Ministry of Education and Science of Ukraine
Kyiv National University of Technologies and Design

T. M. Derkach

ANALYTICAL CHEMISTRY FOR TECHNOLOGISTS

Part 2

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for International Training Programmes

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of Kyiv National University of Technologies and Design

T. M. Derkach

Analytical Chemistry for Technologists

Part 2: Sections 10-18

It is recommended by the Academic Council of
the Kyiv National University of Technology and Design
as lecture notes for students of higher education
in the fields of chemical technology & engineering,
biotechnology & bioengineering, and pharmacy & industrial pharmacy

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T. M. Derkach
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The book presents the basic concepts of analytical chemistry, such as qualitative and quantitative chemical analysis, sampling and sample preparation, statistical data processing, separation methods. Modern physicochemical methods of analysis are considered. The theoretical bases of methods are stated, conditions and their branches are specified practical application. The control questions and tasks presented at the end of each section will help to consolidate the studied material.

The book is intended for undergraduate students majoring in chemical technologies & engineering, biotechnology & bioengineering, and pharmacy & industrial pharmacy. The lecture notes consist of two parts. The first part includes Sections 1-9 and covers introductory topics, equations and equilibrium, classic methods of chemical analysis. The second part includes Sections 10-18 and covers instrumental methods.

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Introduction

Analytical chemistry is a branch of chemistry that deals with the study of theory and practice of methods used to determine the composition of matter. Analytical chemistry is often described as a field of chemistry that is responsible for characterizing the composition of a substance, both qualitatively and quantitatively. However, analytical chemistry and chemical analysis are not the same.

The difference between analytical chemistry and chemical analysis is that analyst chemists work to improve and expand established analytical methods. The characteristic detail of analytical chemistry is not to perform routine analysis on a routine sample, which is more appropriately called chemical analysis. The meaning of the analytical chemistry is to improve established methods, to expand them to new types of samples and to develop new analytical methods for measuring chemical phenomena.

Forty to fifty years ago, the chemical analysis focused on three main areas: qualitative determination; quantitative determination using "classical" methods of titrimetry and gravimetry; structural analysis, which required time-consuming procedures and calculations.

Today, chemists have instrumental methods, automated systems, and computers that make analytical measurements easier, faster, and more accurate. However, the chemist has to have profound understanding principles, areas of practical application and limitations of each method to work without mistakes.

Reviews of daily operations of many industrial and other analytical laboratories in the UK, Europe, Japan and the US have identified the most methods widely used. The textbook describes the techniques and methods commonly used by most analytical laboratories today.

The textbook is written as lectures-presentations, which gradually reveal the analytical process. Regardless of the area where the need for analysis arises, the chemist needs to answer the following questions:

- How should a representative sample be obtained?
- How much material is available for analysis and how many samples should be taken?
- What should be determined? With what accuracy?
- What components are in the sample? Will they have interferences?
- What tools should be used?
- How reliable will the data be?

The answers to these questions and related topics are discussed in Sections 1-3.

Statistical methods of processing the results are given somewhat simplified, but enough to obtain reliable results and use them to assess the correctness of the proposed analysis methods.

The following lectures-presentations contain a description of the principles, tools and application of analytical methods. The lecture notes consist of two parts. The first part includes Sections 1-9 and covers introductory topics, equations and equilibrium, classic methods of chemical analysis. The second part includes Sections 10-18 and covers instrumental methods of chemical analysis.

The material of this textbook may be useful to future professionals as an overview of topics to continue learning at a deeper level.

This knowledge is enough for a specialist to be able to work in analytical laboratories and control the quality of various products.

Nobody can do your learning for you. The most important way to master this course is to work tasks and gain experience in the laboratory. Problem-solving may illustrate how to apply what you have just read. Exercises are the minimum set of problems that apply the most significant concepts of each chapter.

Tables of dissociation constants and pK values for acids and bases, solubility-product constants for compounds, standard reduction potentials, formation constants (or stability constants) for complex ions in aqueous solutions, and densities of acids, alkalis and some other substances are shown in the last chapter of the textbook.

Section 10: Instrumental Analytical Methods

Contents:

- Introduction
- Some important definitions
- General classifications of instrumental methods of analysis
- Principal scheme of an analytical instrument
- Method classification by lectures
- Characteristics of electromagnetic radiation
- Interaction between substance and electromagnetic radiation
- Optical instrumentation methods
- Line, band and continuous spectra
- Atomic and molecular spectra
- Methods based on scattering, refraction and polarisation
- Selecting an optimal method and steps of
- the analytical procedure

Introduction

Unlike classical methods of chemical analysis, which mainly exploit chemical reactions, instrumental methods are based on the use of various electron-optical and electrolytic devices (instruments). Instrument-making industry has been actively developed for the last twenty years.

Analytical instruments provide information on the composition of a sample of matter. Some of such instruments are relatively simple, while others can be very sophisticated.

Instrumental methods provide both qualitative and quantitative data.

Qualitative identification provides information about the identity of species or functional groups in the sample. In other words, an analyte can be identified. Quantitative analysis provides numerical information of analyte (quantitate the exact amount or concentration).

Analytical instrumentation is the study of the separation, identification, and quantification of the chemical components of natural and artificial materials.

There are no good or bad instrumental methods: They are either suitable methods for a given application (and thus correct) or not.

The focus of Section 9 is on general issues - the interaction of electromagnetic radiation with matter. The techniques that use optical materials to disperse and focus the radiation are often identified as optical spectroscopic methods or optical spectroscopy. In this course, we are considering only a limited part of a much broader area of analytical techniques.

Despite the difference in instrumentation, all spectroscopic techniques share several common features. Before we consider individual examples in greater detail, let us take a moment to consider some of these similarities. As you work through Section 9, this overview will help you focus on similarities between different spectroscopic methods of analysis. You will find it easier to understand a new analytical method when you can see its relationship to other similar methods.

You have to renovate some terms from Inorganic chemistry to understand a new subject.

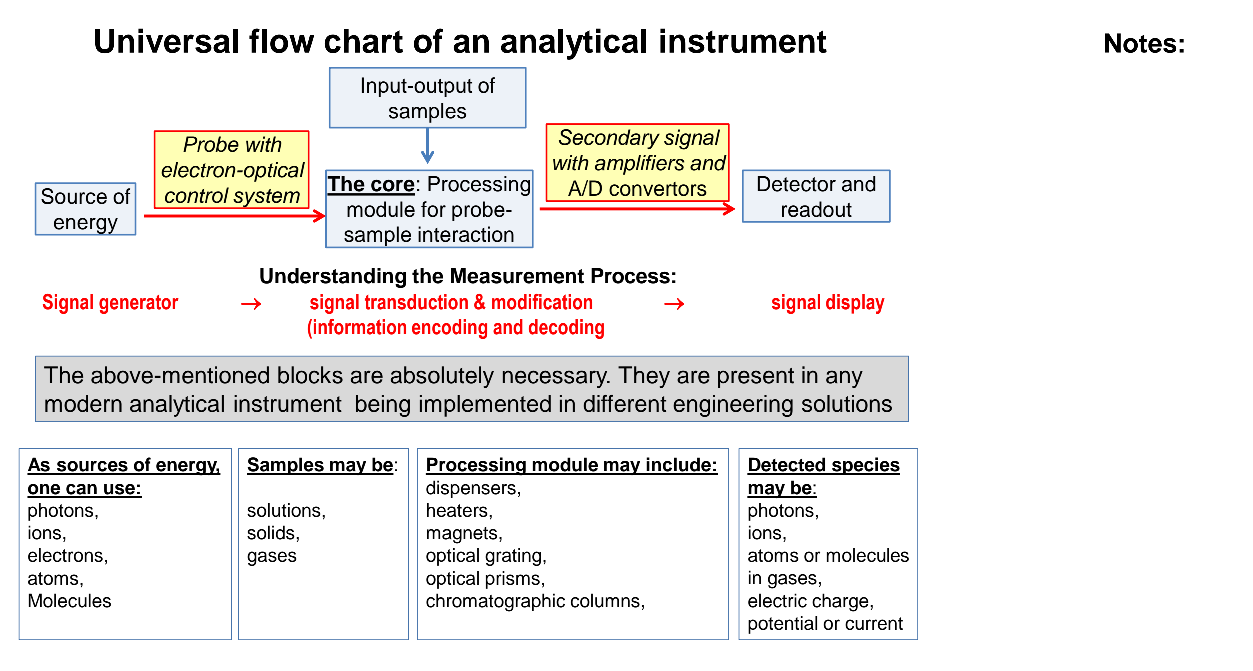
Electromagnetic radiation – The electromagnetic spectrum covers a huge range of wavelengths, frequencies and energies, and many analytical spectrometric techniques involve electromagnetic radiation.

Atomic energy level – energy levels in atoms are defined by quantum numbers, the atoms of each element possessing a characteristic set of discrete levels determined by its atomic and nuclear structure.

Molecular energy levels – Every molecule has several sets of discrete energy levels, which are associated with particular structural and behavioural properties of molecules.

Universal flow chart of an analytical instrument

Notes:



In addition to principal blocks, analytical instrumentation is typically equipped with computers (or microcomputers in less sophisticated devices).

Notes:

Computers are used to:

1. Control the instrument: for example, switch on and off; automate adjustments, sample change and so on.
2. Collect data controlling all necessary parameters and operations during measurements.
3. Analyse and process data: for example, process the recorded spectra such as subtract background, differentiate, integrate, identify and quantify comparing the obtained data with analogs in program libraries.
4. Store and display data in digital format using hard disk drives, monitors and printers.
5. Transfer data: for example, convert to a necessary file format and export data.

General classification of instrumental methods

Notes:

General classification of instrumental methods of analysis is not easy because of a diversity of such methods. We can name at least three important parameters; each of these parameters can be used for classification.

Such parameters are as follows:

1. The physical phenomenon behind the method, among them:

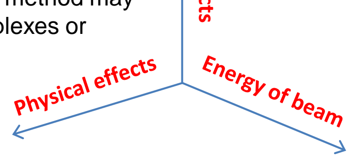
absorption, emission, scattering, refraction, rotation, separation.

2. The energy (or wavelength) of the probing beam:

- photon wavelength can range from 10^{-4} nm for γ-radiation to centimetres or meters for micro- or radio waves;
- the energy of probing particles (electrons, atoms or ions) ranges from few electron volts (eV) to dozens of kilo eV (keV).

3. The object of study: depending on other parameters, a method may be applicable to study nuclei, atoms, molecules, atom complexes or particles of a substance.

Therefore, the complete classification should be displyed in a 3D spaces.



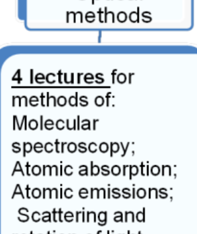
What’s about the classification used in the given course?

Notes:

This course focuses on some selected high-demand and effective methods.
The methods are divided into four blocks (or groups).
They will be delivered for eight lectures: an introduction followed by four lectures for optical methods and by one lecture for each of the other three groups.

Classification of instrumental methods by lectures

- Optical methods



- Electron and ion spectroscopy methods
  - One lecture to illustrate interrelations between various spectroscopies and present details of ICP-MS
- Electrochemical methods
  - One lecture to discuss 7 methods: Potentiometry, Voltametry, Stripping, Amperometry, Coulometry, Electrogravimetry, Conductance techniques
- Separation methods
  - One lecture to illustrate chromatography methods and give examples of hyphenated techniques

We start with optical and spectroscopic methods. All optical methods, as well as spectroscopic ones, have one common word in their titles – “spectro”

What does the word “Spectro” mean?

Notes:

Spectro derives from the underlying base word “spectrum”.

Initially a spectrum referred to the rainbow’s series of colours, ranging from Violet through Indigo, Blue, Green, Yellow, Orange, and Red, which is produced when visible light is split into its component colours.

Now this word is applied to all types of the electromagnetic radiation, ranging from the shortest gamma rays to the longest radio waves.

That is why, instrumental techniques are named infrared spectroscopy, x-ray spectroscopy, etc.

The term “spectrum” is no longer confined to the designation of an electro-magnetic radiation, but also is applied to other similar phenomena, like “mass spectrometer” (device to sort atoms or radicals by their atomic weight).

The use of suffices –scope, -graph, -meter, and –photometer, in combination with spectro-, designates the kind of detector used in the instrument.

- Spectroscope is used to read the reading using unaided eye.
- Spectrograph, if the data are recorded in film.
- Spectrometer, if the data are displayed.
- Spectrophotometer is used to measure the intensity of a signal.

Electromagnetic radiation

Notes:

It is a form of energy that has both wave and particle properties.
Spectroscopy is based on the interaction of various types of light with matter.
It also includes the interaction of electrons, ions, atoms and molecules with matter.
EM radiation is conveniently modeled as waves consisting of perpendicularly oscillating electric and magnetic fields.

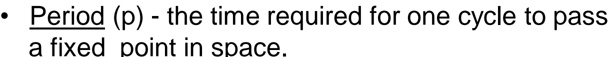
Electric field component
Magnetic field component

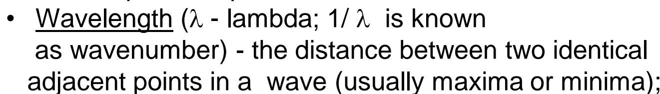
The propagation rate of electromagnetic waves depends on the medium. This speed in vacuum is denoted as c and approximately equals 300,000 km per second

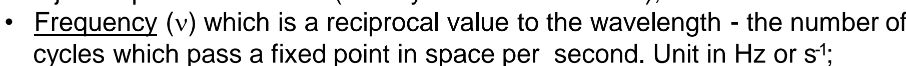
Properties of electromagnetic radiation (light)

Notes:

The main characteristics of electromagnetic radiation as a wave are as follows.







- Period (p) - the time required for one cycle to pass a fixed point in space.
- Wavelength (λ - lambda; $1/\lambda$ is known as wavenumber) - the distance between two identical adjacent points in a wave (usually maxima or minima);
- Frequency (ν) which is a reciprocal value to the wavelength - the number of cycles which pass a fixed point in space per second. Unit in Hz or s^{-1};
- Amplitude (A) - the maximum length of the electric vector in the wave (Maximum height of a wave).

Wavelength
Amplitude
Direction of wave propagation

Light can be represented by discrete particles of energy called photons.
Energy (E) of a photon is connected with its wavelength (or frequency) by the following equation:

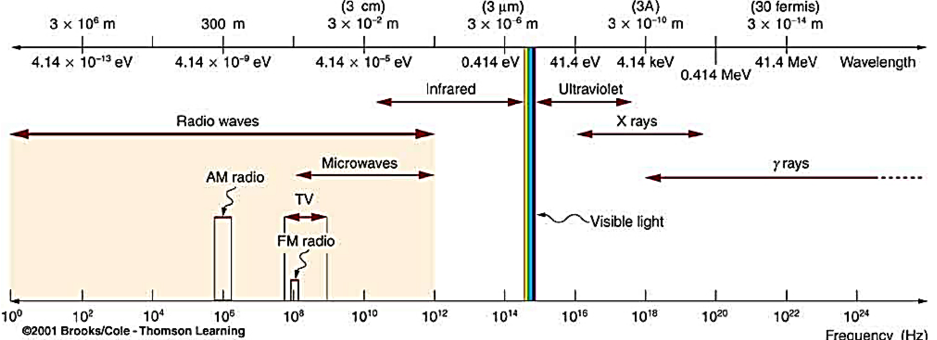
$$E = h\nu = \frac{hc}{\lambda},$$

where E is in Joules, ν - frequency (sec^{-1}), h - Planck's constant equal to 6.626×10^{-34} $J \cdot s^{-1}$

The electromagnetic spectrum covers a very large range of wavelengths, frequencies and energies.

The actual range of wavelengths and energies (in electron-volts) of different kinds of electromagnetic radiation

Notes:



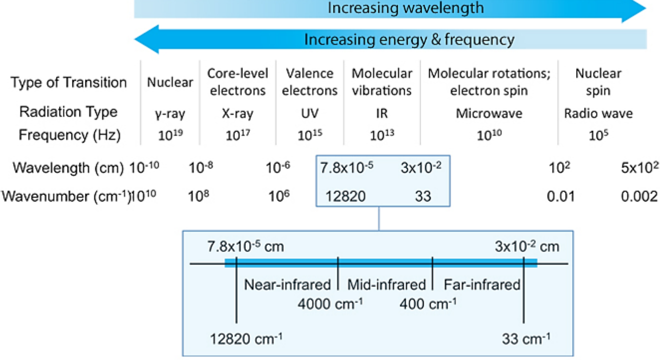
As you see, only a small fraction of the range (350-780 nm) is visible light. The longer length waves correspond to lower energies and lower frequencies: Gamma and X-rays fall into the category of high-energy radiation and radio waves to low-energy one.

Electron-volt (eV) is a unit of energy equal to exactly $1.602176634 \times 10^{-19}$ joules in SI units. One eV is equal to the energy of an electron after passing through the potential 1 volt.

Electromagnetic waves interact with atoms and molecules of substances

Notes:

The radiation may be transmitted, absorbed, scattered, deflected, refracted, reflected or excite fluorescence.



The effect of radiation on the substance depends on the energy of radiation:

- High-energy gamma rays interact with nuclei, may ionise atoms;
- X-ray can break interatomic bonds and interact with electrons in inner shells;
- Ultraviolet and visible radiation interact with valent electrons;
- Infrared and microwave radiation can rotate and vibrate molecules;
- Radio waves interact with nuclear spins.

Summary for applications of optical methods

Notes:

Type of Spectroscopy	Usual Wavelength Range	Usual Wave number Range, cm^{-1}	Type of Quantum Transition
Gamma-ray emission	0.005-1.4 Å	_	Nuclear
X-ray absorption, emission, fluorescence, and diffraction	0.1-100 Å	_	Inner electron
Vacuum ultraviolet absorption	10-180 nm	$1x10^6$ to $5x10^4$	Bonding electrons
Ultraviolet visible absorption, emission, fluorescence	180 -780 nm	$5x10^4$ to $1.3x10^4$	Bonding electrons
Infrared absorption and Raman scattering	0.78-300 mm	$1.3x10^4$ to $3.3x10^1$	Rotation/vibration of molecules
Microwave absorption	0.75-3.75 mm	13-27	Rotation of molecules
Electron spin resonance	3 cm	0.33	Spin of electrons in a magnetic field
Nuclear magnetic resonance	0.6-10 m	$1.7x10^{-2}$ to $1x10^3$	Spin of nuclei in a magnetic field

Types of interaction between electromagnetic radiation and substance

Notes:

Absorption
EMR energy transferred to absorbing molecule (transition from low energy to high energy state).

Emission
EMR energy transferred from emitting molecule to space (transition from high energy to low energy state).

Scattering
redirection of light with no energy transfer.

Refraction
change in direction in the travel of a light beam when it comes at an angle to a boundary (interface) between two transparent media with different densities

Polarisation
the ability of waves to oscillate in more than one direction

Photoluminescence
Spontaneous re-emission of light (at a longer wavelength than that of the incident radiation) resulting from absorption of photons

Instrumental optical methods can be classified

Notes:

By the range of electromagnetic energy:

- **Gamma spectroscopy** (wavelength 10^{-4}-10^{-1} nm) to study nuclei and nucleus reactions;
- **X-ray spectroscopy** (0.1-10 nm) to excite electrons at internal shells;
- **Optical spectroscopy**, including:
  vacuum ultraviolet (10-180 nm) and ultraviolet (180-400 nm) to excite valent electrons,
  visible optical (400-780 nm) to excite valent electrons,
  near infrared (780-2500 nm) and infrared spectroscopy (2,5-25 μm) for excitation of vibrational modes in molecules;
- **Microwave spectroscopy** (0.001-0.1 m) for molecule rotation studies;
- **Radiowaves** (0.01-10 m) for study splitting of unpaired electrons (electron paramagnetic) and nuclear spins (nuclear magnetic resonance) in a magnetic field

By the type of optical phenomena:

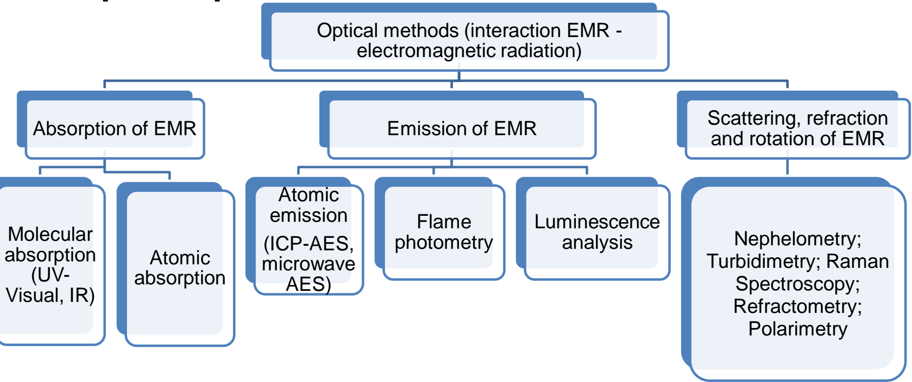
- **Emission spectroscopy** including atomic emission and luminescent spectroscopy;
- **Absorption spectroscopy** including atomic and molecular absorption spectroscopy;
- **Scattering, refraction and rotation spectroscopy.**

By the object (nuclei, atoms or molecules) of research:

- **Nuclear spectroscopy** – analytical Moessbauer spectroscopy;
- **Atomic spectroscopy** – atomic absorption, atomic emission, atomic fluorescence, X-Ray spectroscopy, EPR, NMR;
- **Molecular spectroscopy** – UV-visible, IR-luminiscence, Raman-scattering spectroscopy, microwave spectroscopy

Groups of optical methods for further consideration

Notes:

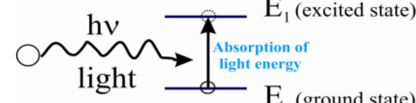


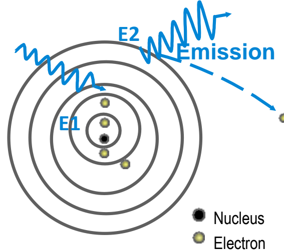
Molecular absorption will be represented by ultra-violet, visible and infra-red spectroscopies.
Atomic absorption – by atomic absorption spectroscopy in flame and graphite furnace variants.
Atomic emission will be studied for the cases of luminesces analysis and atomic emission spectroscopy equipped by flame, inductively coupled and microwave plasma sources.
All other modes (**except for absorption and emission**) of photon-substance interaction will be presented in a set of analytical methods which mainly study liquid solutions

Fundamentals of atomic absorption & emission spectroscopy, and mass-spectrometry

Notes:

Atomic absorption spectroscopy (AAS) techniques rely on the fact that an atomised element will absorb light of a characteristic wavelength, elevating it from the ground state to an excited state.





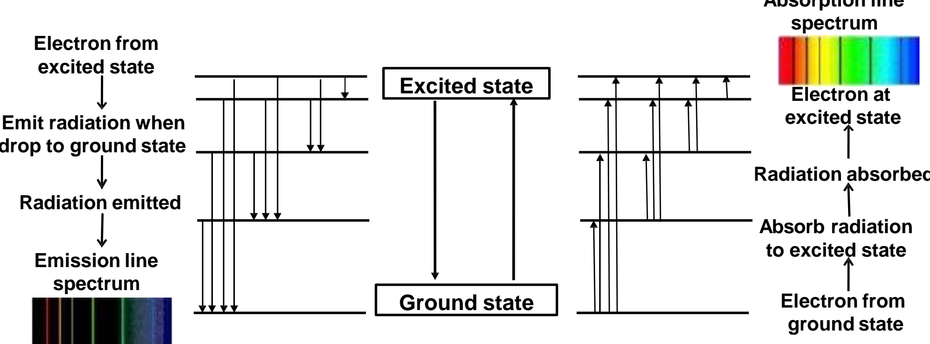
Atomic emission spectroscopy (AES) techniques rely on the fact that once an atom of a specific element is excited, it emits light in a characteristic pattern of wavelengths (an emission spectrum) as it returns to the ground state.

If there is enough energy, the electron will leave the atom completely and leave behind a positively charged ion (ionisation) – this is the basis for **mass-spectrometry (MS)**

- Since different chemicals have different electron shells which are filled, they will each absorb their own particular type of light
- The amount of light energy absorbed is proportional to the number of analyte atoms in the light path.
- The technique is calibrated by introducing known concentrations of analyte atoms into the light path and plotting the absorption versus concentration curve

Emission spectroscopy vs adsorption spectroscopy

Notes:



- Photon energy required for a transition between two electron states is $\Delta E = E_1 - E_o$.
- Absorbing light, electron goes from a low energy (ground state) to a higher energy (excited) state.
- Emitting light of a particular wavelength, the excited electron drops back to the ground state
- Only photons with energies exactly equal to the energy difference ΔE will be either absorbed or emitted

Luminescence

Notes:

In general, luminescence is spontaneous emission of light by a substance not resulting from heat or "cold light".
In particular, photoluminescence is a result of absorption of photons

There are a few types of **photoluminescence**:
Fluorescence results from singlet–singlet electronic relaxation (lifetime: nanoseconds)
Phosphorescence results from triplet–singlet electronic relaxation (lifetime: millisecond to hours)
Raman emission results from inelastic light scattering (lifetime: nanoseconds)

Methods of chemical analysis in which analyte concentration is related to luminescence intensity or some other property of luminescence.
Photoluminescence, particularly **fluorescence**, is the most widely used type of luminescence for chemical analysis.

Emission spectra

Notes:

Radiation from an excited source is conveniently characterised by means of an ***emission spectrum.***

Emission spectrum is a plot of the relative power of the emitted radiation as a function of wavelength or frequency.

There are three types of emission spectra:

1. Line spectra
2. Band spectra
3. Continuum spectra.

Line spectra

Notes:

Line spectra in the UV and visible regions are produced when the radiating species are individual atomic particles that are well separated, in a gas phase.

Electrons in an atom have discrete energies because not all energies are allowed. Only photons of certain energy can interact with the electrons in a given atom.

Transitions of the electrons between electronic levels produce line spectra.

The individual particles in a gas behave independently of one another, and the spectrum consists of a series of sharp lines with widths of about 10^{-3} nm .

Each atom has a specific set of energy levels, and thus a unique set of photon wavelengths with which it can interact.

Emission lines will appear if:

- the atoms are in a low-density gas;
- the atoms are excited into a particular high energy level by an external source

MERCURY
SODIUM
HELIUM
HYDROGEN
750 700 650 600 550 500 450 400
Nanometers

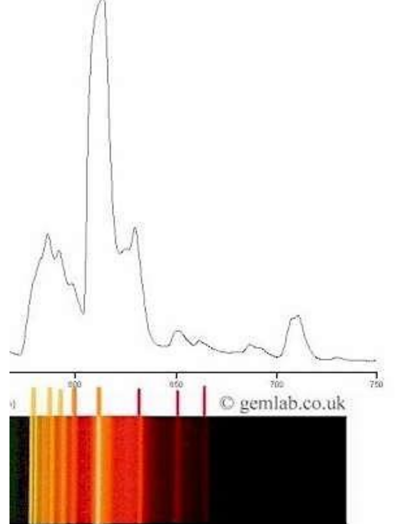
Band spectra

Notes:

Band spectra are often encountered in spectral sources when gaseous radicals or small molecules are present.

They are obtained as a series of closely spaced lines that are not fully resolved by the instrument used to obtain the spectrum.

Bands arise from the numerous quantised vibrational levels that are superimposed on the ground-state electronic energy level of a molecule



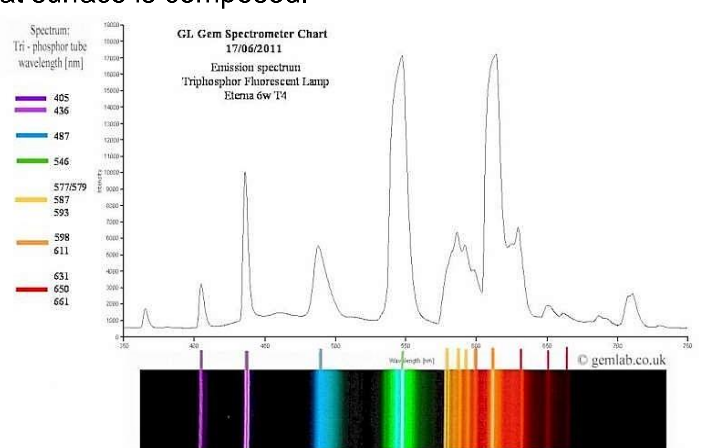
Continuum spectra

Notes:

Continuum radiation is produced when solids are heated to incandescence.

Thermal radiation of this kind, which is called black body radiation, is characteristic of the temperature of the emitting surface rather than the material of which that surface is composed.

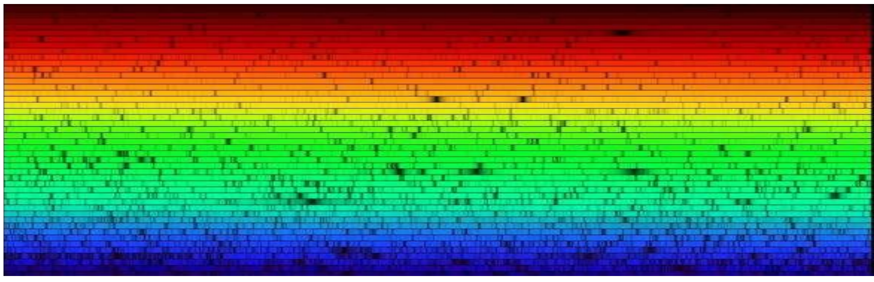
Black body radiation is produced by the innumerable atomic and molecular oscillations excited in the condensed solid by the thermal energy.



Absorption line spectrum

Notes:

A high-resolution spectrum of the Sun shows many dark absorption lines.

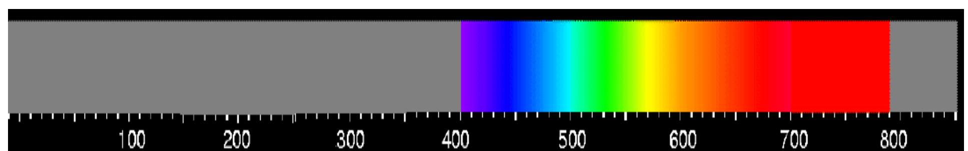


Absorption lines are based on the same physical principle as emission lines: they involve an atom jumping from one particular energy level to another.

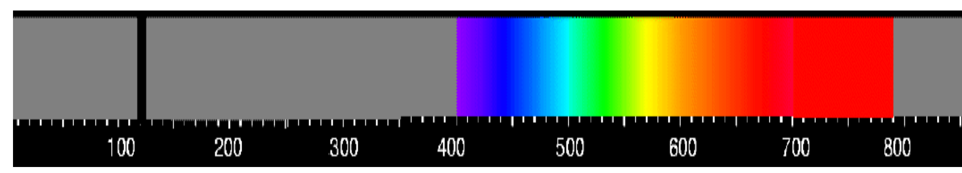
In this case, however, the jumps must be upwards, from a low level to a higher one.

Notes:

If we look at a source of continuous radiation



through a cloud of hydrogen gas,
we will see a dark absorption line at 121 nm.

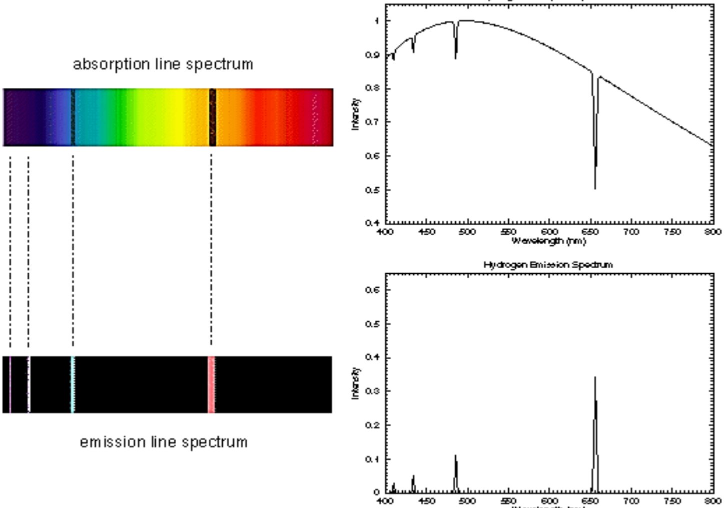


Notes:

The conditions needed to produce line spectra

Absorption lines from an element will appear if:

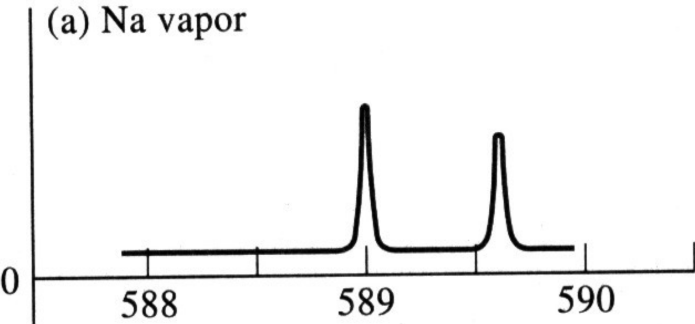
- there are atoms of the element present;
- the atoms are in a low-density gas;
- the atoms spend most of their time in a particular low-energy level;
- the gas lies between us and a source of continuous light (of all wavelengths)



Notes:

Atomic absorption spectra

<u>Absorption spectrum</u> is a plot of the absorbance as a function of wavelength or frequency.



Absorption Spectrum of Na. The two peaks arise from the promotion of a 3s electron to the two 3p states

Molecular spectra

Notes:

The energy, E, associated with the molecular bands:

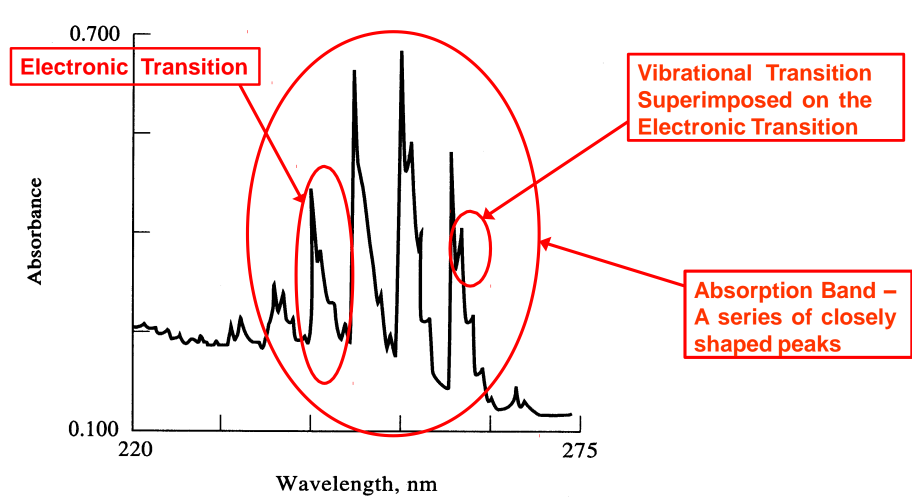
$$E_{total} = E_{electronic} + E_{vibrational} + E_{rotational}$$

In general, a molecule may absorb energy in three ways:

1. By raising an electron (or electrons) to a higher energy level. (electronic)
2. By increasing the vibration of the constituent nuclei. (vibrational)
3. By increasing the rotation of the molecule about the axis. (rotational)

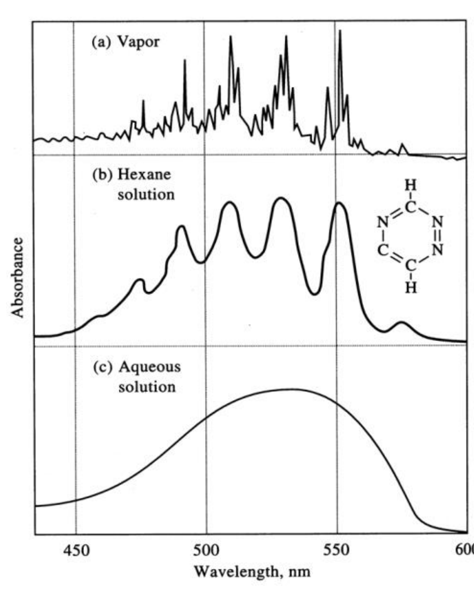
Molecular absorption spectra of benzene gas

Notes:



Molecular absorption spectra in some solvents

Notes:



In solvents, the rotational and vibrational transitions are highly restricted resulting in **broad band absorption** spectra.

Ultraviolet absorption spectra for tetrazine demonstrate:

a) fine structure of spectrum in vapour phase

b) smoothed structure in hexane solution

c) broad band spectrum in aqueous solution

Properties of electromagnetic radiation (*other than absorption and emission*) and related analytical methods

Notes:

Scattering is the base for:

The method of Turbidimetry is involved with measuring the amount of transmitted light (and calculating the absorbed light) by particles in suspension. It allows one to determine the concentration of a substance in solution because amount of absorbed light is dependent on number of particles and size of particles. Measurements are made using light spectrophotometers.

The method of Nephelometry is based on the measurement of scattered light from a cuvette containing suspended particles in a solution. Measurements are made using light spectrophotometer except that the detector is placed at a specific angle from the incident light. The detector is a photomultiplier tube placed at a position to detect forward scattered light.

The method of Raman Spectroscopy is based on the ability of the studied systems (molecules) to inelastic (Raman) scattering of monochromatic light. It provides information on intramolecular and intermolecular vibrations and is used to identify substances.

Properties of light and related analytical methods

Notes:

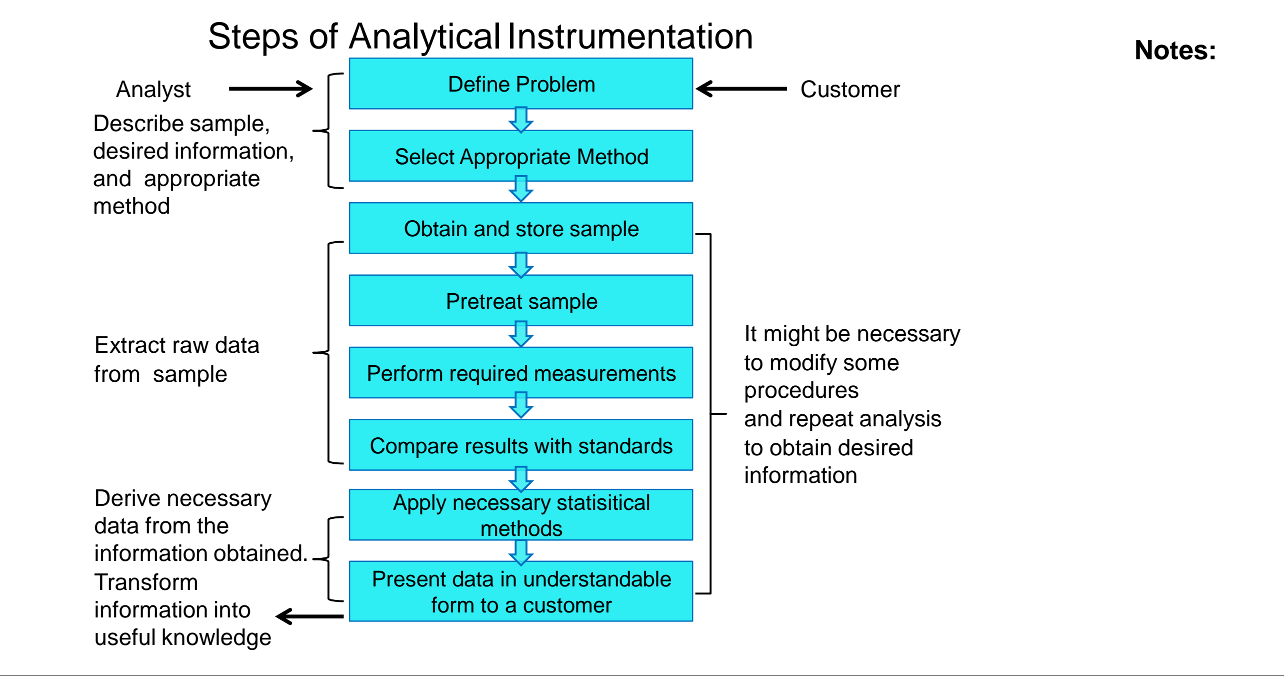
Refraction is the base for:

Refractometry is the analytical method of measuring substances' refractive index (one of their fundamental physical properties) to assess their composition or purity. A refractometer is the instrument used to measure refractive index. Refractometers are best known for measuring liquids, but they are also used to measure gases and some solids (like glasses or gemstones).

Polarisation is the base for:

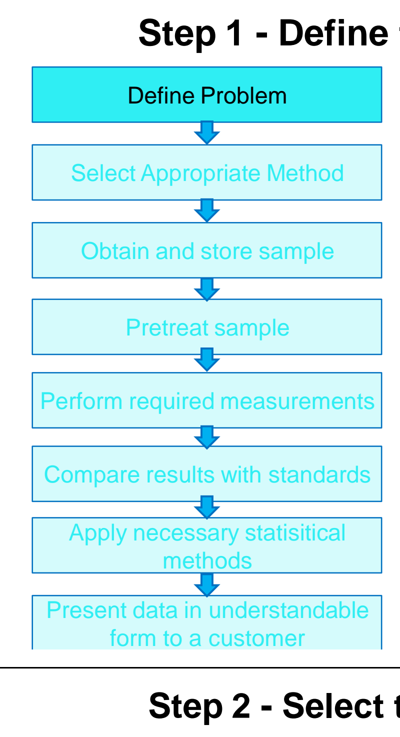
Polarimetry is a method of study of substances based on the measurement of the degree of polarisation of light and optical activity, in other words the magnitude of the angle of rotation of the plane of polarisation of light as it passes through optically active substances (such as for example liquid solutions with chiral molecules). The angle of rotation in solutions depends on their concentration, so polarimetry is widely used to measure the concentration of optically active substances. Changing the angle of rotation when changing the wavelength of light (spectropolarimetry) allows you to study the structure of the substance and determine the amount in the mixture of optically active substances.
This is measured using a polarimeter in which polarised light is passed through a tube of the liquid, at the end of which is another polarizer which is rotated in order to null the transmission of light through it.

Notes:



Step 1 - Define the analytical problem

Notes:



1. Direct interaction with the customer.
2. The analyst should determine the nature of the sample (qualitative or quantitative), the end use of the analytical results, the species to be analyzed, and the information required.
3. Qualitative: elemental composition, oxidation states, functional groups, major components, minor components, complete identification of all species present in the sample, etc.
4. Quantitative: required accuracy and precision, range of an expected analyte concentrations, and detection limits for the analyte.
5. Unique physical and chemical properties of the analyte,
6. Estimate cost (major component of the cost is the time required for the analysis) are other considerations.

Step 2 - Select the appropriate method

Notes:

Define Problem
Select Appropriate Method
Obtain and store sample
Pretreat sample
Perform required measurements
Compare results with standards
Apply necessary statisitical methods
Present data in understandable form to a customer

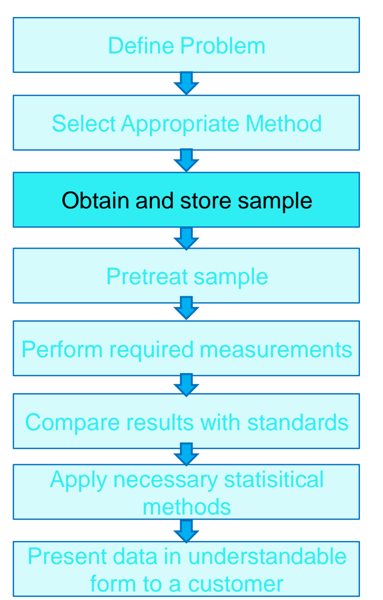
Selecting the method, one need to estimate:
1) strength and limitations of the technique;
2) restrictions due to interferences present in sample;
3) quality of information against cost for obtaining it

What are the key factors to be considered:
What type of information does the method provide?
What are the advantages or disadvantages of the technique versus other methods?
How reproducible and accurate is the technique?
How much or how little sample is required?
How much or how little analyte can be detected (or expected concentrations of analyte)?
What types of samples can be analysed?
Will other sample components cause interference?

Other factors to estimate time and cost of analysis:
Availability of equipment ;
Skill of operator;
Scale of operation (number of samples);
Speed and necessary time for analysis;
Per – sample cost.

