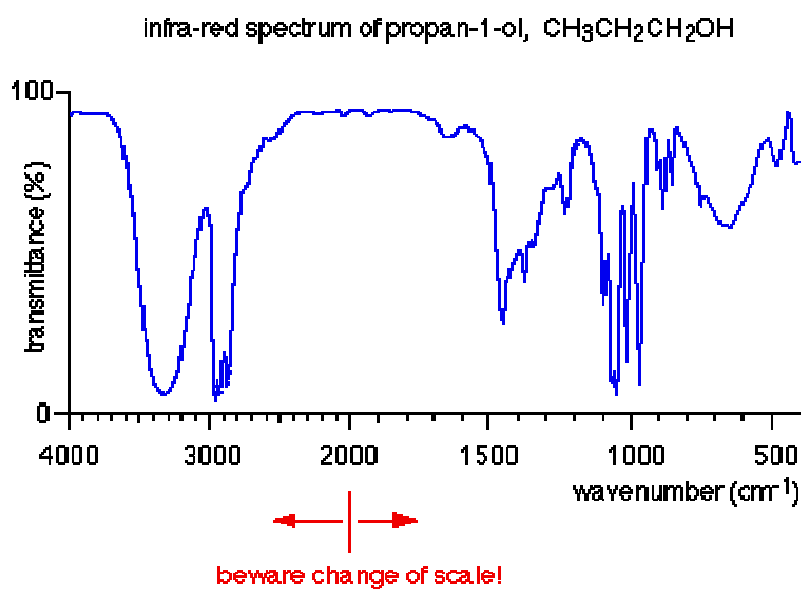
If you extend this to compounds with really massive delocalization, the wavelength absorbed will eventually be high enough to be in the visible region of the spectrum, and the compound will then be seen as coloured.

Infrared Spectroscopy

Infrared radiation consists of a continuous range of frequencies that arise from one vibrational or rotational energy state to another.

What an infra-red spectrum looks like

A graph is produced showing how the percentage transmittance varies with the frequency of the infra-red radiation.



What causes some frequencies to be absorbed?

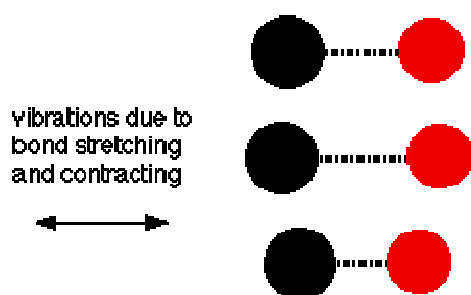
Each frequency of light (including infra-red) has a certain energy. If a particular frequency is being absorbed as it passes through the compound being investigated, it must mean that its energy is being transferred to the compound.

Energies in infra-red radiation correspond to the energies involved in bond vibration

Bond stretching

In covalent bonds, atoms aren't joined by rigid links - the two atoms are held together because both nuclei are attracted to the same pair of electrons. The two nuclei can vibrate backwards and forwards - towards and away from each other - around an average position.

The diagram shows the stretching that happens in a carbon-oxygen single bond. There will, of course, be other atoms attached to both the carbon and the oxygen. For example, it could be the carbon-oxygen bond in methanol, CH_3OH .



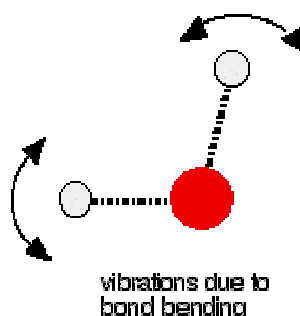
The energy involved in this vibration depends on things like the length of the bond and the mass of the atoms at either end. That means that each different bond will vibrate in a different way, involving different amounts of energy.

Bonds are vibrating all the time, but if you shine exactly the right amount of energy on a bond, you can kick it into a higher state of vibration. The amount of energy it needs to do this will vary from bond to bond, and so each different bond will absorb a different frequency (and hence energy) of infra-red radiation.

Bond bending

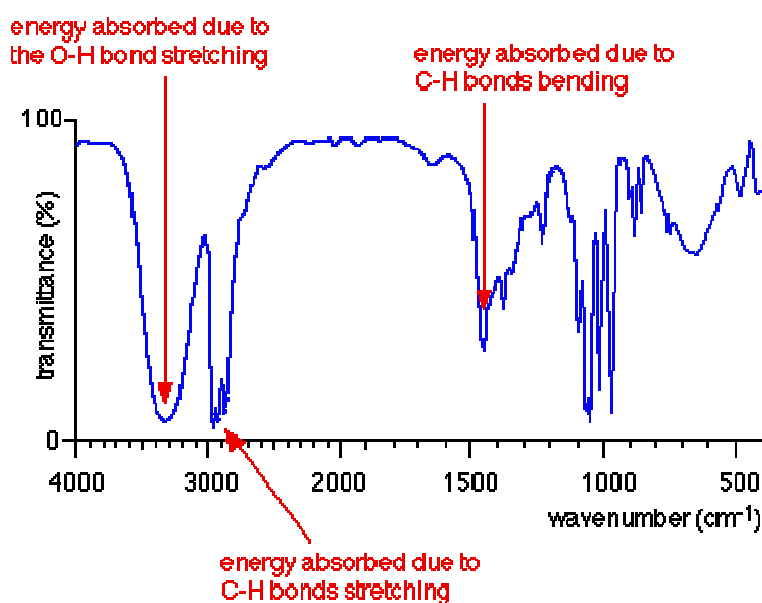
As well as stretching, bonds can also bend. The diagram shows the bending of the bonds in a water molecule. The effect of this, of course, is that the bond angle between the two hydrogen-oxygen bonds fluctuates slightly around its average value. Imagine a lab model of a water molecule where the atoms are

joined together with springs. These bending vibrations are what you would see if you shook the model gently.



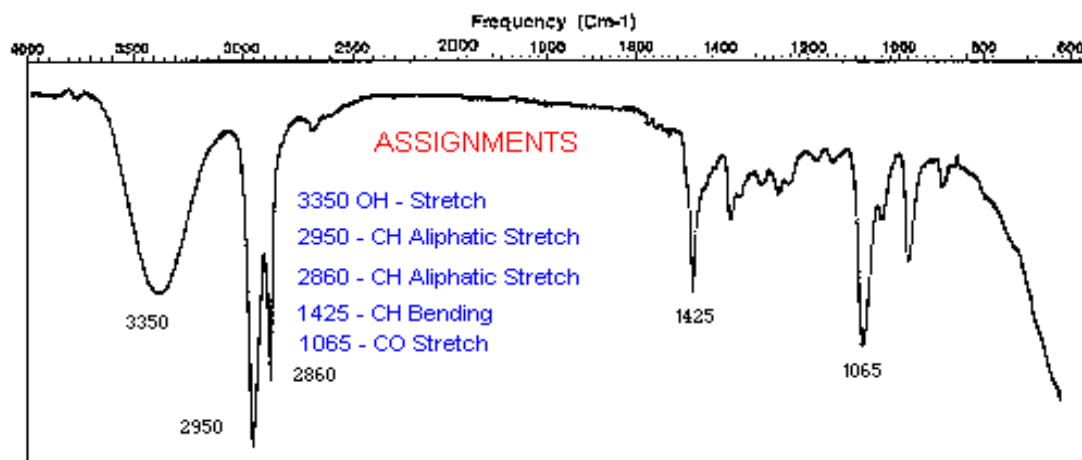
Again, bonds will be vibrating like this all the time and, again, if you shine exactly the right amount of energy on the bond, you can kick it into a higher state of vibration. Since the energies involved with the bending will be different for each kind of bond, each different bond will absorb a different frequency of infra-red radiation in order to make this jump from one state to a higher one.

Look again at the infra-red spectrum of propan-1-ol, $\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$:



In the diagram, three sample absorptions are picked out to show you the bond vibrations, which produced them. Notice that bond stretching and bending produce different troughs in the spectrum.

INTERPRETING AN INFRA-RED SPECTRUM



The interpretation of infrared spectra involves the correlation of absorption bands in the spectrum of an unknown compound with the known absorption frequencies for types of bonds.

CHARACTERISTIC INFRARED ABSORPTION FREQUENCIES		
Bond	Compound Type	Frequency range, cm ⁻¹
C-H	Alkanes	2960-2850(s) stretch
		1470-1350(v) scissoring and bending
	CH₃ Umbrella Deformation	1380(m-w) - Doublet - isopropyl, t-butyl
C-H	Alkenes	3080-3020(m) stretch
		1000-675(s) bend
C-H	Aromatic Rings	3100-3000(m) stretch
	Phenyl Ring Substitution Bands	870-675(s) bend
	Phenyl Ring Substitution Overtones	2000-1600(w) - fingerprint region
C-H	Alkynes	3333-3267(s) stretch
		700-610(b) bend
C=C	Alkenes	1680-1640(m,w)) stretch
C≡C	Alkynes	2260-2100(w,sh) stretch
C=C	Aromatic Rings	1600, 1500(w) stretch
C-O	Alcohols , Ethers , Carboxylic acids , Esters	1260-1000(s) stretch

C=O	Aldehydes , Ketones , Carboxylic acids , Esters	1760-1670(s) stretch
O-H	Monomeric -- Alcohols, Phenols	3640-3160(s,br) stretch
	Hydrogen-bonded -- Alcohols , Phenols	3600-3200(b) stretch
	Carboxylic acids	3000-2500(b) stretch
N-H	Amines	3500-3300(m) stretch
		1650-1580 (m) bend
C-N	Amines	1340-1020(m) stretch
C \equiv N	Nitriles	2260-2220(v) stretch
NO ₂	Nitro Compounds	1660-1500(s) asymmetrical stretch
		1390-1260(s) symmetrical stretch

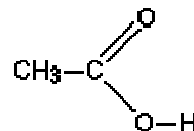
v - variable, m - medium, s - strong, br - broad, w - weak

Examples:

(a) The infra-red spectrum for a simple carboxylic acid

Ethanoic acid

Ethanoic acid has the structure:



You will see that it contains the following bonds:

carbon-oxygen double, C=O

carbon-oxygen single, C-O

oxygen-hydrogen, O-H

carbon-hydrogen, C-H

carbon-carbon single, C-C

The carbon-carbon bond has absorptions, which occur over a wide range of wave numbers in the fingerprint region - that makes it very difficult to pick out on an infra-red spectrum.