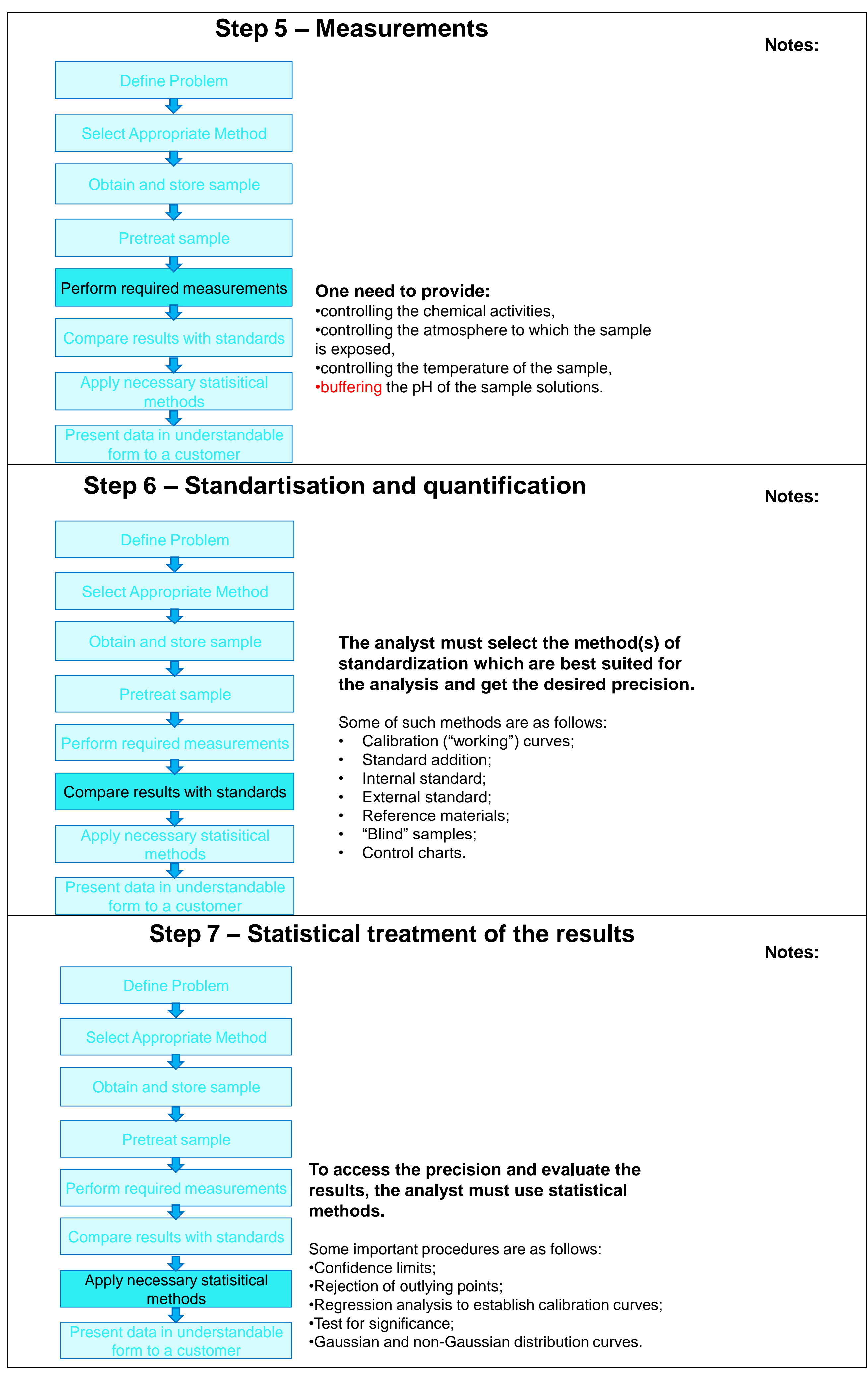
Step 3 - Sampling

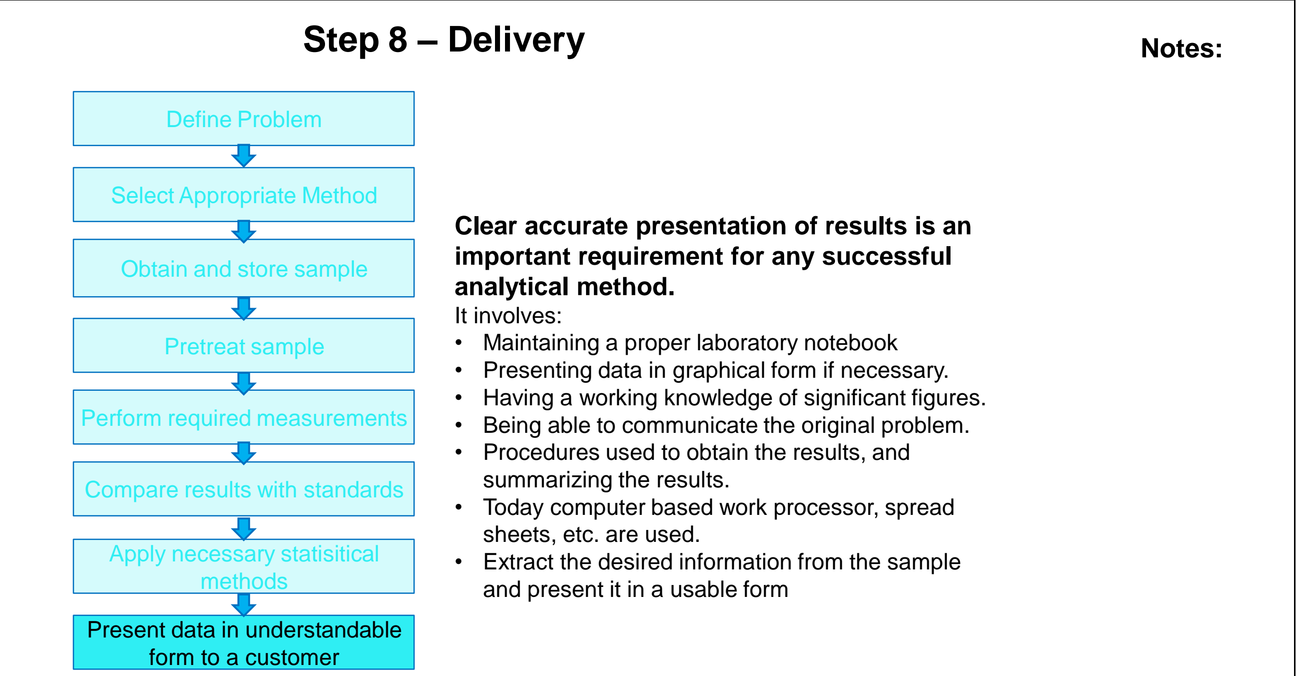
Notes:



Sampling is the most important step in the entire analysis. One need to check:

1. What measure must be taken to obtain the sample required to provide the desired information?
2. Do the field sampling or laboratory sub-sampling procedures assure the integrity of analytical results?
3. Have proper procedures been used to store and preserve both samples and standards?
4. Have all samples been properly labelled and recorded?


Step 5 – Measurements
Notes:
Define Problem
Select Appropriate Method
Obtain and store sample
Pretreat sample
Perform required measurements
Compare results with standards
Apply necessary statisitical methods
Present data in understandable form to a customer
One need to provide:
•controlling the chemical activities,
•controlling the atmosphere to which the sample is exposed,
•controlling the temperature of the sample,
•buffering the pH of the sample solutions.
Step 6 – Standartisation and quantification
Notes:
Define Problem
Select Appropriate Method
Obtain and store sample
Pretreat sample
Perform required measurements
Compare results with standards
Apply necessary statisitical methods
Present data in understandable form to a customer
The analyst must select the method(s) of standardization which are best suited for the analysis and get the desired precision.
Some of such methods are as follows:
• Calibration (“working”) curves;
• Standard addition;
• Internal standard;
• External standard;
• Reference materials;
• “Blind” samples;
• Control charts.
Step 7 – Statistical treatment of the results
Notes:
Define Problem
Select Appropriate Method
Obtain and store sample
Pretreat sample
Perform required measurements
Compare results with standards
Apply necessary statisitical methods
Present data in understandable form to a customer
To access the precision and evaluate the results, the analyst must use statistical methods.
Some important procedures are as follows:
•Confidence limits;
•Rejection of outlying points;
•Regression analysis to establish calibration curves;
•Test for significance;
•Gaussian and non-Gaussian distribution curves.



Tasks to Section 10

1. Give definitions of these terms: instrumental methods of analysis, absorption, emission, wavelength, period, frequency, amplitude, electromagnetic radiation, spectrum, analytical instrumentation, optical methods, electron and ion spectroscopy methods, electrochemical methods, separation methods, spectroscope, spectrograph, spectrometer, spectrophotometer.

2. Describe the main steps of analytical Instrumentation.

3. Explain the origin of the emission spectra (emission) and absorption (absorption) spectra of atoms and molecules from the quantum theory standpoint.

4. What quantities characterize the lines and bands observed in the spectra of radiation and absorption?

5. What are electronic transitions called resonant? Why do resonant lines associated with the transition from the first excited level is used to determine the elements by flame photometry?

6. Determine the frequency in reverse seconds (Hertz), which corresponds to the following wavelengths of electromagnetic radiation: a) 222 nm; b) 17 Å; c) 3.2 cm; d) $1.3 \cdot 10^{-7}$ cm; e) 6.1 µm.

7. Determine the wavenumber (in cm^{-1}) for the following wavelengths: a) 261.5 nm; b) 2615 Å; c) 0.030 cm; d) 8.0 µm. To which region of the spectrum do the values of each of these wavenumbers belong?

8. Determine the wavelengths (in cm) that correspond to the following frequencies of electromagnetic radiation: a) $1.97 \cdot 10^{9}$ Hz; b) $4.86 \cdot 10^{15}$ Hz; c) $7.32 \cdot 10^{19}$ Hz.

9. Determine the wavenumber (in cm^{-1}) for the following frequencies: a) $1.07 \cdot 10^{9}$ Hz; b) $4.5 \cdot 10^{15}$ Hz; c) $7.5 \cdot 10^{19}$ Hz. Determine the region of the spectrum to which they belong.

10. Count how many kilojoules per mole is the energy of O_2 increased when it absorbs ultraviolet radiation with a wavelength of 147 nm? How much is the energy of CO_2 increased when it absorbs infrared radiation with a wavenumber of 2 300 cm^{-1}?

11. What are the wavelength, wavenumber, and name of radiation with an energy of 100 kJ/mol?

Section 11: UV/Vis and IR Spectroscopy

Contents:

- Introduction
- Molecular spectroscopy
- Ultraviolet spectrophotometry
- Visible spectrophotometry
- Infrared spectrophotometry

Introduction

The electromagnetic spectrum covers a very wide range of wavelengths, frequencies and energies. Many analytical spectrometric methods involve the use of electromagnetic radiation. In the textbook, only the most frequently used modern methods are considered.

Electromagnetic radiation-light is a form of energy whose behaviour is described by the properties of both waves and particles. Some properties of electromagnetic radiation, such as its refraction when it passes from one medium to another, are explained best by describing light as a wave. Other properties, such as absorption and emission, are better described by treating light as a particle. The exact nature of electromagnetic radiation remains unclear. Nevertheless; the dual models of wave and particle behaviour provide a useful description for electromagnetic radiation.

An electromagnetic wave is characterized by several fundamental properties, including its velocity, amplitude, frequency, phase angle, polarization, and direction of propagation. Other properties also are useful for characterizing the wave behaviour of electromagnetic radiation. The wavelength is defined as the distance between successive maxima. The frequency and wavelength of electromagnetic radiation vary over many orders of magnitude. For convenience, we divide electromagnetic radiation into different regions—the electromagnetic spectrum—based on the type of atomic or molecular transition that gives rise to the absorption or emission of photons. The boundaries between the regions of the electromagnetic spectrum are not rigid, and overlap between spectral regions is possible.

For ultraviolet and visible electromagnetic radiation the wavelength is usually expressed in nanometres (1 nm = 10^{-9} m), and for infrared radiation, it is given in microns (1 mm = 10^{-6} m). The relationship between wavelength and frequency is $\lambda v=c$. Another functional unit is the wavenumber, v, which is the reciprocal of wavelength $v\lambda=1$. Wavenumbers are frequently used to characterize infrared radiation, with the units given in cm^{-1}.

Section 11 is devoted to the interaction of ultraviolet, visible and infrared radiation with matter. Methods that use the effects of such interactions are called spectroscopy instead of optical spectroscopy for convenience.

Absorption spectroscopy is a method of analysis based on the selective absorption of light by particles, molecules or ions of a substance in solution. In absorption spectroscopy, a photon is absorbed by an atom or molecule that undergoes a transition from a lower energy state to higher energy. The type of transition depends on the energy of the photon.

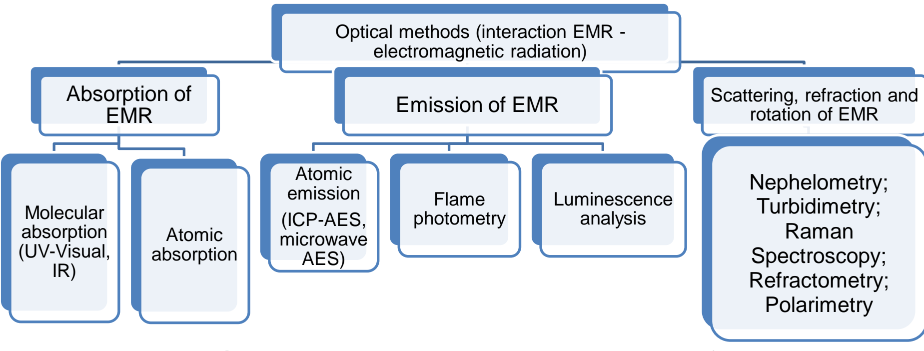
When a molecule absorbs a photon, the energy of the molecule increases, and we say that the molecule is brought to an excited state. If a molecule emits a photon, the energy of the molecule decreases.

The lowest energy state of a molecule is called the ground state. Microwave radiation stimulates the rotation of molecules during its absorption. Infrared radiation stimulates vibration. Visible and ultraviolet radiation move electrons to higher energy orbitals. X-rays and short-wave ultraviolet radiation break chemical bonds and ionize molecules.

When the molecules of the sample absorb light, the irradiation of the light beam decreases because the number of photons passing through the sample decreases. Measuring this reduction of photons, which we call absorption, is a useful analytical signal. Absorption occurs only when the photon energy, hv, corresponds to the energy difference, ΔE, between two energy levels.

The absorption graph, as a function of photon energy, is called the absorption spectrum.

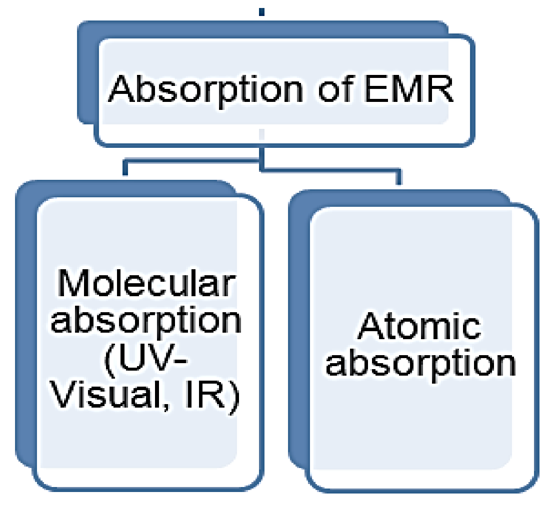
Groups of optical methods for further consideration



- **Molecular absorption** will be represented by ultra-violet, visible and infra-red spectroscopies.
- **Atomic absorption** – by atomic absorption spectroscopy in flame and graphite furnace variants.
- **Atomic emission** will be studied for the cases of luminesces analysis and atomic emission spectroscopy equipped by flame, inductively coupled and microwave plasma sources.
- All other modes (**except for absorption and emission**) of photon-substance interaction will be presented in a set of analytical methods which mainly study liquid solutions

Notes:

Absorption spectroscopy including atomic and molecular absorption spectroscopy



Notes:

Spectrum Region

Region	Wavelength Range
UV	180 – 380 nm
Visible	380 – 780 nm
Near-IR	780 – 2500 nm
Mid-IR	2500 – 50000 nm

Notes:

Notes:

Wavelength Units

Region	Unit	Definition (m)
X-ray	Angstrom unit, Å	10^{-10} m
Ultraviolet/visible	Nanometer, nm	10^{-9} m
Infrared	Micrometer, µm	10^{-6} m

Notes:

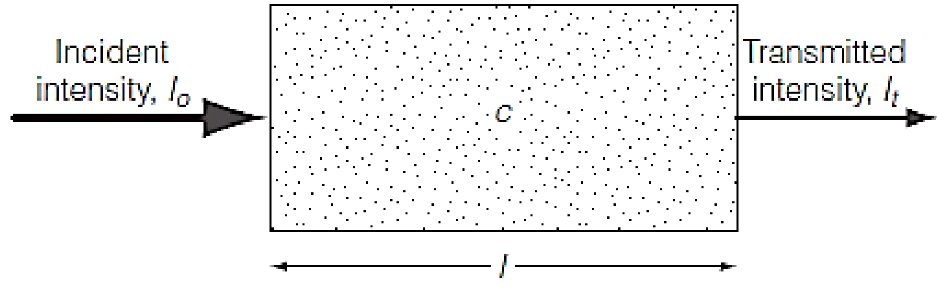
Absorption spectroscopy –

is a method of analysis based on the selective absorption of light by particles, molecules, or ions of a substance in solution.

For **absorption spectrometry** the intensity of the exciting radiation is reduced when it interacts with the atoms or molecules, raising them to higher energy levels. In order to interact, the radiation must come into contact with the species.

Notes:

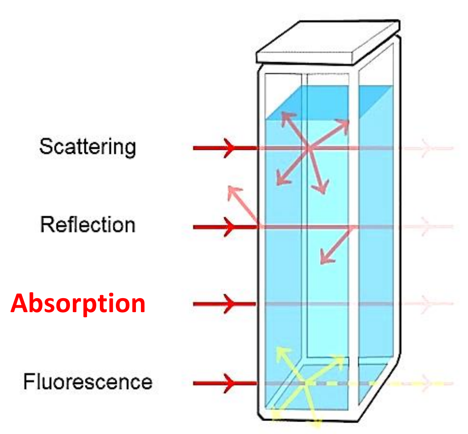
The extent to which it does this will depend on the **concentration** of the active species and on the **path length** through the sample, as shown in the picture.



Absorption

The flow of light that passes through the layer of matter decreases.

EMR energy transferred to absorbing molecule (transition from low energy to high energy state).



Notes:

Molecular absorption

The energy, E, associated with the molecular bands:

$$E_{total} = E_{electronic} + E_{vibrational} + E_{rotational}$$

In general, a molecule may absorb energy in three ways:

1. By raising an electron (or electrons) to a higher energy level **(electronic)**
2. By increasing the vibration of the constituent nuclei **(vibrational)**
3. By increasing the rotation of the molecule about the axis **(rotational)**

Notes:

Energy for Atom and Molecule

Atom

Electronic Energy

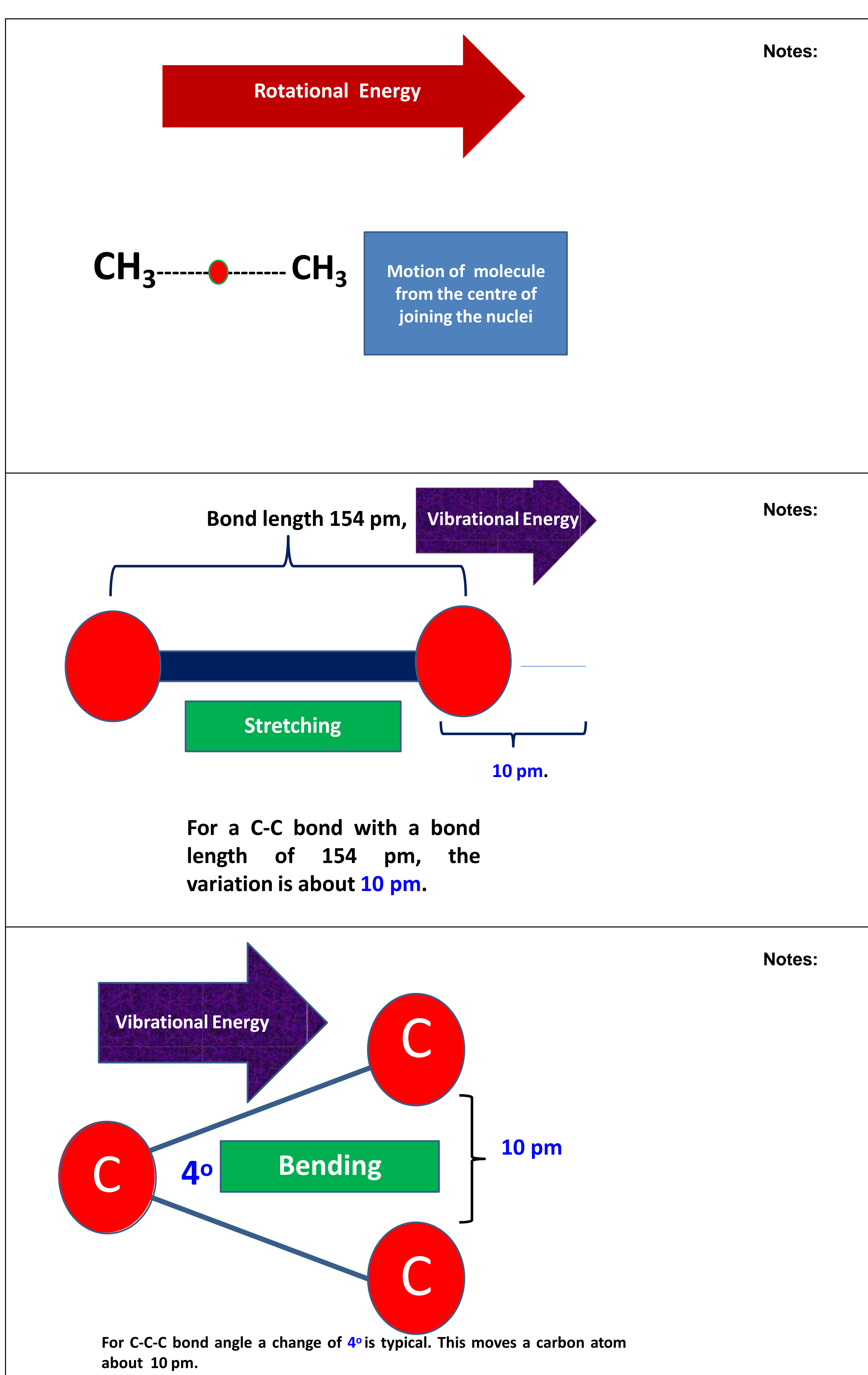
Atom Atom

Electronic Energy

Rotational Energy

Vibrational Energy

Notes:


Notes:
Rotational Energy
CH3-------●-------- CH3
Motion of molecule from the centre of joining the nuclei
Notes:
Bond length 154 pm,
Vibrational Energy
Stretching
10 pm.
For a C-C bond with a bond length of 154 pm, the variation is about 10 pm.
Notes:
Vibrational Energy
C
C
4o
Bending
10 pm
C
For C-C-C bond angle a change of 4o is typical. This moves a carbon atom about 10 pm.

As the radiation of a particular wavelength passes through the sample, the intensity decreases exponentially, and Lambert showed that this depended on the path length, l, while Beer showed that it depended on the concentration, C.

Beers Law: (1760)

When monochromatic light is allow to pass through a solution, the rate of decrease of intensity of incident light with the concentration of solution is directly proportional to intensity of incident light.

OR

Equal fractions of the incident radiation are absorbed by successive layers of solution containing the same no of absorbing species

Notes:

The two dependencies are combined to give the Beer–Lambert absorption law

$$\log (I_o / I_t) = A = \varepsilon C l$$

Incident intensity, I_o

c

Transmitted intensity, I_t

l

where I_o and I_t are the incident and transmitted intensities,
A is the **absorbance,**
and ε **is the molar absorptivity**

The value of ε is most usually quoted for a concentration of 1 M and a path length of 1 cm

Notes:

The Beer-Lambert law applies equally to infrared absorption spectra. Spectra are plotted either as absorbance, A, or as the transmittance, T, against wavelength, frequency or wavenumber, where

$$\mathbf{T = (I_t/I_0)}$$

or sometimes as percentage transmittance = 100 T

It is worth noting the range of values which each of these parameters may take.

A can have any value from 0 to infinity.

T must be between 0 and 1, and ε usually has values from about 1 to 10^6

Notes:

Deviation from Beer-Lambert's Law

Notes:

$$A = \varepsilon \, l \, C$$

Where:

A is the absorbance;

ε is the molar absorptivity;

l is the path length in cm;

C is the concentration in mole/L.

According to the above equation

Absorbance is directly proportional to concentration

$$A = \varepsilon \, l \, C$$

Notes:

The amount of light absorbed by a sample is dependent on the path length, concentration of the sample and a proportionality constant (molar absorptivity).

Amount of light absorbed is dependent on frequency or wavelength

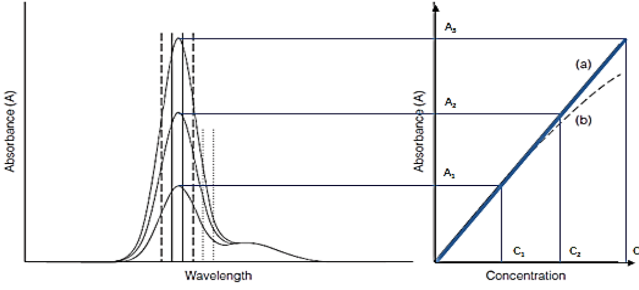
Increasing Fe^{2+} concentration →

Absorbance is directly proportional to concentration Fe^{2+}

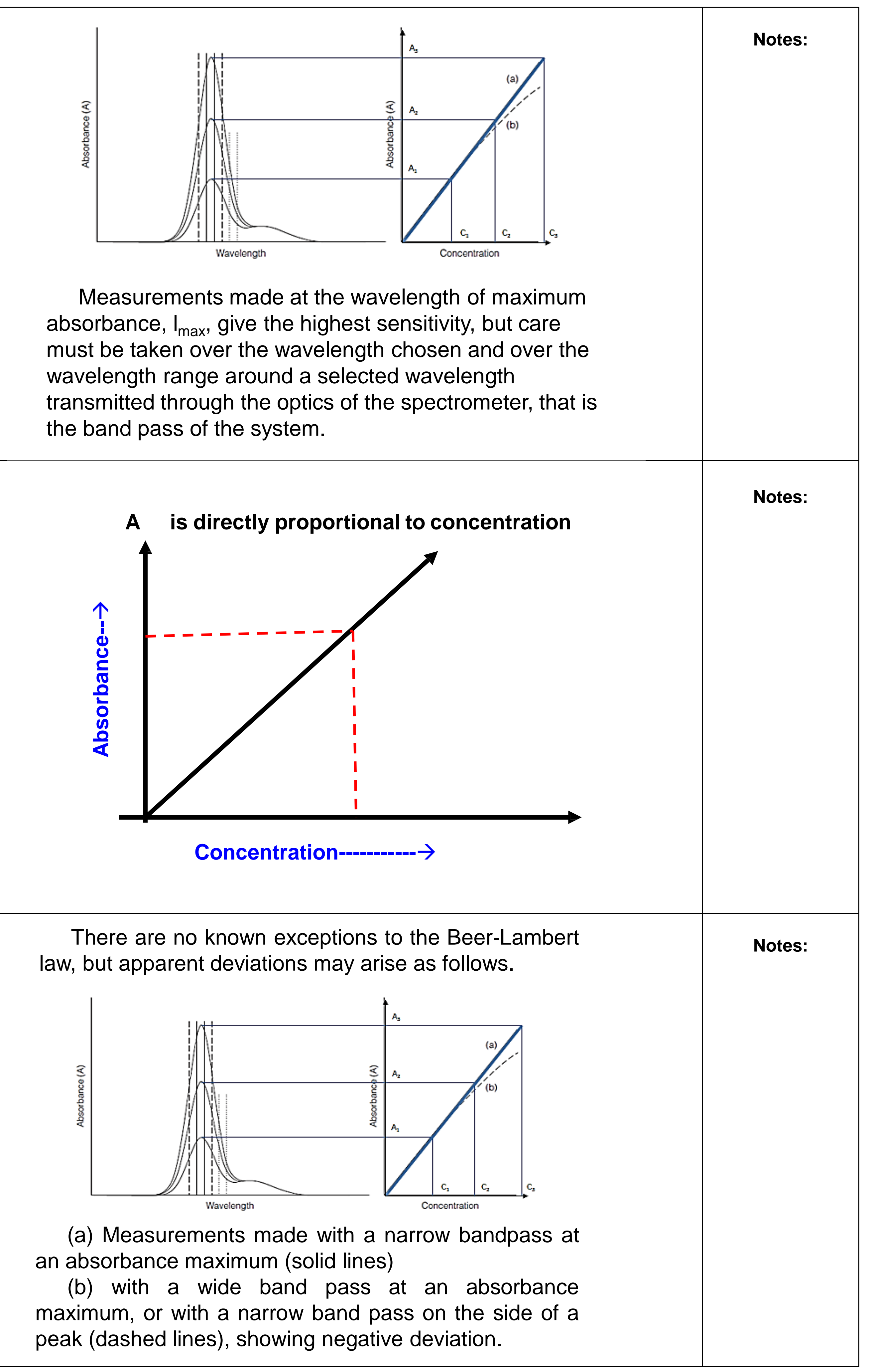
Notes:

Calibration graphs of A against C may be plotted to verify that the Beer-Lambert law applies over the range of concentrations that is to be studied.

It must be appreciated that the absorbance, and hence the transmittance and absorptivity, vary with wavelength, with the highest absorbance giving a peak in the absorption spectrum.



The spectrum and the band passes used for measurement are shown on the left, and the relation of absorbance to concentration is shown on the right.



Measurements made at the wavelength of maximum absorbance, l_{max}, give the highest sensitivity, but care must be taken over the wavelength chosen and over the wavelength range around a selected wavelength transmitted through the optics of the spectrometer, that is the band pass of the system.

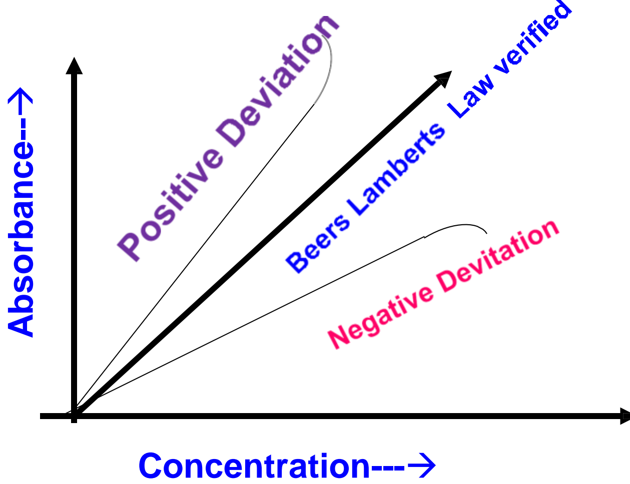
There are no known exceptions to the Beer-Lambert law, but apparent deviations may arise as follows.

(a) Measurements made with a narrow bandpass at an absorbance maximum (solid lines)

(b) with a wide band pass at an absorbance maximum, or with a narrow band pass on the side of a peak (dashed lines), showing negative deviation.

TYPES OF DEVIATION

1) Real Deviation
2) Chemical Deviation
3) Instrumental Deviation

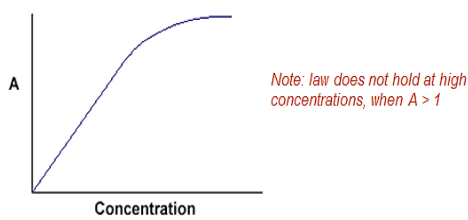


Notes:

Real deviation

At high concentrations the solute may chemically affect the nature of the solvent, and hence affect the value of ε. Dilute solutions will give better linearity of results. So:

- Concentration should be below 0.01M
- At higher concentration average distance between molecule decreases which affect in absorbance.
- Molar absorptivity changes because at higher concentration refractive index of solution changes



Note: law does not hold at high concentrations, when A > 1

Notes:

If chemical equilibria affect the solute species, then, since the nature of the absorbing species is changed, ε would be expected to change.

For example, in the infrared spectra of hydroxyl compounds, such as alcohols, the OH stretching vibration absorbs sharply at around 3600 cm^{-1} in the gas phase spectrum. In the liquid phase, or in solution, hydrogen bonding may occur and the vibrational frequency lowered to around 3300 cm^{-1}.

Notes:

Chemical Deviation:

This deviation occurs when solute present in solution undergoes association, dissociation or ionization.

Association: Diluting or concentrating the solution of Benzyl alcohol in chloroform undergoes polymerization.

$$4C_6H_5CH_2OH \rightarrow (C_6H_5CH_2OH)_4$$

Dissociation $K_2Cr_2O_7$

$$Cr_2O_7^{2-} + H_2O \rightarrow 2HCrO_4 \rightarrow 2H^+ + 2CrO_4^{2-}$$

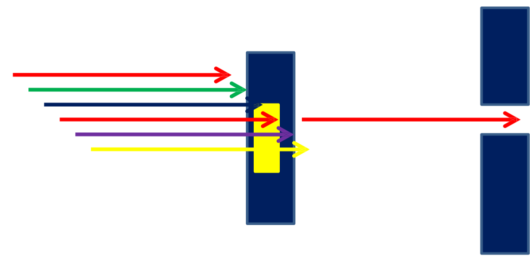
orange yellow

Notes:

Instrumentational deviation

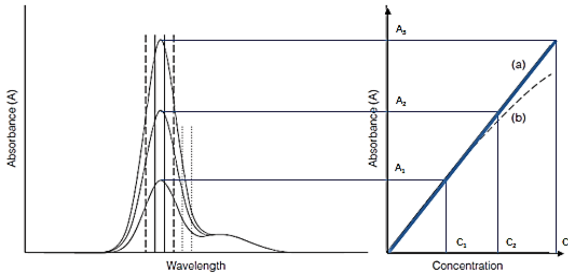
The law is valid If light used is strictly monochromatic. If not deviation may occur.

Deviation may occur if width of slit is not proper which may allow undesired radiation to pass through it.



Notes:

If the radiation is polychromatic, or measured in a part of the spectrum other than at an absorbance maximum, the Beer's law dependence will be affected, giving a negative deviation



Measurements made with a wide band pass at an absorbance maximum, or with a narrow band pass on the side of a peak (dashed lines), showing negative deviation.

Notes:

The **precision** of absorbance measurements depends on the instrumentation used and on the chemical species being determined.

At high absorbances, (A>1), very little radiation reaches the detector, so that a higher amplifier gain is needed.

At very low absorbances, (A<0.1) the instrumental noise becomes very important. Therefore, there is a region where the relative concentration error as a percentage (100 \ dC/C) is at a minimum.

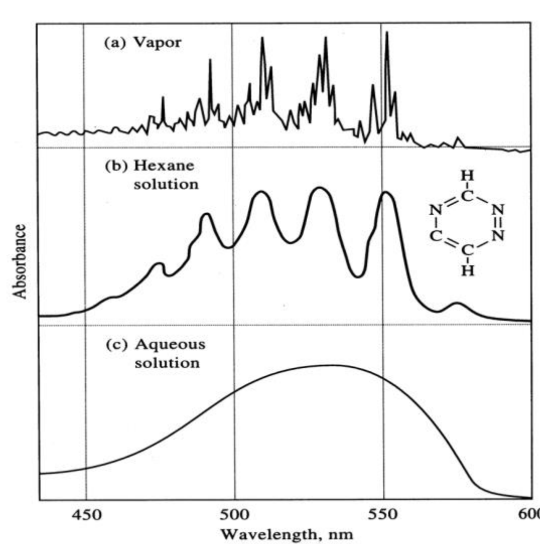
With a photovoltaic detector, the error curve has a narrow minimum, whereas for the photomultiplier detector used in many modern instruments, the curve has a broader minimum, and therefore an extended useful working range.

In practice it is advisable to measure absorbance in the range 0.1<A<1.0

Notes:

Molecular absorption spectra

Notes:

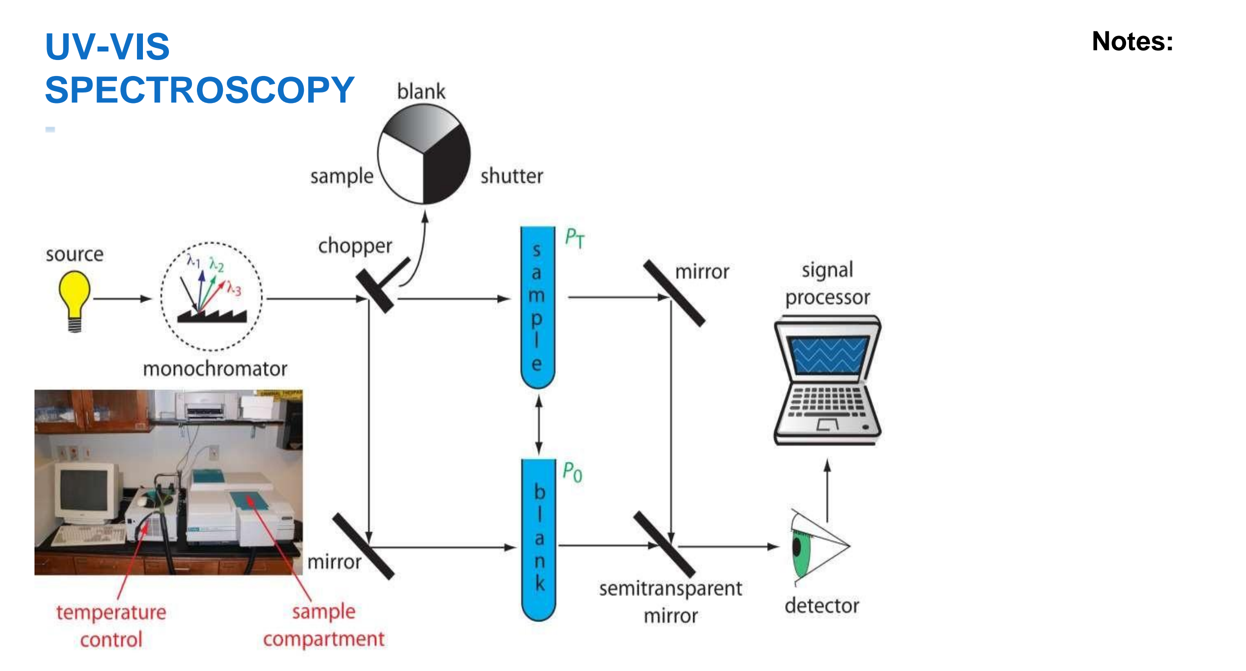


In solvents the rotational and vibrational transitions are highly restricted resulting in broad band absorption spectra

Ultraviolet absorption spectra for 1,2,4,5-tetrazine (a) in the vapour phase, (b) in hexane solution, and (c) in aqueous solution

UV-VIS SPECTROSCOPY

Notes:



Notes:

In Ultra Violet and visible spectroscopy absorption of light is measured in the wavelength region from 200 nm to 800 nm.

The instrument used in Ultra violet and visible region (from 200 nm to 800 nm) is called as **spectrophotometer.**

The instrument used for measurement of absorption of light in visible region (from 400 nm to 800 nm) is called as **colorimeter or photometer.**

Notes:

The spectrophotometer is ubiquitous among modern laboratories.

Ultraviolet (UV) and Visible (VIS) spectrophotometry has become the method of choice in most laboratories concerned with the identification and quantification of organic and inorganic compounds across a wide range of products and processes.

They are equally as relevant in agriculture, chemical industry, geological exploration, food safety, environmental monitoring, and many manufacturing industries to name a few.

Notes:

Modern spectrophotometers are quick, accurate, and reliable. They require only small demands on the time and skills of the operator.

However, the non-specialised end-user who wants to optimise the functions of their instrument, and be able to monitor its performance will benefit from the appreciation of the elementary physical laws governing spectrophotometry, as well as the basic elements of spectrophotometer design.

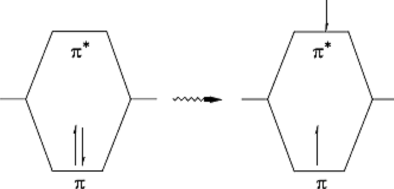
Principle of UV-VIS Spectrometry

Notes:

Ultraviolet light and visible light cause an electronic. Transition of electron from one filled orbital to another of higher Energy unfilled orbital.

These transition occur between the electronic energy levels.

As molecule absorbs energy, an electron is promoted from occupied orbital to an unoccupied orbital of greater potential energy.



Notes:

Ultraviolet absorption spectra arise from transition of electron within a molecule from a lower level to a higher level.

A molecule absorb ultraviolet radiation of frequency (ϑ), the electron in that molecule undergo transition from lower to higher energy level.

The energy can be calculated by the equation,

$$E=h\vartheta$$

Notes:

$$E_1-E_o= h\vartheta$$

$$E_{total} = E_{electronic} + E_{vibrational} + E_{rotational}$$

The energies decreases in the following order:

Electronic >Vibrational > Rotational

Types of Transitions

Notes:

- In U.V spectroscopy molecule undergo electronic transition involving σ, π and n electrons.
- Four types of electronic transition are possible.

σ → σ* transition

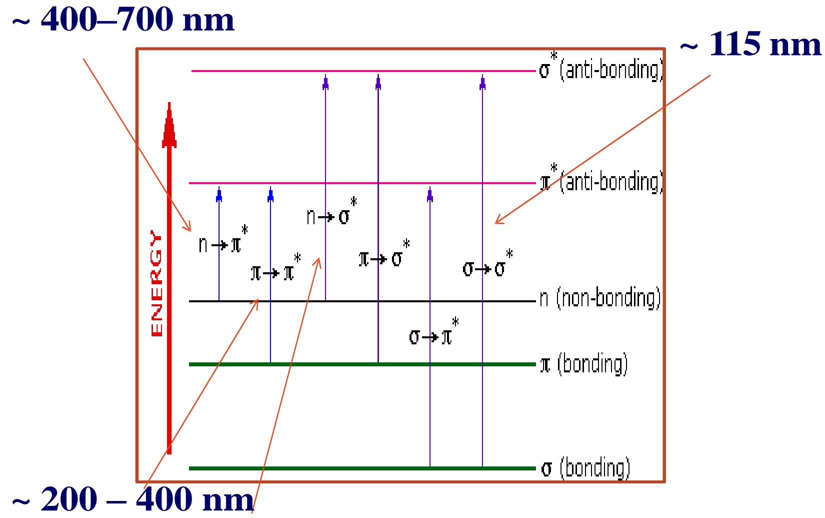
n → σ* transition

n → π* transition

π → π* transition

Transition's Characteristics

Notes:



The absorption spectrum

Notes:

When a sample is exposed to light energy that matches the energy difference between a electronic transition within the molecule, the light energy would be absorbed by the molecule and the electrons would be promoted to the higher energy orbital.

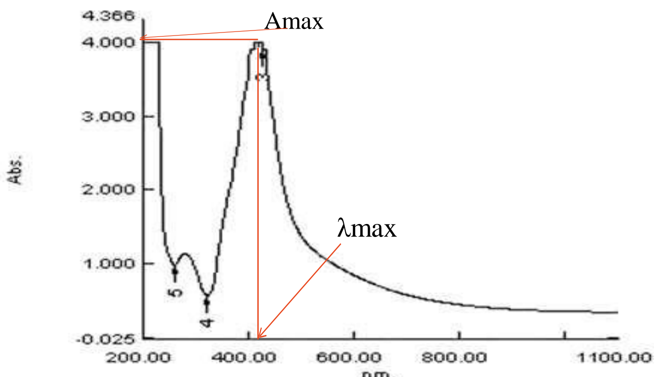
A spectrometer records the degree of absorption by a sample at different wavelengths and the resulting plot of absorbance (A) versus wavelength (λ) is known as a spectrum.

The significant features:

λ_{max} is the wavelength at which there is a maximum absorption

A_{max} is the intensity of maximum absorption

UV-visible spectrum of Silver Nanoparticles showing maximum absorption at 420 nm.



UV-Vis Spectrum of Silver Nanoparticles

Notes:

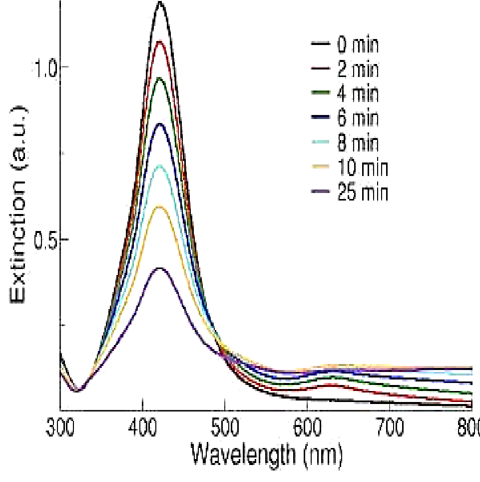
Applications of UV-Vis spectroscopy

- Detection of functional groups
- Detection of extent of conjugation
- Determination of configurations of geometrical isomers
- Determination of the purity of a substance

Notes:

Stability of Nanoparticle

- The optical properties of silver nanoparticles change when particle agglomerate
- When nanoparticle aggregate, the plasmon resonance will be red- shifted to a longer wavelength
- The peak will broaden or a secondary peak will form at longer wavelengths



Notes:

Notes:

Absorption Spectroscopy

(1) Source lamp or heated solid → $h\nu$ → (2) Sample holder → $h\nu$ → (3) Wavelength selector → $h\nu$ → (4) Photoelectric transducer → I → (5) Signal processor and readout

The basic component of the instruments are:

Radiation Source (Tungsten Filament lamp/ hydrogen or deuterium lamp)

Sample cell

Wavelength selector (Filter/ monochromator)

Detector

Notes:

INSTRUMENT

- Colorimeter or Photometer 400-800 nm
- Spectrophotometer 200-800 nm

Distinguish between Photometer and Spectrophotometer

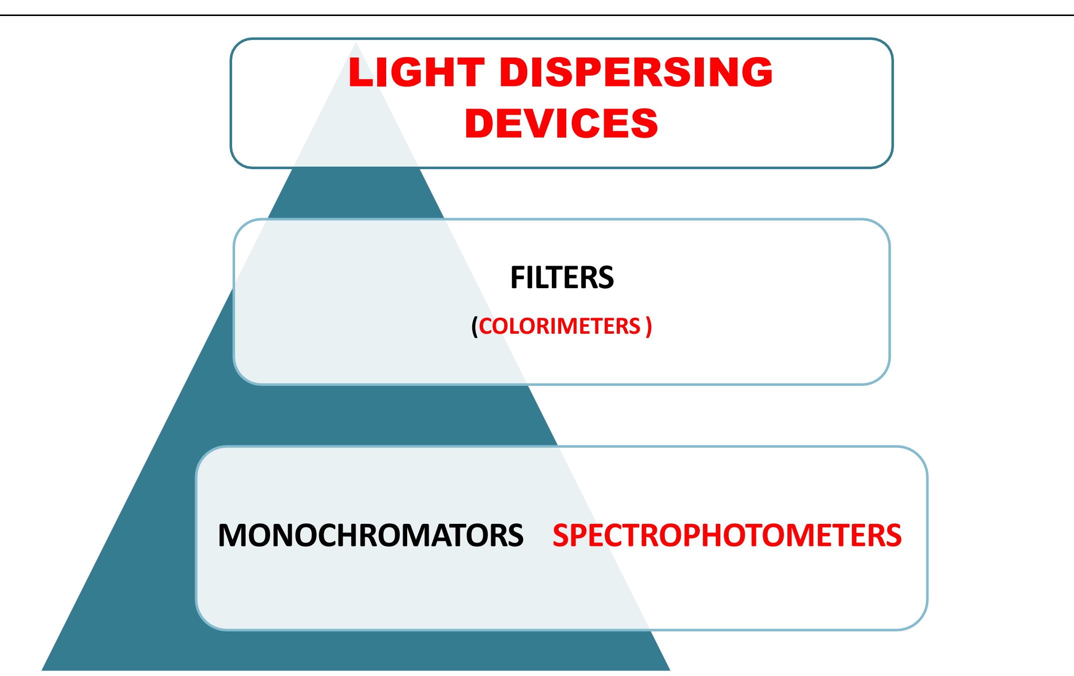
Notes:

PHOTOMETER

- Radiation source is Tungsten filament lamp.
- Absorbance of light is measured in the wavelength 400-800 nm (visible region)
- Filters are used to select monochromatic light.
- Sample cells made from glass.
- Detectors used are photocell or photoemmisive tube.
- Absorbance of coloured solution is measured

SPECTROPHOTOMETER

- Radiation source is Hydrogen or deuterium lamp.
- Absorbance of light is measured in the wavelength 200-800 nm (U.V.& visible region)
- Prism or Gratings are used to select monochromatic light.
- Sample cells made from quartz.
- Detectors used are photomultiplier tube.
- Absorbance of coloured as well as colorless solution can be measured

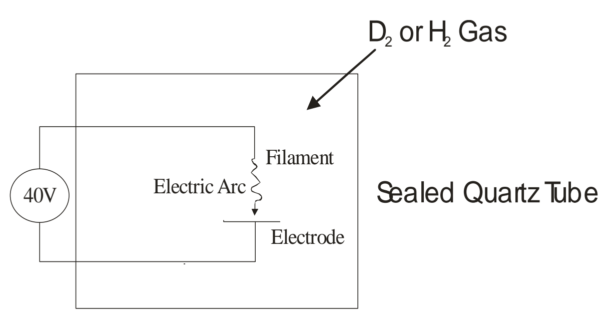


Notes:

Light Sources UV/Vis (~ 200 – 800 nm):

Deuterium & Hydrogen Lamps (UV range)

- continuous source, broad range of frequencies
- based on electric excitation of H_2 or D_2 at Low pressure



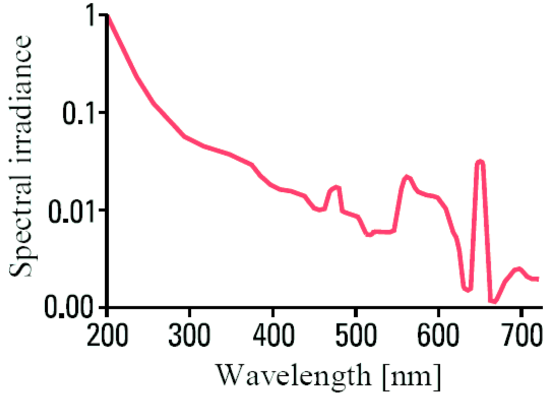
In presence of arc, some of the electrical energy is absorbed by D_2 (or H_2) which results in the disassociation of the gas and release of light

$D_2 + E_{elect} \rightarrow D^*_2 \rightarrow D' + D'' + h\nu$ (light produced)

Excited state

Notes:

- hv will vary continuously from ~ 160 nm up to 375nm (UV range) due to different frequencies going into D' and D''
- need to make cell from quartz since glass absorbs light at $\lambda \leq 350$ nm
- cost ~ $350-$500



Intensity spectrum of deuterium arc lamp

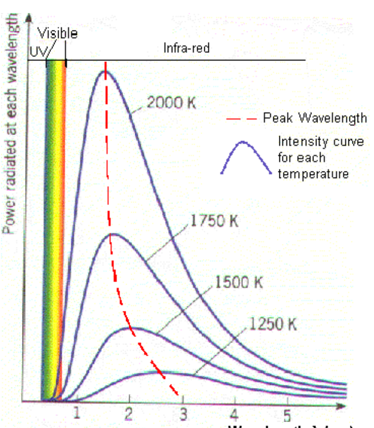
Notes:

Tungsten Filament Lamp (Vis – Near IR)

- continuous source, a broad range of frequencies
- based on black body radiation: heat solid filament to glowing, light emitted will be characteristic of temperature more than the nature of the solid filament

Low pressure (vacuum)

Tungsten Filament



Temperature Dependence of λ

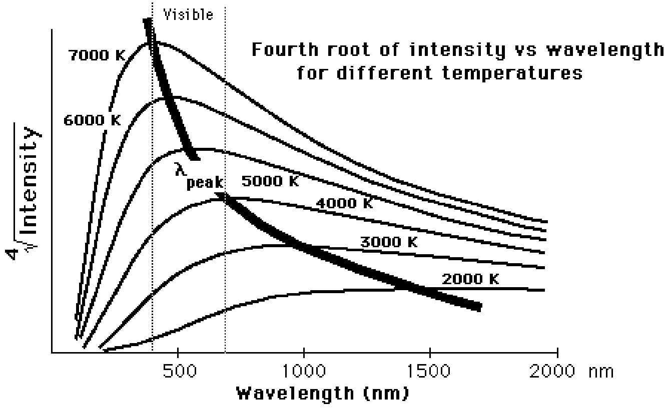
Notes:

Tungsten Filament Lamp (Vis – Near IR)

typical tungsten lamp T ~ 2870K

λ range: 350 – 2500 nm need high temperatures to get high light intensity

cost ~ $10-15



Notes:

Xenon Arc Lamps (UV – Vis Range)

Continuous source, broad range of frequencies

λ range: 250 – 600 nm

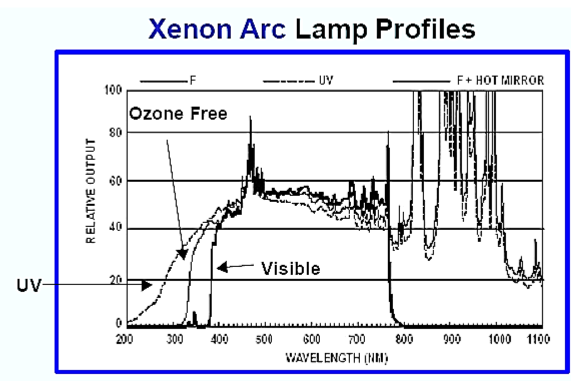
works by passage of current through Xe, causes thermal excitation

Blackbody emission

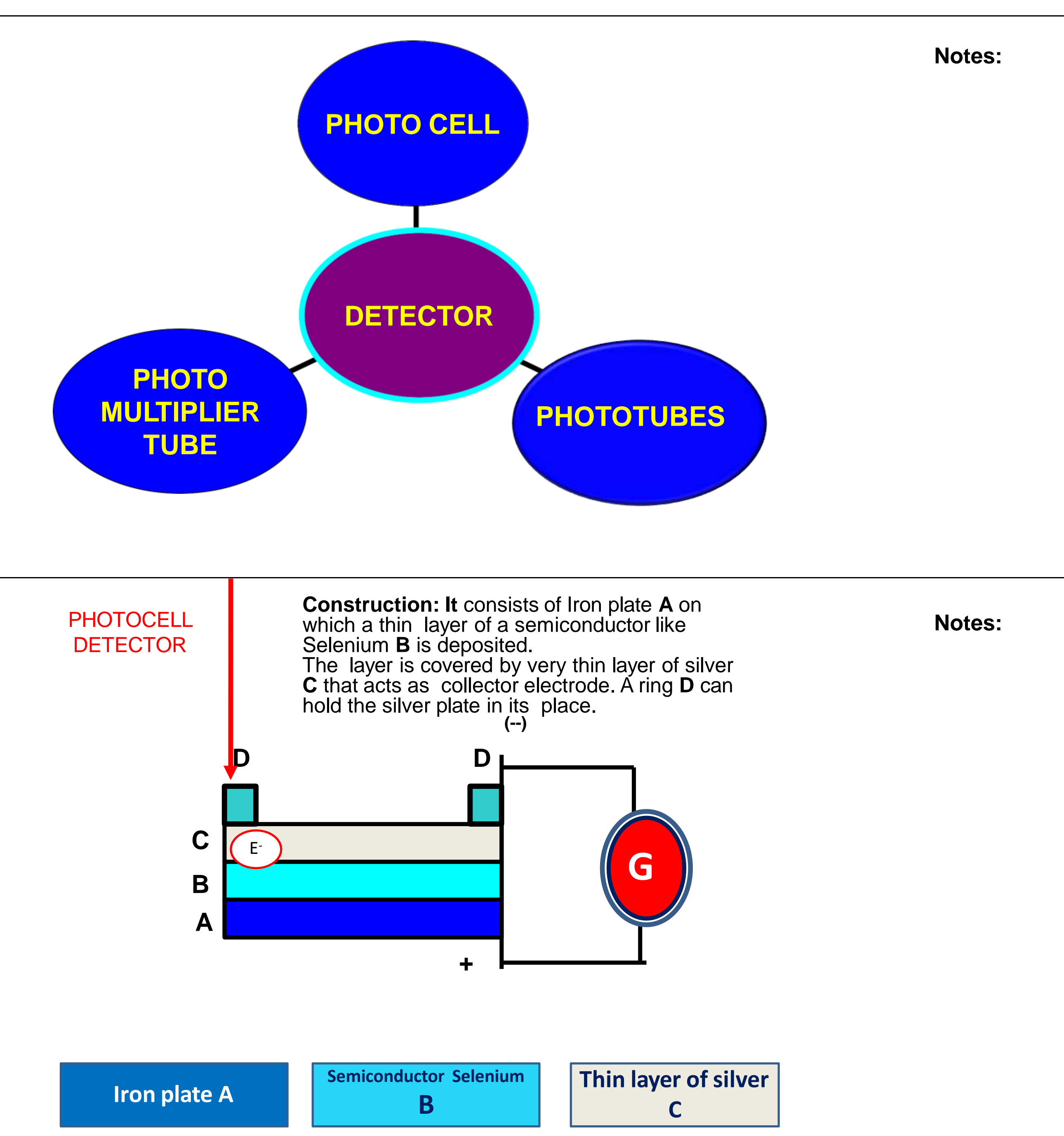
Gives Very intense radiation over frequency range.

developed for search lights during WWII

Problems: higher heat, more stray light, higher cost, shorter lifetimes



Notes:



Working:

Notes:

This cell operates without battery. When the transmitted beam of light passes through thin film of silver metal to selenium layer, electrons released from semiconductor surface

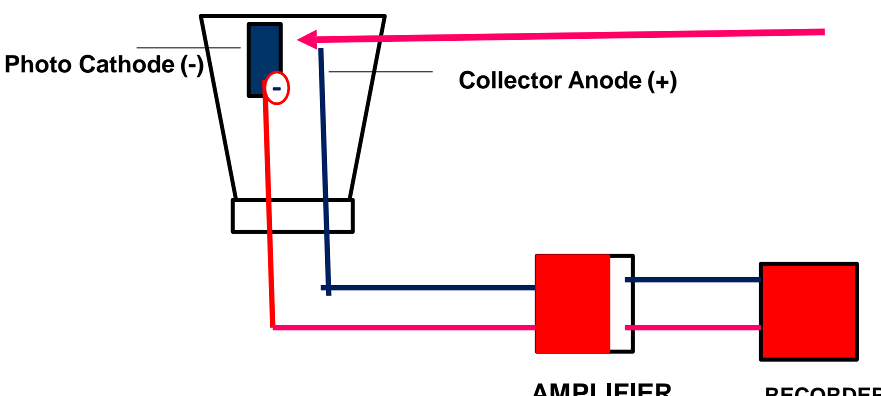
These electrons pass through a hypothetical barrier layer in between silver and selenium layer and are collected by silver electrode.

Thus under the action of light a cell is formed with iron plate as positive electrode

Metal ring as negative electrode. The current flow is detected in galvanometer. This current is directly proportional to absorbance.

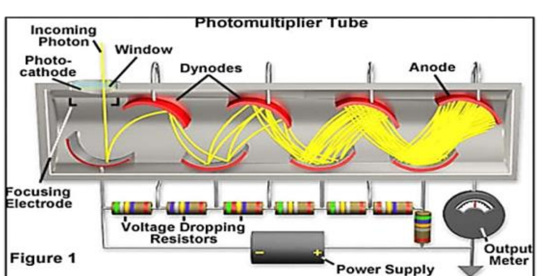
PHOTO TUBE DETECTOR

Notes:



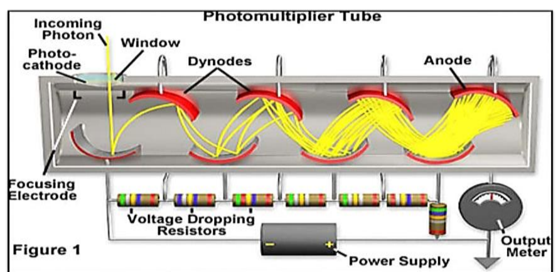
The photomultiplier tube (PMT)

Notes:



- The **photomultiplier** tube is a commonly used detector in UV spectroscopy. It consists of a photo emissive cathode (a cathode which emits electrons when struck by photons of radiation), several dynodes (which emit several electrons for each electron striking them) and an anode.
- A photon of radiation entering the tube strikes the cathode, causing the emission of several electrons.
- These electrons are accelerated towards the first dynode (which is 90V more positive than the cathode).
- The electrons strike the first dynode, causing the emission of several electrons for each incident electron.

- These electrons are then accelerated towards the second dynode, to produce more electrons which are accelerated towards dynode three and so on.

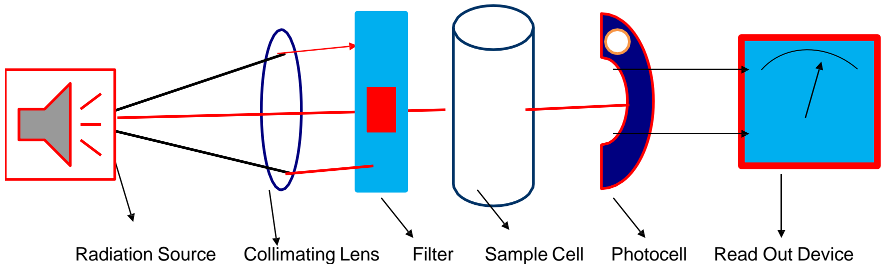


Notes:

- Eventually, the electrons are collected at the anode.
- By this time, each original photon has produced 106 - 107 electrons.
- The resulting current is amplified and measured.
- Photomultipliers are very sensitive to UV and visible radiation.
- They have fast response times.
- Intense light damages photomultipliers;
- they are limited to measuring low power radiation.

Notes:

Single Beam Photometer



Notes:

Working of Single Beam photometer

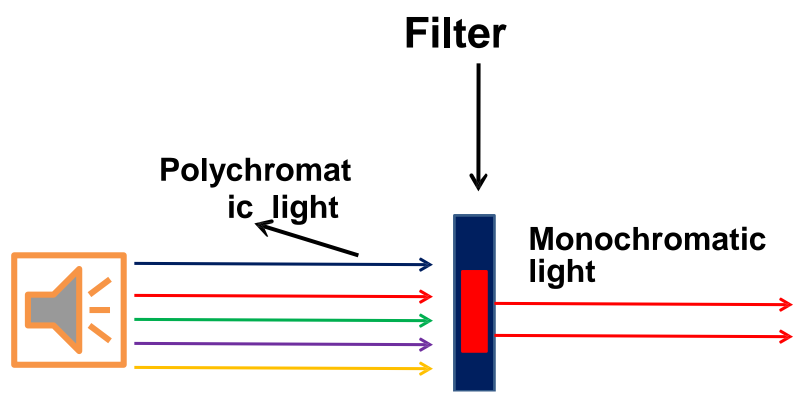
Quantitative analysis can be performed by calibration curve method.

Instrument is adjusted to 0 absorbance or 100% transmittance by Using Blank Solvent at a particular wavelength.

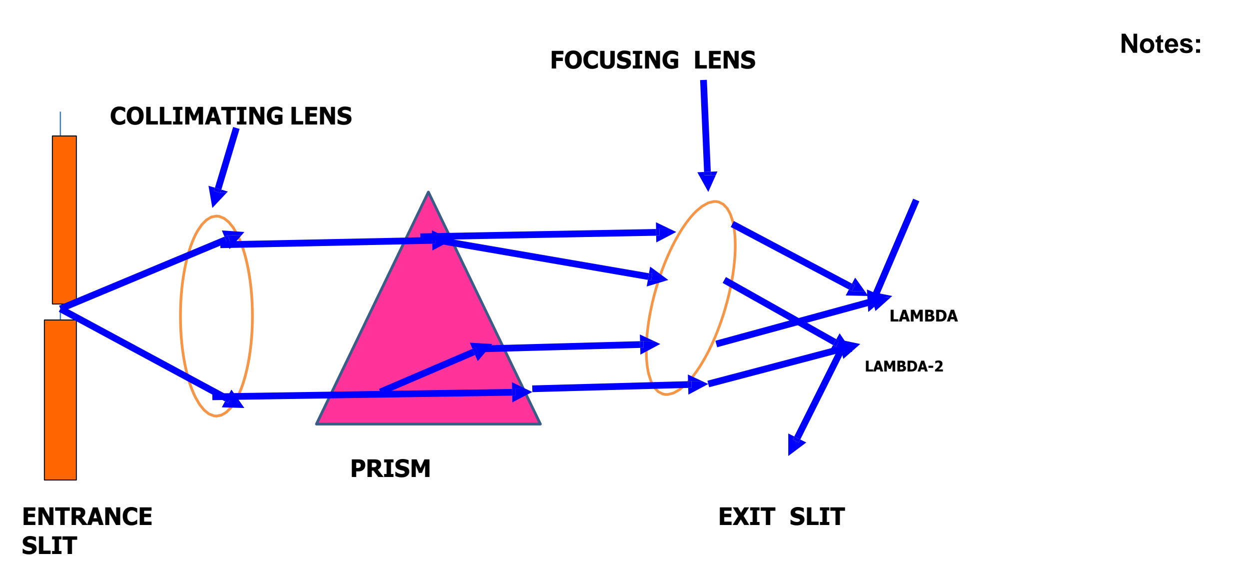
The absorbance of sample solution as well as standard solution are measured.

Notes:

Filter: A filter transmits the monochromatic beam of light and absorbs other light



Notes:



PRISM MONOCHROMATOR

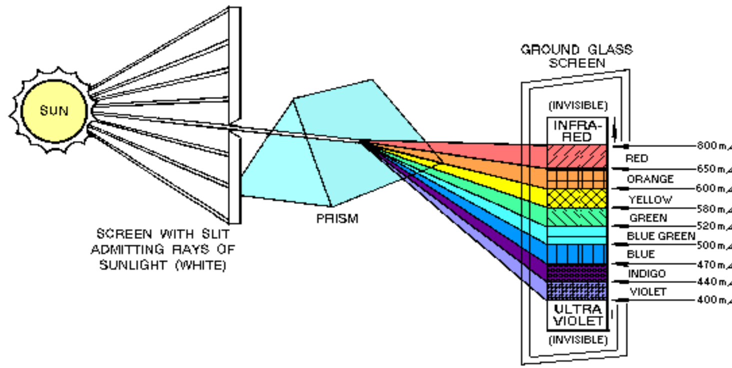
Notes:

Wavelength Selectors:

1. Monochromator
   - separates frequencies (v) from polychromatic light.
   - allows only certain λ's to be selected and used.

Dispersing Monochromator:

a) Prism: based on refraction of light and fact that different λ's have different values of refraction index (h_i) in a medium.

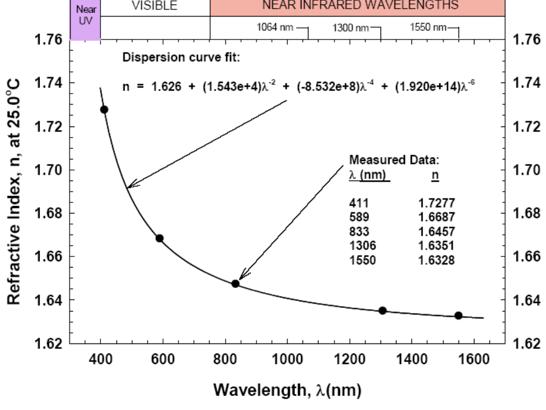


Notes:

Dispersion Curve: change in η_i as a function of λ

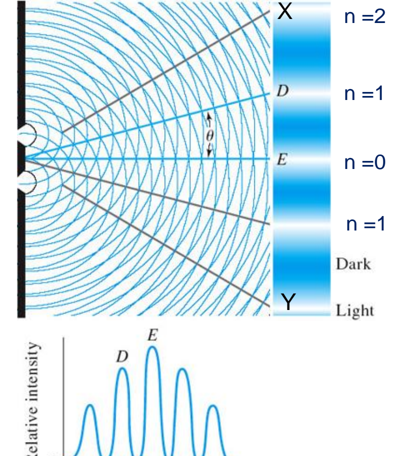
Normally want to work in areas of normal dispersions for prisms. Anomalous dispersion occurs near where substance itself absorbs light.

Refractive Index vs. Wavelength (25°C)
SantoLight Optical Fluid SL-5267
lot# 080901



Grating Monochromator: based on diffraction of light (constructive and deconstructive interference)

a) Transmission Grating: groves or slits placed on a transparent material. Same as earlier example shown in diffraction discussion



Order of Interference (n): $n\lambda = d \sin\theta$

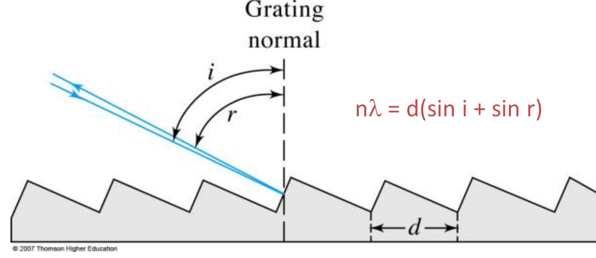
Different λ's will have constructive interference at different points.

Can select desired λ by letting light at different points into instrument.

Notes:

b) Reflection Grating: most commonly used

- grooved surface with reflective coating (Al, Au, Pt)



$n\lambda = d(\sin i + \sin r)$

Now, spacing of slits (d) is distance from one groove to next. Typically have 300-2000 grooves/mm.

Constructive and deconstructive interference occurs because light travels different distances when reflected from each grating

Angle at which constructive interference occurs is now given by: $n\lambda = d(\sin i + \sin r)$

Notes:

Single and double beam instruments:

To determine absorbance both I_o and I must be measured.

$$\text{Absorbance (A)} = \log_{10} I_o/I$$

depends on:

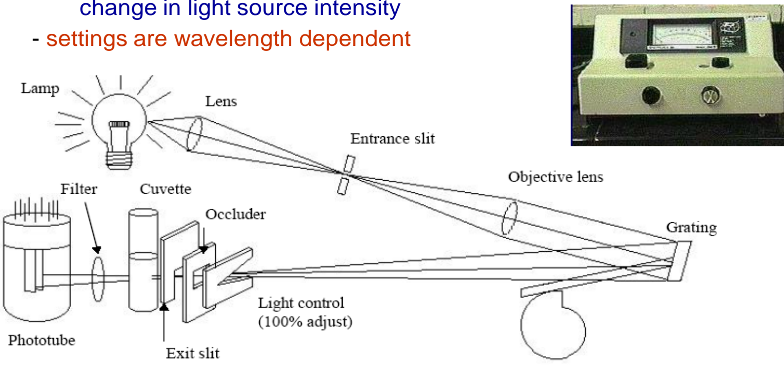
- ♦ intensity of source
- ♦ slit width and λ of monochromator
- ♦ reflectance of the cell
- ♦ sensitivity of the detector

Notes:

Process for a Single Beam Spectrometer:

a) I_o is measured with solvent in the cell
b) I, blank everything except compound to be analyzed
- spectrophotometer is adjusted to read 100% T or 0% A.
- 0%T or A is set by blocking the light beam (P=0)
- settings may change over a period of minutes (drift)
  change in light source intensity
- settings are wavelength dependent

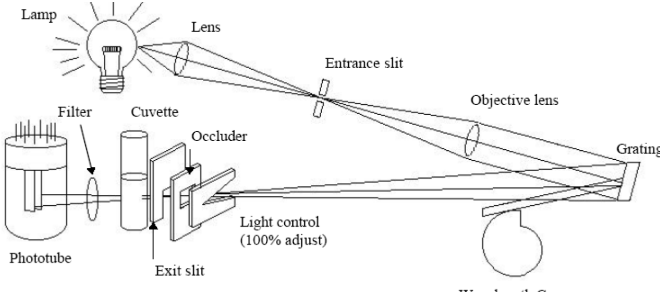
Spectronic 20



Schematic of Spectronic 20

Notes:

- Single beam spectrophotometer (340-625nm) other phototube goes to 950 nm (20 nm band pass)
- Reference phototube electronically adjusts for changes in source intensity
- The λ control turns the grating.
- The 0%T adjust sets the meter to 0%T when the occluder blocks the light beam.
- The 100% T adjusts moves a V-shaped slot into or out of the light beam so the meter reads 100%T

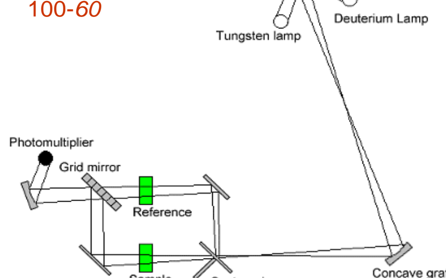


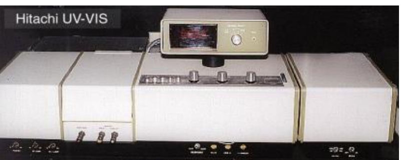
Advantages: cheap, rugged.
Disadvantages: must readjust 100%T at every λ and periodically check for drift, cell is round so path length can vary if don't have cell aligned the same each time

Notes:

Double Beam Spectrometer Decrease/Eliminates Single Beam Problems

Schematic of Hitachi 100-*60*





<u>Selector mirror or beam chopper</u>: mirrors which rotate at 60 cps, reflecting the light alternatively to the reference and sample cells

All light is combined and goes to a single PMT. Output of PMT is 60 cps square wave. P_o and P are measured alternatively at a rate of 60 times per second.

<u>Advantages</u>: λ scanning, little drift – only one PMT.
<u>Disadvantages</u>: more complex and expensive

Notes:

Sample Cell:

- Must be transparent at λ used.
  - quartz or fused silica for UV (< 350 nm)
  - glass or plastic for visible
- best if flat cells, with matched sample and reference cells.
- many automated instruments have flow-through cells with temperature control.

Detectors:

a) earliest detectors were the eye or film.
- ♦ now use devices that convert light to electrical signal

b) for good detector want:
- ♦ high sensitivity
- ♦ good signal to noise ratio
- ♦ constant response over λ range of interest
- ♦ signal ∝ light intensity
- ♦ fast response
- ♦ little or no signal in absence of light (dark current)

Notes:

Applications of U.V. & Visible Spectroscopy

Qualitative Analysis:

Identification of structural groups in molecules.

Spectroscopic analysis of a substance is carried out using radiation of a particular wavelength this wave length is called as ***λ* max**.

The Constituent groups in a molecule absorbed to their characteristic wavelengths.

It is possible to determine a particular group in a molecule by determining its ***λ* max**.

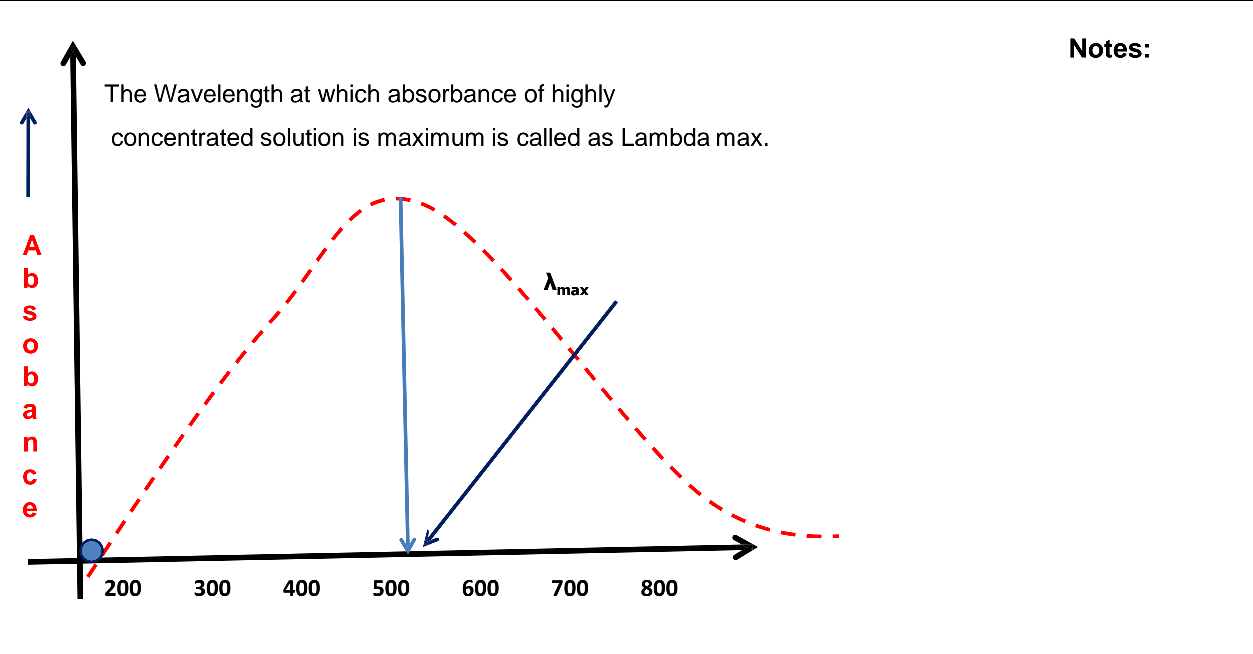
***λ* max** values of important groups are given in following table.

Notes:

Example	Functional Groups	λmax (nm)	Solvents
Acetic Acid	**--COOH**	**208**	**Ethyl Alcohol**
Acetyl Chloride	**--COCl**	**220**	**Hexane**
Acetamide	**--CONH$_2$**	**178**	**Hexane**
Nitromethane	**--NO$_2$**	**201**	**Methyl Alcohol**
Azomethane	**--N=N--**	**338**	**Ethyl Alcohol**
Acetaldehyde	**>C=O**	**290**	**Hexane**
Acetone	**>C=O**	**189**	**Hexane**

Notes:

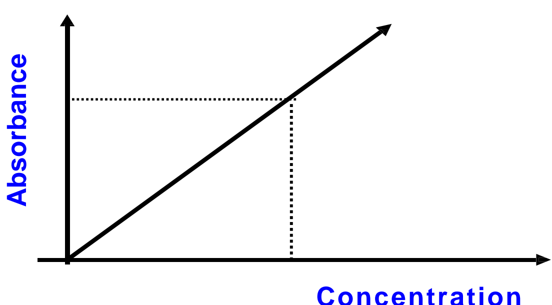
Notes:



Notes:

Applications of U.V. & Visible Spectroscopy

Quantitative Analysis By Calibration Curve Method



Notes:

Other Applications

UV and visible spectroscopy can be used up to concentrations up to 10^{-6} and some times up to 10^{-7} M.

Qualitative analysis can be performed because each absorbing species absorb at different wave length.

It is also used:

in water and air pollution studies;

to study the complexes and in the determination of complex;

in industry to monitor various process controls;

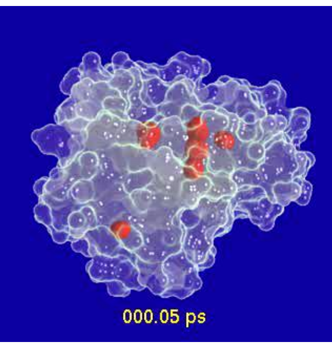
to study to distinguish between cis-and trans isomerism. C – is isomer absorbs at shorter wave length and trans isomer absorb at longer wave length.

Infrared Spectroscopy

Notes:

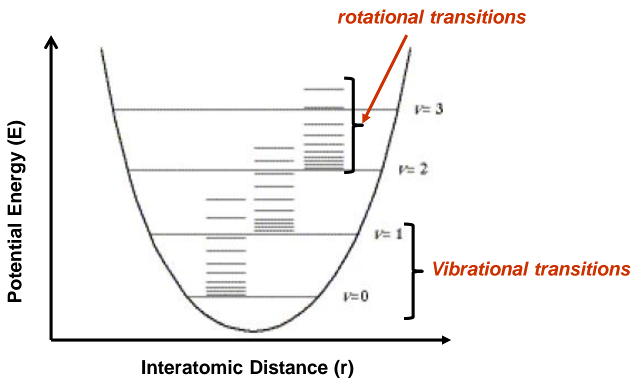
Despite the typical graphical display of molecular structures, molecules are highly flexible and undergo multiple modes of motion over a range of time-frames

Motions involve rotations, translations, and changes in bond lengths, bond angles, dihedral angles, ring flips, methyl bond rotations.



Infrared (IR) spectroscopy: based on IR absorption by molecules as undergo vibrational and rotational transitions.

Notes:



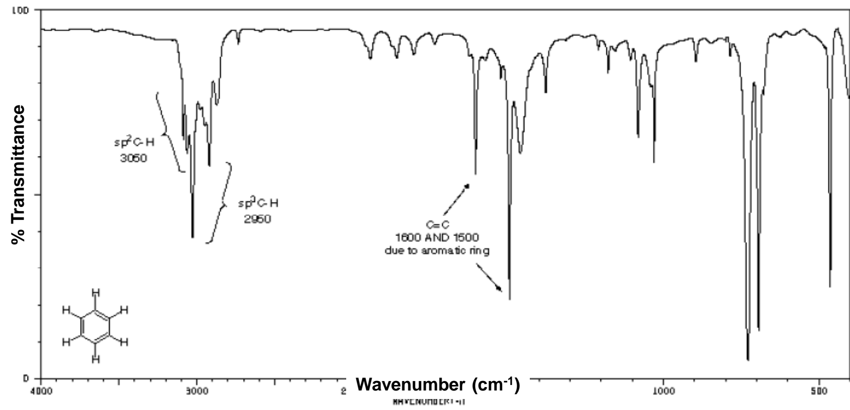
Potential energy resembles classic harmonic oscillator

IR radiation is in the range of 12,800 – 10 cm^{-1} or λ = 0.78 – 1000 μm

- rotational transitions have small energy differences
  ≤ 100 cm^{-1}, λ > 100 μm
- vibrational transitions occur at higher energies
- rotational and vibrational transitions often occur together

Typical IR spectrum for Organic Molecule

Notes:



Typical IR spectrum for Organic Molecule

Notes:

The spectra are usually displayed for convenience as a percentage of transmission (instead of absorption) against the wavenumber (instead of λ).

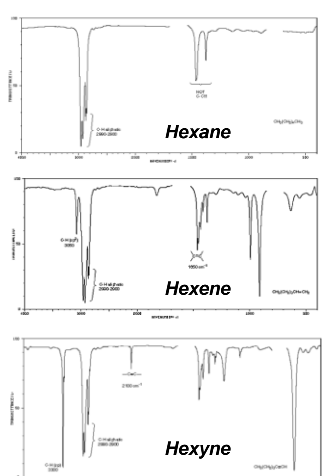
Many more bands than UV visas.

The stripes are also much sharper.

The spectra are different for molecules except for optical isomers

This method is a good quality tool and can be used to analyze identification groups

It is also a quantitative tool because there is a relationship between bandwidth and concentration of compounds present



The spectra of different organic compounds are shown at the figure.
There are several different peaks. They indicate the presence of a double bond and a different groups

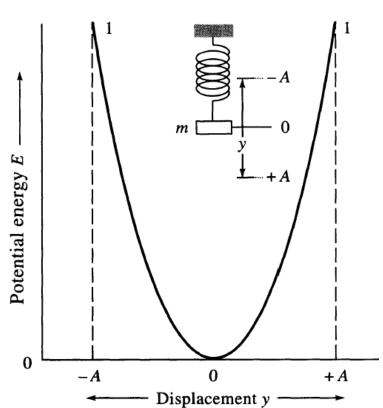
Theory of IR Absorption

Notes:

Molecular Vibrations

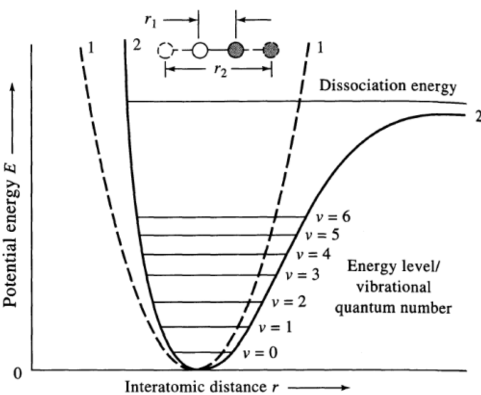
iHarmonic Oscillator Model:

- approximate representation of atomic stretching
- two masses attached by a spring



$$E = \frac{1}{2} ky^2$$

where:
y is spring displacement
k is spring constant



Notes:

Vibrational frequency given by:

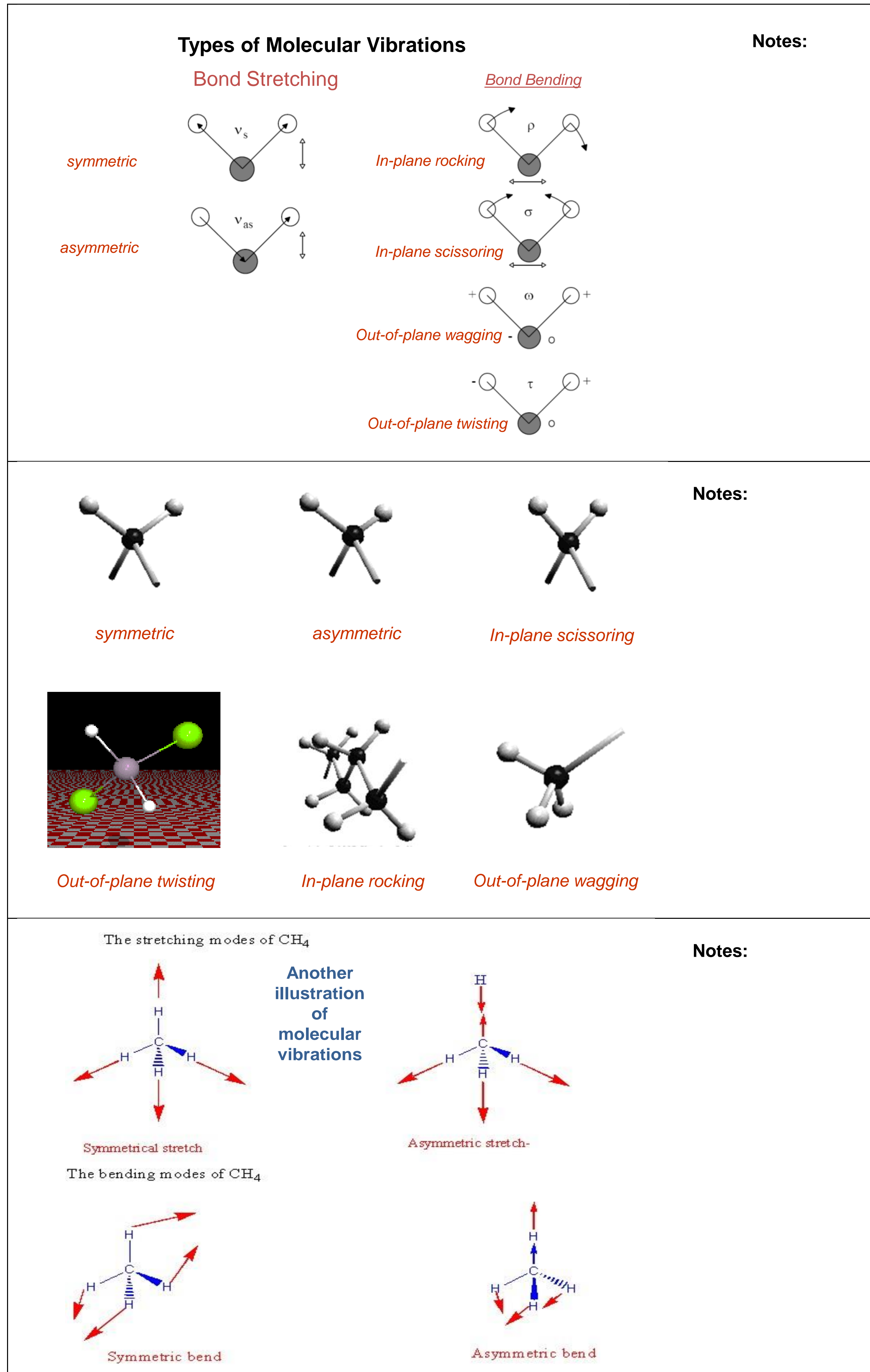
$$\nu = 1/2\pi\sqrt{k/m}$$

where:
ν is frequency
k is force constant (measure of bond stiffness)
μ is reduced mass – m_1m_2/m_1+m_2

If know n and atoms in bond, can get *k:*

Single bonds:
k ~ $3x10^2$ to 8 $x10^2$ N/m (Avg ~ $5x10^2$)
double and triple bonds ~ 2x and 3x k for single bond.

So, vibration n occur in order:
single < double < triple


Types of Molecular Vibrations
Notes:
Bond Stretching
Bond Bending
symmetric
ν_s
asymmetric
ν_{as}
In-plane rocking
ρ
In-plane scissoring
σ
Out-of-plane wagging
ω
Out-of-plane twisting
τ
Notes:
symmetric
asymmetric
In-plane scissoring
Out-of-plane twisting
In-plane rocking
Out-of-plane wagging
Notes:
The stretching modes of CH4
Another illustration of molecular vibrations
Symmetrical stretch
Asymmetric stretch-
The bending modes of CH4
Symmetric bend
Asymmetric bend

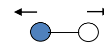
Number of Vibrational Modes:

- for non-linear molecules, number of types of vibrations: 3N-6
- for linear molecules, number of types of vibrations: 3N-5
- why so many peaks in IR spectra
- observed vibration can be less then predicted because
  - symmetry (no change in dipole)
  - energies of vibration are identical
  - absorption intensity too low
  - frequency beyond range of instrument

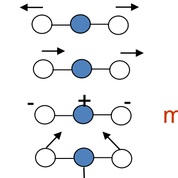
Notes:

Examples:

1) HCl: 3(2)-5 = 1 mode



2) CO_2: 3(3)-5 = 4 modes



moving in-out of plane

You can see examples of different molecules at:
http://www.chem.purdue.edu/gchelp/vibs/co2.html

IR Active Vibrations:

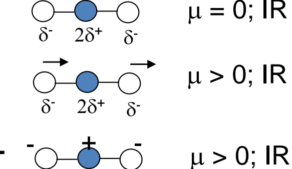
In order for a molecule to absorb IR radiation:

- vibration at the same frequency as in light,
- but also, must have a change in its net dipole moment as a result of the vibration

Notes:

Examples:

CO_2: 3(3)-5 = 4 modes



$\mu = 0$; IR inactive

$\mu > 0$; IR active

$\mu > 0$; IR active

$\mu > 0$; IR active

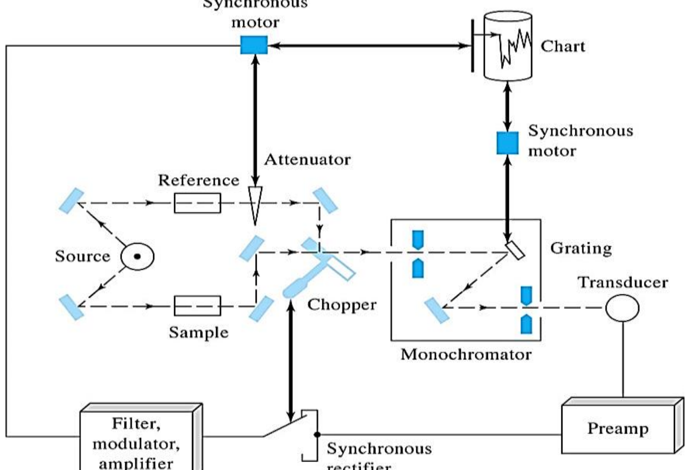
degenerate –identical energy single IR peak

Instrumentation

Notes:

Basic Design

- normal IR instrument similar to UV-vis
- main differences are light source & detector



Universal flow chart of an analytical instrument

Notes:

Source of energy → *Probe with electron-optical control system* → **The core**: Processing module for probe-sample interaction (← Input-output of samples) → *Secondary signal with amplifiers and A/D convertors* → Detector and readout

Understanding the Measurement Process:

Signal generator → signal transduction & modification (information encoding and decoding) → signal display

The above-mentioned blocks are absolutely necessary. They are present in any modern analytical instrument being implemented in different engineering solutions

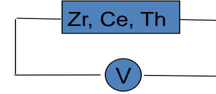
As sources of energy, one can use:	**Samples may be:**	**Processing module may include:**	**Detected species may be:**
photons, ions, electrons, atoms, Molecules	solutions, solids, gases	dispensers, heaters, magnets, optical grating, optical prisms, chromatographic columns	photons, ions, atoms or molecules in gases, electric charge, potential or current

Notes:

Light Source:

The source requirements are as follows:
- must produce IR radiation
- can't use glass since absorbs IR radiation
- several possible types

a) Nernst Glower



- rare earth metal oxides (Zr, Ce, Th) heated electrically
- apply current to cylinder, has resistance to current flow generates heat (1200° – 2200° C)
- causes light production similar to blackbody radiation
- range of use ~ 670 – 10,000 cm^{-1}
- need good current control or overheats and damaged

b) Globar

- similar to Nernst Glower but uses silicon carbide rod instead of rare earth oxides
- similar usable range

c) Incandescent Wire Source

Notes:

- tightly wound nichrome or rodium wire that is electrically heated
- same principal as Nernst Glower
- lower intensity then Nernst Glower or Globar, but longer lifetime

d) CO_2 Laser

- CO_2 laser gas mixture consists of 70% He, 15% CO_2, and 15% N_2
- a voltage is placed across the gas, exciting N_2 to lowest vibrational levels.
- the excited N_2 populate the asymmetric vibrational states in the CO_2 through collisions.
- infrared output of the laser is the result of transitions between rotational states of the CO_2 molecule of the first asymmetric vibrational mode to rotational states of both the first symmetric stretch mode and the second bending mode
- gives off band of ~ 100 cm^{-1}'s in range of 900-1100 cm^{-1}
- small range but can choose which band used & many compounds have IR absorbance in this region
- much more intense than Blackbody sources

e) Others

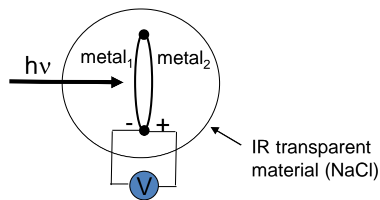
- mercury arc (λ > 50 μm) (far IR)
- tungsten lamp (4000 -12,800cm^{-1}) (near IR)

Detectors. There are two main types in common IR instruments:

a) Thermal Detectors

Thermocouple

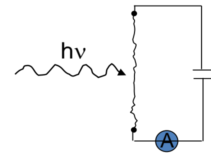
- two pieces of dissimilar metals fused together at the ends
- when heated, metals heat at different rates
- potential difference is created between two metals that vary with their difference in temperature
- usually made with the blackened surface (to improve heat absorption)
- placed in an evacuated tube with a window transparent to IR (not glass or quartz)
- IR "hits" and heats one of the two wires.
- can use several thermocouples to increase sensitivity.