The carbon-oxygen single bond also has an absorption in the fingerprint region, varying between 1000 and 1300 cm^{-1} depending on the molecule it is in.

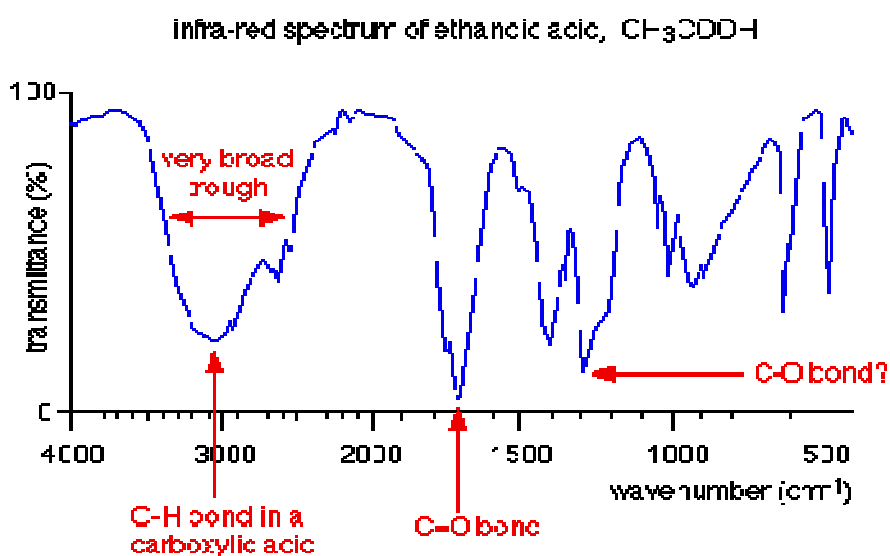
The other bonds in ethanoic acid have easily recognized absorptions outside the fingerprint region.

The C-H bond (where the hydrogen is attached to a carbon which is singly bonded to everything else) absorbs somewhere in the range from 2853 - 2962 cm^{-1} . Because that bond is present in most organic compounds, that's not terribly useful! What it means is that you can ignore a trough just under 3000 cm^{-1} , because that is probably just due to C-H bonds.

The carbon-oxygen double bond, C=O, is one of the useful absorptions, found in the range 1680 - 1750 cm^{-1} . Its position varies slightly depending on what sort of compound it is in.

The other useful bond is the O-H bond. This absorbs differently depending on its environment. It is easily recognized in an acid because it produces a very broad trough in the range 2500 - 3300 cm^{-1} .

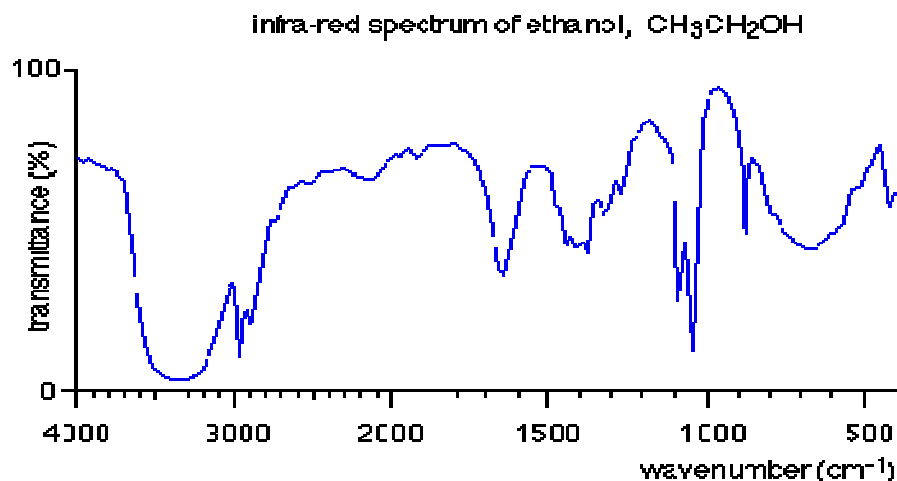
The infra-red spectrum for ethanoic acid looks like this:



The possible absorption due to the C-O single bond is queried because it lies in the fingerprint region. .

(b) The infra-red spectrum for an alcohol

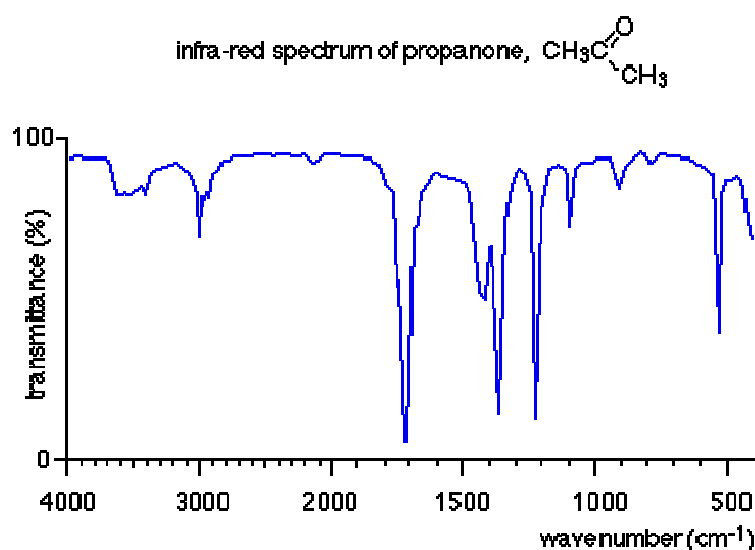
Ethanol



The O-H bond in an alcohol absorbs at a higher wave number than it does in an acid, “between” $3230 - 3550 \text{ cm}^{-1}$. This absorption would be at a higher number still if the alcohol is not hydrogen bonded - for example, in the gas state. All the infrared spectra on this page are from liquids - so that possibility will never apply.

(c) The infra-red spectrum for a ketone

Propanone



You will find that this is very similar to the infra-red spectrum for ethyl ethanoate, an ester. Again, there is no trough due to the O-H bond, and again there is a marked absorption at about 1700 cm^{-1} due to the C=O.

Confusingly, there are also absorptions, which look as if they might be due to C-O single bonds - which, of course, are not present in propanone.

Aldehydes will have similar infra-red spectra to ketones.

CHROMATOGRAPHY

Chromatography is used to separate mixtures of substances into their components. All forms of chromatography work on the same principle.

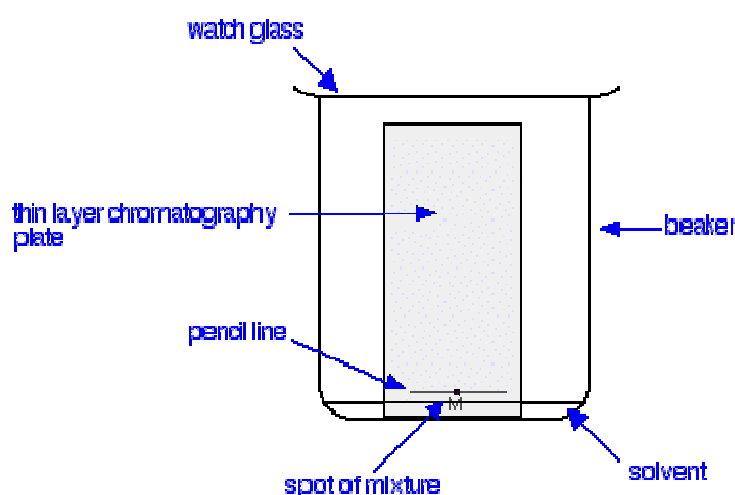
They all have a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates. We'll look at the reasons for this further down the page.

Thin layer chromatography is done exactly as it says - using a thin, uniform layer of silica gel or alumina coated onto a piece of glass, metal or rigid plastic.

The silica gel (or the alumina) is the stationary phase. The stationary phase for thin layer chromatography also often contains a substance which fluoresces in UV light - for reasons you will see later. The mobile phase is a suitable liquid solvent or mixture of solvents.

Producing the chromatogram

Suppose that a particular dye is a mixture of simpler dyes.

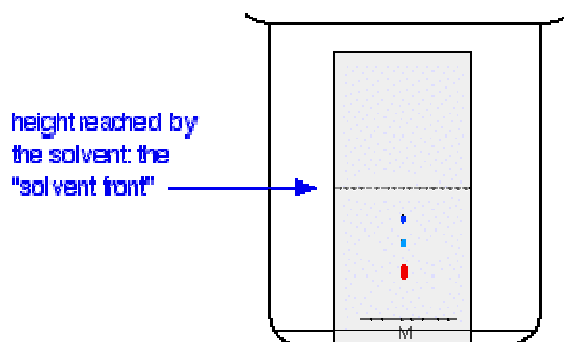


A pencil line is drawn near the bottom of the plate and a small drop of a solution of the dye mixture is placed on it. Any labelling on the plate to show

the original position of the drop must also be in pencil. If any of this was done in ink, dyes from the ink would also move as the chromatogram developed.

When the spot of mixture is dry, the plate is stood in a shallow layer of solvent in a covered beaker. It is important that the solvent level is below the line with the spot on it.

As the solvent slowly travels up the plate, the different components of the dye mixture travel at different rates and the mixture is separated into different colored spots.



The diagram shows the plate after the solvent has moved about half way up it.