Notes:

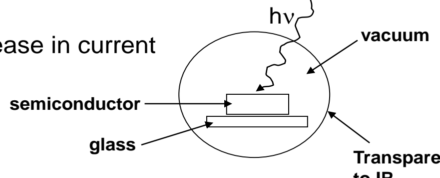
Bolometer

- strips of metal (Pt, Ni) or semiconductor that has a large change in resistance to current with temperature
- as light is absorbed by blackened surface, resistance increases and current decreases
- very sensitive



b) Photoconducting Detectors

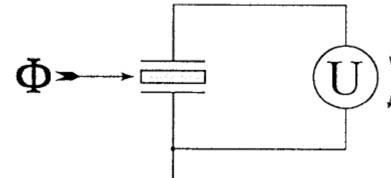
- thin film of semiconductor (ex. PbS) on a nonconducting glass surface and sealed in a vacuum.
- absorption of light by semiconductor moves from non-conducting to conducting state
- decrease in resistance → increase in current
- range: 10,000 -333 cm^{-1} at room temperature

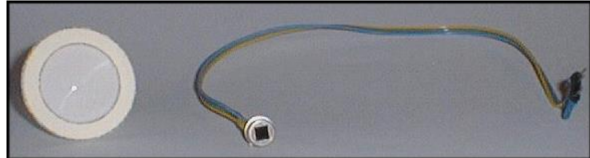


Notes:

c) Pyroelectric Detectors

- pyroelectric (ceramic, lithium tantalate) material get polarized (separation of (+) and (-) charges) in presence of an electric field.
- temperature-dependent polarization
- measure the degree of polarization related to the temperature of the crystal



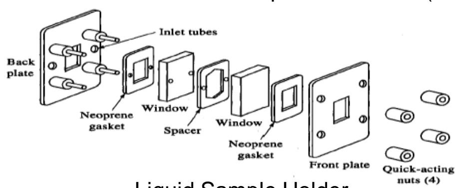


Notes:

Other Components

Sample Cell

- must be made of IR transparent material (KBr pellets or NaCl)

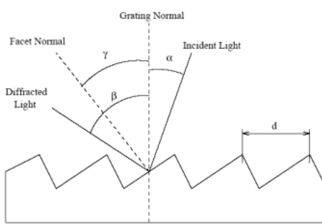


Liguid Sample Holder

NaCl pellets

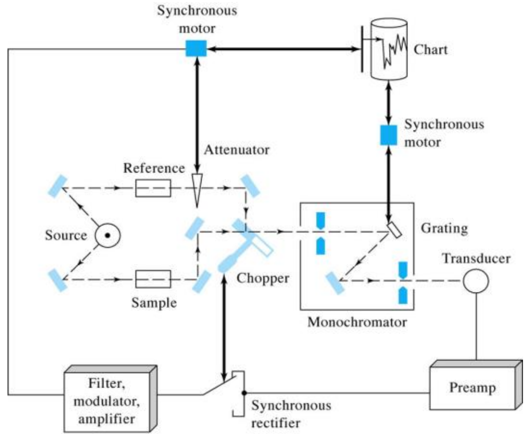
Monochromator

- reflective grating is common
- can't use glass prism, since absorbs IR



Notes:

Overall Instrument Design



Need chopper to discriminate source light from background IR radiation

Monochromator after sample cell

Not done in UV-Vis since letting in all hν to sample may cause photodegradation (too much energy)

IR lower energy

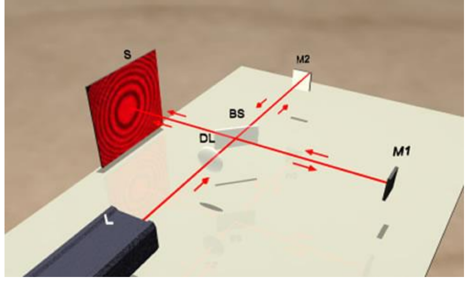
The advantage is that allows monochromator to be used to screen out more background IR light

Problems:
Source weak, need long scans
Detector response slow – rounded peaks

Notes:

Fourier Transfer IR (FTIR) – alternative to Normal IR

Based on Michelson Interferometer



Principal:

1) light from source is split by central mirror into two beams of equal intensity

2) beams go to two other mirrors, reflected by central mirror, recombine and pass through sample to detector

3) two side mirrors.

One fixed and other movable:

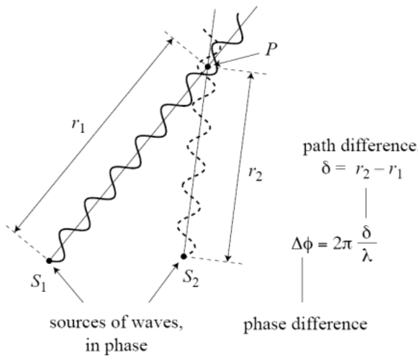
a) move second mirror, light in two-paths travel different distances before recombined;

b) constructive & destructive interference

c) as mirror is moved, get a change in signal

Notes:

Remember: Destructive Interference can be created when two waves from the same source travel different paths to get to a point.



This may cause a difference in the phase between the two waves.

- If the paths differ by an integer multiple of a wavelength, the waves will also be in phase.
- If the waves differ by an odd multiple of half a wave then the waves will be 180 degrees out of phase and cancel out.

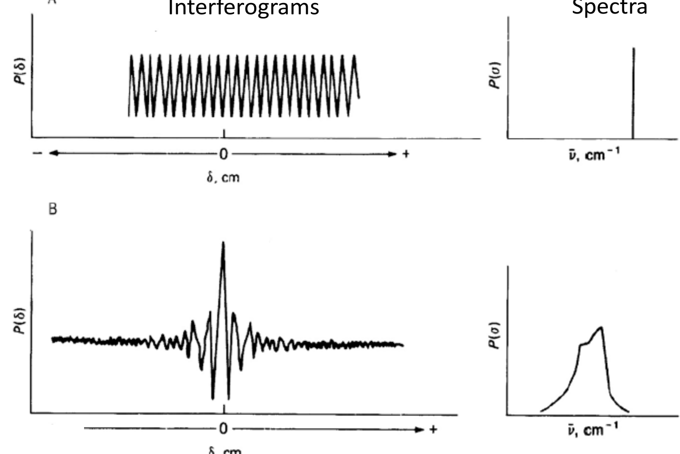
Notes:

- observe a plot of Intensity vs. Distance (interferograms)
- convert to plot of Intensity vs. Frequency by doing a Fourier Transform

$$I(x) = \int_0^{\infty} B(\nu)(1 + \cos 2\pi\nu x)d\nu$$

- resolution $\Delta\nu = 1/\Delta\delta$ (interval of distance traveled by mirror)

Fourier transform pairs for (A) a monochromatic source and (B) a broadband source



Notes:

Advantages of FTIR compared to Normal IR:

1) much faster, seconds vs. minutes
2) use signal averaging to increase signal-to-noise (S/N)

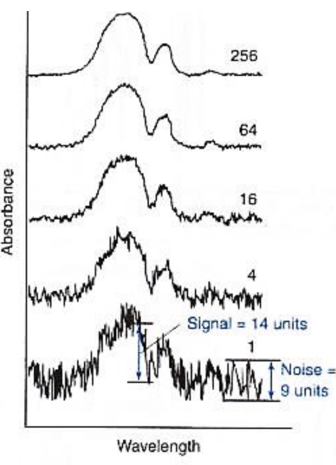
$$increase\ S/N \propto \sqrt{number\ scans}$$

3) higher inherent S/N – no slits, less optical equipment, higher light intensity
4) high resolution (<0.1 cm^{-1})

Disadvantages of FTIR compared to Normal IR:

1) single-beam, requires collecting blank
2) can't use thermal detectors – too slow

In normal IR, scan through frequency range. In FTIR collect all frequencies at once.



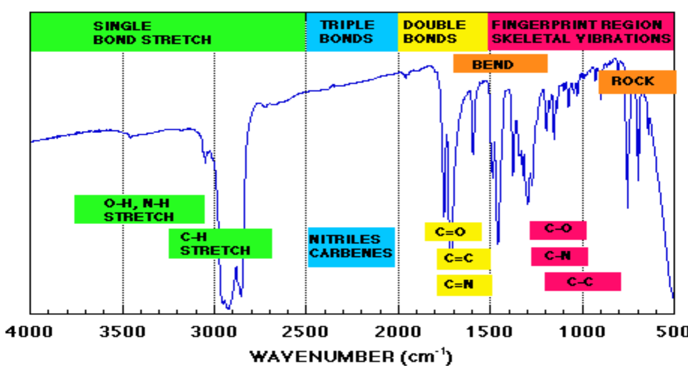
Notes:

Application of IR

Qualitative Analysis (Compound Identification) is the main application. Use of IR, with NMR and MS, in the late 1950s revolutionized organic chemistry. It is decreased the time to confirm compound identification 10-1000 fold.

General Scheme

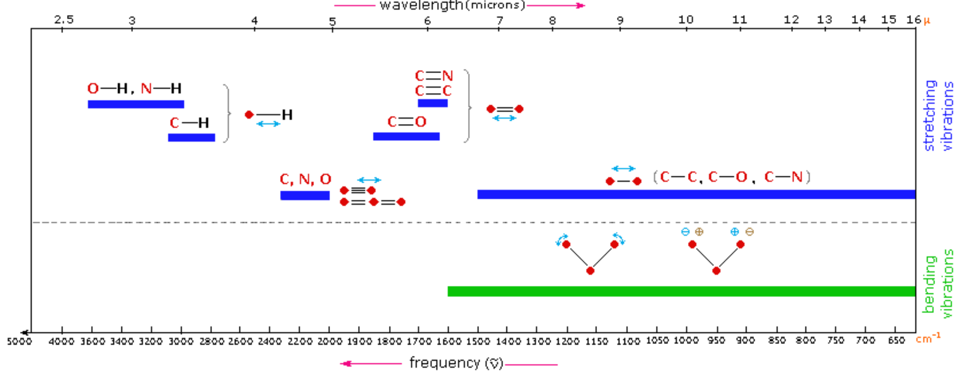
Examine what functional groups are present by looking at group frequency region from 3600 cm^{-1} to 1200 cm^{-1}



Notes:

Group Frequency Region

- approximate frequency of many functional groups (C=O, C=C, C-H, O-H) can be calculated from atomic masses & force constants
- positions changes a little with neighboring atoms, but often in same general region
- serves as a good initial guide to compound identity, but not positive proof.



Notes:

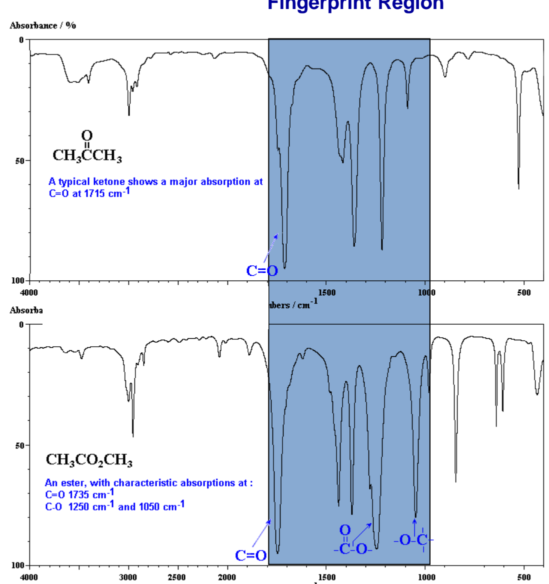
Abbreviated Table of Group Frequencies for Organic Groups

Bond	Type of Compound	Frequency Range, cm^{-1}	Intensity
C-H	Alkanes	2850-2970	Strong
C-H	Alkenes	3010-3095 675-995	Medium strong
C-H	Alkynes —C≡C—H	3300	Strong
C-H	Aromatic rings	3010-3100 690-900	Medium strong
0-H	Monomeric alcohols, phenols Hydrogen-bonded alchohols, phenols Monomeric carboxylic acids Hydrogen-bonded carboxylic acids	3590-3650 3200-3600 3500-3650 2500-2700	Variable Variable, sometimes broad Medium broad
N-H	Amines, amides	3300-3500	medium
C=C	Alkenes	1610-1680	Variable
C=C	Aromatic rings	1500-1600	Variable
C≡C	Alkynes	2100-2260	Variable
C-N	Amines, amides	1180-1360	Strong
C≡N	Nitriles	2210-2280	Strong
C-O	Alcohols, ethers,carboxylic acids, esters	1050-1300	Strong
C=O	Aldehydes, ketones, carboxylic acids, esters	1690-1760	Strong
NO_2	Nitro compounds	1500-1570 1300-1370	Strong

Notes:

Fingerprint Region
(1200-700 cm^{-1})

- region of most single bond signals
- many have similar frequencies, so affect each other & give pattern characteristics of overall skeletal structure of a compound
- the exact interpretation of this region of spectra seldom possible because of complexity
- complexity → uniqueness

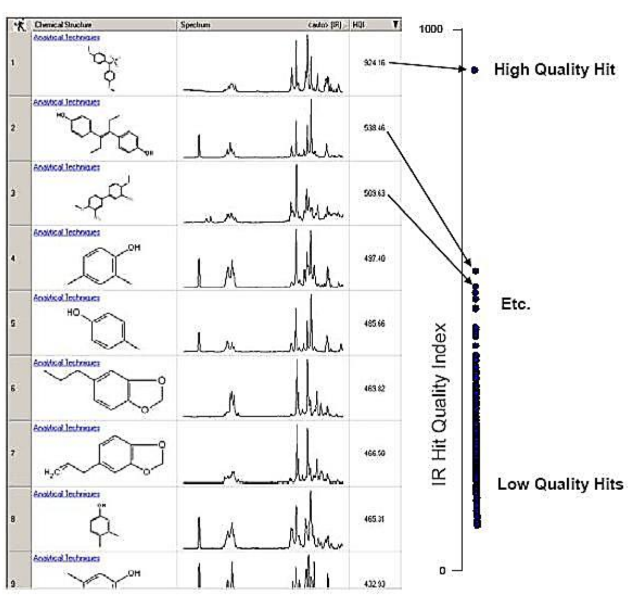


Notes:

Computer Searches

- many modern instruments have reference IR spectra on file (~100,000 compounds)
- matches based on location of strongest band, then 2nd strongest band, etc overall skeletal structure of a compound
- exact interpretation of this region of spectra seldom possible because of complexity
- complexity → uniqueness

Bio-Rad SearchIT
database of ~200,000 IR spectra



Notes:

Quantitative Analysis

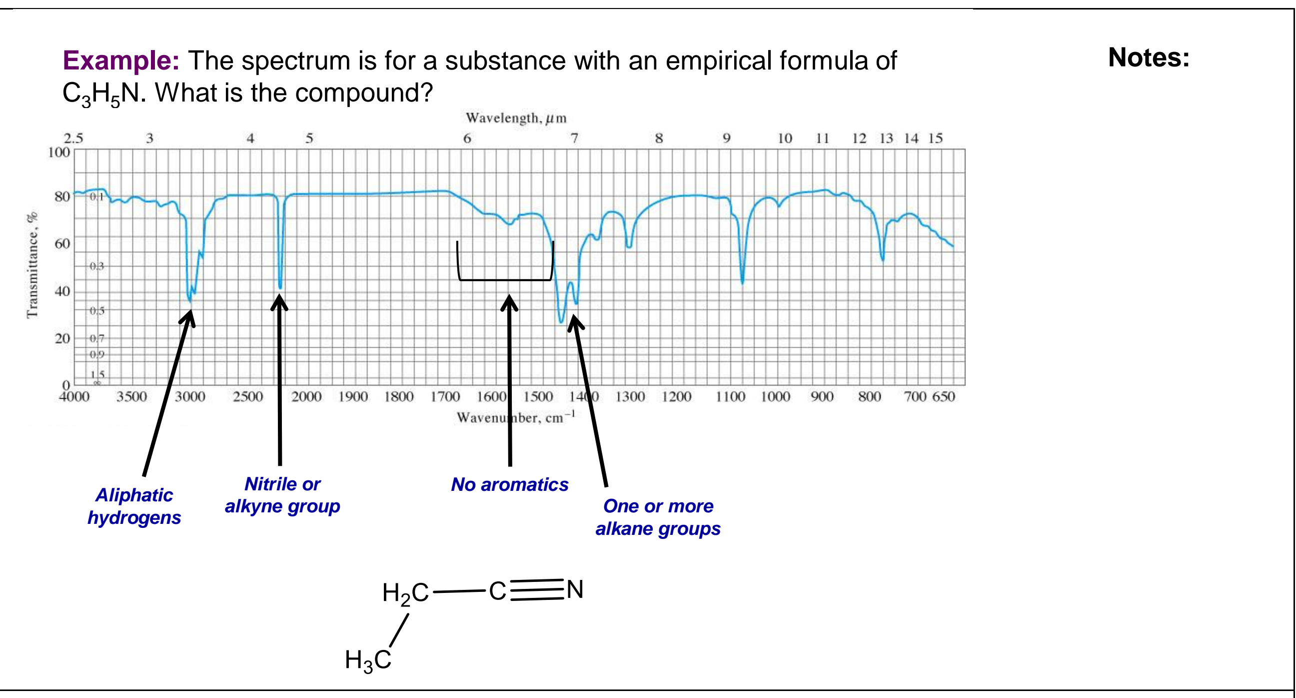
- not as good as UV/Vis in terms of accuracy and precision
  - ► more complex spectra
  - ► narrower bands (Beer's Law deviation)
  - ► limitations of IR instruments (lower light throughput, weaker detectors)
  - ► high background IR
  - ► difficult to match reference and sample cells
  - ► changes in ε (A=εbc) common
- potential advantage is good selectivity, since so many compounds have different IR spectra
  - ► one common application is determination of air contaminants.

Contaminants	Concn, ppm	Found, ppm	Relative error, %
Carbon Monoxide	50	49.1	1.8
Methylethyl ketone	100	98.3	1.7
Methyl alcohol	100	99.0	1.0
Ethylene oxide	50	49.9	0.2
chloroform	100	99.5	0.5

Notes:

Example: The spectrum is for a substance with an empirical formula of C_3H_5N. What is the compound?

Notes:



Summary

Notes:

Region	Source	Sample holder	Detector
Ultraviolet	Deuterium lamp	Quartz/fused silica	Phototube, Photo Multiplier tube, diode array
Visible	Tudngsten lamp	Silicate Glass/ Quartz	Phototube, photo Multiplier tube, diode array
Infrared	Nernst glower (rare earth oxides or silicon carbide glowers)	Salt crystals (crystalline sodium chloride)	Thermocouples, bolometers

Tasks to Section 11.

1. Give definitions of these terms: selective absorption, absorption spectrometry, molecular absorption, molar absorptivity, absorption spectra, calibration graph, photometer, spectrophotometer, monochromator, phototube, photomultiplier tube.

2. A sample has a per cent transmittance of 50%. What is its absorbance?

3. What is the %T for a sample if its absorbance is 1.27?

4. A $5.00 \cdot 10^{-4}$ M solution of an analyte is placed in a sample cell with a pathlength of 1.00 cm. When measured at a wavelength of 490 nm, the solution's absorbance is 0.338. What is the analyte's molar absorptivity at this wavelength?

5. Pure hexane has negligible ultraviolet absorbance above a wavelength of 200 nm. A solution prepared by dissolving 25.8 mg of benzene (C_6H_6, FM 78.11) in hexane and diluting to 250.0 mL had an absorption peak at 256 nm and absorbance of 0.266 in a 1.0-cm cell. Find the molar absorptivity of benzene at this wavelength.

6. A sample of hexane contaminated with benzene had an absorbance of 0.070 at 256 nm in a cuvette with a 5.0-cm pathlength. Find the concentration of benzene in mg/L.

7. Standard solutions of 0.1, 0.25, 0.5, 1.0 and 1.5 mL volumes, containing 1 mg/mL of phosphorus, were added to 25 mL volumetric flasks. Ammonium molybdate solution was then added and brought the volume to the mark. The optical density of these solutions was determined as follows:

V, mL	1.0	2.5	5.0	10.0	15.0
$C_{(P)}$, mg/mL					
A	0.03	0.07	0.13	0.24	0.36

An alloy sample of 0.50 g mass was dissolved in a 100 ml flask. The solution was analysed in the same way as the standard solutions; the value of its optical density was obtained to be Ax = 0.16. Plot a calibration graph and determine the phosphorus content (in %) in the alloy.

9. The volumes of standard solutions containing 1.25 mg/mL of manganese, listed in the table below, were added to 25 mL volumetric flasks. The volumes were brought to the mark. The prepared solutions were analysed, and the following data were obtained:

V, mL	1.0	2.0	3.0	4.0	5.0
$C_{(Mn)}$, mg/mL					
A	0.20	0.40	0.60	0.80	0,97

A 0.50 g sample of ore was dissolved in a 250 ml flask, and it was analysed in the same way as the standard solutions. Its optical density was measured to be Ax = 0.320. Plot a graph of the dependence A versus C_{Mn} and determine the content (in %) of manganese in the ore.

Section 12: Atomic Absorption Spectroscopy

Contents:

- Introduction
- Principles of atomic spectroscopy
- Atomic absorption spectroscopy
- Instrumentation in atomic absorption spectroscopy
- Quantitative applications
- Evaluation of atomic absorption spectroscopy
- Selection of the proper atomic spectroscopic technique

Introduction

At the end of the nineteenth century, spectroscopy was limited to the absorption, emission, and scattering of visible, ultraviolet, and infrared electromagnetic radiation. Since its introduction, spectroscopy has expanded to include other forms of electromagnetic radiation – such as X-rays, microwaves, and radio waves – and other energetic particles – such as electrons and ions.

Atomic absorption spectroscopy methods are based on the fact that elements in an atomized state absorb light at a characteristic wavelength. In this case, they pass from the ground state to the excited state.

In the process of absorption, the electron moves from the primary energy level to the higher due to photon excitation. It occurs as a result of irradiation with light with a specific frequency that satisfies the condition $E^* - E_0 = h\nu$. In this case, the intensity of the excitatory light of this frequency decreases.

The amount of absorbed light energy is proportional to the number of analyte atoms in the path of radiation propagation.

As in molecular absorption spectroscopy, atomic absorption spectrometry (AAS) has a law similar to the Bouguer-Lambert-Behr law. The decrease in the intensity of excitable light is characterized by the magnitude of "atomic absorption" or "absorption". Absorption will increase with increasing concentration of the test substance.

Comparison with the method of molecular absorption shows that the sensitivity of the atomic absorption method is much higher.

For atomisation, the sample requires a temperature of 2000-3500 C. In this temperature range, more than 90% of the atoms are unexcited. Under such conditions, the atoms and molecules surrounding the atoms of the studied element have almost no effect on the amount of its absorption. This fact, along with the small number of absorption lines, causes high selectivity of the atomic absorption method.

Measurements in atomic absorption spectroscopy are carried out by the method of calibration graph or the addition technique. Calibration is performed by introducing known concentrations of analyte atoms along the path of radiation propagation and plotting the concentration against absorption. Special standard samples of solutions are used for calibration.

Atomic Spectroscopy

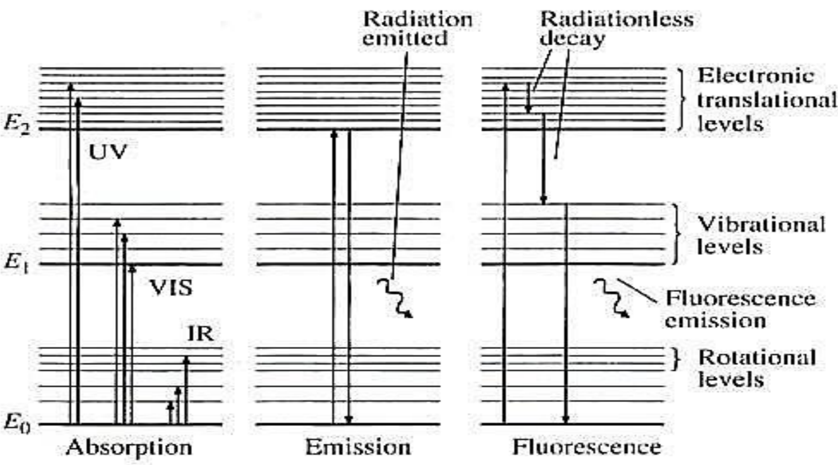
technique for determining the elemental composition of an analyte by its electromagnetic or mass spectrum

atomic emission, atomic absorption, atomic fluorescence

Atomic spectroscopic methods normally are classified according to the type of spectral process involved and the method of atomization

Notes:

Three types of spectroscopy:
absorption, emission, fluorescence

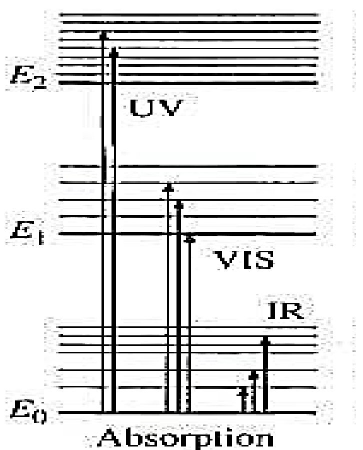


Notes:

Atomic absorption is a technique in which the absorption of light by free gaseous atoms in a plasma, flame, or furnace is used to measure the concentration of atoms.

In the process of absorption, the electron passes from the basic energy level to the higher one due to photonic excitation under the influence of light irradiation with a certain frequency, which satisfies the condition $E^* - E_0 = h\nu$

In this case, the intensity of the excitation light of this frequency decreases.



E_0 = Ground level;
E_1 , E_2 = Excited states;
Energy spacing:
vibration>rotation>translation

Notes:

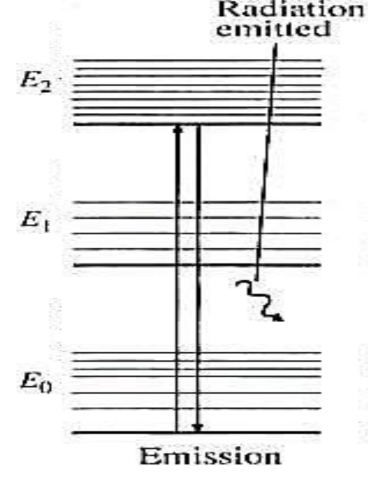
Atomic emission

A technique in which the emission of light by thermally excited atoms in a flame or furnace is used to measure the concentration of atoms.

Atoms are excited in a thermal way (flame, arc, spark, plasma sources).

After ~ 10^{-7} s, the excited electron returns to its ground state, in which case light with frequency v is emitted according to the expression

$$E_i - E_0 = hv$$



E_0 = Ground level;
E_1 , E_2 = Excited states;
Energy spacing:
vibration>rotation>>translation

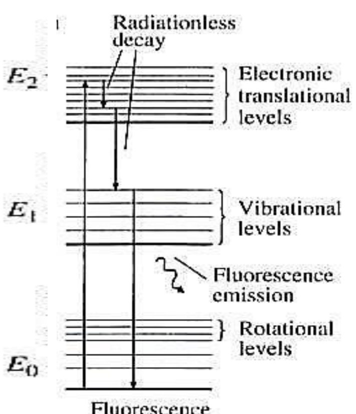
Notes:

Atomic fluorescence

A technique in which electronic transitions of atoms in a flame, furnace, or plasma are excited by light, and the fluorescence is observed at a right angle to the incident beam.

The atomic fluorescence method is based on photon excitation of electrons.

Fluorescence - radiation associated with the return of the excited electron to the ground state, is detected at right angles to the direction of the exciting radiation.



E_0 = Ground level;
E_1 , E_2 = Excited states;
Energy spacing:
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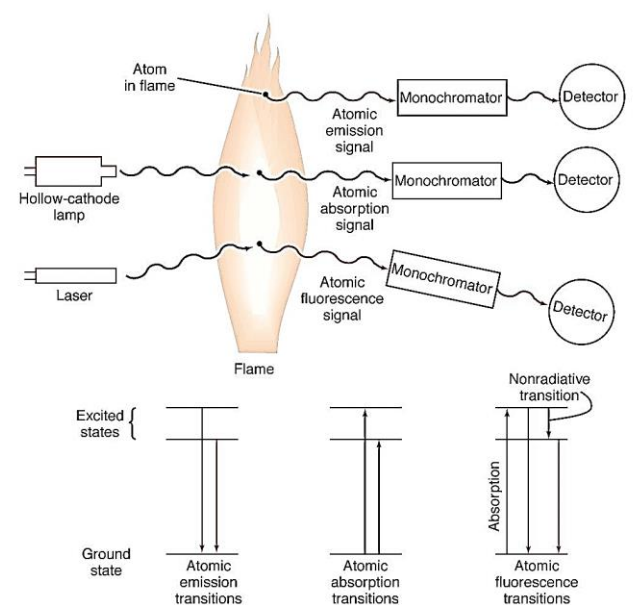
Notes:

Schematic representation of absorption, emission, and fluorescence.



Notes:

Classification of atomic spectroscopic methods

Atomization method	Typical atomization temperature, °C	Types of spectroscopy	Common name and abbreviation
Inductively coupled plasma	6000-8000	Emission Mass	Inductively coupled plasma atomic emission spectroscopy, ICPAES Inductively coupled plasma mass spectrometry, ICP-MS
Flame	1700-3150	Absorption Emission Fluorescence	Atomic absorption spectroscopy, AAS Atomic emission spectroscopy, AES Atomic fluorescence spectroscopy, AFS
Electrothermal	1200-1300	Absorption Fluorescence	Electrothermal AAS Electrothermal AFS
Direct-current plasma	5000-10000	Emission	DC plasma spectroscopy, DCP
Electric arc	3000-8000	Emission	Arc-source emission spectroscopy
Electric spark	Varies with time and position	Emission Mass	Spark-source emission spectroscopy Spark-source mass spectroscopy

Notes:

Atomic absorption spectrometry

Atomic absorption is the process that occurs when a ground state atom absorbs energy in the form of electromagnetic radiation at a specific wavelength and is elevated to an excited state. The atomic absorption spectrum of an element consists of a series of resonance lines, all originating with the ground electronic state and terminating in various excited states. Usually the transition between the ground state and the first excited state is the line with the strongest absorptivity, and it is the line usually used.

Transition between the ground state and excited state occur only when the incident radiation from a source is exactly equal to the frequency of a specific transition. Part of the energy of the incident radiation I_o is absorbed.

Atomic absorption is determined by the difference in radiant power of the resonance line in the presence and absence of analyte atoms in the flame. The width of the line emitted by the light source must be narrower than the width of the absorption line of the analyte in the flame.

Notes:

Just like in molecular absorption spectroscopy, a law similar to the Bouguer-Lambert-Behr law applies in atomic absorption (AA) spectrometry.

$$A = \lg (I_0 / I) = kbC,$$

where
A is the value that characterizes the absorption of light (optical density);
I_0 is the intensity of excitation radiation;
I is the intensity of the radiation that has passed;
k is the absorption coefficient;
b is the thickness of the absorbent layer;
C is the concentration of the element to be determined.

Notes:

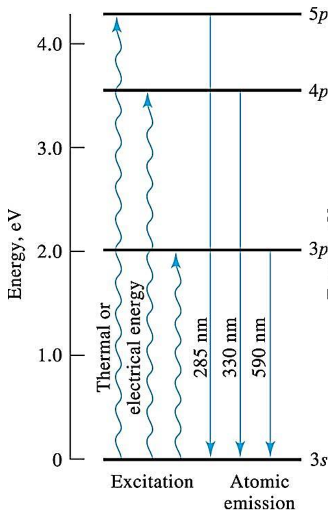
The relationship between light absorption and concentration is linear. The absorption coefficient is proportional to the probability of this transition. Of course, the highest values of k correspond to the transition from the basic to the nearest level (the so-called **"resonance line"**).

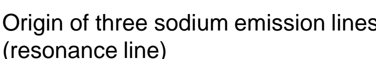
Notes:

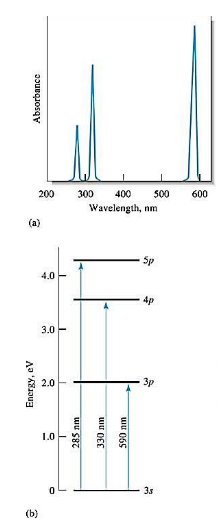
For example, for sodium, the transition is 3s → 3p (589 nm).

A further transition of 3s → 4p (330 nm) is already 100 times less likely, so the limit of sodium determination by the atomic absorption method for line with 330 nm is 100 times higher than the 589 nm line.

If C is expressed in grams of atoms per liter, then for almost all elements k = 107 ÷ 109. Comparison with the photometric method, where the maximum value of the molar absorption coefficient 105, shows that the sensitivity of the atomic absorption method is much higher.







(a) Partial absorption spectrum for sodium vapor.

(b) Electronic transitions responsible for the absorption lines in (a)

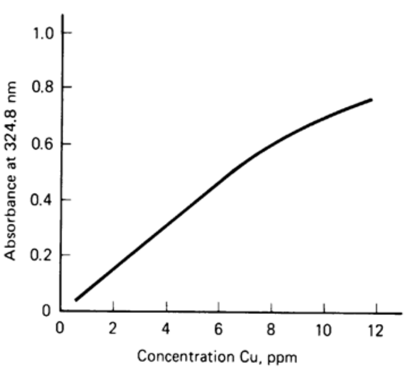
Methodology:

Establishing a relationship between absorbance and concentration

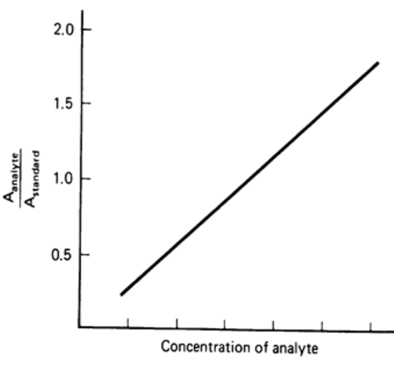
Standard curve method

Standard addition method

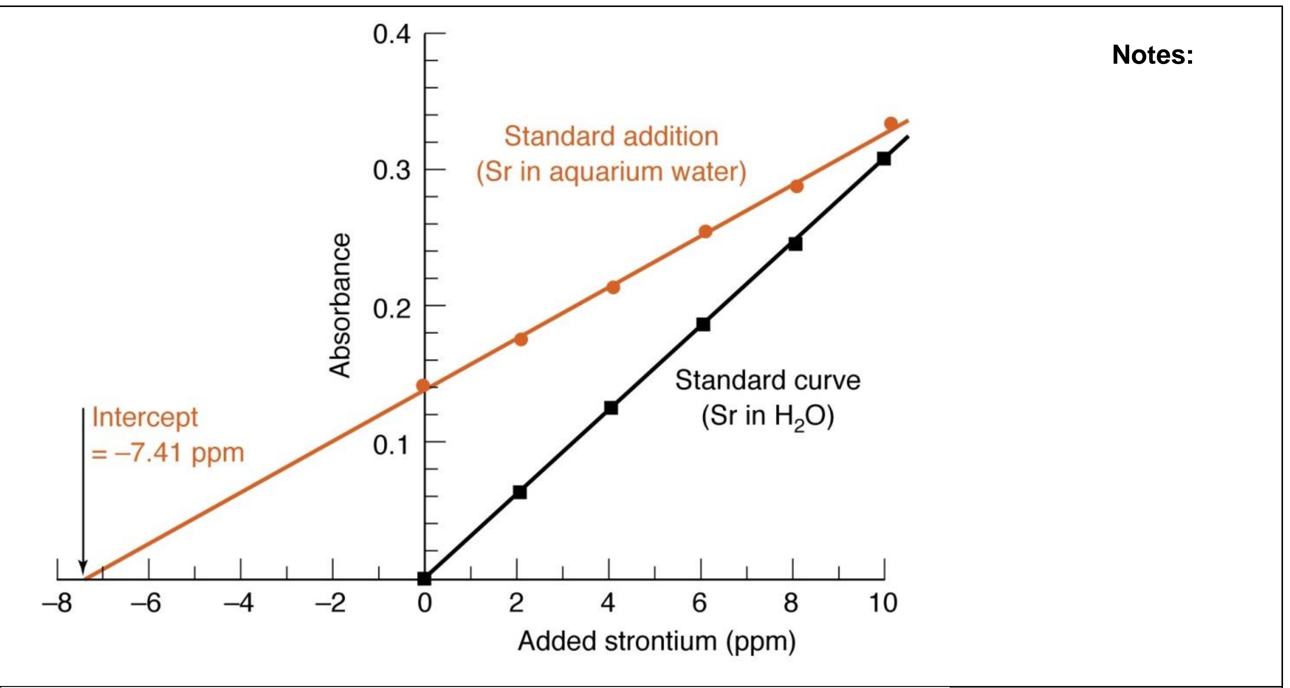
Internal standard method



Standard calibration curve for copper



Internal standard calibration curve



Notes:

Atomic absorption analysis equipment

Notes:

The first AA spectroscopy experience was conducted by Walsh and published in 1955. Subsequently, flame AA spectrometry has been widely developed and has long been one of the main methods of analysis in various fields of science and technology.

The most well-known foreign companies that manufacture equipment for atomic absorption analysis –

Perkin-Elmer (USA),

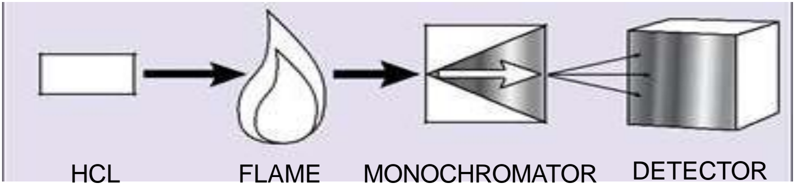
Varian-Techtron (Australia),

Hitachi (Japan).

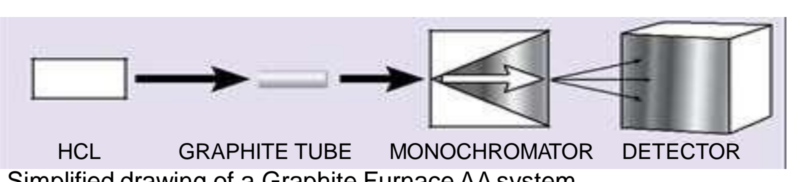
AAS-type Carl Zeiss (Jena, Germany) appliances are widely used.

Different atomic spectroscopy systems

Notes:



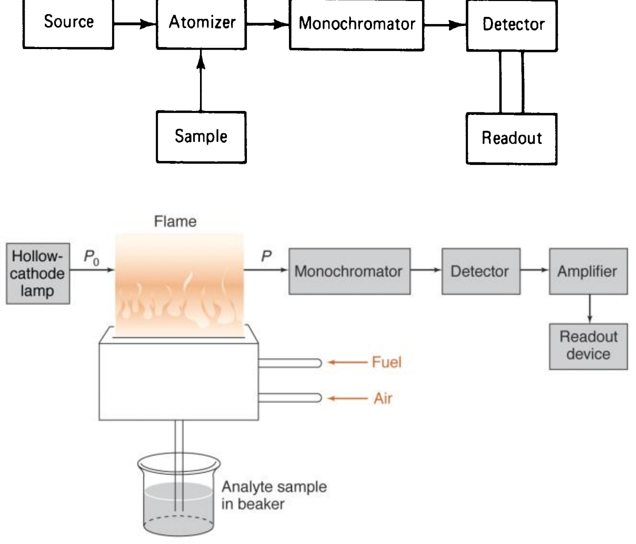
Simplified drawing of a Flame AA system.

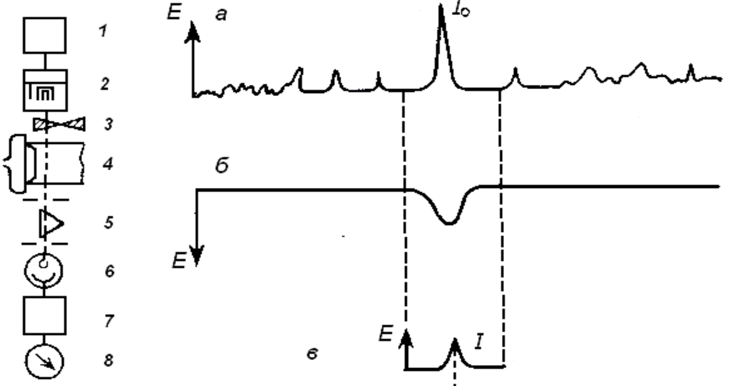


Simplified drawing of a Graphite Furnace AA system.

Basic components of an atomic spectrophotometer

Notes:

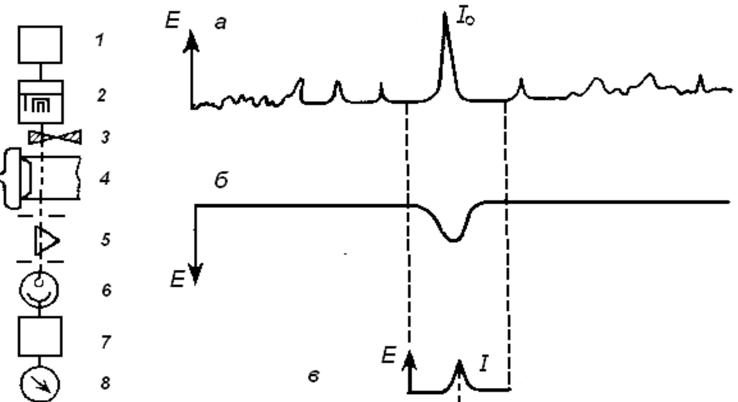




Notes:

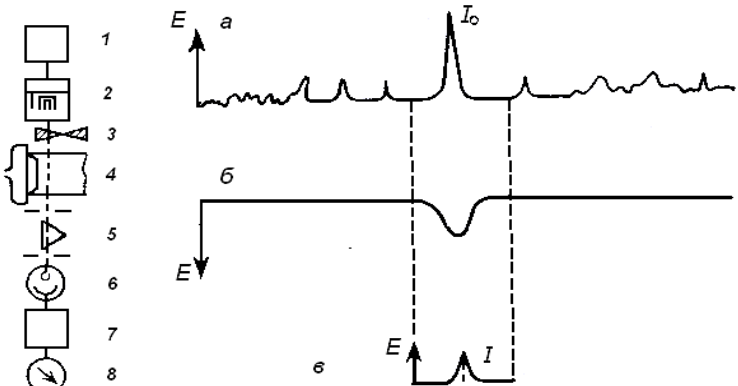
Schematic diagram of AA method

The source (2) emits a linear spectrum containing the desired element line. In the atomizer (4) (using flame and non-flame atomizers), the sample is converted into an atomic vapor that absorbs light of the corresponding wavelength. As a result of the atomic absorption, the initial intensity of I_0 decreases to I.



Notes:

The monochromator (5) emits a narrow spectral band (usually 0.2–2 nm) within which the measured spectral line of the determining element is located. The modulator (3) interrupts the flow of light from the source by mechanical or electrical means.



The detector (6) converts the light beam into an electrical signal to record the absorption value. The analytical signal measuring device (8) is synchronized with the modulator and responds only to the intermittent source signal.
In this way, the radiation of the atomizer is eliminated - it is constant in time and causes a constant current in the detector, to which the device does not respond.

Notes:

Notes:

A modern spectrophotometer is an analytical unit consisting of individual units and devices.

At the request of the experimenter, depending on the nature of the analytical task, different variants of analytical installations are mounted, using one or another combination of individual instruments and blocks.

Notes:

In any spectrometer, it is necessary to have the same set of components to produce and analyze the spectrum.

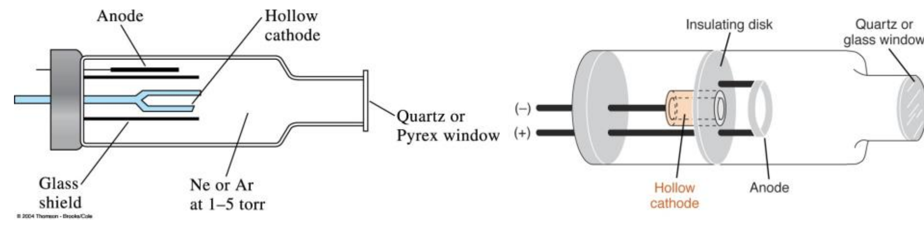
While each region of the spectrum and each particular technique requires its own specific modules, the basic parts of each set are the same:

the **source,**
the **sample,**
the **dispersion element,**
the **detector**
and the **display** or **data processor**.

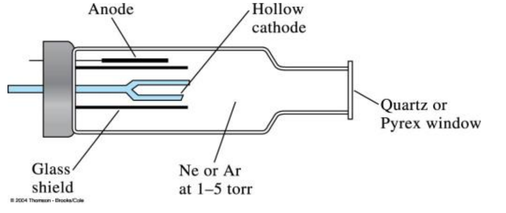
Sources

Hollow cathode lamp

These lamps consist of a cylindrical metallic cathode(the same element as that being analyzed) and tungsten anode sealed in a glass tube containing **neon** or argon at a pressure of about 1 to 5 torr. When high voltage is applied between the anode and cathode, the filler gas is ionized and positive ions are accelerated toward the cathode. They strike the cathode with enough energy to "sputter" metal atoms from the cathode surface into the gas phase. The free atoms are excited by collisions with high-energy electrons and then emit photons to return to the ground state. This radiation has the same frequency as that absorbed by analyte atoms in the flame or furnace.



Notes:



A hollow cathode lamp

Notes:

In order to measure the magnitude of the atomic absorption A, the two conditions formulated by Walsh are required:

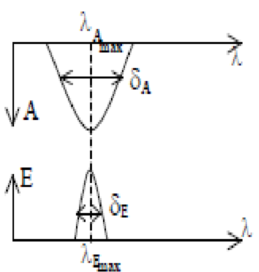
• $\lambda_{Emach} = \lambda_{Amah}$, that is, the wavelength corresponding to the maximum absorption of atomic vapors λ_{Amah} must be equal to the wavelength of the maximum radiation intensity of the source λ_{Emach};

• $\delta A \geq 2\delta E$, ie the half-width of the atomic vapor absorption line δA must be at least twice the half-width of the source line δE.

Notes:

These conditions can be illustrated.

If the first condition is not fulfilled, atomic absorption does not occur at all. Unless the second Walsh condition is satisfied, only a small fraction of the source radiation is absorbed by the atoms (because the contour of the emission line is wider than the contour of the absorption line). This leads to a sharp deterioration in the sensitivity of the atomic absorption determination.

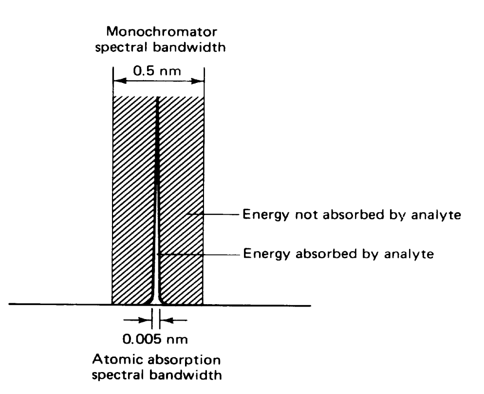


The half-width of the atomic absorption line is less than 0.01 nm. Therefore, the half-width of the corresponding radiation band should be less than 0.005 nm.

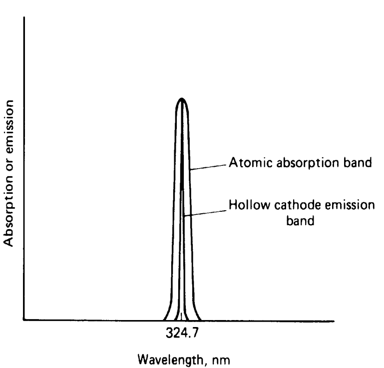
Notes:

Atomic absorption bandwidths are so narrow, generally in the range 0.002 to 0.005 *nm*. The narrowest band of wavelengths that can be isolated from a continuum with best monochromator is about 0.5 *nm*.

At a proper conditions, the bandwidth of emitted radiation with hollow cathode lamp is even narrower than the atomic absorption bandwidth.

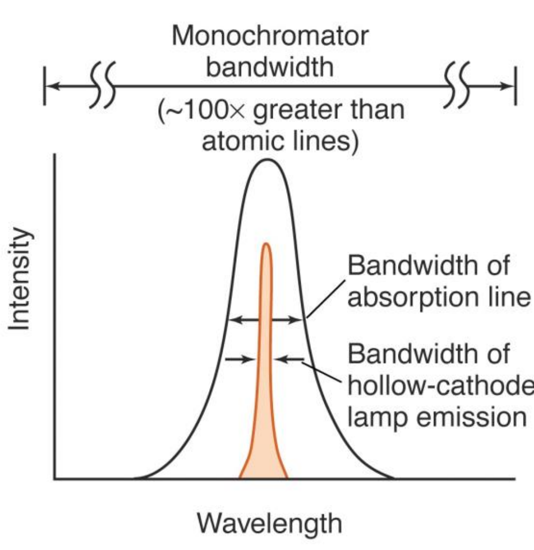


Comparison of atomic absorption and monochromator spectral bandwidths.



Relative line widths for copper emission and absorption.

Notes:



Notes:

Line broadening

The linewidth of the source must be narrower than the linewidth of the atomic vapour for Beer's law to be obeyed.

Doppler broadening :

The wavelength of radiation emitted or absorbed by a fast moving atom decreases if the motion is toward a detector and increases if the atom is receding from the detector. The linewidth, $\Delta\nu$, due to the Doppler effect, is given approximately by

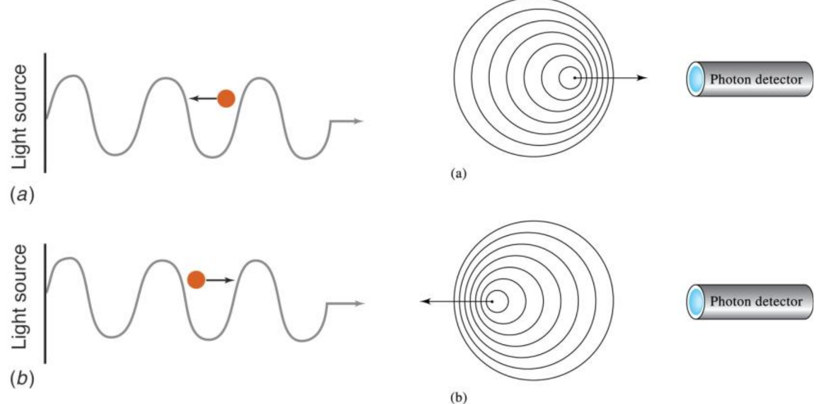
$$\Delta\nu \approx \nu(7\times10^{-7})(T/M)^{-1/2}$$

where is the frequency (Hz) of the peak, T is temperature (K), M is the mass of the atom.

Pressure broadening:

Pressure or collisional broadening arises from collisions of emitting or absorbing species with other atoms or ions in the heated medium.

Notes:



Cause of Doppler broadening.

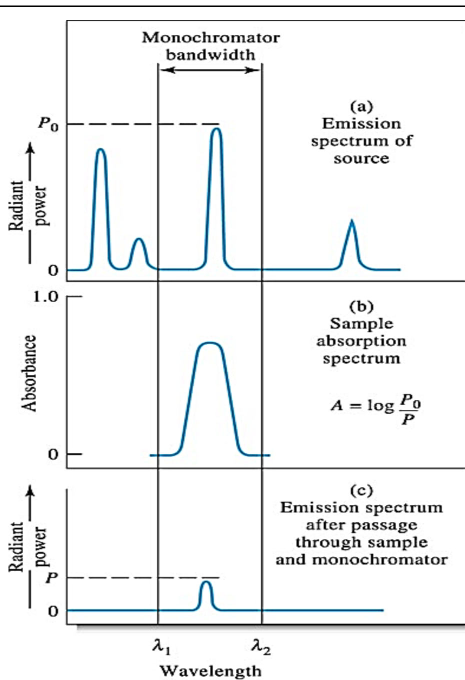
(a) When an atom moves toward a photon detector and emits radiation, the detector sees wave crests more often and detects radiation of higher frequency. (b) When an atom moves away from a photon detector and emits radiation, the detector sees crests less frequently and detects radiation of lower frequency. The result in an energetic medium is a statistical distribution of frequencies and thus a broadening of spectral lines.

Notes:

Atomic absorption of a narrow emission line from a source.

The source lines in (a) are very narrow. One line is isolated by a monochromator. The line is absorbed by the broader absorption line of the analyte in the flame (b) resulting in attenuation (c) of the source radiation.

Since most of the source radiation occurs at the peak of the absorption line, Beer's law is obeyed.



Notes:

At present, the market for atomic absorption devices offers spectrometers with a single source of radiation for all determining elements. Continuous spectrum lamps are used as the source.

The most critical in the choice of such a spectral source is its radiation in the region below 220 nm.

Notes:

They use arc lamps with inert gases, which have a higher emission in the ultraviolet range. Most often - high and ultra high-pressure xenon lamps (initial pressure - about 20 atm).

The radiation spectrum of such lamps is continuous in the range of 190-700 nm, with a maximum of about 500 nm. In the region of 210 nm and less, the intensity of their radiation is significantly reduced, which complicates the determination of As, Se.

Notes:

The disadvantages of high-pressure xenon lamps with short arc are the continuous chaotic movement of the hot cathode spot, which requires its fast and constant focus.

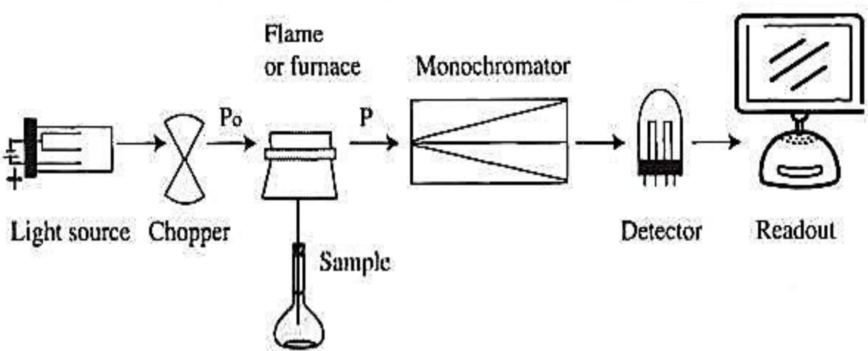
There is a blackening of the bulb of the lamp during operation, which reduces the intensity of the continuous spectrum by 25% after 1000 hours. work. The lamp life is limited (about 1000-1500 hours).

The lamps are of high cost (according to various figures from 1000 to 2650 €).

Notes:

Flame and Flameless Atomic Absorption

Basic instrument components for a single-beam atomic absorption spectrometer



Notes:

Atomic absorption spectrometers are subdivided into

single-channel and multi-channel,

depending on the number of elements determined simultaneously,

as well as

single-beam and double-beam,

depending on the optical scheme.

Notes:

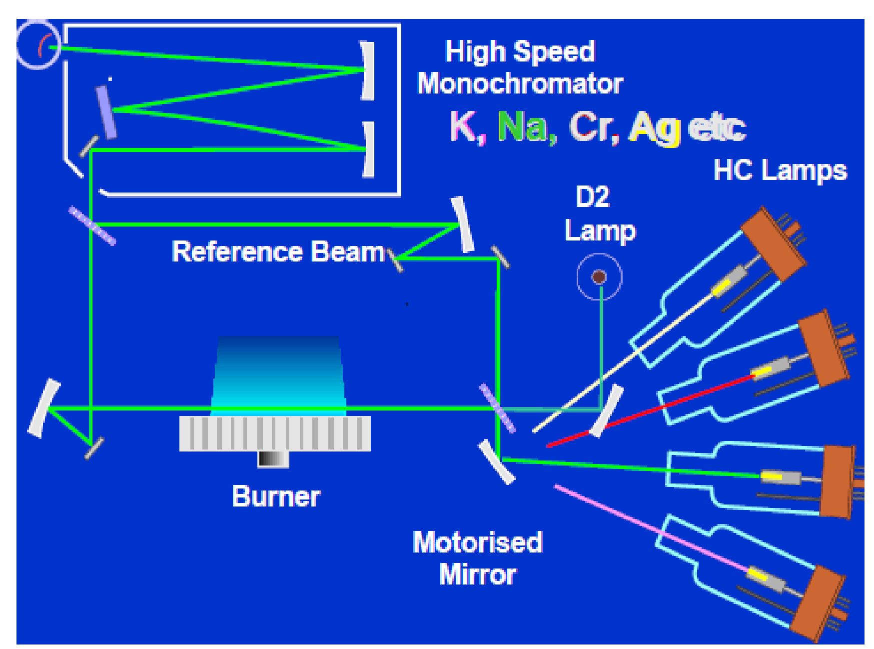
Modern spectrometers are multi-channel

For example, in a two-channel device, the system of rotating mirrors alternately directs light streams from the source A (first channel) and source B (second channel) to the atomizer. Two-channel monochromators are tuned to the corresponding lines of the two elements that are defined. They can be measured almost simultaneously.

However, only in very rare cases can one find the conditions that are optimal for the simultaneous determination of two elements.

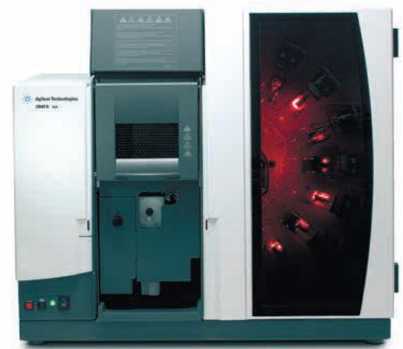
Therefore, multi-element definitions can be called semi-quantitative.

An internal standard method is used to improve the accuracy of multi-element measurements.



Notes:

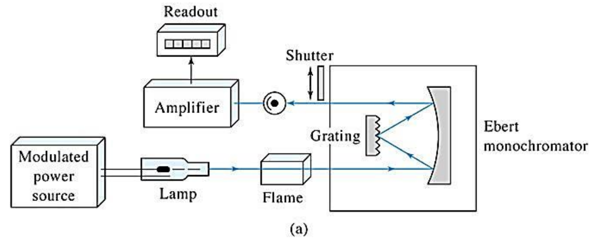
Examples of modern devices include
the Agilent 55B AA / 280FS AA / 280Z AA / AA Duo
spectrophotometer



Notes:

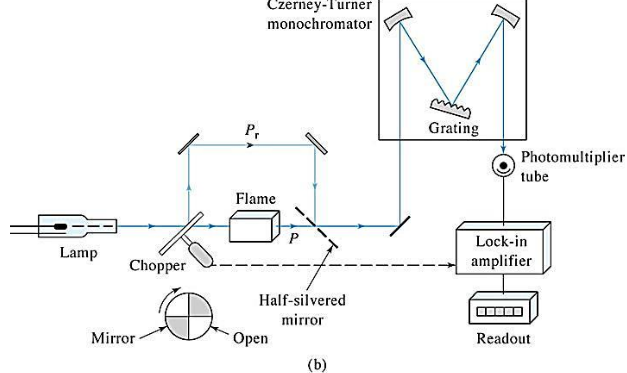
Single beam

The components may be arranged so that **one beam of radiation** only passes along the spectrometric path. Comparisons are then made by interchanging a sample and reference.



Double beam

To correct for changes related to the source and detector, and in order to speed up the analysis, double-beam instruments automatically pass beams through **both the sample and a reference**.



Notes:

Single beam

The simplest type of spectrometer employs a single source to supply radiation to the sample and then to the background in turn.

The advantages of this system are that only a single set of components is required and that a complex sampling device may be incorporated.

The main disadvantage is that correcting for the background spectrum, due to the solvent, matrix or interferences must be done separately, adding to the analysis time.

Notes:

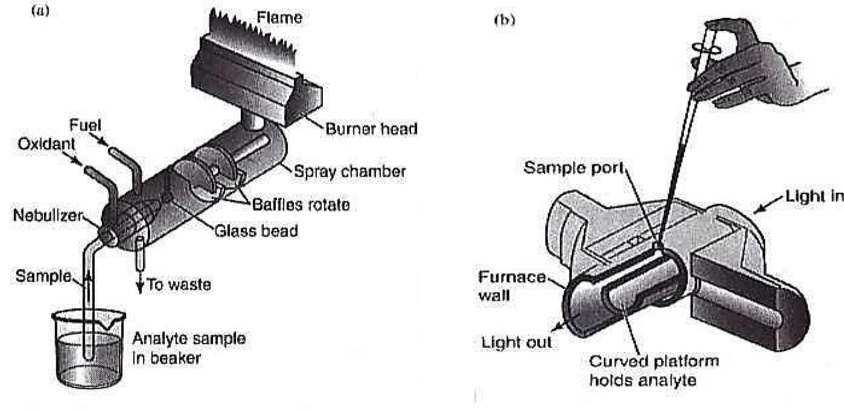
Double beam

In order to make rapid, accurate comparisons of a sample and a reference, double-beam instruments are used. Since it is essential that the two beams are as similar as possible, a single source is used and the optics arranged to pass equal intensities of the beam through the sample area and through the reference area, and then to disperse and detect them alternately.

The source is reflected equally onto mirrors so that beams pass through the sample and reference areas. These beams are then selected alternately by a rotating mirror and each beam follows a common path to the diffraction grating, which disperses the radiation and directs it onto the detector. The width of the beams is controlled by slits, which determine the resolution.

Notes:

Flame and Flameless Atomic Absorption
Basic instrument components: Nebulizer and atomizer



Notes:

Flame Atomic Absorption Spectrometry (FAAS)

In flame atomic absorption spectrometry, either an air/acetylene or a nitrous oxide/acetylene flame is used to evaporate the solution and dissociate the sample into its component atoms. When light from a hollow cathode lamp passes through the cloud of atoms, the atoms of interest absorb the light from the lamp. This is measured by a detector, and used to calculate the concentration of that element in the original sample.

Notes:

Flame Atomic Absorption Spectrometry (FAAS)

Notes:

The use of a flame limits the excitation temperature reached by a sample to a maximum of approximately 2600ºC (with the N_2O/acetylene flame). For many elements this is not a problem. Compounds of the alkali metals, for example, and many of the heavy metals such as lead or cadmium and transition metals like manganese or nickel are all atomized with good efficiency with either flame type, with typical FAAS detection limits in the sub-ppm range.

However, there are a number of refractory elements like V, Zr, Mo and B which do not perform well with a flame source. This is because the maximum temperature reached, even with the N_2O/acetylene flame, is insufficient to break down compounds of these elements. As a result, flame AAS sensitivity for these elements is not as good as other elemental analysis techniques.

Atomic Absorption Spectroscopy

Notes:

– commonly used for elemental analysis
– expose sample to flame or high-temperature
– characteristics of flame impact use of atomic absorption spectroscopy

Flame AAS:

- simplest atomization of gas/solution/solid
- laminar flow burner - stable "sheet" of flame
- flame atomization best for reproducibility (precision) (<1%)
- relatively insensitive - incomplete volatilization, short time in flame

Notes:

The **sample** must be examined with as little change as possible, and sometimes measurements can be made directly with no sample preparation.

Very often, a solution of the sample in a solvent suited to the spectrometric investigation is required.

The term 'spectrometry' indicates measurements made after separating the radiation using a device to **disperse** it.

The sample is generally volatilized by a flame or furnace.

Sample Atomization – expose sample to flame or high-temperature

Need to break sample into atoms to observe atomic spectra

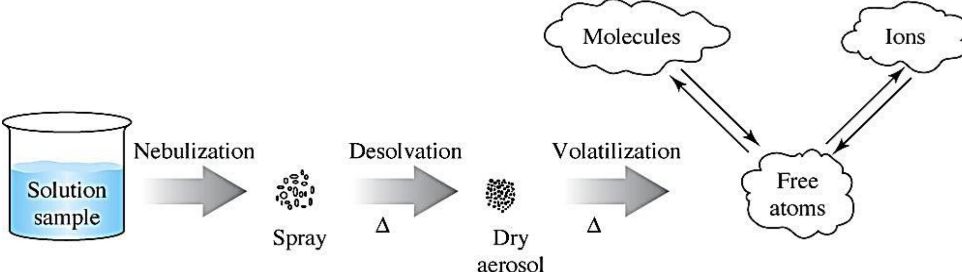
Basic steps:

a) nebulization – solution sample, get into fine droplets by spraying thru thin nozzle or passing over vibrating crystal.

b) desolvation - heat droplets to evaporate off solvent just leaving analyte and other matrix compounds

c) volatilization – convert solid analyte/matrix particles into gas phase

d) dissociation – break-up molecules in gas phase into atoms.



Notes:

Processes occurring during atomization

Solution of analyte

↓ ← Nebulization

Spray(small droplets)

↓ ← Desolvation

Solid/Gas aerosol

↓ ← Volatilization

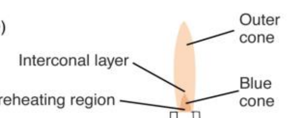
Gaseous molecules → Excited molecules → $h\nu$ molecular

↕ ← Dissociation(reversible)

Atoms → Excited atoms → $h\nu$ atomic

↕ ← Ionization(reversible)

Atomic ions → Excited ions → $h\nu$ atomic



a) Schematic diagram of a premix burner

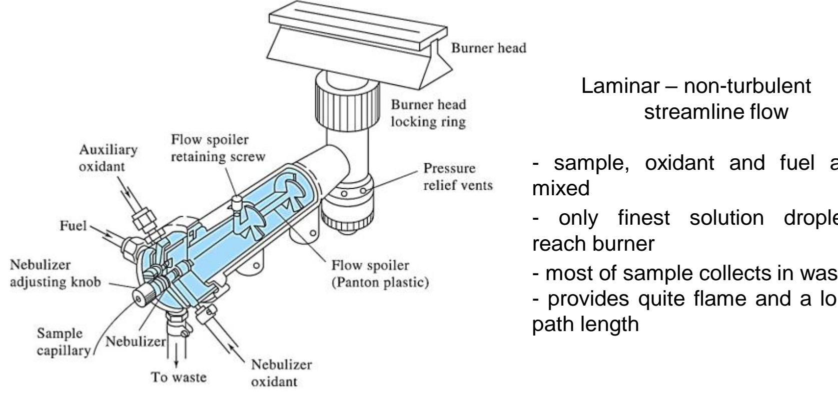
b) b) End view of flame.

Notes:

a) Atomizer

Laminar Flow Burner

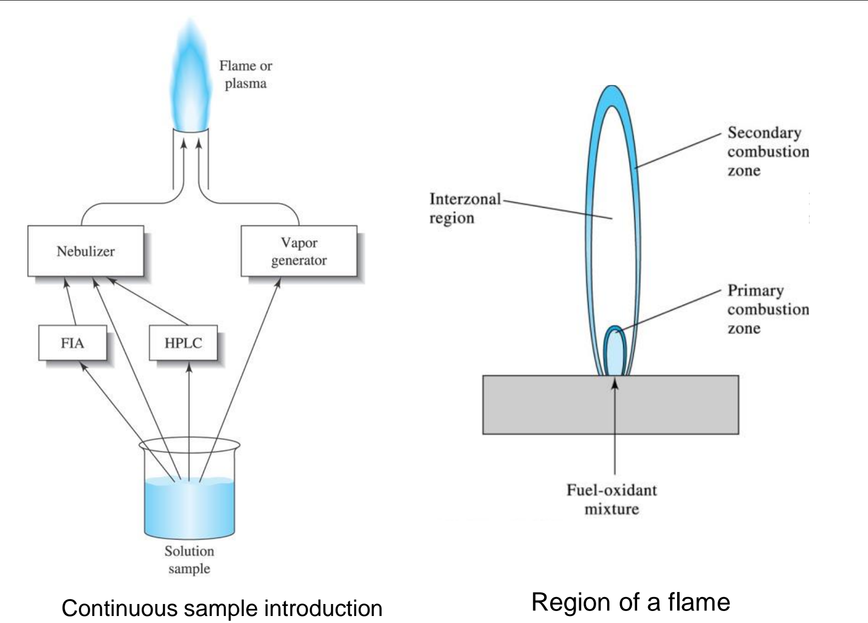
- adjust fuel/oxidant mixture for optimum excitation of desired compounds
- usually 1:1 fuel/oxidant mix but some metals forming oxides use increase fuel mix
- different mixes give different temperatures.



Laminar – non-turbulent streamline flow

- sample, oxidant and fuel are mixed
- only finest solution droplets reach burner
- most of sample collects in waste
- provides quite flame and a long path length

Notes:



Continuous sample introduction methods.

Region of a flame

Notes:

Properties of flames

Fuel	Oxidant	Temperature °C	Maximum burning velocity(cm s^{-1})
Natural gas	Air	1700~1900	39~ 43
Natural gas	Oxygen	2700~2800	370 ~ 390
Hydrogen	Air	2000~2100	300 ~ 440
Hydrogen	Oxygen	2550~2700	900 ~1400
Acetylene	**Air**	2100~2400	158 ~266
Acetylene	Oxygen	3050~3150	1100 ~ 2480
Acetylene	Nitrous oxide	2600~2800	285

Notes:

Comparison of detection limits for various elements by flame absorption and flame emission methods

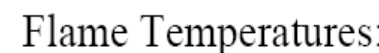
Flame emission more sensitive	Sensitivity about the same	Flame absorption more sensitive
Al, Ba, Ca, Eu,	Cr, Cu, Dy, Er,	Ag, As, Au, B,
Ga, Ho, In, K,	Gd, Ge, Mn, Mo,	Be, Bi, Cd, Co,
La, Li, Lu, Na,	Nb, Pd, Rh, Sc,	Fe, Hg, Ir, Mg,
Nd, Pr, Rb, Re,	Ta, Ti, V, Y,	Ni, Pb, Pt, Sb,
Ru, Sm, Sr, Tb	Zr	Se, Si, Sn, Te,
Tl, Tm, W, Yb		Zn

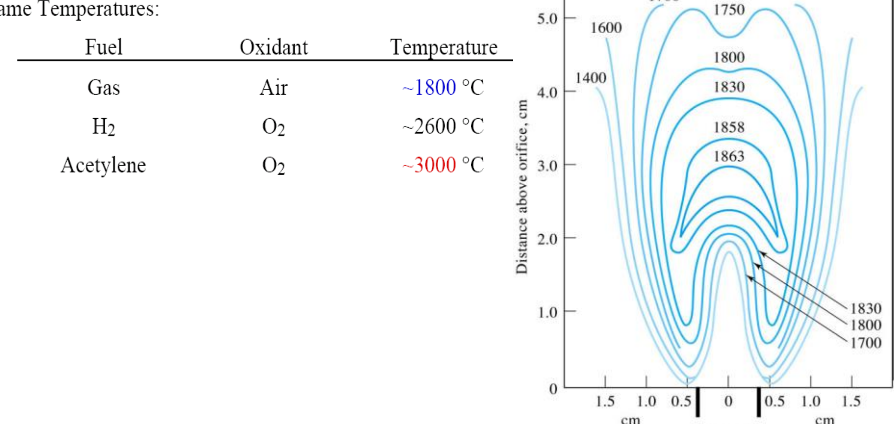
Notes:

Different mixes and flow rates give different temperature profile in flame

- gives different degrees of excitation of compounds in path of light source

Notes:





Fuel	Oxidant	Temperature
Gas	Air	~1800 °C
H_2	O_2	~2600 °C
Acetylene	O_2	~3000 °C

Types of Flame/Flame Structure – selection of correct flame region is important for optimal performance

Notes:

a) primary combustion zone – blue inner cone (blue due to emission from C_2, CH and other radicals)

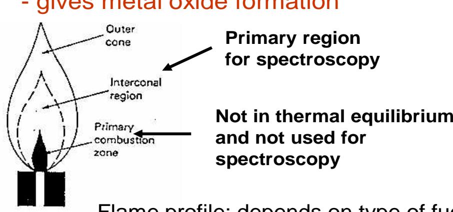
- not in thermal equilibrium and not used

b) interconal region

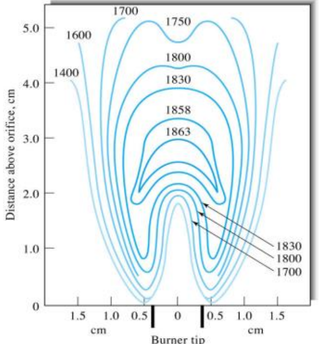
- region of highest temperature (rich in free atoms)
- often used in spectroscopy
- can be narrower in some flames (hydrocarbon) tall in others (acetylene)

c) outer cone

- cooler region
- rich in O_2 (due to surrounding air)
- gives metal oxide formation



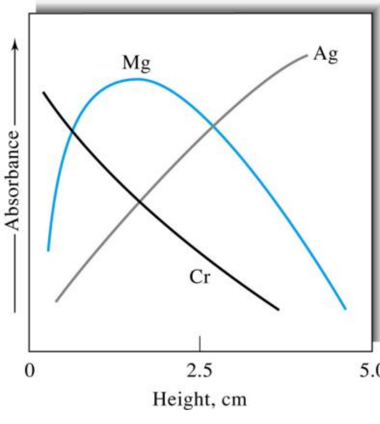
Flame profile: depends on type of fuel and oxidant and mixture ration



Temperature varies across flame – need to focus on correct part of flame

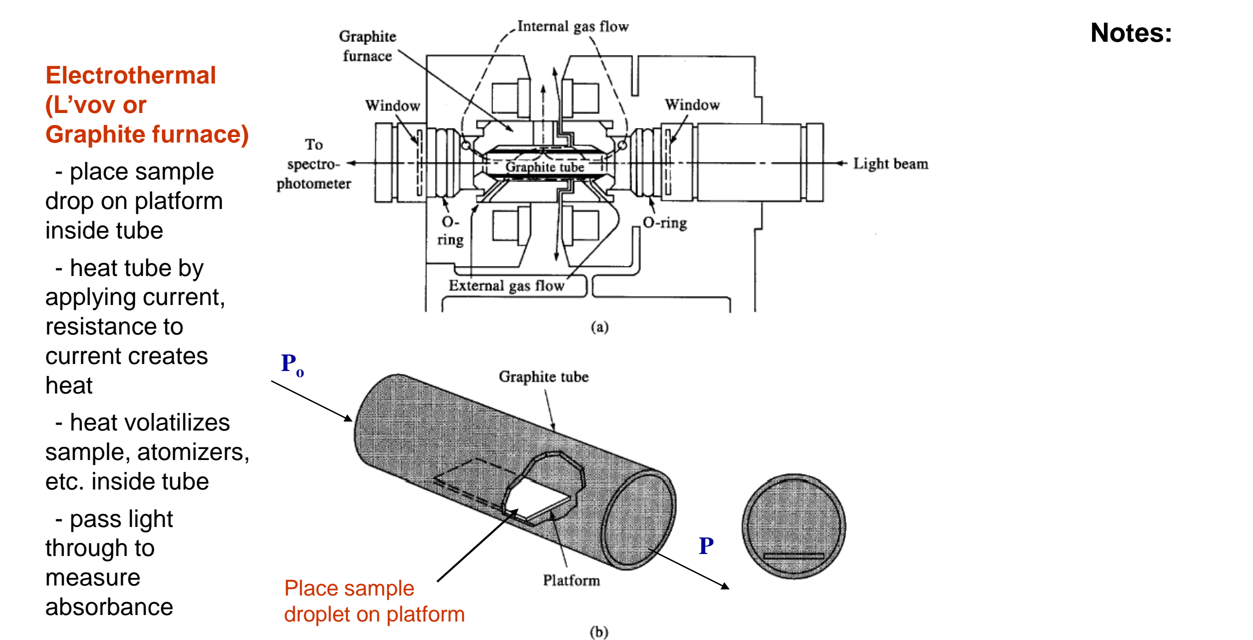
Notes:

Most sensitive part of flame for AAS varies with analyte



Consequences:

- sensitivity varies with element;
- must maximize burner position;
- makes multi-element detection difficult.



Notes:

Graphite Furnace Atomic Absorption Spectrometry (GFAAS)

This technique is essentially the same as flame AA, except the flame is replaced by a small, electrically heated graphite tube, or cuvette, which is heated to a temperature up to 3000°C to generate the cloud of atoms.

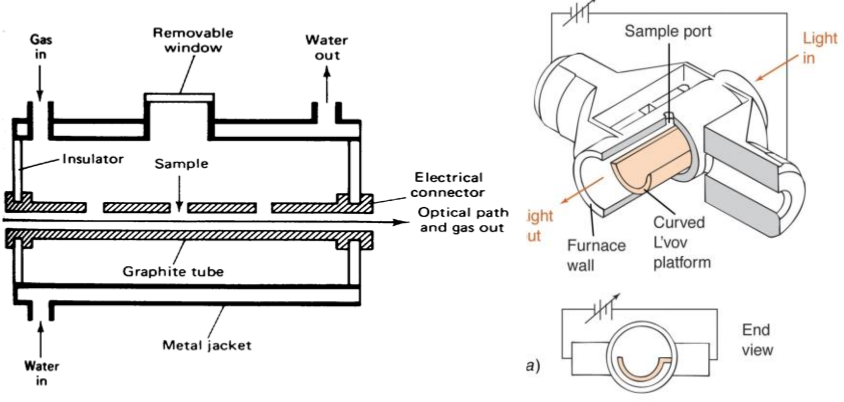
The higher atom density and longer residence time in the tube improve furnace AAS detection limits by a factor of up to 1000x compared to flame AAS, down to the sub-ppb range.

However, because of the temperature limitation and the use of graphite cuvettes, refractory element performance is still somewhat limited.

Notes:

Electrothermal atomization : the graphite furnace

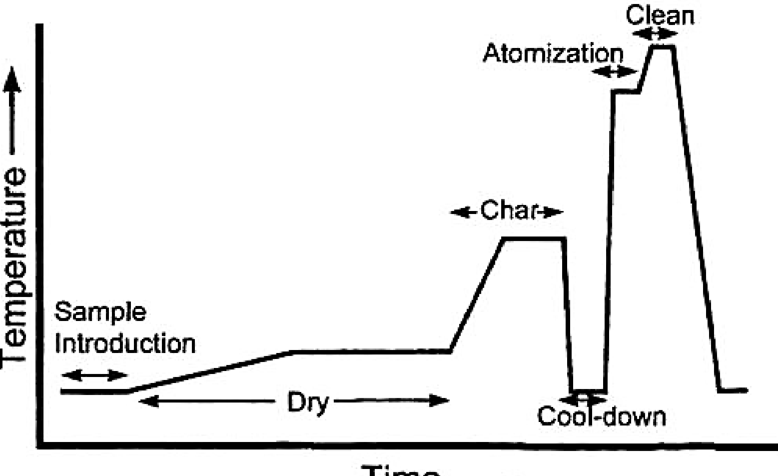
The electrically heated furnace offers greater sensitivity than that afforded by flames and requires a smaller volume of sample. The main part of the atomizer is a small graphite tube about 5 cm in length and 1 cm in diameter. The maximum recommended temperature for a graphite furnace is 2550°C for not more than 7 sec.



Notes:

Schematic diagram of a graphite furnace program

Notes:



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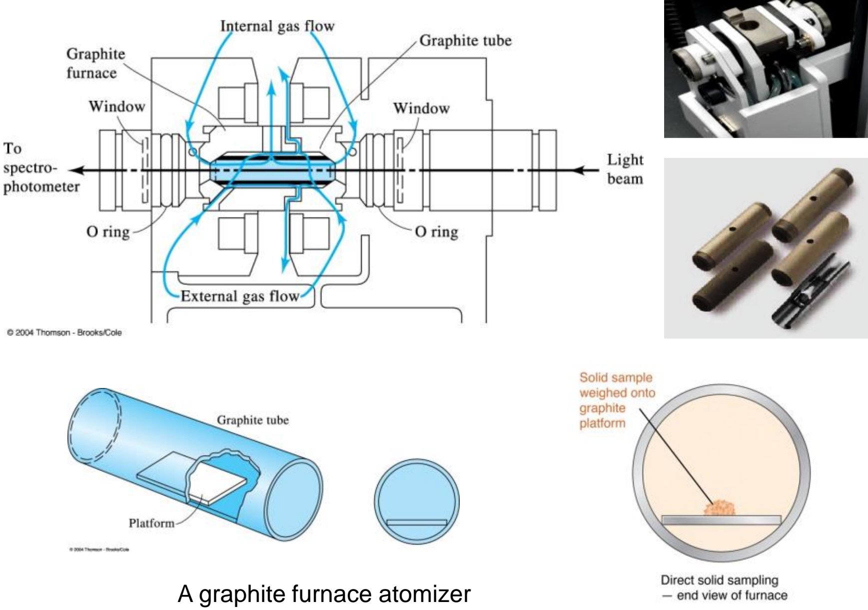


A graphite furnace atomizer

Features of the Electrothermal (nonflame) atomizers

Notes:

1. Only small amounts (10^{-8} to 10^{-11} g absolute) of analyte are required.

2. Solid can be analyzed directly, often without any pretreatment.

3. Small amounts of liquid samples, 5 to 100μl, are needed.

4. Background noise is very low.

5. Sensitivity is increased because the production of free analyte is more efficient than with a flame.

Notes:

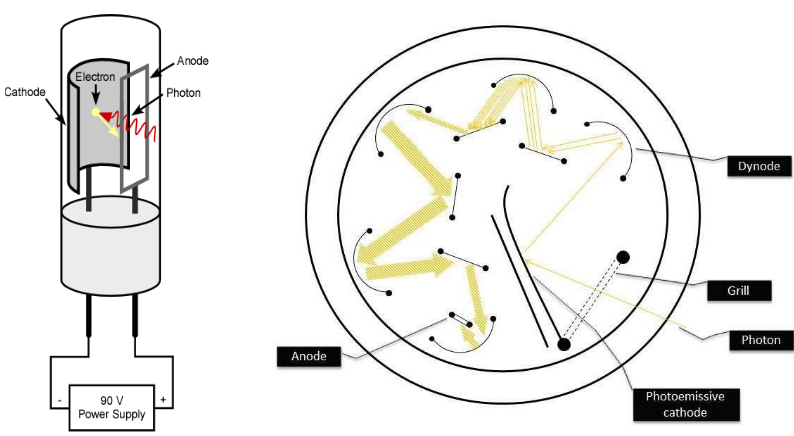
After interacting with the sample, the resulting beam of radiation or ions must be detected.

As with the source, each spectral region and technique requires its own type of detector.

Notes:

Flame and Flameless Atomic Absorption

Detectors used in atomic spectroscopy – traditional phototube, photomultiplier tube



Notes:

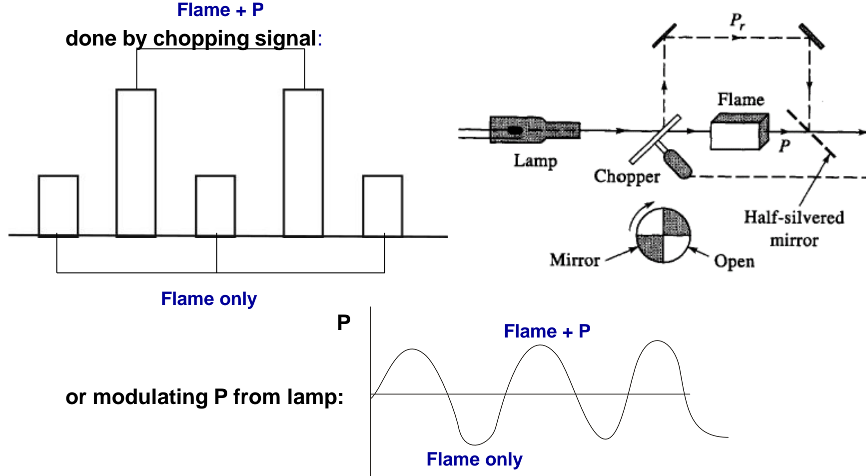
The detector converts the incident light energy into an electrical signal. Photomultipliers are used in atomic absorption analysis.

Through the quartz window of the photomultiplier light enters the cathode K1, covered with a thin layer of matter containing an element which is easily ionized (Cs, Rb, Li, etc.).

The light quanta knock out electrons from the cathode, which are routed at high speed to the next electrode K2. Each electron ejects 2-5 electrons (depending on the applied voltage). The latter, in turn, are sent to the next electrode K3, knocking out new electrons, etc. Atomic absorption spectrometers use photomultipliers with 9-13 electrodes that amplify the current from the first photocathode 106 times.

Source Modulation (spectral interference due to flame)

- problem with working with flame in AA is that light from flame and light source both reach detector
- measure small signal from large background
- need to subtract out flames to get only light source signal (P/P_o)



Notes:

Background correction

Background signal arises from absorption, emission, or scatter by everything in the sample besides analyte ((the matrix), as well as absorption, emission, scatter by the flame, the furnace, or the plasma.

Background correction methods :

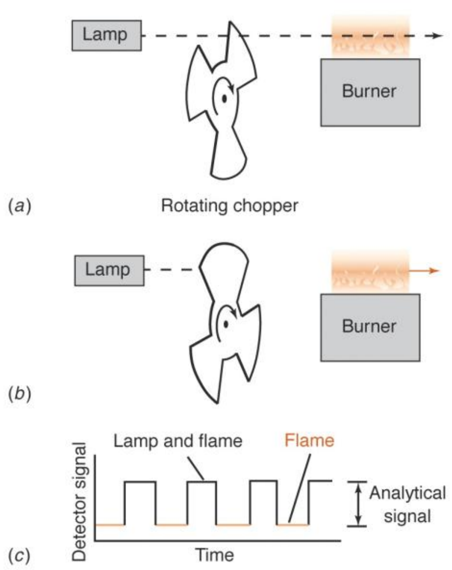
Beam chopping

D_2 lamp – The difference between absorbance measured with the hollow cathode and absorbance measured with the D_2 lamp is the absorbance due to analyte.

Zeeman – When an atomic vapor is exposured to a strong magnetic field (0.1 to 1 tesla), a splitting of electronic energy levels of the atoms takes place, which leads to formation of several absorption lines for each electronic transition.

Smith-Hieftje – based on the self-reversal, or self-absorption, behavior of radiation emitted from hollow cathode lamps when they are operated at high currents.

Notes:



A beam chopper for subtracting the signal due to flame background emission. Resulting square-wave signal.

Notes:

Notes:

Types of interference

Spectral: unwanted signals overlapping analyte signal

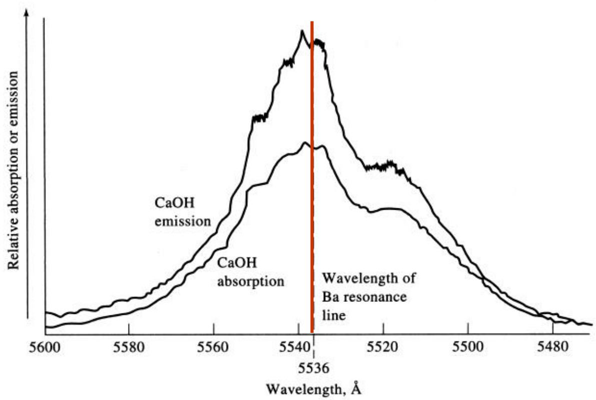
Chemical: chemical reactions decreasing the concentration of analyte atoms

Ionization: ionization of analyte atoms decreases the concentration of neutral atoms.

Notes:

Corrections For Spectral Interferences Due to Matrix

- molecular species may be present in flame
- problem if absorbance spectra overlap since molecular spectrum is much broader with a greater net absorbance
- need way of subtracting these factors out



Notes:

Methods for Correction

1) Two-line method

- monitor absorbance at two λ close together

- one line from sample one from light source
- second λ from impurity in HCL cathode, Ne or Ar gas in HCL, etc

- second λ must not be absorbed by analyte

- absorbed by molecular species, since spectrum much broader

- A & ε are ~ constant if two λ close

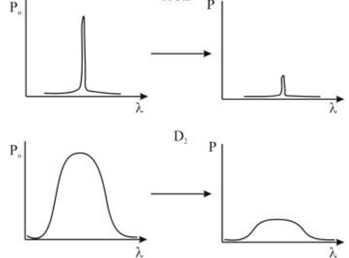
- comparing $A_{\lambda 1}$, $A_{\lambda 2}$ allows correction for absorbance for molecular species

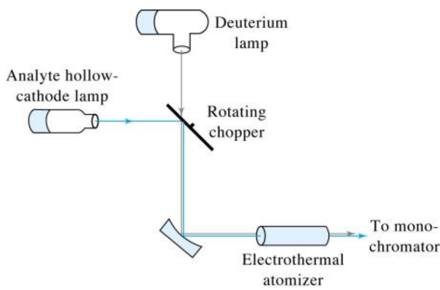
$A_{\lambda 1}$ (atom&molecule) – $A_{\lambda 2}$ (molecule) = A (atom)

Problem: Difficult to get useful second λ with desired characteristics

2) Continuous source method

- alternatively, place the light from HCL or a continuous source D_2 lamp through the flame
- HCL → absorbance of atoms + molecules
- D_2 → absorbance of molecules





advantage:

-available in most instruments

-easy to do

disadvantage:

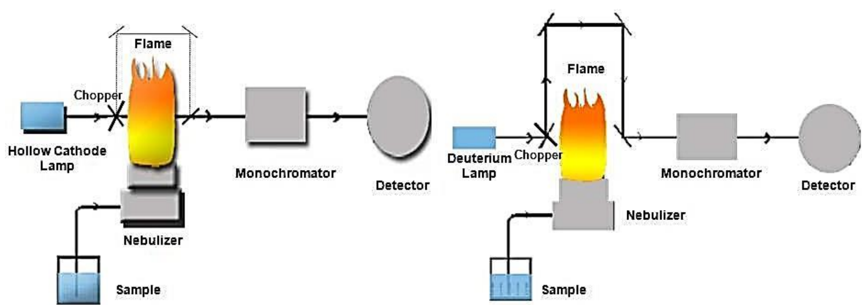
-difficult to perfectly match lamps (can give + or – errors)

Notes:

BACKGROUND CORRECTION METHODS

Continuum Deuterium Source Background Correction

- common background correction technique in FAAS
- significant at lower wavelength range (180 nm - 420 nm)



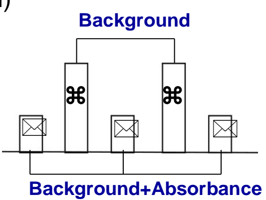
D2 Lamp Background Correction Schematic

Notes:

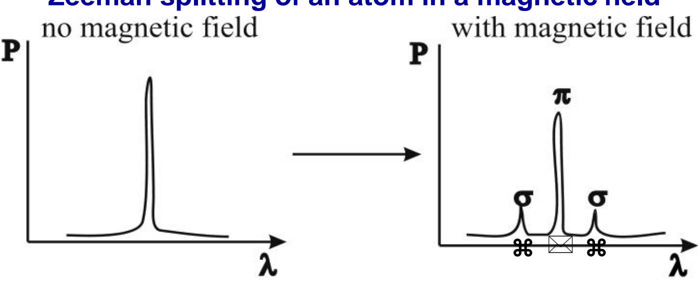
Zeeman Effect

- placing gaseous atoms in magnetic field causes non-random orientation of atoms
- not apparent for molecules
- splitting of electronic energy levels occurs (~ 0.01 nm)
- the sum of split absorbance lines → original line
- only absorb light with the same orientation
- can use Zeeman effect to remove the background

place flame polarized light through sample in magnetic field get absorbance (atom+molecule) or absorbance (molecule) depending on how light is polarized



Zeeman splitting of an atom in a magnetic field



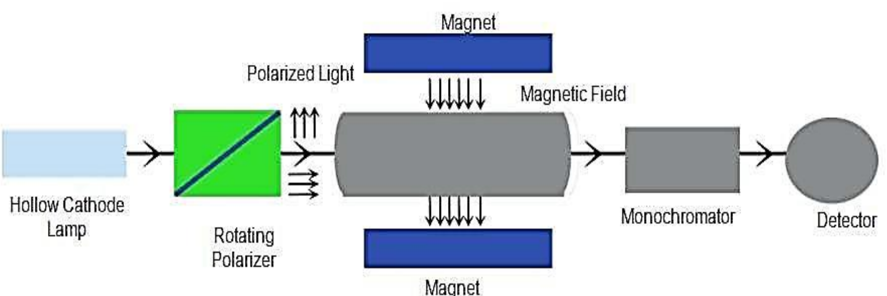
Notes:

BACKGROUND CORRECTION METHODS

Zeeman Background Correction

Mainly used in GFAAS

Notes:



Zeeman Background Correction Schematic

Chemical Interference - more common than spectral interference

Notes:

1) Formation of Compounds of Low Volatility

- Anions + Cations → Salt

$Ca^{2+} + SO_4^{2-} \rightarrow CaSO_4$ (s)

- Decreases the amount of analyte atomized → decreases the absorbance signal
- Avoid by:

◷ increase temperature of flame (increase atom production)

◷ add "releasing agents" – other items that bind to interfering ions

eg. For Ca^{2+} detection add Sr^{2+}

$Sr^{2+} + SO_4^{2-} \rightarrow SrSO_4$ (s)

increases Ca atoms and Ca absorbance

◷ add "protecting agents" – bind to analyte but are volatile

eg. For Ca^{2+} detection add $EDTA^{4-}$

$Ca^{2+} + EDTA^{4-} \rightarrow CaEDTA^{2-} \rightarrow$ Ca atoms

2) Formation of Oxides/Hydroxides

M + O ◷ MO *non-volatile & intense molecular absorbance*

M + 2OH ◷$M(OH)_2$

M is analyte

Avoid by:

◷ increase temperature of flame (increase atom production)

◷ use less oxidant

3) Ionization

Notes:

M ◷ $M^+ + e^-$ affects Group 1 and 2 elements (Ba, Ca, Sr, Na, K)

M is analyte

Avoid by: Use of Low Temperature Flame or Use of Ionization Buffer Ionization buffer/suppressor/suppressant prevents analyte ionization e.g. Addition of a 0.1% KCl soln to blank, standard, and sample

◷ lower temperature

◷ add ionization suppressor – creates high concentration of e^- suppresses M^+ by shifting equilibrium.

Degree of ionization of metals at flame temperatures

Element	Ionization Potential, eV	Fraction Ionized at the Indicated Pressure and Temperature: $p = 10^{-4}$ atm, 2000 K	$p = 10^{-4}$ atm, 3500 K	$p = 10^{-6}$ atm, 2000 K	$p = 10^{-6}$ atm, 3500 K
Cs	3.893	0.01	0.86	0.11	>0.99
Rb	4.176	0.004	0.74	0.04	>0.99
K	4.339	0.003	0.66	0.03	0.99
Na	5.138	0.0003	0.26	0.003	0.90
Li	5.390	0.0001	0.18	0.001	0.82
Ba	5.210	0.0006	0.41	0.006	0.95
Sr	5.692	0.0001	0.21	0.001	0.87
Ca	6.111	3×10^{-5}	0.11	0.0003	0.67
Mg	7.644	4×10^{-7}	0.01	4×10^{-6}	0.09

Notes:

MATRIX INTERFERENCES

a physical interference and can either suppress or enhance absorbance signal of analyte.

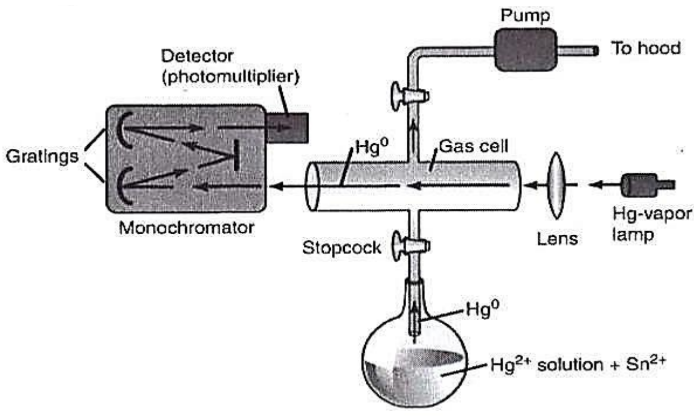
Causes:

1. Differences in viscosity and surface tension.
2. Preparation in different solvents.
3. Measurement at different temperatures.
4. Presence of organic species.
5. Different atomization rate in flame.