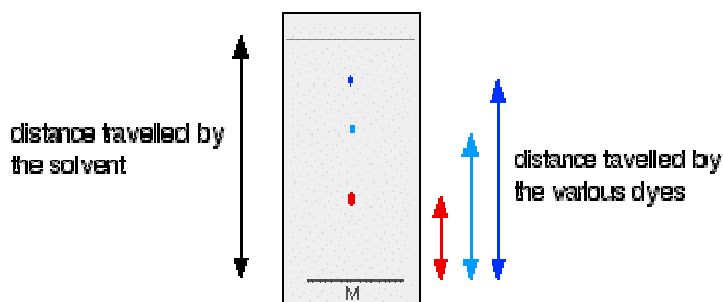
The solvent is allowed to rise until it almost reaches the top of the plate. That will give the maximum separation of the dye components for this particular combination of solvent and stationary phase.

Measuring R_f values

Individual spots the distance traveled by the solvent, and the distance travel these measurements.

When the solvent front gets close to the top of the plate, the plate is removed from the beaker and the position of the solvent is marked with another line before it has a chance to evaporate.

These measurements are then taken:



The R_f value for each dye is then worked out using the formula:

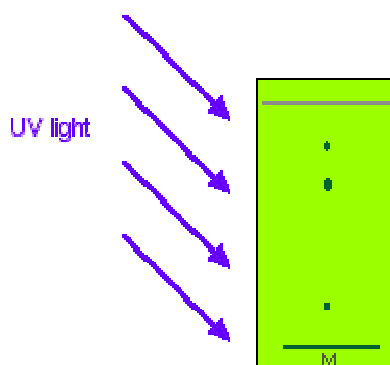
$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

For example, if the red component traveled 1.7 cm from the base line while the solvent had traveled 5.0 cm, then the R_f value for the red dye is:

$$R_f = \frac{1.7}{5.0} = 0.34$$

If you could repeat this experiment under exactly the same conditions, then the R_f values for each dye would always be the same. For example, the R_f value for the red dye would always be 0.34. However, if anything changes (the temperature, the exact composition of the solvent, and so on), that is no longer true.

For colourless substances the stationary phase on a thin layer plate often has a substance added to it which will fluoresce when exposed to UV light. That means that if you shine UV light on it, it will glow.

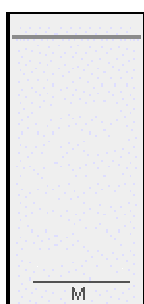


While the UV is still shining on the plate, you obviously have to mark the positions of the spots by drawing a pencil circle around them. As soon as you switch off the UV source, the spots will disappear again.

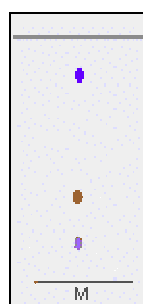
Showing the spots up chemically

In some cases, it may be possible to make the spots visible by reacting them with something which produces a coloured product. A good example of this is in chromatograms produced from amino acid mixtures.

The chromatogram is allowed to dry and is then sprayed with a solution of ninhydrin. Ninhydrin reacts with amino acids to give coloured compounds, mainly brown or purple.



before spraying with ninhydrin



after spraying with ninhydrin

In another method, the chromatogram is again allowed to dry and then placed in an enclosed container (such as another beaker covered with a watch glass) along with a few iodine crystals.

The iodine vapour in the container may either react with the spots on the chromatogram, or simply stick more to the spots than to the rest of the plate. Either way, the substances you are interested in may show up as brownish spots.

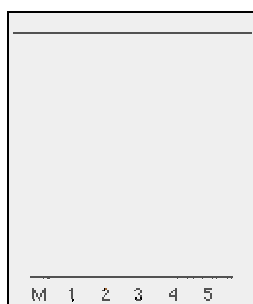
Using thin layer chromatography to identify compounds

Suppose you had a mixture of amino acids and wanted to find out which particular amino acids the mixture contained. For simplicity we'll assume that

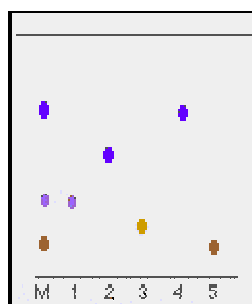
you know the mixture can only possibly contain five of the common amino acids.

A small drop of the mixture is placed on the base line of the thin layer plate, and similar small spots of the known amino acids are placed alongside it. The plate is then stood in a suitable solvent and left to develop as before. In the diagram, the mixture is M, and the known amino acids are labelled 1 to 5.

The left-hand diagram shows the plate after the solvent front has almost reached the top. The spots are still invisible. The second diagram shows what it might look like after spraying with ninhydrin.



before spraying with ninhydrin



after spraying with ninhydrin

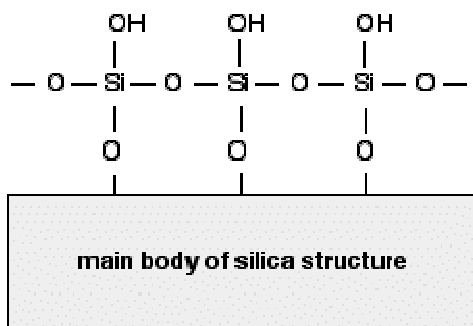
There is no need to measure the R_f values because you can easily compare the spots in the mixture with those of the known amino acids - both from their positions and their colours.