Notes:

Cold Vapor Atomic Absorption (CVAA) Spectroscopy for Hg

- Free Hg atoms exist at room temperature, no requirement for heating
- Sample may contain Hg^0, Hg^{2+} or Hg_2^{2+}
- Reduction with a strong reducing agent (e.g. $SnCl_2$ or $NaBH_4$)



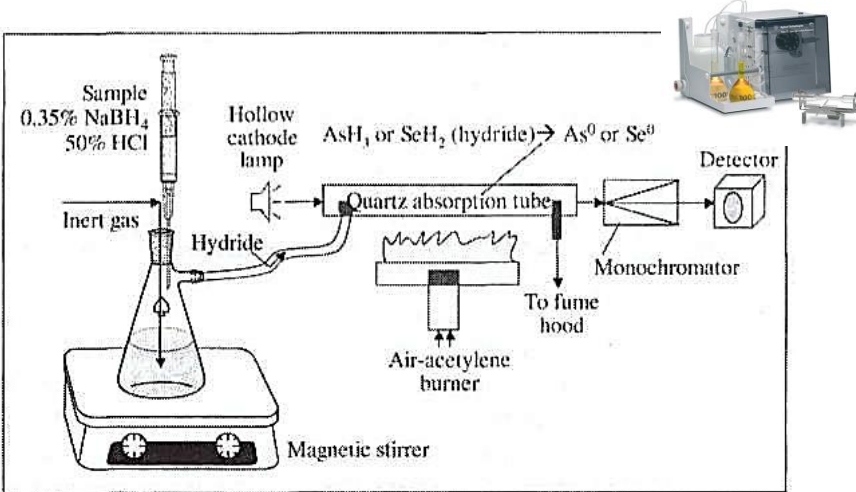
Schematic diagram of cold vapor mercury analyzer

Notes:

Hydride Generation Atomic Absorption (HGAA) Spectroscopy

- AsH_3 and SeH_3 generated by reaction samples containing As and Se with $NaBH_4$
- Allso used for Pb, Sn, Bi, Sb, Te, Ge, Se determination

VGA 77



Atomic Spectroscopy

Selection of the Proper Atomic Spectroscopic Techniques

Important factors:

- Detection limit
- Working range
- Sample throughput
- Cost
- Interferences
- Ease of use
- Availability of proven methodology

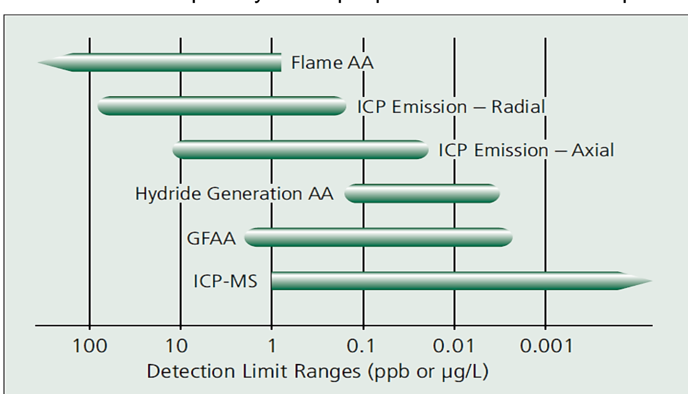
	Flame AA	GFAA	ICP-OES	ICP-MS
How Many Elements?				
Single	■			
Few		■		
Many			■	■
What Levels?				
High ppb	■		■	
Sub ppb		■	■	■
Sub ppb-ppm				■
Sub ppt				■
How Many Samples?				
Very few	■	■		
Few	■	■	■	■
Many			■	■
How Much Sample?				
> 5 mL	■	■	■	■
< 1-2 mL		■		

Notes:

Atomic Spectroscopy

Comparison of Detection Limits and Working Range

- Low detection limit is essential for trace analysis
- Without low level capability – sample pre-concentration is required

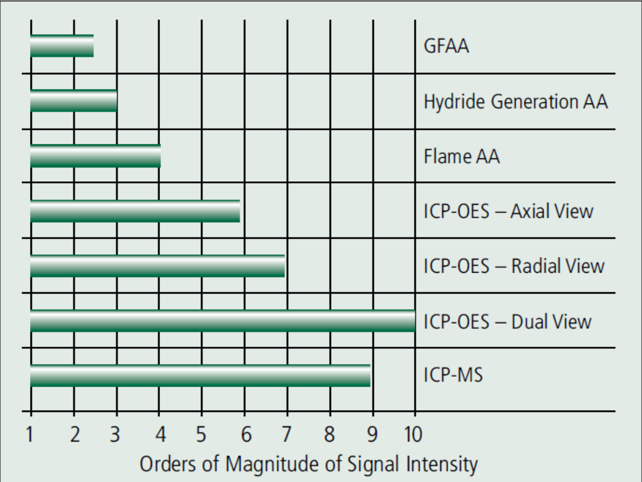


Typical detection limit ranges for the major atomic spectroscopy techniques.

Notes:

Comparison of Detection Limits and Working Range

- Ideal working range minimizes analytical effort and potential errors



Typical analytical working ranges for the major atomic spectroscopy techniques.

Notes:

Comparison of Interferences and Other Considerations
Atomic spectroscopy interfernces nterference
4 types: spectral, chemical, ionization, physical/matrix

Notes:

Technique	Type of interferences	Method of compensation
FAA	Ionization Chemical Physical (self-absorption)	Ionization buffer Releasing agent or nitrous- acetylene flame Dilution, matrix matching, or method of additions
GFAA	Physical and chemical Molecular absorption Spectral	Stabilized temperature platform furnace (STPF) condition Zeeman or Continuum source background correction Zeeman background correction
ICP-OES	Spectral Matrix	Background correction or the use of alternative analytical lines, IECs or MSF Internal standardization
ICP-MS	Mass overlap Matrix	Interelement correction, use of dynamic reaction cell (DRC) technology, use of alternate mass values or higher mass resolution Internal standardization

Tasks to Section 12

1. Give definitions of these terms: atomic emission, atomic absorption, atomic fluorescence, monochromator, hollow cathode lamp, flame and flameless atomic absorption, atomizer, electrothermal atomization, detector.

2. Name the ways to eliminate chemical influences in atomic absorption determinations.

3. What are matrix effects? In what ways are they eliminated?

4. What is non-selective absorption? Describe the main ways to correct it.

5. Name the basic principles of choosing the conditions of atomic absorption analysis and the development of experimental methods.

6. List the areas of application and possibilities of atomic absorption spectroscopy.

7. Why should the composition of the standards used in atomic absorption analysis be as close as possible to the composition of the analysed sample?

8. Explain the possible reasons for the nonlinear dependence of atomic absorption on concentration in the AAS method. Suggest your algorithm of actions of the analyst during the analysis of the object, if the obtained dependence of absorption on concentration is nonlinear.

9. The dependence of the atomic absorption of caesium chloride on the concentration, which has a nonlinear shape, is obtained. Explain the possible reasons for this dependence on the method of AAS. Suggest another, better method for determining caesium.

10. It is necessary to determine Vanadium by the AAS method. Suggest different variants of this method if: a) a pure solution of $VOCl_2$ is investigated; 6) the investigated solution of $VOCl_2$ contains an excess of foreign salts (KCl, NaCl, etc.); c) the test sample contains thermally stable forms of Vanadium (e.g., oil sample).

11. Review the latest journals in analytical chemistry and find an example of the application of methods of flame and electrothermal atomic absorption spectroscopy for each of the objects: a) food; 6) drugs; c) biological objects (tissues, blood, urine, hair, etc.); d) soils; e) wastewater. For each of them, give a literature reference and indicate the conditions for determining any element.

12. The concentration of Cu was determined by atomic absorption analysis. The 200-mL sample of the caustic solution with Cu was acidifying with 20 mL of concentrated HNO3, adding 1 mL of 27% w/v H2O2, and boiling for 30 min. The resulting solution was diluted to 500 mL, filtered, and analyzed by flame atomic absorption using matrix-matched standards. The results for a typical analysis are shown in the following table.

solution	mg Cu/L	absorbance
blank	0.000	0.007
standard 1	0.200	0.014
standard 2	0.500	0.036
standard 3	1.000	0.072
standard 4	2.000	0.146
sample		0.027

Determine the concentration of Cu in the caustic suspension.

Section 13: Emission Spectroscopy

Contents:

- Introduction
- Backgrounds
- Flame emission spectroscopy
- Inductively coupled plasma – atomic emission spectroscopy
- Microwave plasma - atomic emission spectroscopy
- Emission spectroscopy for solid samples
- Luminescence spectroscopy
- Comparison and selection of proper technique

Introduction

Atomic spectroscopy is a technique for determining the elemental composition of an analyte by its electromagnetic or mass spectrum.

Atomic emission spectroscopy is a technique in which the emission of light by thermally excited atoms in a flame or furnace is used to measure the concentration of atoms.

Methods of atomic emission spectroscopy are also known as optical emission spectroscopy.

When the atoms of samples are excited to higher electronic energy levels in flames, they emit radiation in the visible and UV regions of the electromagnetic spectrum. Emission intensities may be measured to analyse for metals, especially alkali and alkaline earth elements.

A flame atomic emission spectrometer or flame photometer incorporates a burner, monochromator or filters, a detector and a method of introducing the sample solution into the flame. The technique is used primarily for the quantitative determination of alkali and alkaline earth metals in clinical, biological and environmental samples.

Early atomic emission instruments used an electric arc or spark excitation. The higher energy of these sources produced a very significant number of emission lines throughout the visible and UV regions. The simultaneous measurement of a large number of elements is possible.

Luminescence is the spontaneous emission of light by a substance not resulting from heat; or "cold light". It is thus a form of cold body radiation. It can be caused by chemical reactions, electrical energy, subatomic motions or stress on a crystal. It distinguishes luminescence from incandescence, which is light emitted by a substance as a result of heating.

Emission spectrum for the analytical luminescence method is a graph of luminescence intensity versus luminescence wavelength (or frequency/wavenumber), obtained with a fixed excitation wavelength.

A graph of luminescence is measured at a fixed wavelength versus excitation frequency or wavelength. It closely corresponds to an absorption spectrum because the luminescence is generally proportional to the absorbance.

There are many types of luminescence. They are as follows:

- Chemiluminescence is the emission of light as a result of a chemical reaction;
- Crystalloluminescence is produced during crystallisation;
- Electroluminescence is a result of an electric current passed through a substance;
- Mechanoluminescence is a result of mechanical action on a solid;
- Photoluminescence is a result of the absorption of photons;
- Radioluminescence is a result of bombardment by ionising radiation;
- Thermoluminescence is the re-emission of absorbed energy when a substance is heated.

The following types of photoluminescence are frequently used in analytical chemistry:

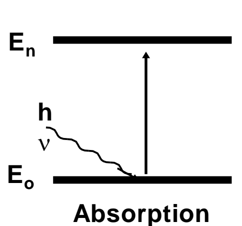
– Fluorescence is photoluminescence as a result of singlet–singlet electronic relaxation (typical lifetime: nanoseconds);

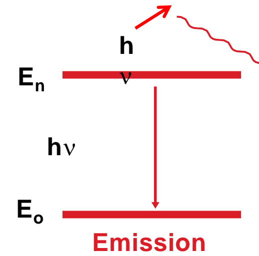
– Phosphorescence is photoluminescence as a result of triplet–singlet electronic relaxation (typical lifetime: milliseconds to hours).

Atomic fluorescence spectroscopy is a technique in which electronic transitions of atoms in a flame, furnace, or plasma are excited by light. The fluorescence is observed at a right angle to the incident beam.

Three types of spectroscopy

Notes:



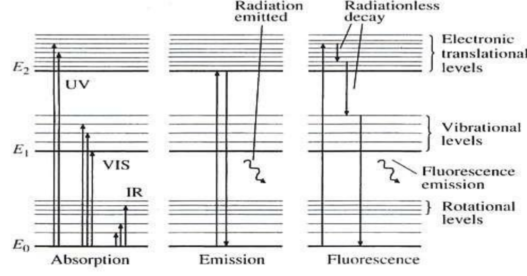


Absorption spectrum:
When absorbance is plotted as a function of wavelength

Emission spectrum:
When the relative power of the emitted radiation is plotted as a function of wavelength.

Photoluminescence is one of many forms of luminescence (light emission). Photon is absorbed and excites electrons to a higher energy level in an atom. Light is emitted from any form of matter after the absorption of photons.

Photoluminescence spectrum is measured as an emission spectrum of electromagnetic radiation resulting from the phenomenon of photoluminescence



E_0 = Ground level; E_1, E_2 = Excited states
Energy spacing: vibration > rotation >> translation

Key characteristics

Notes:

- The study of radiation emitted by excited monatomic species because relaxation of atoms in the excited state results in emission of light.
- Produces line spectra in the UV-VIS and the vacuum UV regions.
- Used for qualitative identification of elements present in the sample.
- Also for quantitative analysis from ppm levels to percent.
- Multielement technique.
- Can be used to determine metals, metalloids, and some nonmetals simultaneously.
- Emission wavelength and energy are related by $\Delta E = hc/\lambda$.

Five basic components of optical instruments

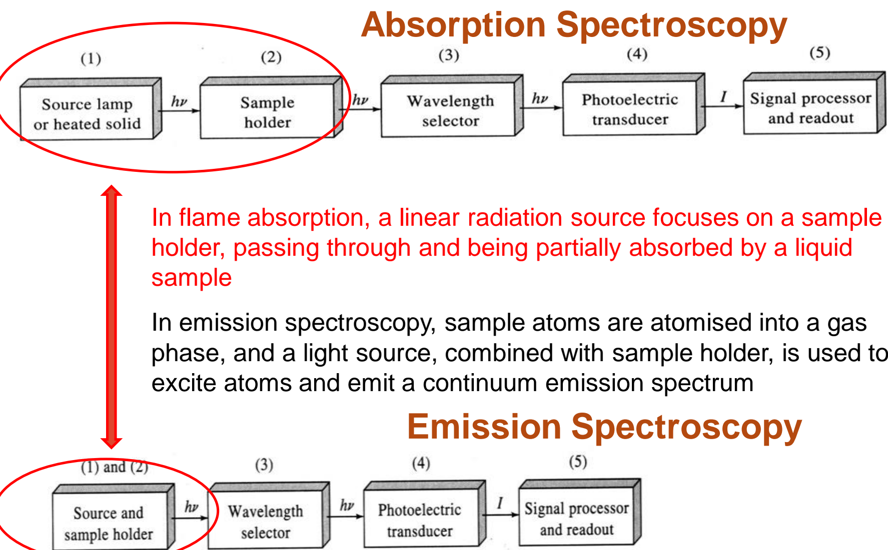
Notes:

1. **Source**
   A stable source of radiant energy at the desired wavelength range.
2. **Sample Holder**
3. **Wavelength Selector**
   A device that isolates a restricted region of the EM spectrum used for measurement (monochromators, prisms, & filters).
4. **Photoelectric Transducer** (Detector)
   Converts the radiant energy into a useable signal (usually electrical).
5. **Signal Processor & Readout**
   Amplifies or attenuates the transduced signal and sends it to a readout device such as a meter, digital readout, chart recorder, computer, etc.

Positions 3 to 5 are practically same in emission and absorption instruments while constructions and roles of positions 1 and 2 are different

The difference in sources between absorption and emission instruments

Notes:



In flame absorption, a linear radiation source focuses on a sample holder, passing through and being partially absorbed by a liquid sample

In emission spectroscopy, sample atoms are atomised into a gas phase, and a light source, combined with sample holder, is used to excite atoms and emit a continuum emission spectrum

Spectrometer and spectrophotometer - difference

Notes:

SPECTROMETER

is an instrument that provides information about the intensity of radiation as a function of wavelength or frequency.

SPECTROPHOTOMETER

is a spectrometer equipped with one or more exit slits and photoelectric transducers that permits the determination of the ratio of the radiant power of two beams as a function of wavelength as in absorption spectroscopy.

Notes:

Wavelength selectors

Wavelength selectors provides a limited, narrow, continuous group of wavelengths called a **band**.

Two types of wavelength selectors:

Filters

A) Interference filters
B) Absorption filters

Monochromator

Monochromator – wavelength selector that can continuously scan a broad range of wavelengths.
Used in most scanning spectrometers including UV, visible, and IR instruments

Radiation transducers

Notes:

Early detectors in spectro-instruments were the human eye, photographic plates or films.
Modern instruments contain devices that convert the radiation to an electrical signal.

Two types of radiation transducers

Photon detectors

Commonly useful in ultraviolet, visible and near infrared instruments.

Several types of photon detectors are available:
1. Vacuum phototubes
2. Photomultiplier tubes
3. Photovoltaic cells
4. Silicon photodiodes
5. Diode array transducers
6. Photoconductivity transducers

Thermal detectors

Used for infrared spectroscopy because photons in the infrared region lack the energy to cause photoemission of electrons.

Three types of thermal detectors:
1. Thermocouples
2. Bolometers
2. Pyroelectric transducers

Atomic Emission Spectroscopy (AES)

Notes:

Atomic processes are similar to atomic absorption with flame now being used for atomisation and excitation of the sample for light production

Degree of Excitation Depends on Boltzmann Distribution:

$$\frac{N_1}{N_0} = \frac{P_1}{P_0}\exp\left(-\frac{\Delta E}{kT}\right)$$

Where N_1 and N_o – are the number of atoms in excited and ground states;
k – Boltzmann constant (1.28×10^{-23} J/K);
T – temperature;
ΔE – energy difference between ground and excited states;
P_1 and P_o – number of states having equal energy at each quantum level

Increase temperature leads to increase in N_1/N_o (more excited atoms)
The emission signal increases with increase in temperature

Notes:

Emission spectroscopy needs good temperature control to get reproducible signal. For example, temperature difference of 10° (from 2500 → 2510°C) results in a 4% change in N_1/N_o for Na (sodium)

Temperature Dependence Comparison between AA and AES:

- AA is relatively temperature independent. Need heat only to get atoms, not atoms in excited state;
- AA looks at ~ 99.98% of atoms;
- AES uses only small fraction (0.02%) of excited atoms.

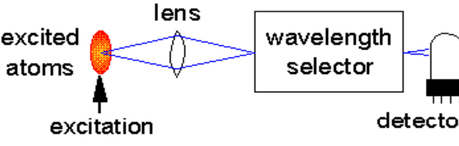
Comparison of AA and AES Applications and detection limits:

AES - emission from multiple species simultaneously;
AAS – absorption of a single line;
Some elements are better by AA others better by AES.

Flame Emission More Sensitive	Sensitivity About the Same	Flame Absorption More Sensitive
Al, Ba, Ca, Eu, Ga, Ho, In, K, La, Li, Lu, Na, Nd, Pr,Rb, Re, Ru, Sm, Sr, Tb, Tl, Tm, W, Yb	Cr, Cu, Dy, Er, Gd, Ge, Mn, Mo, Nb, Pd, Rh, Sc, Ta, Ti, V, Y, Zr	Ag, As, Au, B, Be, Bi, Cd, Co, Fe, Hg, Ir, Mg, Ni, Pb, Pt, Sb, Se, Si, Sn, Te, Zn

Light sources for emission spectroscopy

Similar to AA, but no need for external light source or chopper.
Flame acts as sample cell (sample holder) & light source.



Atomisation Sources:

Source	Temperature (°C)
Flame	1700-3150
Inductive coupled argon plasma (ICP)	4,000-6,000
Microwave-induced argon plasma (MIP)	2000-3000
Glow-discharge plasma (GD)	Non thermal
Electro thermal vaporization (ETV) *	1200-3000
Direct current argon plasma (DCP)	4000-6000
Electric Arc/Spark	4,000-5,000/40,000

Currently, first three sources are widely used in modern instruments: They will be discussed in detail.

* Electrothermal usually not used – too slow and not as precise

Notes:

Flame emission spectrometry. Theory and principles

1. In flame emission spectrometry, the sample solution is nebulised (converted into a fine aerosol) and introduced into the flame where it is desolvated, vaporised and atomized, all in rapid succession.

2. Subsequently, atoms and molecules are raised to excited states via thermal collisions with the constituents of the partially burned flame gases. Upon their return to a lower or ground electronic states, the excited atoms and molecules emit radiation characteristic of the sample components.

3. The emitted radiation passes through a monochromator that isolates the specific wavelength for the desired analysis. A photodetector measures the radiant power of the selected radiation, which is then amplified and sent to a readout device.

Notes:

Flame emission spectrometry. Theory and principles

4. Combustion flames provide a means of converting analytes in solution to atoms in the vapour phase.

5. Flame supplies energy necessary to move the electrons of the free atoms from the ground state to excited states.

6. The intensity of emitted radiation provides the basis for analytical determination because the emission is proportional to the number of excited atoms, which is proportional to the total number of atoms in flame or sample concentration

Notes:

Processes in the flame

When a metallic salt solution is introduced in the form of a fine spray at a controlled rate into the flame of the burner, the following events take place:

Desolvation: The sample containing metal particles is dehydrated by the heat of the flame and the solvent is evaporated

Vaporisation: The heat of the flame vaporises the sample constituents. No chemical changes take place at this stage. A solvent is vaporised leaving particles of solid salt.

Atomisation: At this stage, the metal ions that were in the solvent are reduced to metal atoms, for example, $Mg^{2+}_{(aq)} + 2e \rightarrow Mg_{(g)}$

By the heat of flame and action of the reducing gas (fuel), molecules and ions of the sample species are decomposed and reduced to give atoms.

Notes:

Processes in the flame (continued)

Excitation: The atoms at this stage are able to absorb energy from the heat of the flame. The amount of energy absorbed depends on the electrostatic forces of attraction between the negatively charged electrons and positively charged nucleus. As electron absorbs energy, they move to higher energy levels and are in the excited states.

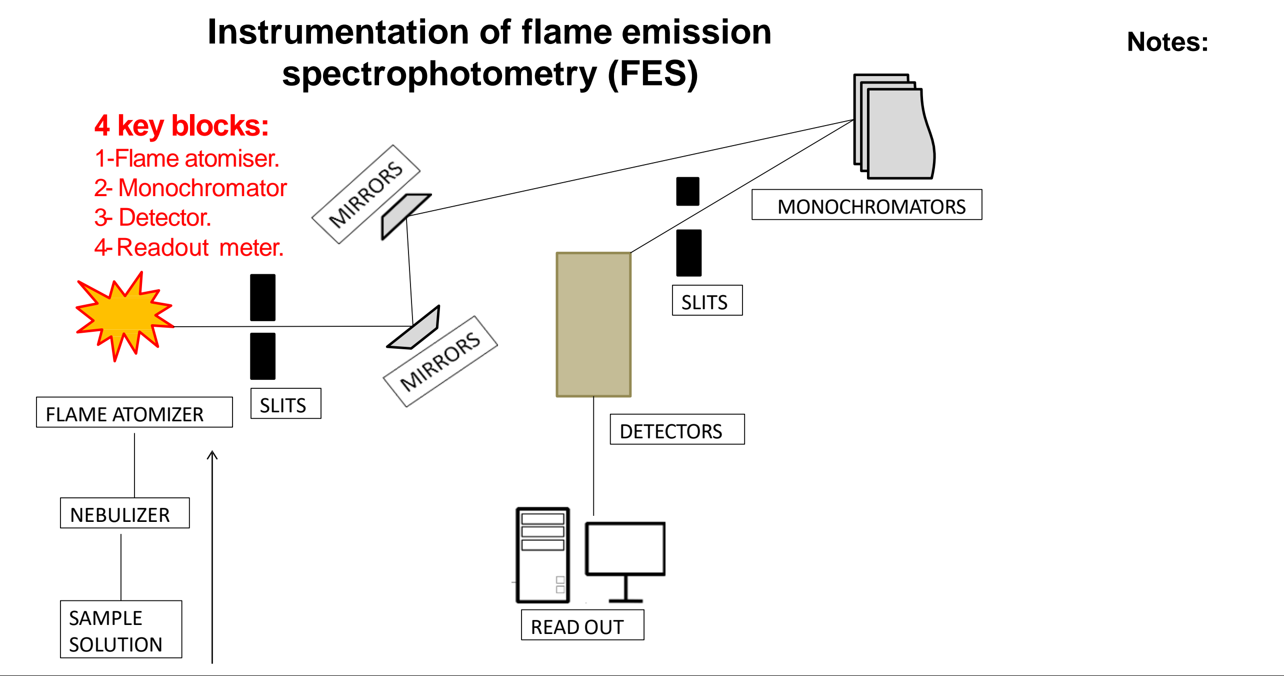
Emission of radiation: Electrons in the excited states are very unstable and move back down to the ground state quickly. As they do so, they emit the energy in the form of radiation of characteristic wavelength, which is measured by detector.

For some metals, this radiation corresponds to the wavelength of visible light and is observed as the characteristic colour of the flame.

As electrons from different energy levels are able to emit light as they relax, the flame colour observed will be a mixture of all different wavelengths emitted by different electrons in the metal atom under investigation.

Notes:

Instrumentation of flame emission spectrophotometry (FES)



Notes:

First component: Flame atomiser

Notes:

The role of an atomiser is to generate the vapours of analyte which get excited by the thermal energy of the flame and then emit characteristic radiation that is measured.

The flame atomizer assembly consists of two components.

The prior is a nebuliser where the sample in the form of a solution is drawn in and converted into a fine aerosol.

It is then passed onto the second component – the burner along with air or oxygen and fuel gas. In the flame a number of processes occur that convert the analyte into excited species.

Nebuliser

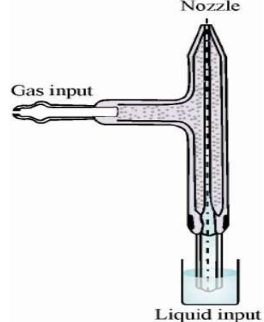
Notes:

Nebuliser is a device used for sample introduction into the flame.

The process is called nebulisation and consists of thermal vaporisation and dissociation of aerosol particles at high temperature producing small particle size with high residence time.

There are known a few nebulisation methods such as:

- Pneumatic (is the most commonly used for introducing liquid samples)
- Ultrasonic
- Electrothermal
- Hydride generation (used only for certain elements).



Concentric pneumatic nebuliser consists of a fine capillary surrounded by concentric tube with a small orifice near one end of a capillary. The capillary dipped into a solution of analyte while the outer tube is connected to a high-pressure gas supply. The analyte is sucked into the capillary by the high pressure gas stream flowing around the tip of the capillary using the Bernoulli effect (so-called aspiration process). The high-velocity gas breaks up the liquid into various sized fine droplets.

Burner and flame

Notes:

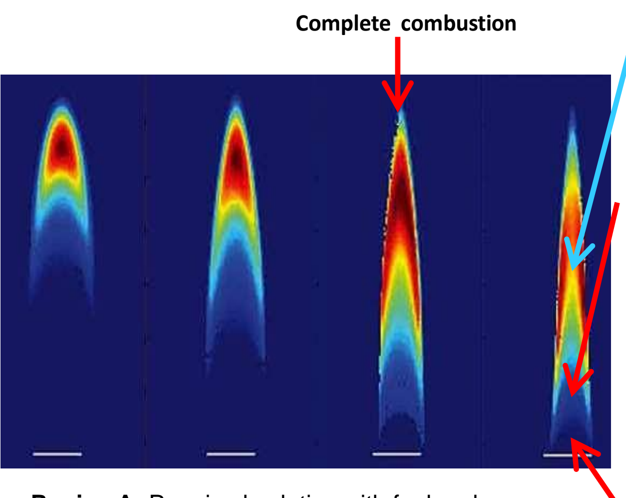
A flame is the most generally useful atomiser for atomic spectroscopy despite the developments in electrothermal atomisation. A satisfactory flame source must:

1. Have a proper temperature.
2. The temperature should be constant and non-fluctuating through-out the operation.
3. The spectrum of the flame should not interfere with the emission or absorption lines of the analytes.
4. For FES and AAS a premixed, laminar-flow flame is employed that rests upon a slot burner.
5. The flame burner head is aligned so that it intersects the light path of the spectrophotometer.
6. Titanium burner heads provide maximum corrosion resistance when analysing any type of sample

Fuel: burning aid or burning component.
Oxidant: whose presence with the fuel will compensate for complete combustion of fuel, resulting in high-efficiency flame.

Flames are not uniform in composition, length, or cross section: The structure of a premixed flame

Notes:



Region D: In this region gases approach thermal equilibrium. The conditions in this region are optimum for most AAS measurements.

Region C: Initiates combustion. Gases emerging from this region consists mainly of CO_2, CO, H_2O and N_2, if air is used as oxidant. **In this region** the conc. of radicals is too high for the gases to achieve thermal equilibrium. The intense emission of radiation from flame contents can create noise problems.

Region A: Premixed solution with fuel and oxidant. Unburnt hydro-carbon gas mixture

Region B: The mixture is heated by energy from region C.

Characteristics of common premixed flames

Notes:

Fuel	Oxidant	Temperature (Celsius)	Burning velocity (cm per sec)
Acetylene	Air	2400	160-266
Acetylene	Nitrous oxide	2800	260
Acetylene	Oxygen	3140	800-2480
Hydrogen	Air	2045	324-440
Hydrogen	Nitrous oxide	2690	390
Hydrogen	Oxygen	2660	900-3680
Propane	Air	1925	43

Fuel: burning aid or burning component.
Oxidant: whose presence with the fuel provides complete combustion of fuel, resulting in high efficiency flame.

Notes:

Effect of flame temperature

1. The temperature of the flame determines its utility in both AAS and FES.
2. The exact temperature depends on the fuel/oxidant ratio and is generally highest for a stoichiometric mixture.
3. Temperatures high enough to cause ionisation of the analyte atoms are usually undesirable in both methods unless an ionisation buffer is added to the sample.
4. Many of the interferences due to the formation of refractory oxides can be overcome or minimised by the use of the proper oxidant–fuel system, particularly the nitrous oxide–acetylene system.

Burning parameters

Notes:

Burning Velocity. Flame propagation rate or burning velocity is important. If it exceeds approximately 40 cm s^{-1}, the flame likely will flashback into the mixing chamber and an explosion will result.

Flame Profile. The concentration of excited and unexcited atoms in a flame varies in different parts of the flame envelope.

Observation Site. The region that is viewed within the flame is important. For example, the emission lines of boron (249.7 nm) and antimony (259.8 nm) are either absent or very weak in the outer mantle of a stoichiometric flame, but they appear in high concentrations in the reaction zone (blue cone) of a fuel-rich flame.

Notes:

Ionisation Buffer

When flame or plasma temperatures are high enough to cause ionisation of the analyte atoms, an ionisation buffer must be incorporated into the sample solution.

- To suppress the ionisation of metal, another easily ionisable element (denoted an ionisation or radiation buffer) is added to the sample, but it must be an element which will not add any spectral line interference.
- Often easily ionised elements such as K, Cs, or Sr are added.

Notes:

Releasing and Shielding Agents

Releasing and shielding agents provide a chemical means for overcoming some vaporization interferences.

These agents may either combine with the interfering substance or deny the analyte to the interfering substance by mass action.

Calcium and magnesium can be shielded by complexing the calcium and magnesium with EDTA. Once in the flame, the EDTA is destroyed.

Second component: Monochromator

A grating or a prism monochromator are used.

The role of the monochromator is to disperse the radiation coming from the flame and falling on it.

The dispersed radiation from the exit slit of the monochromator goes to the detector.

If a low-temperature flame is used, the spectral lines from only a few elements are emitted. For routine analysis, a filter can be used as a monochromator for such a case.

Filters are made from materials which are transparent in a small selective wavelength range.

One need to select a filter which is transparent to emission in a wavelength range where spectral lines of the studied element are observed.

In such a case, a condensed lens id employed to collect the emitted light and send the rays through the filter as an approximately parallel beam to reach the detector.

Filters have been designed to determine Li, Na, K, Ca and some other elements.

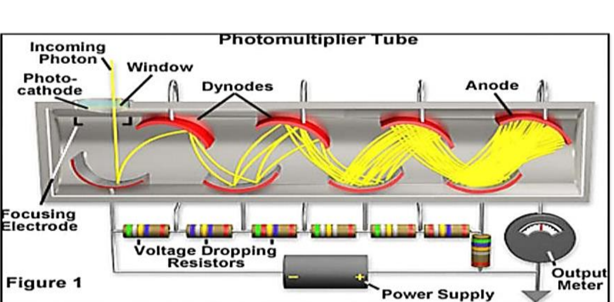
Notes:

Third component: Detector

The function of a detector is to measure the intensity of radiation falling on it.

Photoemissive cells or photomultiplier tubes are used for this purpose.

Similar detectors are also used in UV-VIS spectroscopy



Notes:

Notes:

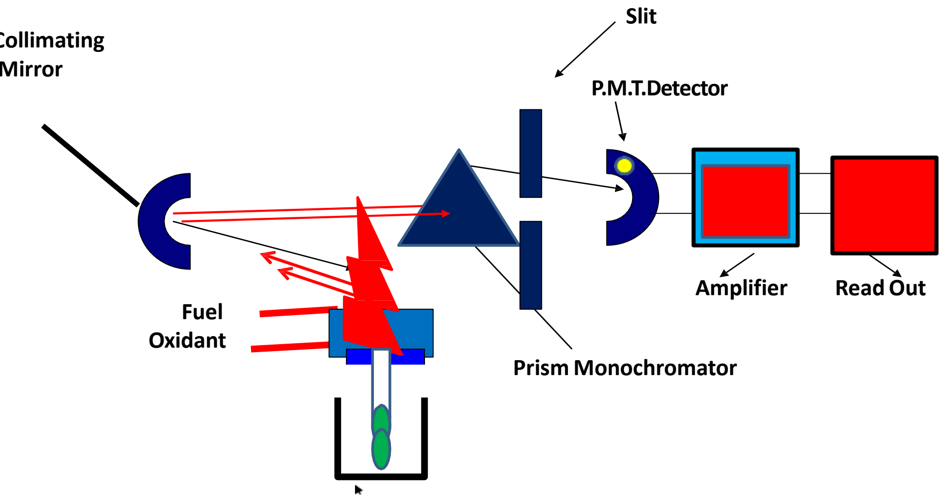
Fourth component: Amplifier and Readout Device

The output from the detector is suitably amplified and displayed on a readout device like a meter or digital display.

The amplifier can be changed so as to be able to analyse samples of varying concentrations.

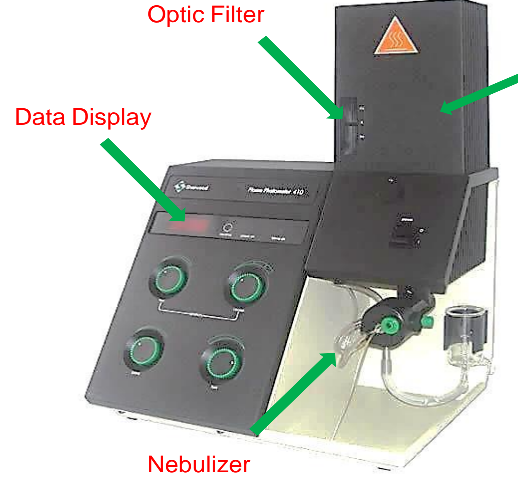
Nowadays, the instruments have microprocessor-controlled electronics that provides outputs compatible with the printers and computers thereby minimising the possibility of operator error in transferring data.

Notes:



Flame emission spectrometer (FES)

Notes:



Flame

Flame Photometer M-410 (Sherwood Scientific, UK)

Flame emission spectrometers (flame photometers) are probably the simplest type of atomic spectrometers.

They are normally designed to make measurements up to 10 different elements, usually those from groups 1 and 2 of the periodic table.

This is achieved using interchangeable coloured filters or different gratings which isolate an area of the spectrum containing the chosen emission line.

Qualitative applications

Notes:

1. Flame photometric methods are widely used for the determination of alkali and alkali earth metals in samples that are easily prepared as aqueous solutions.
2. Some of these elements can be detected visually by the colour in the flame (Na gives yellow flame)
3. The best way is to use flame photometer with a filter or monochromator to separate radiation with the wavelengths characteristic of the different metals from other radiations present.
4. If characteristic radiation is detected, it will indicate the presence of the metal in the sample. This method is simple and quite reliable if carried out with care.
5. However, this method does not provide information about the molecular structure of the compounds. None of the radiating elements, such as carbon, hydrogen, halides, can be detected.

Quantification: calibration curve method

Notes:

1. The intensity of the spectral line being measured is directly proportional to the solution concentration of the analyte.
2. Quantitative measurements are made by reference to a previously prepared calibration line or by the method of standard addition.
3. The response linearity of most instruments is restricted to concentrations between **10 and 100 ppm** which is fairly limiting.
4. **Typical elements that this technique is used for are Ca, Ba, K, Li, Na, Mg, Al.**
5. One of the quantification methods involves the preparation of calibration curve by measuring the intensity of emission of a series of solutions of different concentrations prepared by using a standard solution and plotting a graph between emission intensity versus concentration of the ionic species of the element of interest.

It is important to measure all intensities under identical conditions.

Quantification: calibration curves (continued)

Notes:

6. The calibration curve method helps in finding the concentration of unknown samples. However, it is difficult to prepare standards for some samples.
7. This may occur when the samples contain high of variable concentrations of matrix materials or when the samples contain solids whose effect on absorption is hard to duplicate.

n such cases we need to resort to any of the following two methods:

1. standard addition method
2. Internal standard method.

Quantification: standard addition method

Notes:

1. In this method, a known amount of a standard solution is added to identical aliquots of the sample and the absorbance is measured.
2. The first reading is the absorbance of sample alone and the second reading is the absorbance of a sample containing analyte plus, a known amount of analyte and so on. Increasing amounts of a standard solution of the salt of the element to be determined are added to a series of solutions of the sample.
3. The intensity of emission for all these solutions is then measured. A curve of intensity versus concentration of the added element is obtained and extrapolated to zero value of intensity to give the concentration of the element in the sample.

Quantification: internal standard method

Notes:

1. In this method, a constant amount of another metal which is not present in the sample is added to both the unknown sample and a series of standard solutions of the element to be determined.
2. The is called internal standard, for example, Li is added in the determination of Na metal. Since both the element and the internal standard are in the same solution, the emission readings ate the wavelengths of both the internal standard and the element to enclosed are simultaneously determined.
3. The intensity ratio for the two elements is then plotted against the concentration of the standard solution. From the observed ratio for the sample, the concentration of the element in it can be determined.

Limitations of Flame Emission Spectroscopy

Notes:

- **As natural gas and air flame is employed for excitation the temperature is not high enough to excite transition metals, therefore the method is selective towards detection of alkali and alkali earth metals**

- The low temperature makes this method susceptible to certain disadvantages, most of them related to interference and the stability of the flame and aspiration conditions. Fuel and oxidant flow rates and purity, aspiration rates, solution viscosity, affect these. It is therefore very important to measure the emission of the standard and unknown solutions under identical conditions.

Limitations of Flame Emission Spectroscopy

Notes:

- The relatively low energy available from the flame leads to a relatively low intensity of the radiation from the metal atoms, particularly those that require a large amount of energy to become excited.

- **Flame photometry is a means of determining the total metal concentration of a sample; it tells us nothing about the molecular form of the metal in the original sample.**

- **Only liquid samples can be used. In some cases, lengthy steps are necessary to prepare liquid samples.**

Notes:

Applications of Atomic Emission Spectroscopy

- Quantitative Analysis of Soil Extracts with ICP-OES.
- Analysis of Chlorine, Bromine and Iodine in water.
- Waste Water and Soil Analysis.
- Analysis of Incineration Solvents.
- Analysis of metals in analytical grade solutions and reagents.

Notes:

Multielemental Analysis

Atomic emission spectroscopy is ideally suited for multielemental analysis because all analytes in a sample are excited simultaneously.

It is possible to analyse three or four analytes per minute.

- Atomic emission spectroscopy is used for the regulation of alkali metals in the pharmaceutical processes.
- It is also used for detection of trace metals in different samples.
- Atomic emission spectroscopy is applicable in the smelting process of ores, in the process of extraction of metals.
- Atomic emission spectroscopy helps in the detection of elements in the given sample and hence for structure elucidation.
- It is also used for analysing motor oils

Notes:

Hotter sources for emission spectroscopy

The flame is not an ideal excitation source for atomic emission.

Hotter sources can be used, like plasma.

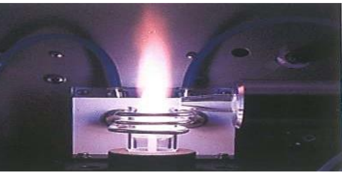
What is a plasma? A hot partially ionised gas. The hottest part of the inductively coupled

plasma is at temperatures of 7000 K - 10000 K

The use of high temperature plasma for sample atomisation/excitation increases the fraction of excited atoms and gives rise to an increase in emission signal, allowing more types of atoms to be detected.

We will discuss the following techniques:

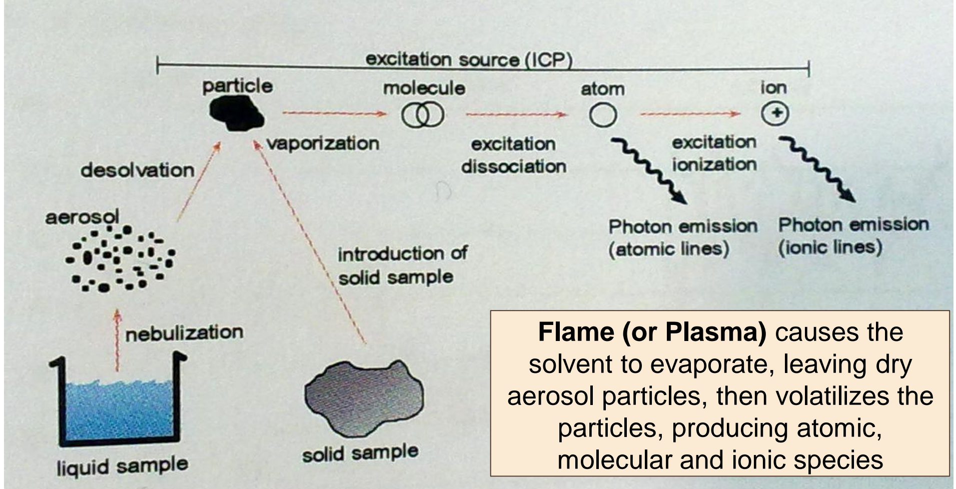
Inductively coupled plasma atomic optical emission spectroscopy (ICP-OES)



Microwave plasma atomic emission spectroscopy (MP-AES)

Notes:

Schematic diagram of the processes in the ICP



Flame (or Plasma) causes the solvent to evaporate, leaving dry aerosol particles, then volatilizes the particles, producing atomic, molecular and ionic species

ICP-AES: Basics

Notes:

ICP-AES measures the intensity of light emitted by atoms or ions of the elements of interest at specific wavelengths;

ICP spectrometers use emission to detect and quantify elements in a sample;

ICP-AES uses the argon plasma (6000º-10000º C) for atomisation and excitation of the sample atoms;

It is capable of measuring both atomic and ionic emission so more wavelengths can be monitored;

ICP-AES determines majority of elements except gases and some non-metals (C, N, F, O, H) – up to 73 elements in 5 minutes.

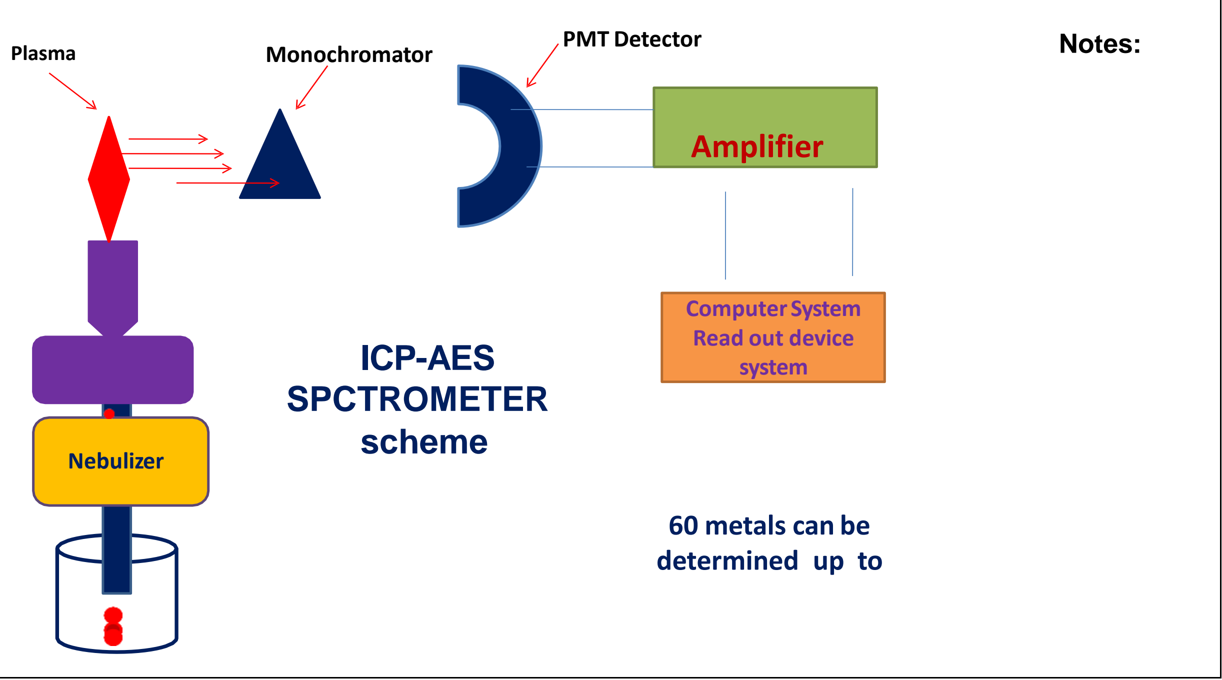
Advantages:

- Can use with gas, liquid or solids sample;
- Simultaneous multi-element analysis;
- Fastest sample throughput;
- Wide dynamic range (from sub-ppb to % level);
- Tolerates complex matrices;
- Few inter-element interferences;
- Precision & accuracy (0.3 – 3%);
- Low argon gas consumption;
- Safe (no flammable gas).

Limitations:

- Higher initial costs than AAS or MP-AES;
- More spectral interferences compared with MP-AES;
- Not as sensitive as graphite furnace AAS or ICP-MS;
- No isotope determination.

Notes:



Plasma Sources

- The ICP torch consists of three concentric quartz tubes, surrounded at the top by a radio-frequency induction coil.
- The sample is mixed with a stream of Ar using a spray chamber nebulizer similar to that used for flame emission and is carried to the plasma through the torch's central tube.
- Plasma formation is initiated by a spark from a Tesla coil.
- An alternating radiofrequency current in the induction coils creates a fluctuating magnetic field that induces the argon ions and electrons to move in a circular path.
- The resulting collisions with the abundant unionized gas give rise to resistive heating, providing temperatures as high as, 10,000 K at the base of the plasma, and between 6000 and 8000 K at a height of 15–20 mm above the coil, where emission is usually measured.