In this example, the mixture contains the amino acids labelled as 1, 4 and 5.

The stationary phase - silica gel

Silica gel is a form of silicon dioxide (silica). The silicon atoms are joined via oxygen atoms in a giant covalent structure. However, at the surface of the silica gel, the silicon atoms are attached to -OH groups.

So, at the surface of the silica gel you have Si-O-H bonds instead of Si-O-Si bonds. The diagram shows a small part of the silica surface.



The surface of the silica gel is very polar and, because of the -OH groups, can form hydrogen bonds with suitable compounds around it as well as van der Waals dispersion forces and dipole-dipole attractions.

The other commonly used stationary phase is alumina - aluminium oxide. The aluminium atoms on the surface of this also have -OH groups attached. Anything we say about silica gel therefore applies equally to alumina.

What separates the compounds as a chromatogram develops?

As the solvent begins to soak up the plate, it first dissolves the compounds in the spot that you have put on the base line. The compounds present will then tend to get carried up the chromatography plate as the solvent continues to move upwards.

How fast the compounds get carried up the plate depends on two things:

- How soluble the compound is in the solvent. This will depend on how much attraction there is between the molecules of the compound and those of the solvent.
- How much the compound sticks to the stationary phase - the silica gel, for example. This will depend on how much attraction there is between the molecules of the compound and the silica gel.

Suppose the original spot contained two compounds - one of which can form hydrogen bonds, and one of which can only take part in weaker van der Waals interactions.

The one which can hydrogen bond will stick to the surface of the silica gel more firmly than the other one. We say that one is adsorbed more strongly than the other. Adsorption is the name given to one substance forming some sort of bonds to the surface of another one.

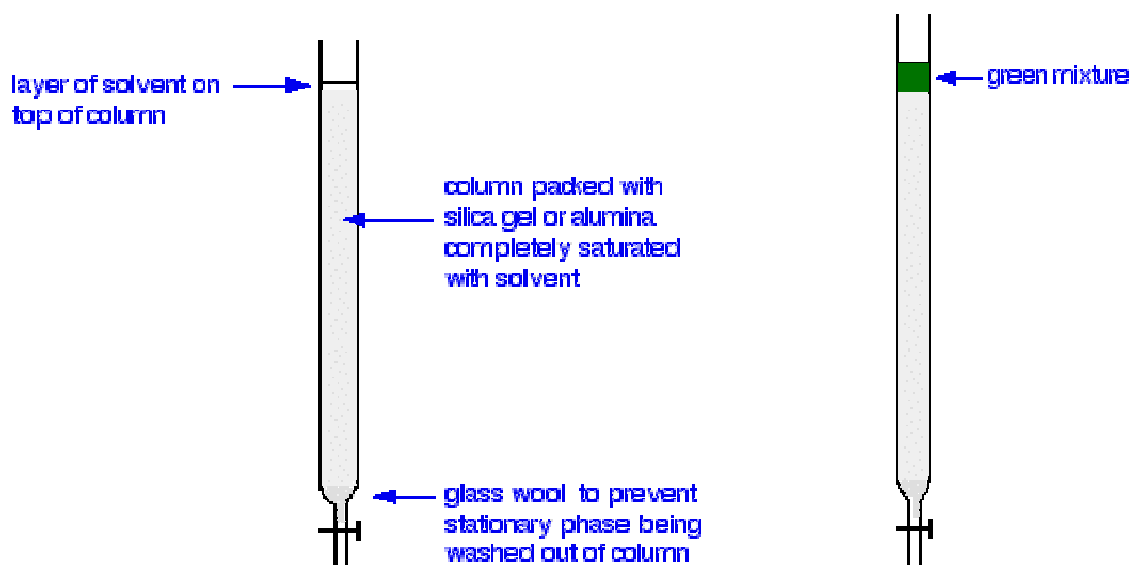
Adsorption isn't permanent - there is a constant movement of a molecule between being adsorbed onto the silica gel surface and going back into solution in the solvent.

Obviously the compound can only travel up the plate during the time that it is dissolved in the solvent. While it is adsorbed on the silica gel, it is temporarily stopped - the solvent is moving on without it. That means that the more strongly a compound is adsorbed, the less distance it can travel up the plate.

In the example we started with, the compound which can hydrogen bond will adsorb more strongly than the one dependent on van der Waals interactions, and so won't travel so far up the plate.

COLUMN CHROMATOGRAPHY

In thin layer chromatography, the stationary phase is a thin layer of silica gel or alumina on a glass, metal or plastic plate. Column chromatography works on a much larger scale by packing the same materials into a vertical glass column.



The process of washing a compound through a column using a solvent is known as elution. The solvent is sometimes known as the eluent.