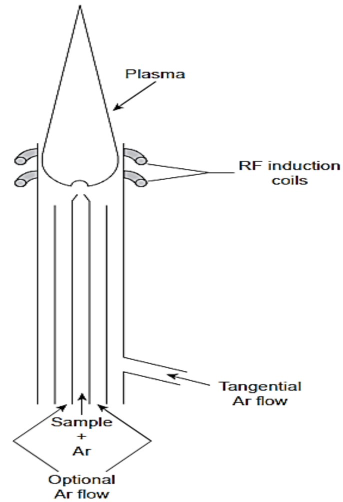
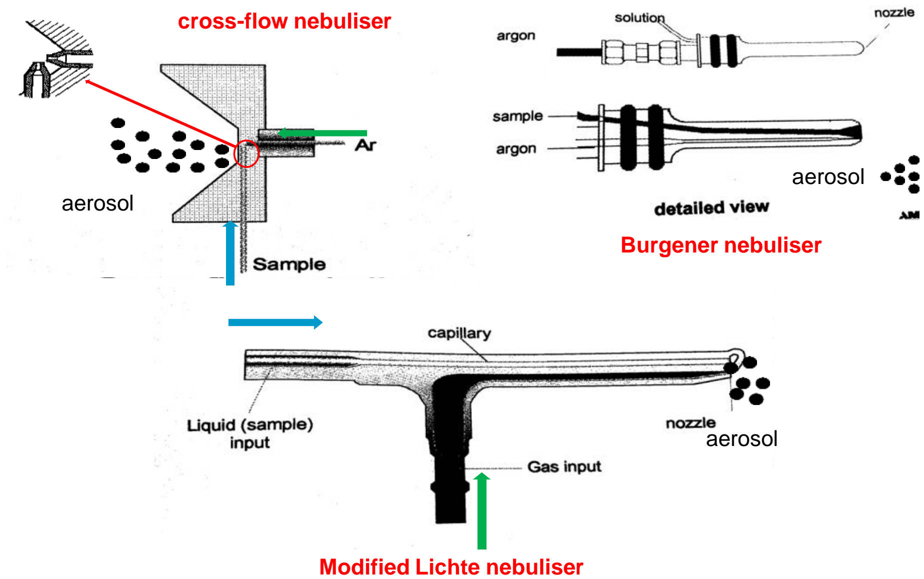


Diagram of an inductively coupled plasma torch

Notes:

ICP-AES: different nebuliser types

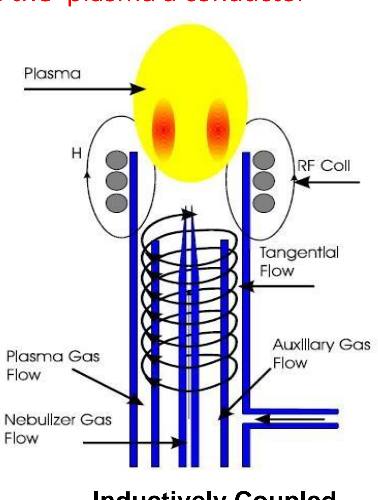


Notes:

ICP-AES: PLASMA

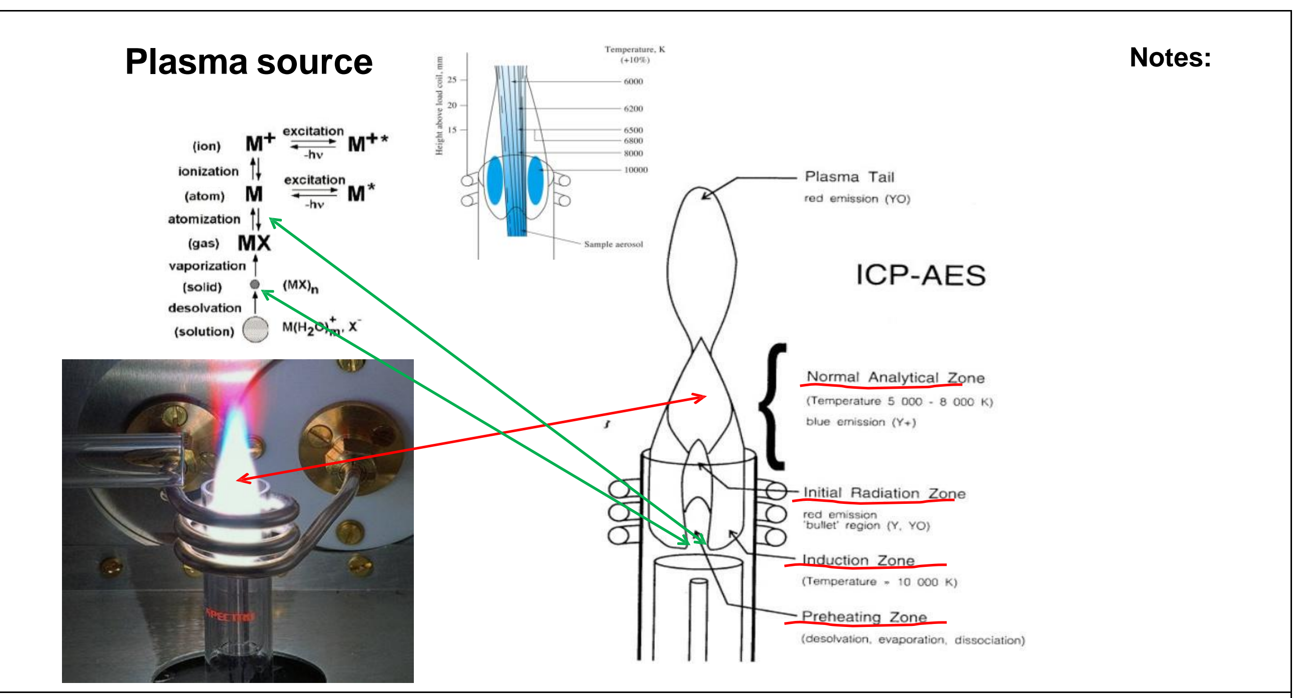
A **plasma** is a hot, partially ionized gas. It contains relatively high concentrations of ions and electrons that make the plasma a conductor

- The plasmas used in atomic emission are formed by ionizing a flowing stream of argon, producing argon ions and electrons.
- Argon ions, once formed in a plasma, are capable of absorbing sufficient power from an external source to maintain the temperature at a level at which further ionisation sustains the plasma indefinitely.
- The plasma temperature is about 10,000 K. The high temperatures in a plasma result from resistive heating that develops due to the movement of the electrons and argon ions.



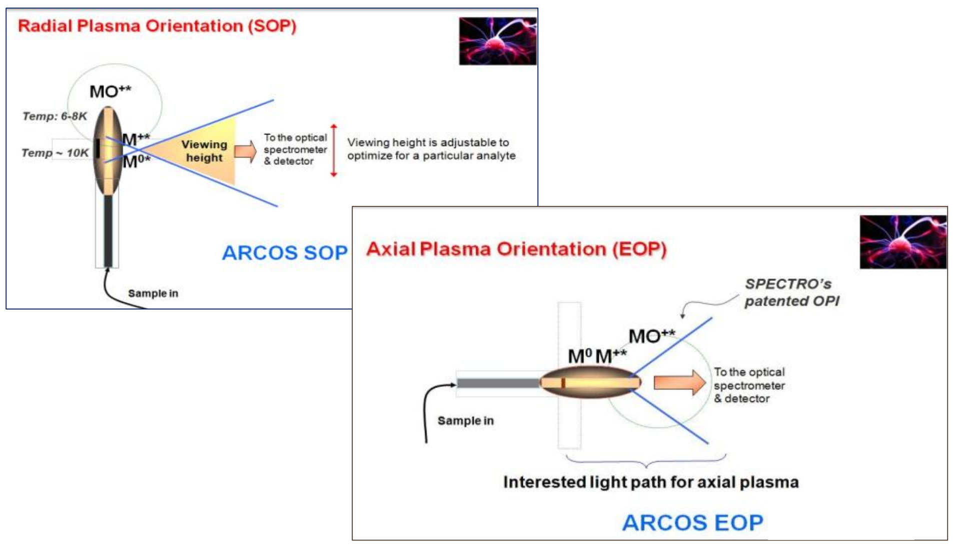
Inductively Coupled Plasma Source

Notes:



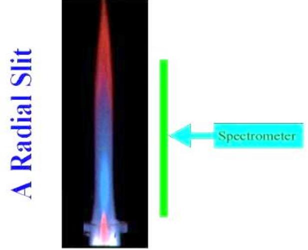
Two basic plasma configurations: RADIAL (SOP) AND AXIAL (EOP)

Notes:

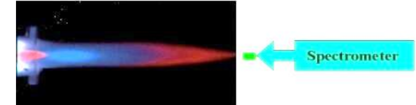


ICP-AES: RADIAL (SOP) AND AXIAL (EOP)

Notes:



An Axial Slit



SOP: Side-on-Plasma

- ❑more suitable for hard matrices (concentrated samples);
- ❑alkali metals (Na, K, Li) calibration is more linear;
- ❑less spectral interferences;
- ❑lower sensitivity (Limit-of-Detection is higher)

EOP: End-on-Plasma

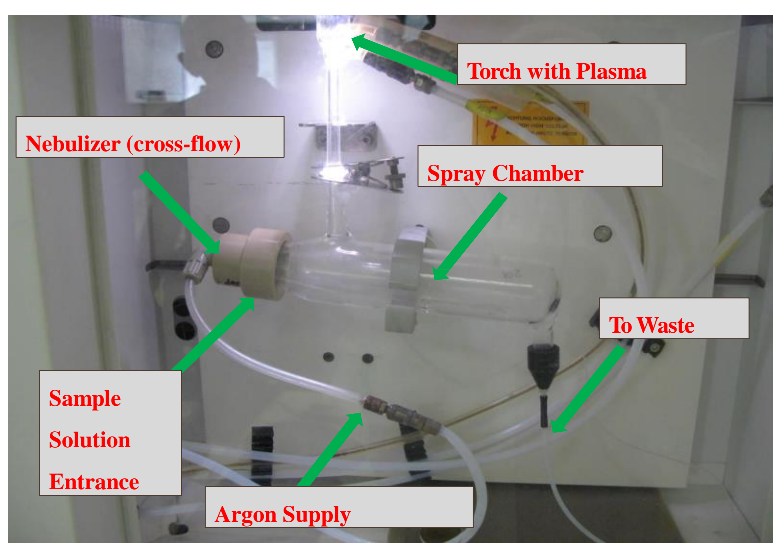
- ❑more suitable for light matrices;
- ❑alkali metals (Na, K, Li) calibration is less linear;
- ❑more spectral interferences;
- ❑higher sensitivity (Limit-of-Detection is lower)

ICP-AES SPECTROMETER

Notes:

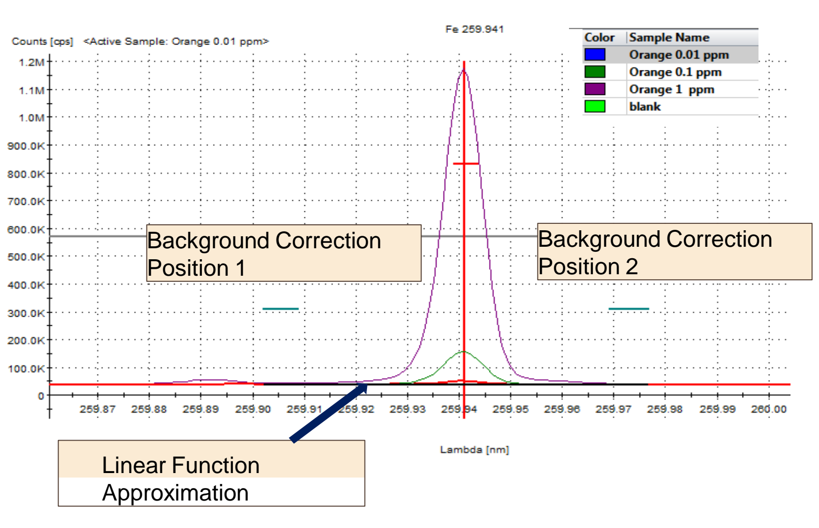
ICP-AES: SAMPLE INTRODUCTION SYSTEM

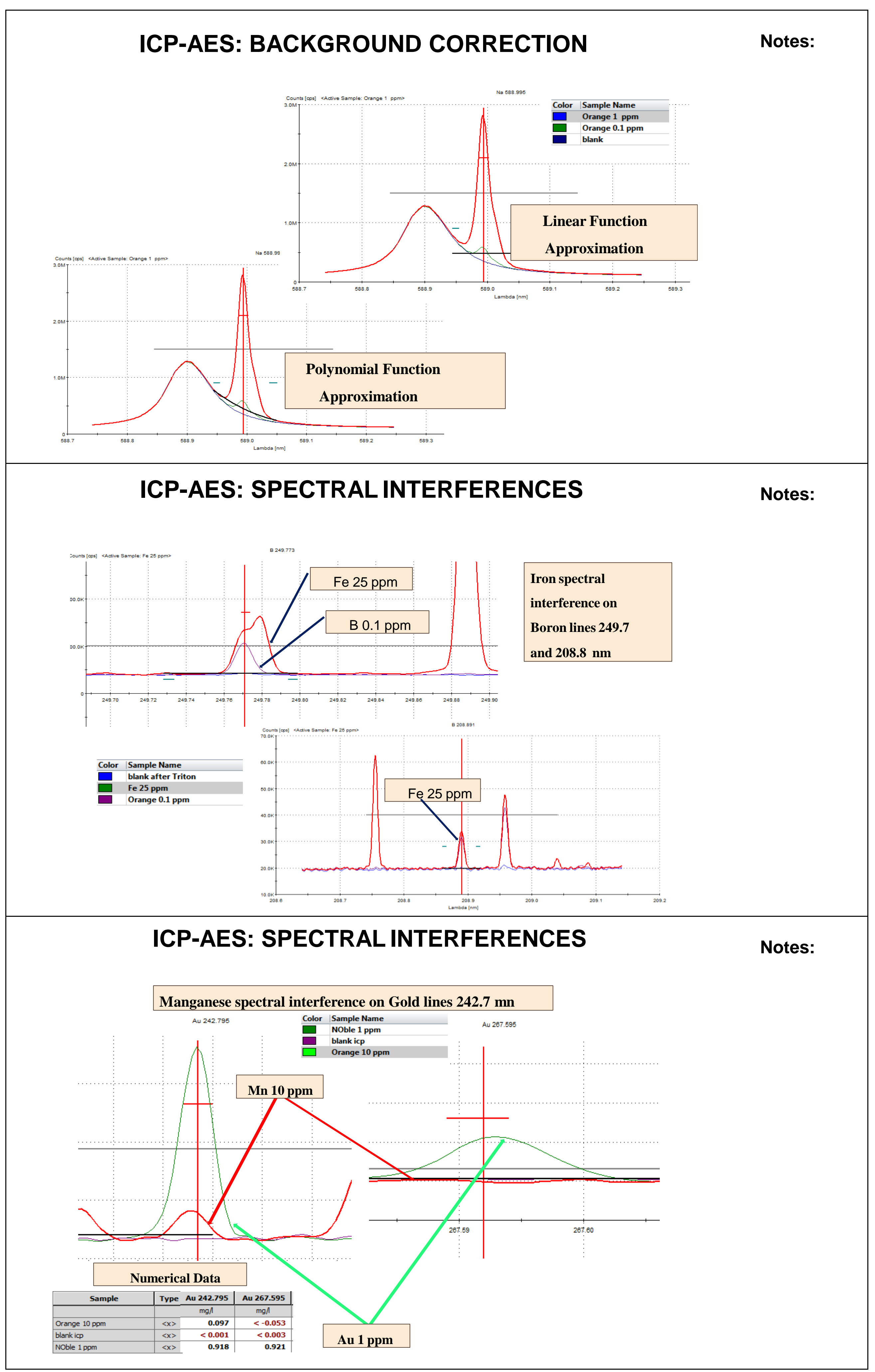
Notes:



ICP-AES: BACKGROUND CORRECTION

Notes:

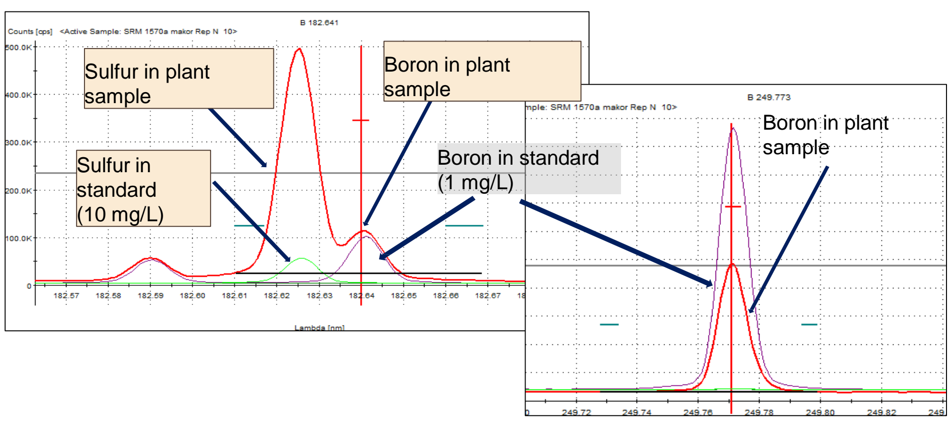




Sample	Type	Au 242.795	Au 267.595
		mg/l	mg/l
Orange 10 ppm	<x>	0.097	< -0.053
blank icp	<x>	< 0.001	< 0.003
NOble 1 ppm	<x>	0.918	0.921

ICP-AES: SPECTRAL INTERFERENCES

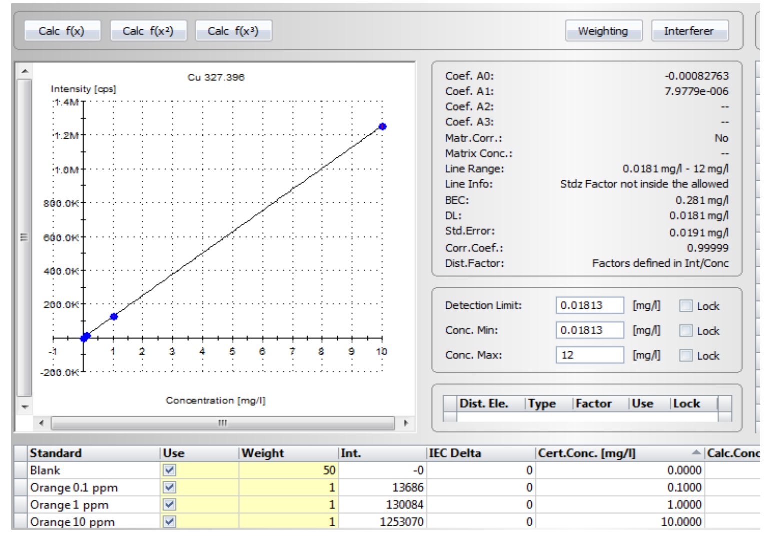
Notes:



Sulfur spectral interference on Boron line 182.6 nm

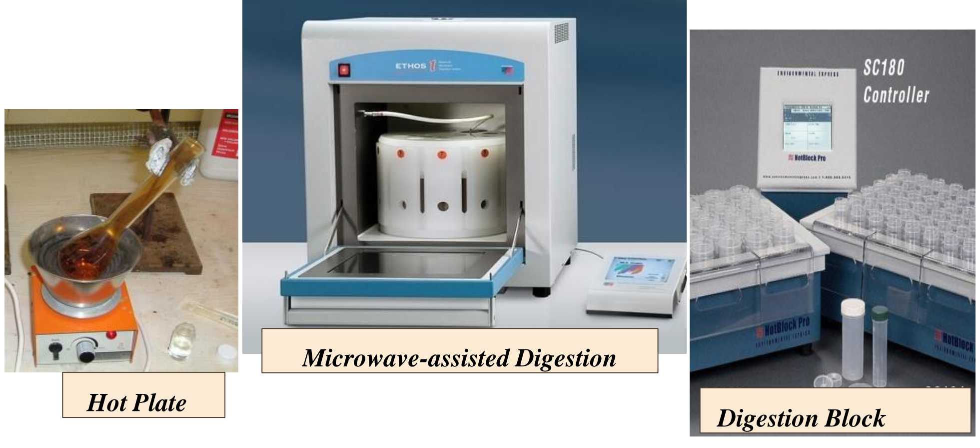
ICP-AES: CALIBRATION CURVE

Notes:



ICP-AES: SAMPLE PREPARATION

Notes:



Hot Plate *Microwave-assisted Digestion* *Digestion Block*

Most samples have to be prepared for analysis by ICP.
Solid samples are solubilized.
Organic matter is "mineralized" i.e. converted to inorganic compounds.

ICP-AES: fields of application

Environmental Analysis: trace metals and other elements in waters, soils, plants, composts and sludges;

Clinical Analysis: metals in biological fluids (blood, urine);

Pharmaceuticals: traces of catalysts used; traces of poison metals (Cd, Pb etc);

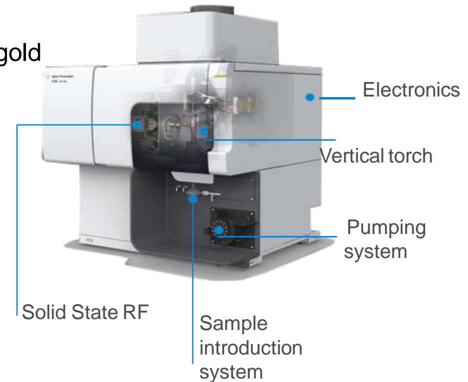
Industry: trace metal analysis in raw materials; noble metals determination.

Forensic science: gunshot powder residue analysis, toxicological examination (e.g., thallium Tl determination)

Notes:

ICP-OES: some examples of applications

- Monitoring of water/wastewater/solid wastes
- Determination of trace elements in water
- Mercury monitoring in environmental samples
- Quantitative analysis of multiple elements in water/soil/sediment environment samples
- Analysis of soil – analysis of micronutrient content (Agriculture)
- Determination of precious metals and gold



Notes:

ICP-OES: Analysis of Milk Powder

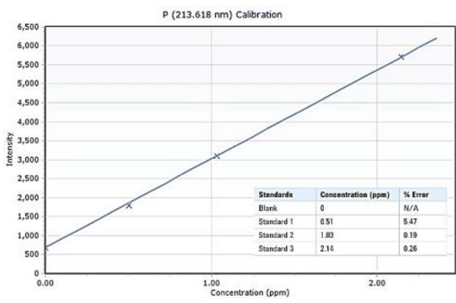
Analysis of NIST milk powder 8435 SRM using the 5100 SVDV ICP-OES

Element	Certified value (mg/kg)	Measured value (mg/kg)	Recovery (%)
Major nutrients			
K 766.491	13630	13070	96
Ca 315.887	9220	9750	106
P 213.618	7800	7160	92
Na 589.592	3560	3530	99
S 181.792	2650	2650	100
Minor and trace nutrients			
Mg 279.078	814	749	92
Zn 202.548	28.0	28.9	103
Sr 421.552	4.35	4.37	101
Fe 259.940	1.8	1.9	107
Cu 327.395	0.46	0.46	100
Mo 204.598	0.29	0.27	92
Mn 257.610	0.17	0.18	103

Notes:

Notes:

ICP-OES: Analysis of Biodiesel Oil



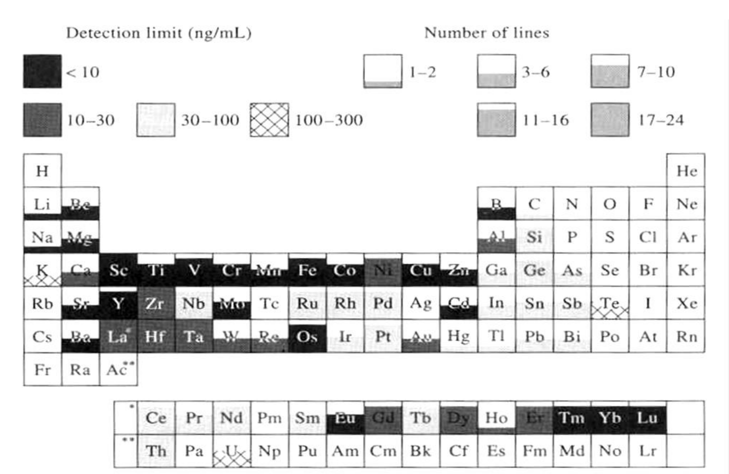
Calibration curve for P 213.618 nm line, using FBC background correction, shows excellent linearity across the calibrated range, with a correlation coefficient of 0.99986.

Element	λ(nm)	Background correction used	Calibration range (mg/kg)	Correlation coefficient	MDL (ppm
Ca	422.673	Fitted	0-2	0.99995	0.004
K	766.491	FACT	0-2	0.99996	0.008
K	766.491	Fitted	0-2	0.99935	0.048
Mg	279.553	Fitted	0-2	0.99994	0.0004
Na	588.995	FACT	0-2	0.99991	0.002
Na	588.995	Fitted	0-2	0.99996	0.048
P	213.618	Fitted	0-2	0.99996	0.013
S	181.972	Fitted	0-2	0.99967	0.31

Agilent 5100 ICP-OES wavelengths and calibration parameters. All results are shown in solutions.

Notes:

Detection power of ICP-AES



Periodic table characterising the detection power and number of useful emission lines of ICP with a pneumatic nebuliser. The degree of shading indicated the range of detection limits for the useful lines. The area of shading indicate the number of useful lines

Notes:

Summary: Atomic Spectroscopy Techniques

	AAS		MP-AES	ICP-OES	ICP-MS	
	FAAS	GFAAS			SQ	QQQ
Detection Limits	100's ppb	10's-100's ppt	ppb – 10's ppb	100's ppt-ppb	<ppt	<ppt
Measurement mode	Sequential	Sequential	Sequential	Simultaneous	Sequential (MS)	Sequential (*MS/MS for difficult interference problems)
Maximum samples/day	100-200 (~6 elements)	50-100 (~2 elements)	300-500 (~10 elements)	2000-2500 (50+ elements)	750-1000 (~50 elements)	500-750 (~50 elements)
Working dynamic range	3-4	2-3	4-5	7-8	10-11	9
Operator skill required	Low	Mid	Low	Mid	High	Highest

Microwave Plasma Atomic Emission Spectroscopy

Notes:

Nitrogen plasma is used to desolvate, atomize, and excite the atoms in the liquid sample that has been nebulized into it.

The nitrogen plasma is considerably hotter (up to 5,000° K) than the air-acetylene flame used in AA.

The atomic emission is quite strong for most elements, leading to improved detection capability and linear dynamic range over flame AA for most elements.

The intensity of the light emitted is measured using optical detection at the wavelengths characteristic of the elements of interest.

MP-AES

Advantages

- Safe (no flammable gas)
- Low operating costs as nitrogen can be extracted from compressed air using a nitrogen generator
- No lamps required for analysis
- Identification and quantitation of virtually all metals and many metalloids.
- Better performance than flame AAS

Limitations

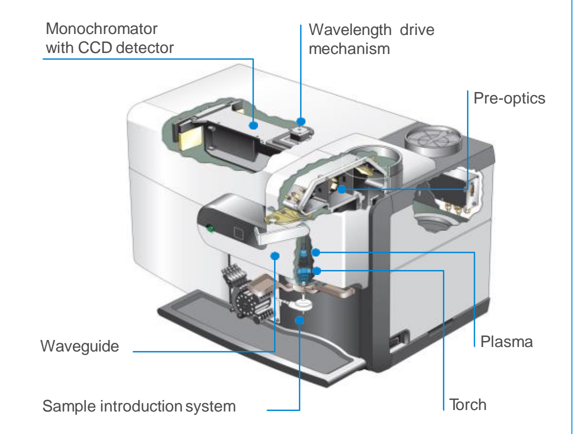
- Higher initial cost than AAS
- More interferences compared with flame AA (including spectral interferences)
- Not as sensitive as graphite furnace AAS or ICP-MS
- Not as productive as ICP-OES
- No isotope determination

Microwave Plasma Atomic Emission Spectroscopy

Notes:

Key Applications

- Trace elements in geological samples
- Metals in soil extracts
- Major elements in food and beverages
- Analysis of petroleum
- Analysis of waste water

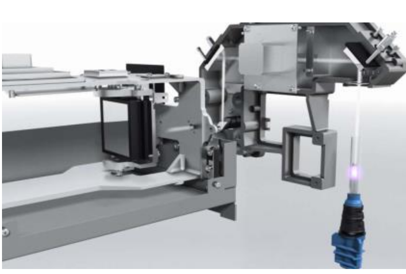


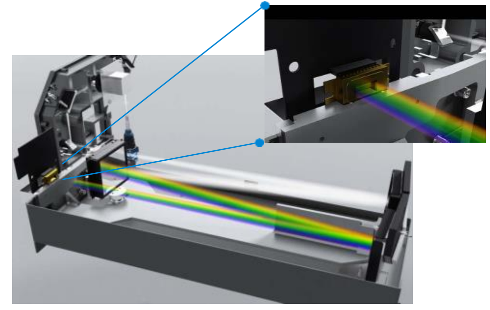
Microwave Plasma Atomic Emission Spectroscopy

Notes:

How Does It Work?

- Axial emission from the nitrogen plasma is directed into the fast-scanning monochromator optics
- Wavelength-specific emissions are detected using a high-efficiency CCD





MP-AES: Determination of Nutrients in Soil Multielement Testing

Notes:

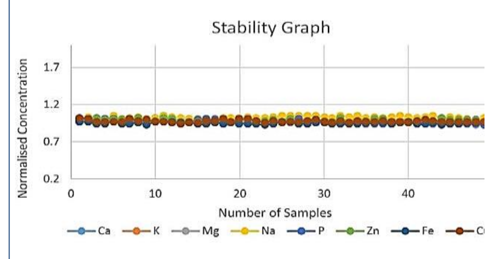
	Cu		Fe		Mn		Zn	
Wavelength (nm)	324.754	324.7	259.94	372	257.61	280.1	213.857	213.9
Technique	MP-AES	FAAS	MP-AES	FAAS	MP-AES	FAAS	MP-AES	FAAS
				Measured conc. µg/g				
SSTD-Trail 1	1.44	1.42	7.76	8.44	24.26	26.22	0.64	0.62
SSTD-Trail 1	1.46	1.45	7.96	8.24	24.40	25.96	0.64	0.64
SSTD-Trail 1	1.44	1.42	8.08	8.64	23.70	26.50	0.62	0.58
Av. µg/g	1.45	1.43	7.93	8.44	24.12	26.23	0.63	0.61
Standard dev.	0.01	0.02	0.16	0.20	0.37	0.27	0.01	0.03

MP-AES results for Cu, Fe, Mn, and Zn in DTPA extraction of soil, compared to FAAS

MP-AES: Measuring Major and Minor Elements in Milk

Notes:

Element	Certified Values (g/kg)	Uncertainty (g/kg)	Result (g/kg)	Recovery (%)
Ca	13.9	0.7	14.21	102
K	17	0.8	16.66	98
Mg	1.26	0.07	1.31	104
Na	4.19	0.23	4.25	101
P	11	0.6	11.27	102
	Certified Values (g/kg)	**Uncertainty (mg/kg)**	**Result (mg/kg)**	**Recovery (%)**
Zn	44.9	2.3	45.89	102
Fe	53	4	50.51	95
Cu	5	0.23	5.13	103



Determination of Ca, K, Mg, Na, P, Fe, Zn and Cu in TMAH, Triton X-100, EDTA and ionizatior buffer by MP-AES 4200

Notes:

Methods for introduction of solid samples

Direct sample insertion
Electro-thermal vaporizers
Arc and spark (DC – direct current and AC - Alternating current) ablation
Laser ablation
Glow discharge technique (GD)

Methods for introduction of solid samples

Notes:

Direct sample insertion:

In this technique, the sample is physically placed into the atomiser.

Electro-thermal vaporizers:

It is same as in the solution samples injection but heating is carried out by conductive heating of the sample and then the inert gas carries the vapourised sample into the atomizer.

Arc and spark ablation:

Electrical discharges of various types are often employed to introduce solid samples into atomizers.

Interaction of the discharge with the surface of a solid sample creates a plume that is made up of a particulate and vaporized sample that is then transported into the atomizer by the flow of an inert gas.

This process of sample introduction is called **ablation**. For arc and spark ablation the sample should be electrically conducting or is to be mixed with a conductor.

Methods for introduction of solid samples

Notes:

Laser ablation:

It is similar to arc and spark ablation but instead a focused laser beam is directed onto the surface of the solid sample for ablation to take place.

Both conducting and non-conducting solids, inorganic and organic samples and powder of metallic powder is suitable for introduction.

Laser beam permits analysis of small areas on the surface of solids.

Methods for introduction of solid samples

Notes:

Glow discharge technique:

Here both sample introduction and sample atomisation takes place simultaneously.

A glow discharge takes place in a low pressure atmosphere of argon gas between a pair of electrodes maintained at a DC potential of 250-1000V.

The applied potential results in argon break down into positively charged argon ions and electrons, these argon ions accelerates towards the cathode surface that contains the sample. Ejection of neutral sample atoms occurs by a process called sputtering.

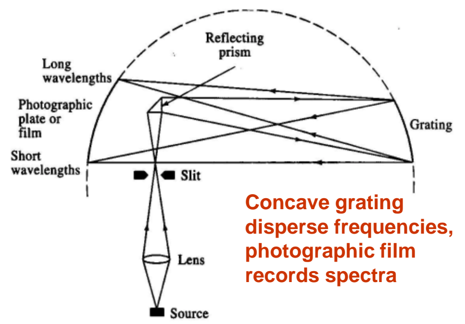
The atomic vapour is produced in the glow discharge which is mixture of atoms and ions which is further subjected to identification.

Example of arc & spark emission spectroscopy

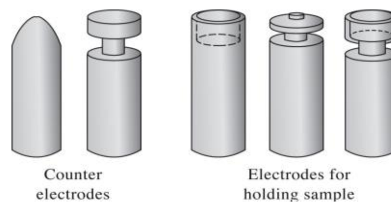
Notes:

- involves use of electrical discharge to give high temperature environment;
- higher fraction of atoms exist in the excited state, giving rise to an increase in emission signal and allowing more types of atoms to be detected;
- can be used for solids, liquids or gas phase samples
- types of discharge used:
  - ✓ DC arc: high sensitivity, poor precision
  - ✓ DC spark: intermediate sensitivity and precision
  - ✓ AC spark: low sensitivity, high precision

Because of difficulty in reproducing the arc/spark conditions, all elements of interest are measured simultaneously by use of appropriate detection scheme.



Concave grating disperse frequencies, photographic film records spectra



Arc created by electrodes separated by a few mm, with an applied current of 1-30 A

Advantages and limitations of plasma sources and AC arc and spark for solid samples

Notes:

Advantages of AC spark:
- Reproducible
- Less material is consumed
- High concentration solution can be used
- Heating effect is less which is useful for analysis of low melting materials.
- No interference

Advantages of AC Arc:
- Best source for qualitative analysis
- Stable
- Reproducible

Arc and Spark Excitation Sources are largely displaced by plasma-AES!!

Advantages of plasma:
Simultaneous multi-element Analysis – saves sample amount
Some non-metal determination (Cl, Br, I, and S)
Concentration range of several decades (10^5 – 10^6)
Disadvantages of plasma:
Very complex Spectra - hundreds to thousands of lines
High resolution and expensive optical components
Expensive instruments, highly trained personnel required

Fluorescent spectroscopy
Basics

Notes:

Fluorescent spectroscopy differs from other spectroscopic techniques because of the recorded spectral dependence is a function of two variables – **the excitation wavelength λ_{ex} and emission wavelength λ_{em}.**

If λ_{ex} is kept constant and λ_{em} is scanned, the measured luminescence spectrum is emission (**spectral dependence of the luminescent intensity on the wavelength**).

If you are scanning λ_{ex} at constant λ_{em}, you measure excitation spectrum (**spectral dependence of the excitation efficiency on the excitation wavelength**).

Luminescence methods

Luminescence methods include studies using fluorescence and phosphorescence.

Fluorescence is the emission of visible light by a substance that has absorbed light of a different wavelength.

The emitted photon usually has a longer wavelength.

Phosphorescence is related to fluorescence in emitting a photon, however, a phosphorescent material does not immediately re-emit the radiation it absorbs.

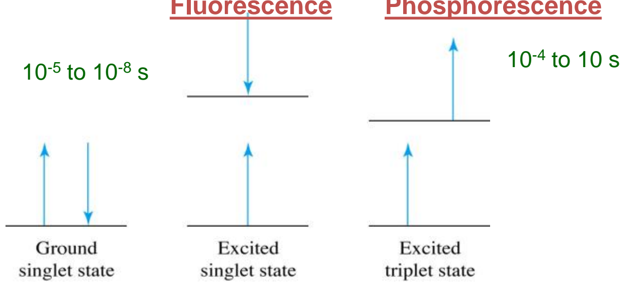
As the excitation of the molecule is due to the absorption of a photon (light), these types of luminescence are called **photoluminescence**.

Chemiluminescence is based on chemical reactions which cause an electronically excited species that emits light as it returns to ground state.

Notes:

Fluorescence and phosphorescence

Fluorescence involves transitions from ground state to single state and back.
Phosphorescence involves transitions from ground state to triplet state and back



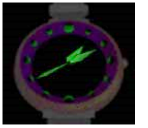
Spins paired
No net magnetic field

Spins unpaired
net magnetic field

Example of Phosphorescence Wrist watch with luminous details

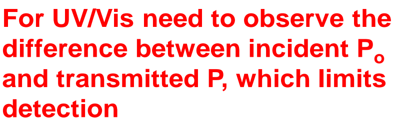
0 sec

1 sec

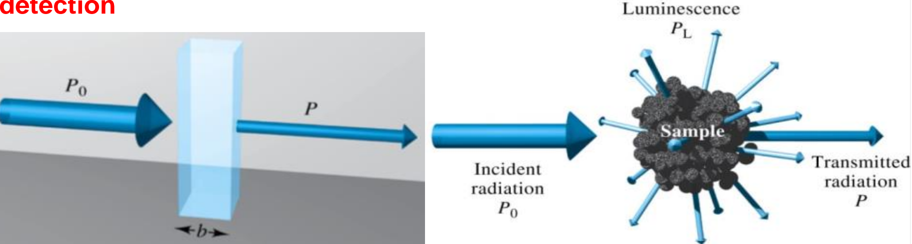

640 sec

Notes:

Detection limits of method


For UV/Vis need to observe the difference between incident P_o and transmitted P, which limits detection

For fluorescence, one need to observe only amount of emitted P_L



Method	Mass detection limit (moles)	Concentration detection limit (molar)	Advantages
UV-Vis	10^{-13} to 10^{-16}	10^{-5} to 10^{-8}	Universal
fluorescence	10^{-15} to 10^{-17}	10^{-7} to 10^{-9}	Sensitive

Notes:

FACTORS AFFECTING FLUORESCENCE AND PHOSPHORESCENCE

Notes:

The common factors affecting the fluorescence are as follows.

- Temperature
- pH
- Dissolved oxygen
- Solvent

The essential components of an instrument used to measure fluorescence of the sample are:

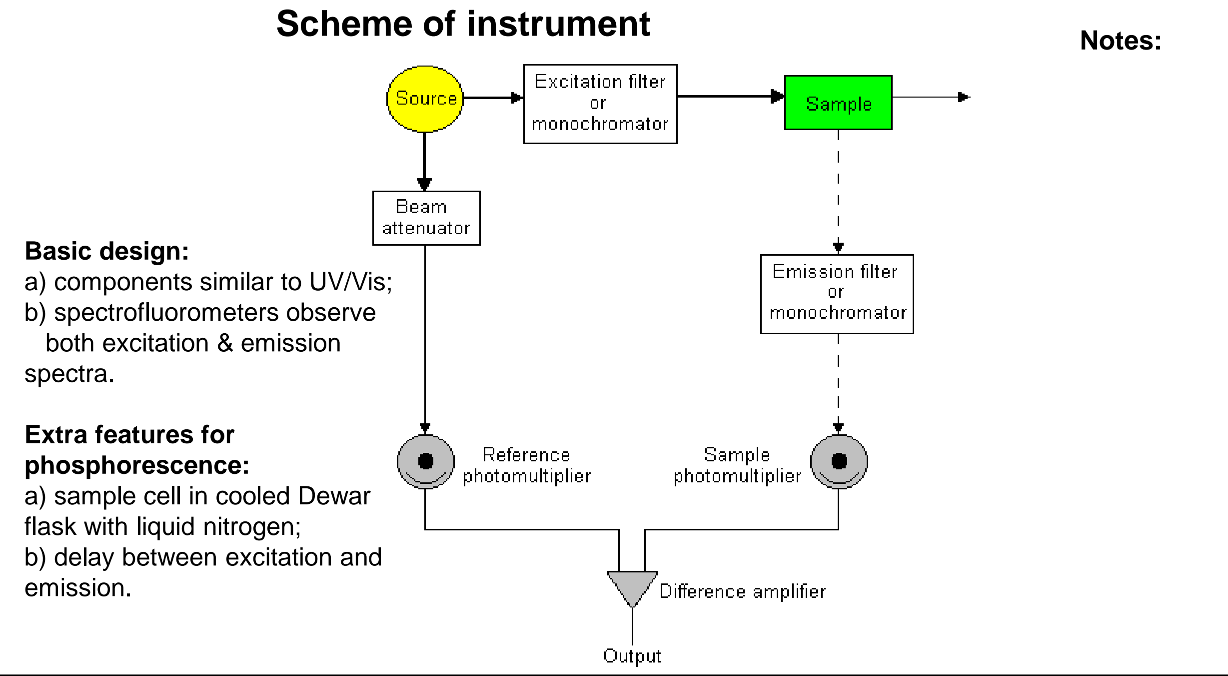
- Excitation light sources
- Filters or Monochromators
- Sample holder
- Detector
- Readout device

Scheme of instrument

Notes:

Basic design:
a) components similar to UV/Vis;
b) spectrofluorometers observe both excitation & emission spectra.

Extra features for phosphorescence:
a) sample cell in cooled Dewar flask with liquid nitrogen;
b) delay between excitation and emission.



Instrumentation

Notes:

Light sources

- Commonly employed sources in fluorescence spectrometry have spectral outputs either as a continuum of energy over a wide range or as a series of discrete lines.
- An example of the first type is the tungsten- halogen lamp and of the latter, a mercury lamp.

Detectors

- All commercial fluorescence instruments use photomultiplier tubes as detectors and a wide variety of types are available.
- The material from which the photocathode is made determines the spectral range of the photomultiplier; generally two tubes are required to cover the complete UV-visible range.

Read-out devices

- The output from the detector is amplified and displayed on a readout device which may be a meter or digital display.
- It should be possible to change the sensitivity of the amplifier in a series of discrete steps to compare samples of widely differing concentration.
- A continuous sensitivity adjustment is also useful so that the display can be made to read directly in concentration units.

Fluorescence Spectra

Excitation Spectra (a):
measure fluorescence or phosphorescence at a fixed wavelength while varying the excitation wavelength.

Emission Spectra (b)
measure fluorescence
or phosphorescence over a range of wavelengths using a fixed excitation wavelength.

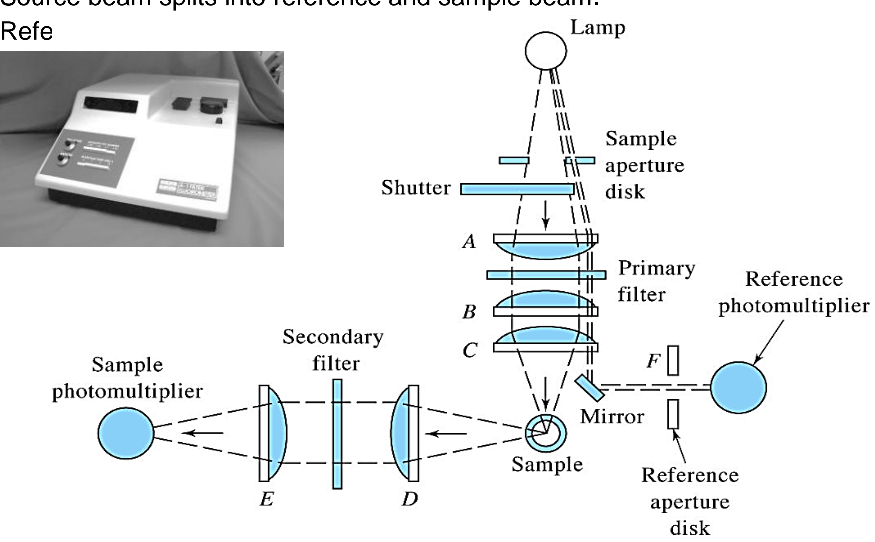
Phosphorescence bands are usually found at longer (>l) then fluorescence because excited triple state is lower energy then excited singlet state.

Excitation wavelength, nm
300 350 400
Fluorescence intensity
(a)
Fluorescence intensity
(b)
300 350 400
Emission wavelength, nm

Notes:

Fluorometers

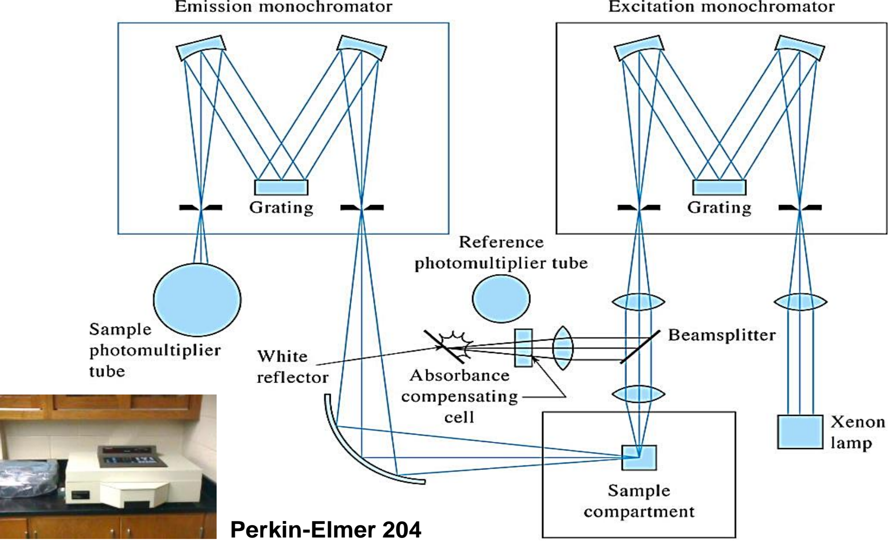
Fluorometers are simple, low cost and compact.
Source beam splits into reference and sample beam.
Refe



Notes:

Spectrofluorometers

Spectrofluorometers are equipped with two grating monochromators and record excitation and emission spectra. Quantitative analysis is possible.



Perkin-Elmer 204

Notes:

Applications

Notes:

Fluorescent measuring is the most widely used as methods for analysis and monitoring of chemical and biochemical reactions and kinetic studies for the fast reactions of electron-excited molecules.

Applications of luminescence spectroscopy for analytical purposes embrace identification of substances, detection of low concentrations of substances, control the changes of studied matter, determination the purity of compounds.

Also, luminescence studies are used to measure kinetics of conventional chemical reactions. The high sensitivity allows detecting the small degree of substances conversion and sometimes it is possible to establish the mechanism of a chemical reaction.