- The polar solvent will compete for space on the silica gel or alumina with the blue compound. Any space temporarily occupied by solvent molecules on the surface of the stationary phase isn't available for blue molecules to stick to and this will tend to keep them moving along in the solvent.
- There will be a greater attraction between the polar solvent molecules and the polar blue molecules. This will tend to attract any blue molecules sticking to the stationary phase back into solution.

The net effect is that with a more polar solvent, the blue compound spends more time in solution, and so moves faster.

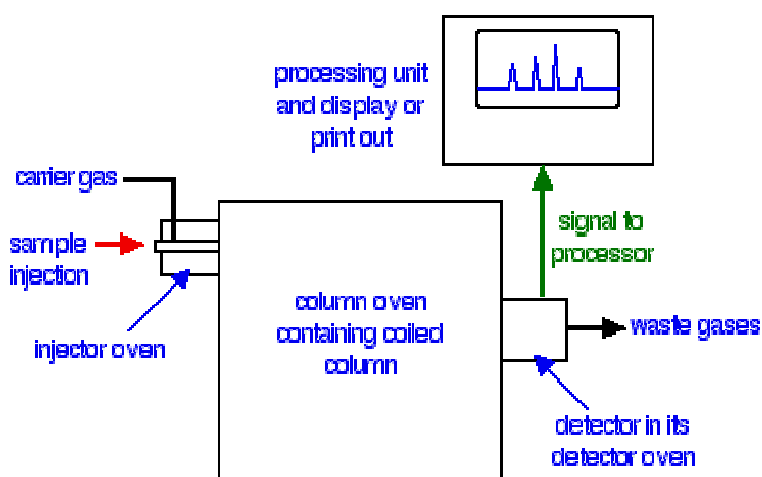
GAS-LIQUID CHROMATOGRAPHY

Gas-liquid chromatography (often just called gas chromatography) is a powerful tool in analysis. It has all sorts of variations in the way it is done

In gas-liquid chromatography, the mobile phase is a gas such as helium and the stationary phase is a high boiling point liquid absorbed onto a solid.

How fast a particular compound travels through the machine will depend on how much of its time is spent moving with the gas as opposed to being attached to the liquid in some way.

A flow scheme for gas-liquid chromatography



The packing material

There are two main types of column in gas-liquid chromatography. One of these is a long thin tube packed with the stationary phase; the other is even thinner and has the stationary phase bonded to its inner surface.

The column is typically made of stainless steel and is between 1 and 4 metres long with an internal diameter of up to 4 mm. It is coiled up so that it will fit into a thermostatically controlled oven.

The column is packed with finely ground *diatomaceous earth*, which is a very porous rock. This is coated with a high boiling liquid - typically a waxy polymer.

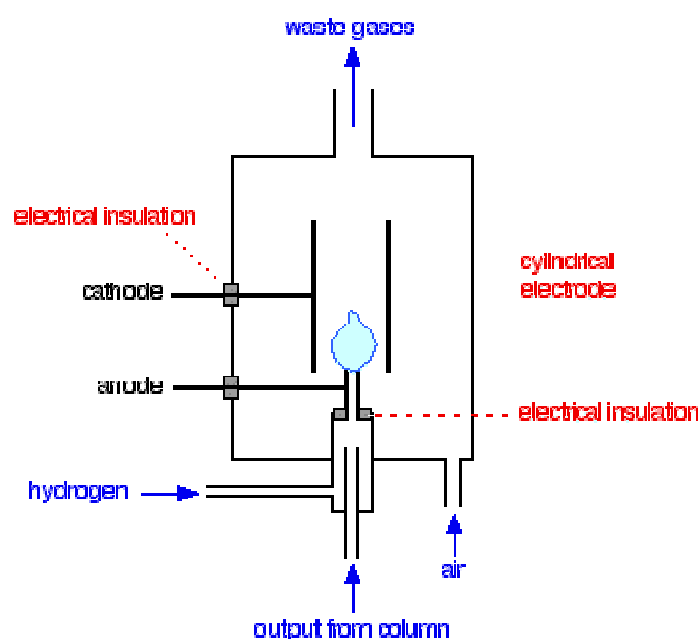
Retention time

The time taken for a particular compound to travel through the column to the detector is known as its *retention time*. This time is measured from the time at which the sample is injected to the point at which the display shows a maximum peak height for that compound.

Different compounds have different retention times. For a particular compound, the retention time will vary depending on:

- the boiling point of the compound. A compound which boils at a temperature higher than the column temperature is going to spend nearly all of its time condensed as a liquid at the beginning of the column. So high boiling point means a long retention time.
- the solubility in the liquid phase. The more soluble a compound is in the liquid phase, the less time it will spend being carried along by the gas. High solubility in the liquid phase means a high retention time.
- the temperature of the column. A higher temperature will tend to excite molecules into the gas phase - either because they evaporate more readily, or because they are so energetic that the attractions of the liquid no longer hold them. A high column temperature shortens retention times for everything in the column.

At the beginning, compounds which spend most of their time in the gas phase will pass quickly through the column and be detected. Increasing the temperature a bit will encourage the slightly "stickier" compounds through. Increasing the temperature still more will force the very "sticky" molecules off the stationary phase and through the column.

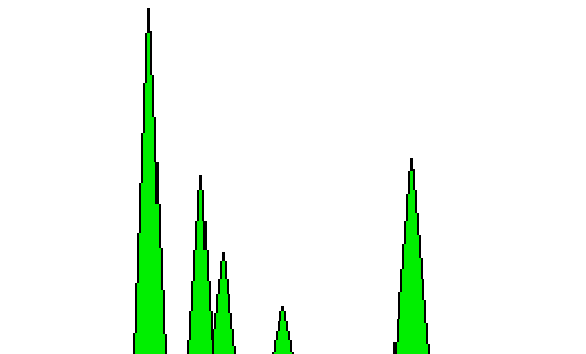


Interpreting the output from the detector

The output will be recorded as a series of peaks - each one representing a compound in the mixture passing through the detector. As long as you were careful to control the conditions on the column, you could use the retention times to help to identify the compounds present - provided, of course, that you (or somebody else) had already measured them for pure samples of the various compounds under those identical conditions.

But you can also use the peaks as a way of measuring the relative quantities of the compounds present. This is only accurate if you are analysing mixtures of similar compounds - for example, of similar hydrocarbons.

The areas under the peaks are proportional to the amount of each compound which has passed the detector, and these areas can be calculated automatically by the computer linked to the display.



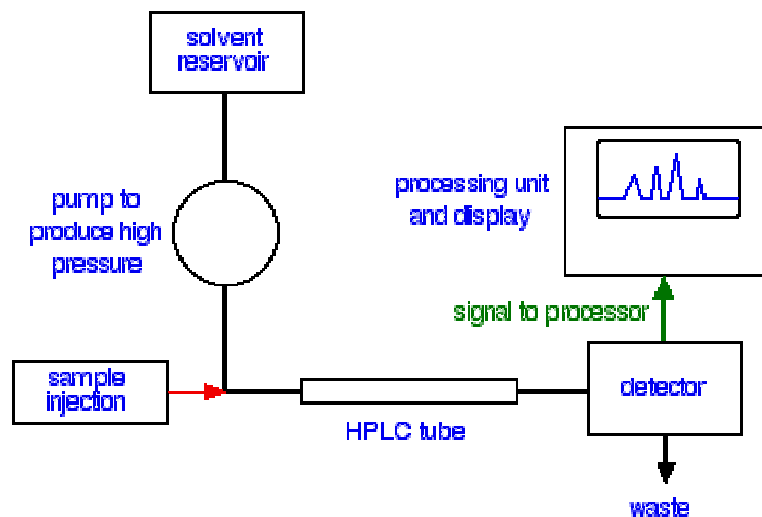
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY HPLC

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster.

It also allows you to use a very much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture.

The other major improvement over column chromatography concerns the detection methods which can be used. These methods are highly automated and extremely sensitive.

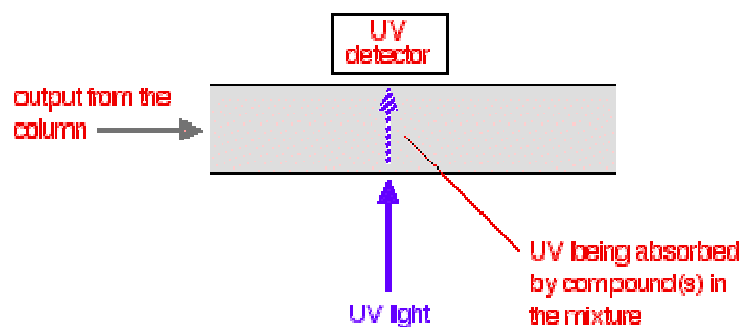
A flow scheme for HPLC



Retention time

Different compounds have different retention times. For a particular compound, the retention time will vary depending on:

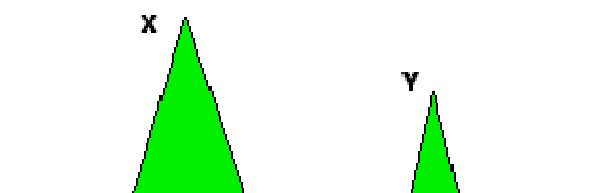
- the pressure used (because that affects the flow rate of the solvent)
- the nature of the stationary phase (not only what material it is made of, but also particle size)
- the exact composition of the solvent
- the temperature of the column



Interpreting the output from the detector

The output will be recorded as a series of peaks - each one representing a compound in the mixture passing through the detector and absorbing UV light.

The area under the peak is proportional to the amount of X which has passed the detector, and this area can be calculated automatically by the computer linked to



In the diagram, the area under the peak for Y is less than that for X. That may be because there is less Y than X, but it could equally well be because Y absorbs UV light at the wavelength you are using less than X does. There might be large quantities of Y present, but if it only absorbed weakly, it would only give a small peak.