Comparison of emission methods (ICP-AES, ICP-MS, FES, AAS) and selection of proper techniques

Notes:

A very wide spectrum of applications, some of them are as follows:

- **Clinical Analysis:** metals in biological fluids (blood, urine);
- **Environmental Analysis:** trace metals and other elements in waters, soils, plants, composts and sludges;
- **Pharmaceuticals:** traces of catalysts used; traces of poison metals (Cd, Pb etc);
- **Industry:** quality control in technological processes; trace metal analysis in raw materials; noble metals determination.
- **Forensic science:** gunshot powder residue analysis, toxicological examination (e.g., thallium determination)

Summary of Atomic Spectroscopy

Notes:

Technique	Strengths	Limitations	Applications
Flame AA – Flame Atomic Absorption Spectroscopy	Very easy-to-use Widely accepted Extensive application information available Relatively inexpensive	Low sensitivity Single-element analytical capability Cannot be left unattended (flammable gas)	Ideal for laboratories analysin large numbers of samples for a limited number of elements and for the determination of major constituents and higher concentration analytes
GFAA – Graphite Furnace Atomic Absorption Spectroscopy	Exceptional detection limits Well-documented applications May be left unattended	Limited analytical working range Sample throughput somewhat less than other techniques	Ideal for laboratories analysin a limited number of elements and requiring excellent detection limits
ICP-OES – Inductively Coupled Plasma Optical Emission Spectroscopy	Best overall multi-element atomic spectroscopy technique Excellent sample throughput Very wide analytical range Good documentation available for applications May be left unattended Easy-to-use	Higher initial investment	Ideal for laboratories analysin multiple elements in a moderate or large number of samples

Chemical and Ionisation interferences

Notes:

REFRACTORY COMPOUND FORMATION

- compounds that cannot be broken down in flame
- e.g. Ca signal is depressed due to formation of Ca sulfate or Ca phosphate
- e.g. Mg signal is depressed in the presence of Al. Al forms heat stable compound with Mg.

Solution:

- Use of Hotter flame
- Use of Releasing agents such as chlorides of La and Sr.
- Use of Protective agent such as EDTA and 8-Hydroxyquinolone

IONIZATION INTERFERENCES

- affects Group 1 and 2 elements (Ba, Ca, Sr, Na, K)

Solution:

- Use of Low Temperature Flame or Use of Ionization Buffer
- Ionization buffer/suppressor/suppressant prevents analyte ionization
- e.g. Addition of a 0.1% KCl soln to blank, standard, and sample.

Matrix interferences

Notes:

MATRIX INTERFERENCES

- a physical interference that can either suppress or enhance absorbance signal of analyte.

Causes:

- Differences in viscosity and surface tension.
- Preparation in different solvents.
- Measurement at different temperatures.
- Presence of organic species.
- Different atomization rate in flame.

Solution:

- by diluting the sample
- or by using a peristaltic pump,
- by using an internal standard
- or by using a high solids nebulizer.

Notes:

ICP-OES versus FAAS

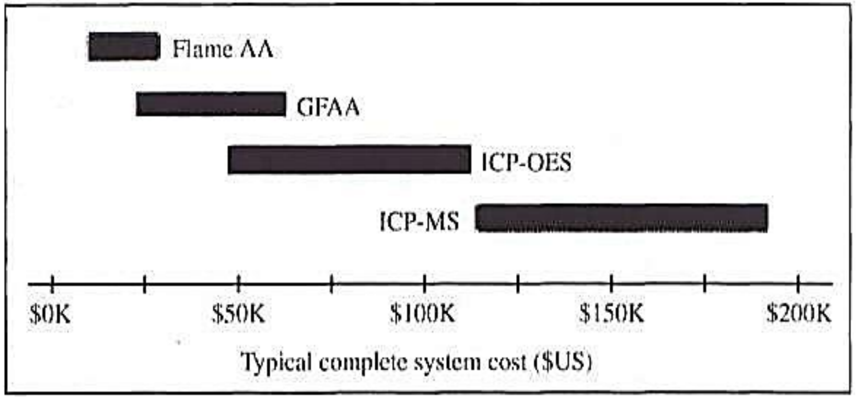
ICP-OES has become the dominant instrument for routine analysis of metals

ICP-OES compared to FAAS:

- Lower interferences (due to higher temperatures);
- Spectra for most elements can be recorded simultaneously under the same conditions;
- Higher temperature allows compounds (e.g. metal oxides) to be measured;
- Determination of non metals (e.g. Cl, Br, I, S).

Costs of spectroscopic equipment

Notes:

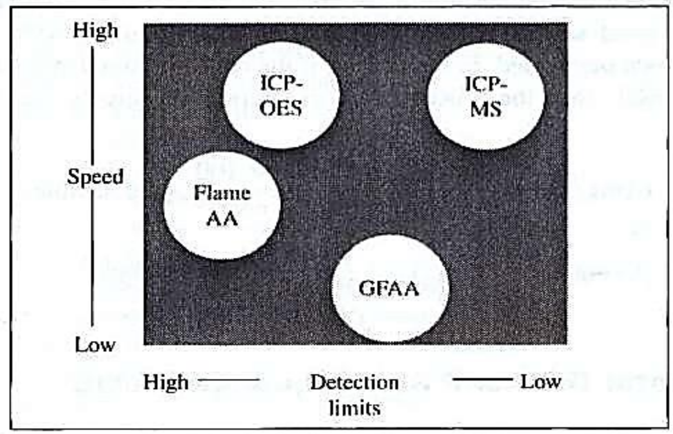


Typical relative prices for atomic spectroscopy systems

Method comparison in productivity

Notes:

ICP-OES and ICP-MS are multi-element techniques favoured when there is a large number of samples and cost is not a concern

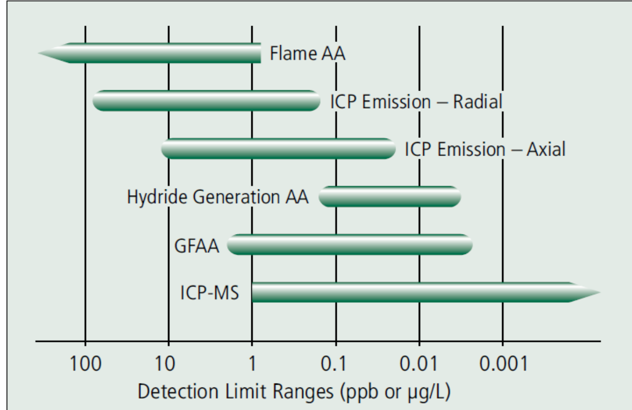


General selection guide for atomic spectroscopy instrumentation based on sample throughput and concentration range

Detection limit

Notes:

- Low detection limit is essential for trace analysis
- Without low level capability – sample pre-concentration is required

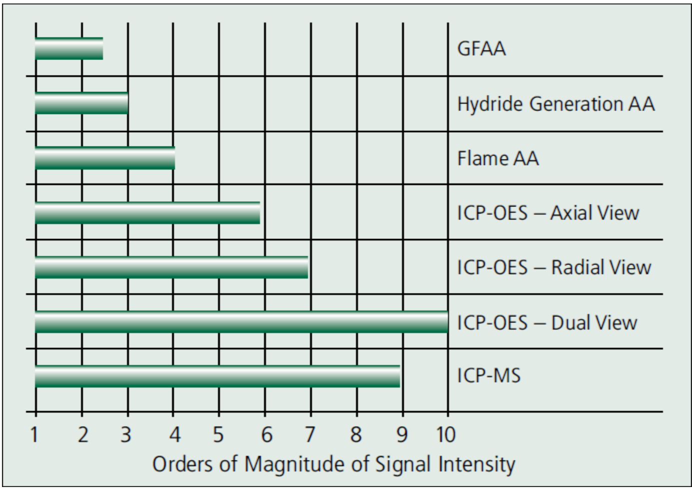


Typical detection limit ranges for the major atomic spectroscopy techniques.

Working range

Notes:

Ideal working range minimizes analytical effort and potential errors



Typical analytical working ranges for the major atomic spectroscopy techniques.

Selection of proper spectroscopic techniques

Notes:

Important factors:

Detection limit

Working range

Sample throughput

Cost

Interferences

Ease of use

Availability of

proven methodology

	Flame AA	GFAA	ICP-OES	ICP-MS
How Many Elements?				
Single	■			
Few		■		
Many			■	■
What Levels?				
High ppb	■		■	
Sub ppb		■	■	■
Sub ppb-ppm				■
Sub ppt				■
How Many Samples?				
Very few	■	■		
Few	■	■	■	■
Many			■	■
How Much Sample?				
> 5 mL	■	■	■	■
< 1-2 mL		■		

Tasks to Section 13

1. Give definitions of these terms: flame emission spectroscopy, inductively coupled plasma, microwave plasma, ionisation buffer, matrix modifier, interference, ionization interference, chemical interference, photoluminescence, fluorescence, phosphorescence.

2. Which technique, atomic absorption or atomic emission, is flame temperature stability more critical? Why?

3. State the advantages and disadvantages of a furnace compared with a flame in atomic absorption spectroscopy.

4. State the advantages and disadvantages of the inductively coupled plasma compared with a flame in atomic spectroscopy.

5. Li was determined by atomic emission with the method of standard addition. Prepare a standard addition graph to find the concentration of Li and its uncertainty in pure unknown. The Li standard contained 1.62 g Li/mL.

Unknown (mL)	Standard (mL)	Final volume (mL)	Emission intensity (arbitrary units)
10.00	0.00	100.0	309
10.00	5.00	100.0	452
10.00	10.00	100.0	600
10.00	15.00	100.0	765
10.00	20.00	100.0	906

Section 14: Methods based on the phenomena of light scattering

Contents:

- Introduction
- Analytical methods based on the phenomena of light scattering
- Raman spectroscopy.
- Nephelometry and turbidimetry.
- Polarimetry.
- Reflectometry.
- Refractometry

Introduction

Different types of interaction of light with matter create a basis for the development of analytical methods. In Section 14, we will focus on the effects of scattering, refraction, and polarisation.

Atoms or molecules which are exposed to light absorb light energy and re-emit light in different directions with different intensity. This phenomenon is an example of scattering. It is a general physical process, where quanta of some form, such as light, sound or moving particles, are forced to deviate from a straight trajectory by localized non-uniformities in the medium through which they pass. In conventional use, this also includes the deviation of reflected radiation from the angle predicted by the law of reflection. Reflections of radiation that undergoes scattering are often called diffuse reflections and unscattered reflections are called specular (mirror-like) reflections.

Scattering may also refer to particle-particle collisions between molecules, atoms, electrons, photons and other particles. The types of non-uniformities which can cause scattering, sometimes known as scatterers or scattering centres, are too numerous to list. However, a small sample includes particles, bubbles, droplets, density fluctuations in fluids, defects in monocrystalline solids etc.

Refraction is the change in the direction of a wave passing from one medium to another or from a gradual change in the medium. For light, refraction follows the following law. For a given pair of media, the ratio of the sines of the angle of incidence and angle of refraction is equal to the ratio of phase velocities in the two media. Equivalently, it is equal to the ratio of the indices of refraction of the two media. The refractive index of materials varies with the wavelength of light. Thus the angle of the refraction also varies correspondingly.

Polarization is the ability of waves to oscillate in more than one direction, determines the geometric orientation of oscillations.

Light is an electromagnetic wave that consists of a coupled oscillating electric field and a magnetic field that is always perpendicular to each other. With linear polarization, the fields oscillate in one direction. With circular or elliptical polarization, the fields rotate at a constant speed in the plane as the wave moves.

Light or other electromagnetic radiation from many sources, such as the sun, flames, and incandescent lamps, consists of shortwave trains with an equal mixture of polarizations. It is called unpolarized light. Polarized light can be produced by passing unpolarized light through a polarizer, allowing only one polarization to be transmitted. The most common optical materials (e.g. glass) are isotropic. They do not affect the polarization of light passing through them. However, some materials — those that exhibit light reflection, dichroism, or optical activity — can alter the polarization of light.

Raman spectroscopy is a molecular spectroscopy method based on the interaction of light with matter. It allows you to get an idea of the structure of the material or its characteristics, and in this respect is similar to the method of IR Fourier spectroscopy (FTIR). Raman spectroscopy is based on scattered light; while IR spectroscopy is based on light absorption. Both methods are complementary to each other. Raman spectroscopy provides information on intramolecular and intermolecular vibrations and helps to provide a more complete picture of the reaction.

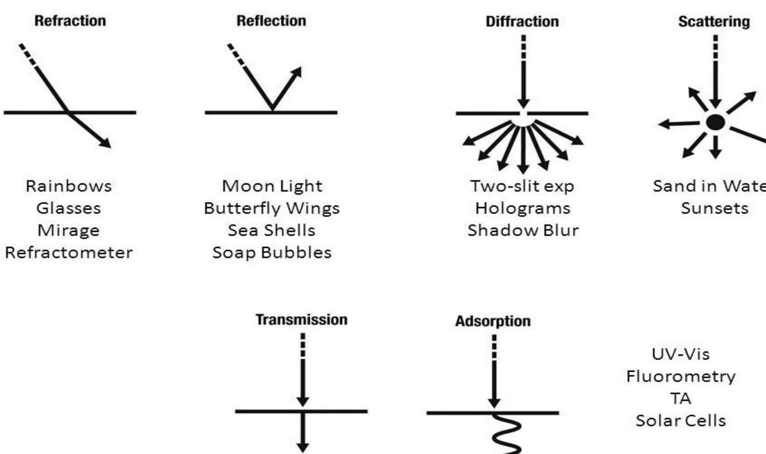
Nephelometry and Turbidimetry are analytical techniques used to measure scattered light. The amount of light scattered is proportional to the concentration of insoluble particle.

Polarimetry is an instrumental analytical method using the rotation of polarized light by some substances as a measure of their concentration in a solution.

Reflectometry uses the reflection of waves at surfaces and interfaces to detect or characterize objects.

Different types of interaction of light with matter create basis for the development of different analytical methods

Notes:



UV-Vis
Fluorometry
TA
Solar Cells

Below, we will focus on the effects of scattering, refraction, reflection and polarisation

Notes:

Raman spectroscopy

(light scattering)

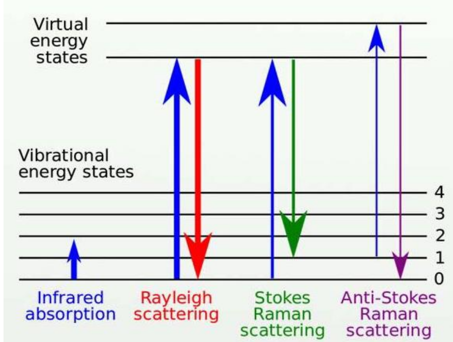
The process of scattering and technique were named after **Sir Chandrasekhar Venkat Raman**, who discovered it and was awarded the Nobel Prize in Physics in 1930

The nature of Raman scattering

Notes:

When light interacts with molecules in a gas, liquid, or solid, the vast majority of photons are scattered, having the same energy as the incident photons (so called **Rayleigh** scattering).

Some photons (one in 10 million) after scattering acquire a frequency different from the frequency of the incident photon.

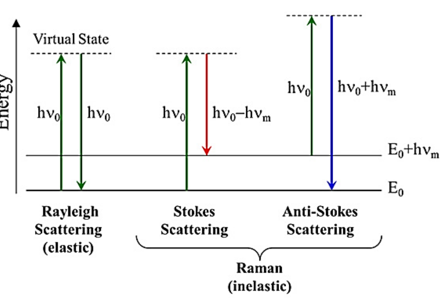


Photons with higher and lower energies are scattered and called **anti-Stocks and Stocks**

This process is called inelastic scattering or the **Raman effect**.

The process of Raman scattering consists in:

- A molecule, interacting with a photon, can transform into a virtual state with a higher energy.
- There are several likely scenarios for a molecule to exit this state.
- In one of them, the molecule can go into a state with a level of vibrational energy, which differs from the initial level, while emitting a photon with a different energy.
- The difference between the energy of the incident photon and the energy of the scattered photon is called the Raman shift.

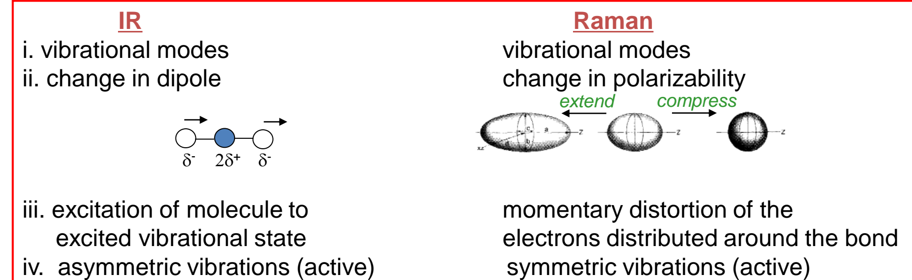


- Observed frequency shifts are related to vibrational changes in the molecule → associated with IR absorbance
- Raman scattering spectrum looks like inversed IR absorbance spectrum
- But the mechanisms of Raman & IR are different

Notes:

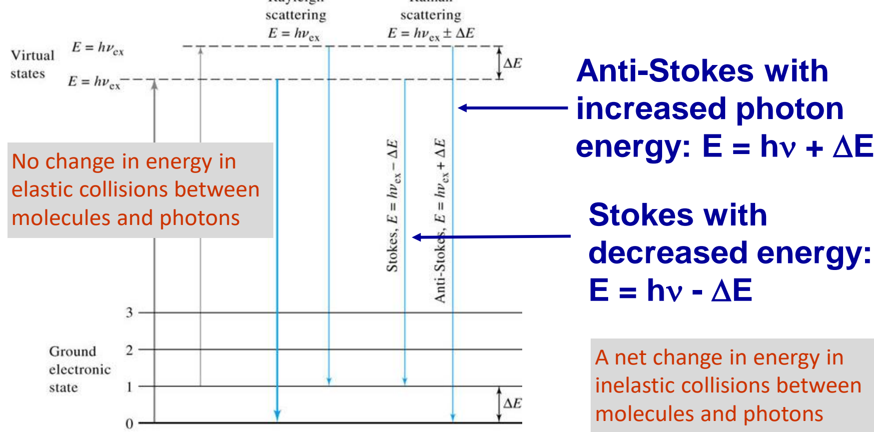
Mechanisms of Raman compared to IR

- Unlike IR Fourier spectroscopy, which shows changes in dipole moments, Raman spectroscopy shows changes in the polarizability of molecular bonds.
- The interaction of light with a molecule can cause deformation of its electron cloud. This deformation is called a change in polarizability.
- At certain energy, transitions accompanied by changes in the polarizability of molecular bonds, active Raman modes arise.
- ***Raman frequency range*: 4000 -50 cm^{-1}(Stokes and anti-Stokes)**



Notes:

Two Types of Raman Scattering are possible

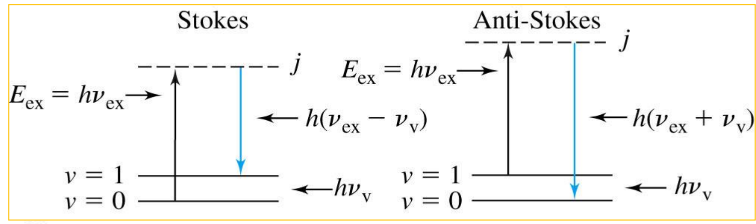


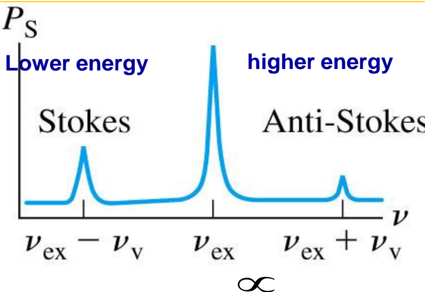
$\pm\Delta E$ – the energy of the first vibration level of the ground state – IR vibration absorbance

Raman frequency shift and IR absorption peak frequency are identical

Notes:

Notes:





Intensity of Raman lines are 0.001% intensity of the initial source

$\propto$

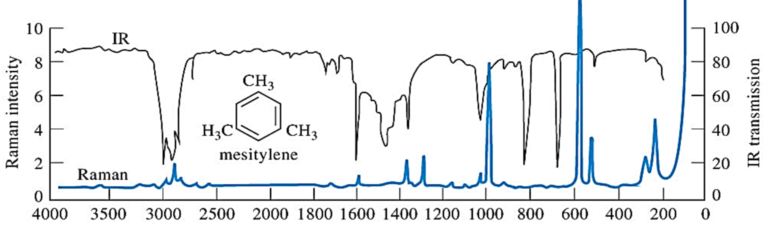
Probability of Emission and Observed Intensity are as follows:

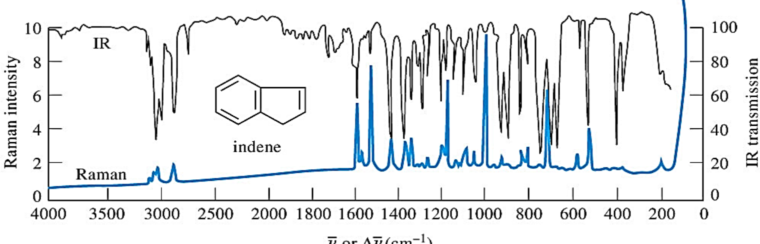
Raleigh scattering >> Stokes >> anti-Stokes

due to difference in population of energy levels of vibrational transitions

Comparison of Raman and IR Spectra

Notes:

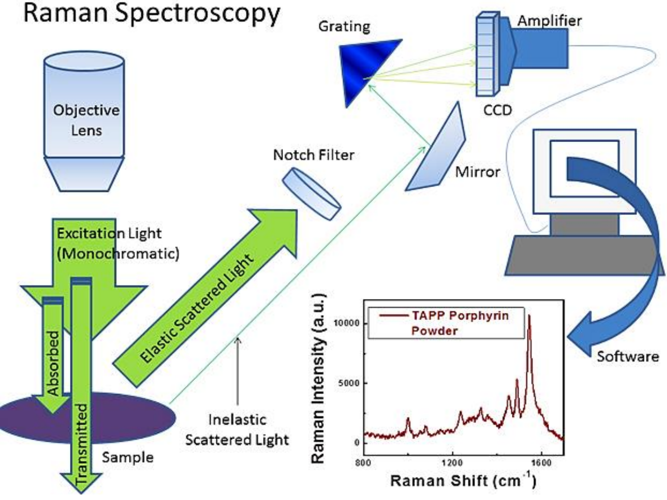




Raman and IR spectra are complimentary. For some molecules, Raman is active and IR inactive, and vice versa. In some cases, both can be active simultaneously

Instrumentation

Notes:



A modern compact Raman spectrometer consists of several main components, including a laser, which serves as a source of excitation of molecules to induce Raman scattering.

Instrumentation (continued)

Notes:

- Modern Raman spectrometers use solid-state lasers with wavelengths of 532, 785, 830 and 1064 nm.
- Lasers with shorter wavelengths have a larger scattering area, so the signal, as a result, is more powerful, but fluorescence more often occurs at such lengths.
- Visible source allows using of glass/quartz sample cells & optics.
- Fibre optic cables are used to transmit laser energy.
- To eliminate Rayleigh and anti-Stokes scattering, band-pass or edge filters are used, and the remaining light subjected to Stokes scattering is transmitted to the dispersion element — usually a holographic grating.
- UV/Vis type detectors (photomultiplier tubes) are typical.
- The light enters the detector, after which the Raman spectrum is built.
- Since the Raman effect is weak (only 0.001% of a light source), the optical components of spectrometer must be optimized and well-aligned.

Applications

Notes:

a) Qualitative Information

i. characteristic regions for different groups as in IR
ii. Raman correlation charts available
iii. Good for aqueous based samples
iv. Useful for a variety of samples, organic, inorganic & biological

b) Quantitative Information – *not routinely used*

i. fewer technical problems than IR, fewer peaks
ii. Interference from fluorescence
iii. Higher cost
iii. Signal weak – require modified Raman methods

1) Resonance Raman spectroscopy allows detection of 10^{-3} ->10^{-7}M by using lasers light with wavelength approaching *electronic* absorption

2) Surface enhanced Raman spectroscopy places samples on metal or rough surfaces that increase Raman scattering

In general: IR tends to detect well polar functional groups (R-OH, C=O, etc.)
Raman well detects aromatic & carbon backbone (C=C, $-CH_2-$, etc.)

Raman does not "see" many common polar solvents and can be used with aqueous samples – advantage over IR

Nephelometry and turbidimetry (light scattering). Backgrounds

Notes:

When particles are suspended in a solution in a cuvette, they make the solution unclear (turbid). Incident light entering the cuvette will be subjected to three reactions:

1. Some of the light will be absorbed (blocked) by the particles
2. Some will be transmitted through the cuvette
3. Some will be scattered or reflected in various directions.
4. The scattered light is at the same wavelength as the incident light

Nephelometry and Turbidimetry are analytical techniques used to measure scattered light.

The amount of light scattered is proportional to the concentration of insoluble particle.

The two techniques differs only in the manner of measuring the scattered radiation.

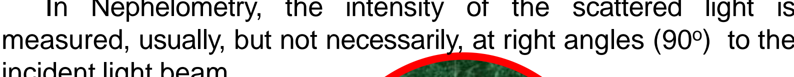
Instrumentation

Notes:

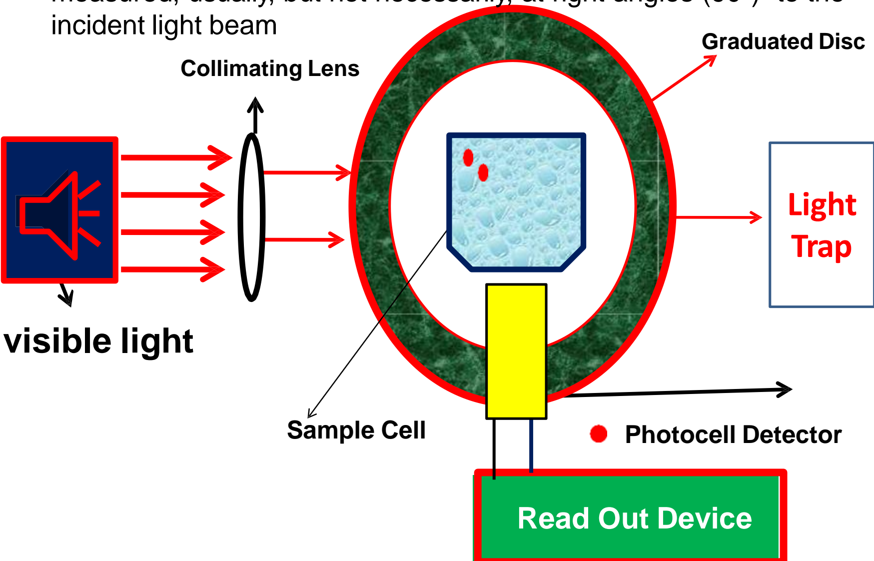
- The principal concern of light scatter instrumentation are:
  - Excitation intensity;
  - Wavelength;
  - Distance of the detector from the sample cuvette;
  - Minimization of external stray light.
- The basic components of the Nephelometer include:
  - Light source;
  - Collimating optics: including light scattering optics, detector optical filter and a detector.
- **Operationally, the optical components used in turbidimeters and nephelometers are similar to those used in fluorometers and photometers.**

Nephelometer

Notes:



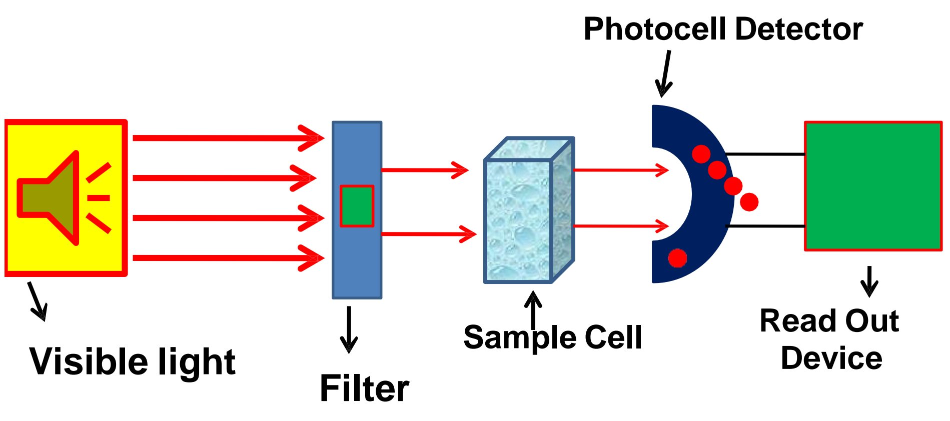
In Nephelometry, the intensity of the scattered light is measured, usually, but not necessarily, at right angles (90°) to the incident light beam



Turbidimeter

Notes:

In turbidimetry, the intensity of light transmitted through the medium, the unscattered light, is measured at 180° from the incident light beam.



Factors that influence light scattering

Notes:

1. Particle size & shape
2. The concentration of particles: is directly proportional to the light scattering intensity
3. The molecular weight of particles: directly proportional to the light scattering intensity
4. Wavelength dependence: the intensity of light scattering is inversely proportional to the wavelength of the incident light
5. The distance of observation (Set-up): scattered light intensity is inversely proportional to the distance from the light scattering particles to the detector
6. Polarization of incident light:
   - The total light scattered by small particles is less when excited by polarized light than by nonpolarized light;
   - Light scattering intensity from small particles excited by nonpolarized light shows symmetric angular dependence of light scattering about the 90 degrees axis;
   - For larger particles, it is dissymmetrical and the dissymmetry increases even further as the particle size increases;
   - The dissymmetry and the change in the angular dependence of light scattering with the change in the size of the particle are very useful for characterization and differentiation of various classes of macromolecules and cells.

Difference between nephelometry and turbidimetry

Notes:

Nephelometry	Turbidimetry
1. Mercury arc lamp.	1. Tungsten / Deuterium lamp
2. Rectangular cuvette used.	2. Semi octagonal cuvette
3. Scattered light is measured.	3. Light transmitted is measured
4. Detectors may be placed at 90°, 70° or 37° depending on the angle at which most scattered light are found.	4. Measured in straight line
5. PMT (photomultiplier tube) is detector	5. Photocell is detector

Selection of a wavelength

- If both solution and suspended particles are colourless, then use any wave length in the visible range.
- If the solution is coloured but the particles are not coloured, then use a wave length that gives minimum absorption for the solution.
- If the particles are coloured and the solution is colourless then use a wavelength that gives maximum absorption with the particles.
- If both solution and particles are coloured then use two wavelengths; one that gives minimum absorbance for the solution and the other one maximum absorbance for the particles. Subtract the solution absorbance from the particles absorbance.

Tyndall Effect

Notes:

- The Tyndall Effect is is light scattering by particles in a colloid or in a very fine suspension, while showing no light scattering in a true solution.
- This effect is used to determine whether a mixture is a true solution or a colloid.
- Under the Tyndall effect, the longer-wavelength light is more transmitted while the shorter-wavelength light is more scattered.
- The Tyndall effect is seen when light-scattering particulate matter is dispersed in an otherwise light-transmitting medium, when the diameter of an individual particle is the range of roughly between 40 and 900 nm, i.e. somewhat below or near The wavelengths of visible Light (400–750 nm).

Comparison of two methods

Notes:

Turbidity can be measured on most routine analysers by a spectrophotometer (absorbed light)

- Reduced sensitivity and precision.
- Extent of light scattering increases as wavelength increases

The intensity of scattered light is normally measured by Nephelometer. Fluorometers are often used to perform Nephelometric measurements

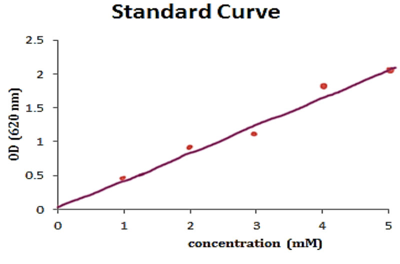
For higher sensitivity and for applications that determine the size and number of particles in suspension, laser light nephelometers is used.

The choice of method used is dependent upon the amount of light scattered by suspended particles present in solution. Since the amount of scattered light is far greater than the transmitted light in a turbid suspension, nephelometry offers higher sensitivity than turbidimetry.

- **Highly concentrated suspensions – turbidimetry**
- **Low concentration – nephelometry (more accurate results)**

Quantification of light scattering measurement:

Notes:



- The amount of light scatter is measured and compared to the amount of scatter from known mixtures (Standards).
- The amount of the unknown is determined from a standard curve.
- Because the absorbance is dependent on both number and size of particles, the standard solution which is used for the standard curve must have similar size in suspension as unknown.

Because some precipitation and settlement of particles may occur with time, in order to obtain good accuracy it is important to:

a) mix the sample well prior to placing the cuvette in the instrument, and,

b) keep the same time for measurement of every sample throughout the measurement.

Kinetic reactions (measurement of the progress of reaction with time) provides higher degree of accuracy, sensitivity, precision and less time than end-point reactions (measuring the reaction at the start and finish of the reaction).

Advantage of Nephelometry over Turbidimetry:

Notes:

1. Higher signal to noise ratio (Uniform scattering)
2. Higher sensitivity (lower detection limit)
3. High precision over turbidimetry

Disadvantages of light scattering techniques:

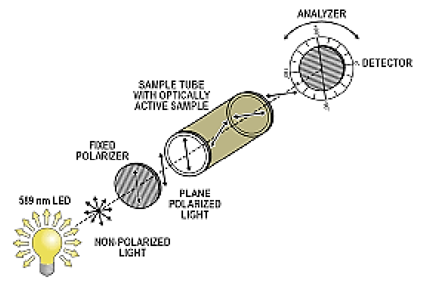
- High cost
- Easily damaged
- They require high power supply

Application

Notes:

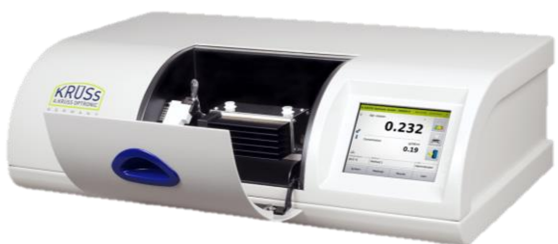
- Analysis of water: clarity, concentration of ions
- Determination of CO_2
- Determination of inorganic substances:
  Sulphate – barium chloride
  Ammonia – Nesslers reagent
  Phosphorus – Strychine molybedate
- Biochemical Analysis
- Quantitative Analysis – (ppm level)
- Miscellaneous: water treatment plants, sewage work, refineries, paper industry
- Atmospheric pollution: Smokes & fogs
- Determination of molecular Weight of high polymers
- Phase titration

Notes:



Polarimetry

(rotation of polarisation plane)



What does polarised light mean?

Notes:

- Light is an electromagnetic wave, and the electric field of this wave oscillates perpendicularly to the direction of propagation.
- Light is called unpolarized if the direction of this electric field fluctuates randomly in time

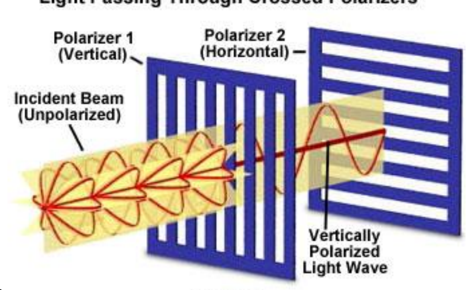


Figure 1

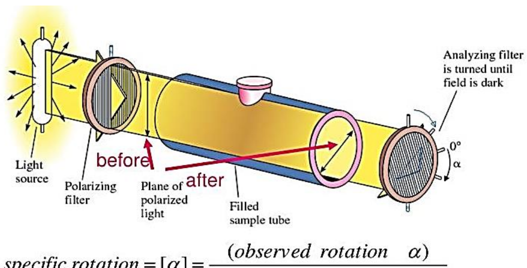
- Many common light sources such as sunlight, halogen lighting, LED spotlights, etc. produce unpolarized light.
- If the direction of the electric field of light is well defined, it is called polarized light.
- The most common source of polarized light is a laser.

Definition

Notes:

A polarizer is a device through which only light waves oscillating in a single plane may pass.

A polarimeter is an instrument used to determine the angle through which plane-polarized light has been rotated by a given sample.



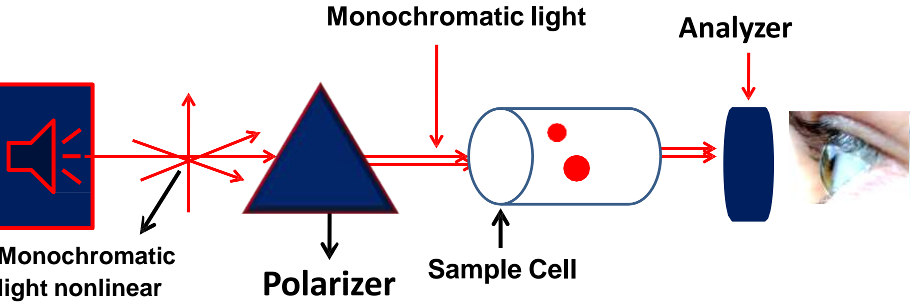
$$specific\ rotation = [\alpha] = \frac{(observed\ rotation\ \ \alpha)}{(length,\ dm)(concentration)}$$

Concentration: pure liquid in g/mL; solution in g per 100 mL of solvent

An analyzer is the component of a polarimeter that allows the angle of rotation of plane-polarized light to be determined.

Scheme of polarimeter

Notes:



- Monochromatic (single wavelength) light, is polarised by a fixed polariser next to the light source.
- A sample cell holder is located in line with the light beam.
- It is followed by a movable analyser (second rotatable polariser) and an eyepiece through which the light intensity can be observed.
- In modern instruments an electronic light detector takes the place of the human eye.

Operation of polarimeter

Notes:

Polarimetry is an instrumental analytical method using rotation of polarized light by some substances as a measure of their concentration in a solution.

- Compounds that rotate the plane of polarized light are termed optically active.
- Unpolarized light from the light source is first polarized.
- This polarized light passes through a cell which can contain sample substance.
- In the absence of a sample, the light intensity at the detector is at a maximum when the analyser (second movable polarizer) is set parallel to the first polarizer ($\alpha = 0^o$).
- If the analyzer is turned 90° to the plane of initial polarization, all the light will be blocked from reaching the detector.
- If an optical active substance is in a sample tube, the plane of the polarized light waves is rotated.
- The rotation is noticed by looking through the analyser as a change in intensity of illumination.
- To reach the same illumination as was without an optical active sample the analyser must be turned around for an angle α.
- Readings are taken in degrees (angle).

Operation of polarimeter (continued)

Notes:

- Chemists use polarimeters to investigate the influence of compounds (in the sample cell) on plane-polarized light. Samples composed only of achiral molecules (e.g. water or hexane), have no effect on the polarized light beam.
- Each enantiomer of a stereoisomeric pair is optically active and has an equal but opposite-in-sign specific rotation. Specific rotations are useful in that they are experimentally determined constants that characterize and identify pure enantiomers.
- If a single enantiomer is examined (all sample molecules being right-handed, or all being left-handed), the plane of polarization is rotated in either a clockwise (positive) or counter-clockwise (negative) direction.
- Enantiomer, rotating polarized light in a clockwise direction, is named dextrorotatory or (+), and its mirror-image partner with counter-clockwise rotation is named levorotatory or (–).
- The prefixes dextro and levo come from the Latin dexter, meaning right and left, and are abbreviated d and l respectively.

Operation of polarimeter (continued)

Notes:

- If equal quantities of each enantiomer are examined, using the same sample cell, then the magnitude of the rotations will be the same, with one being positive and the other negative.
- A 50:50 mixture of enantiomers has no observable optical activity. Such mixtures are called racemates or racemic modifications, and are designated (±).
- When chiral compounds are created from achiral compounds, the products are racemic unless a single enantiomer of a chiral co-reactant or catalyst is involved in the reaction.
- To be absolutely certain whether an observed rotation is positive or negative it is often necessary to make a second measurement using a different amount or concentration of the sample.
- Since it is not always possible to obtain or use samples of exactly the same size, the observed rotation is usually corrected to compensate for variations in sample quantity and cell length.

Notes:

TYPICAL APPLICATIONS OF POLARIMETRY	ANALYSED SUBSTANCES
PHARMACEUTICAL INDUSTRY Determination of the concentration of sugar as an ingredient of pharmaceutical agents. Purity control and content determination. Determination of the stereochemical composition and mutarotation. Characterisation of new synthetic substances	Sugar, amino acids and proteins, blood sera, vitamins, steroids, antibiotics, hormones, painkillers, amphetamines etc.
CHEMICAL INDUSTRY Purity control and concentration determination. Monitoring of chemical processes during the production of optically active substances characterisation tests in research laboratories. Reaction kinetic analyses	Biopolymers, synthetic polymers, glycerinaldehydes, various hydrocarbons etc.
FOOD AND BEVERAGE INDUSTRY Characterisation, quality and purity control of raw materials and end products. Determination of the sugar concentration in beverages and candies. Routine analysis with high sample throughput	Sugar, lactic acid, starch (polysaccharide) in food and feed, aromas, lactose in milk, glucose in wine, sugar composition in honey etc.

TYPICAL APPLICATIONS OF POLARIMETRY | ANALYSED SUBSTANCES

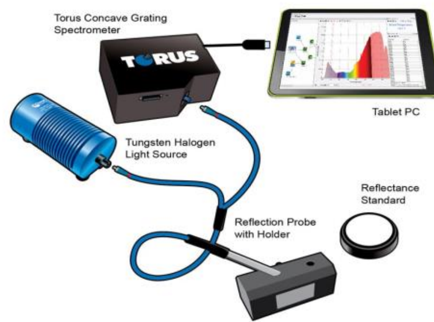
Notes:

TYPICAL APPLICATIONS OF POLARIMETRY	ANALYSED SUBSTANCES
SUGAR INDUSTRY Determination of the sugar concentration in raw materials, preliminary, intermediate and end products. Monitoring of chemical processes, e.g. during the manufacture of invert sugar. Purity control	sugar cane, beet pulp, molasses, refined sugar, syrup, invert sugar etc.
MANUFACTURERS OF AROMAS, FRAGRANCES AND ESSENTIAL OILS Quality control of raw materials and additives. Monitoring of the production of intermediate and end products	Essential oils such as orange, lavender, lime and pepper mint oil, glyceric acid, aromas and perfumes for the food and cosmetics industry etc.
HOSPITALS AND PHARMACIES Incoming/outgoing goods inspection. Control of pharmaceutical products according to pharmacopoeias	Pharmaceutical agents as well as raw materials and additives

Notes:

Reflectance

(light reflection)



Definition

Notes:

Reflectometry uses the reflection of waves at surfaces and interfaces to detect or characterize objects.

There are many different forms of reflectometry.

They can be classified in several ways by the:

- used radiation (electromagnetic, ultrasound, particle beams),
- geometry of wave propagation (unguided versus wave guides or cables),
- involved length scales (wavelength and penetration depth versus size of the investigated object),
- method of measurement (continuous versus pulsed, polarization resolved),
- application domain.

We will focus on some methods based on the principles of optical reflectance spectrophotometry

Backgrounds

Without reflected light, our eyes would be unable to see the colour or texture of objects. The human eye does amazing things with reflected light, using it to identify shapes and patterns, and even sense the distance of an object. To a spectrometer, however, reflection is simply the fraction of light reflected from a surface as a function of wavelength.

When properly measured, spectral reflectance can yield much of the same information as the eye, but it does so more quantitatively and objectively.

Reflectance measurements can measure the colour of a sample, or examine differences between objects for sorting or quality control. The samples may be automotive parts, paint, coffee beans, dyed human hair or lizards, making it challenging to choose the right system.

Notes:

Reflectance spectrophotometry principles

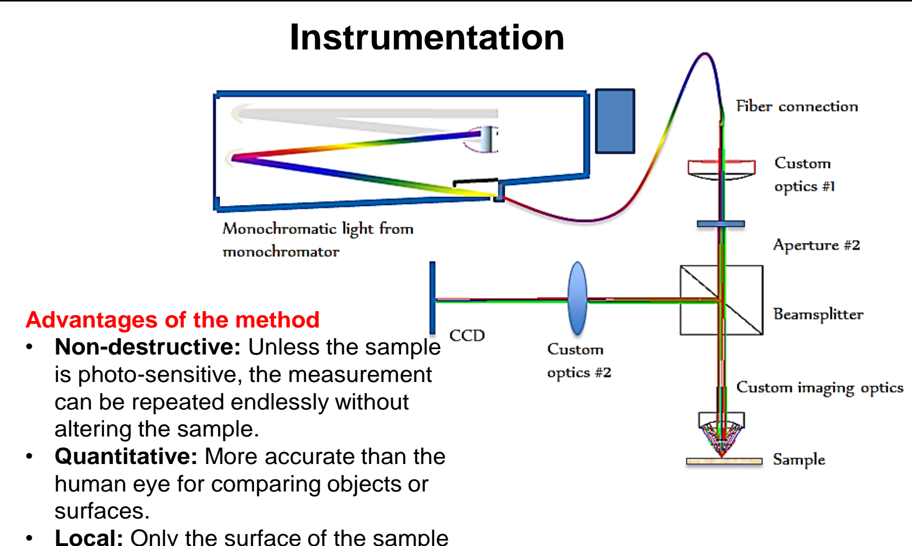
- In reflectance photometry, diffused light illuminates a reaction mixture in a carrier and the reflected light is measured.
- Alternatively, the carrier is illuminated and the reaction mixture generates a diffuse reflected light which is measured.
- The intensity of the reflected light from the reagent carrier is compared with the intensity of light reflected from a reference surface.
- The reflected light intensity is non linear in relation to concentration of analyte.

$$D_R = \log (R_o / R_{test})$$

- Kubelka-Munk or Clapper-Williams transformation equation used to convert the data into linear format.
- Reflectance photometry is used as the measurement method with dry-film chemistry systems.
- The Electro-optical components used in reflectance photometry are essentially the same as that used in absorbance photometry.

Notes:

Instrumentation



Advantages of the method

- **Non-destructive:** Unless the sample is photo-sensitive, the measurement can be repeated endlessly without altering the sample.
- **Quantitative:** More accurate than the human eye for comparing objects or surfaces.
- **Local:** Only the surface of the sample is measured, not its interior.

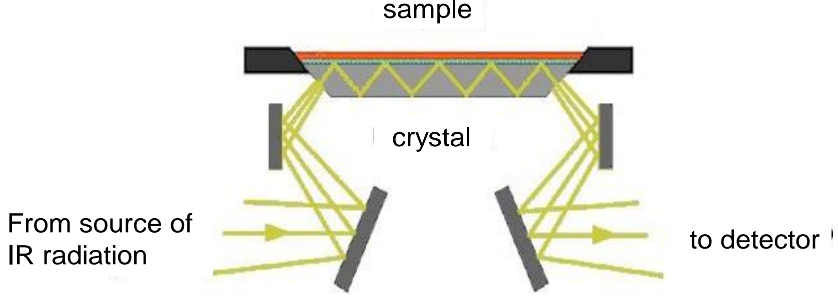
Components are similar to those in absorbance photometry except that the geometry of the system is modified so that the light source & the detector are on one side of the sample.

Notes:

Disturbed total internal reflection

It is used to record the spectra of liquids, gels, pastes, sample surfaces (usually films and plastics) and to study surface transformations

Range - 4000 - 630 cm-1 (crystal - ZnSe)



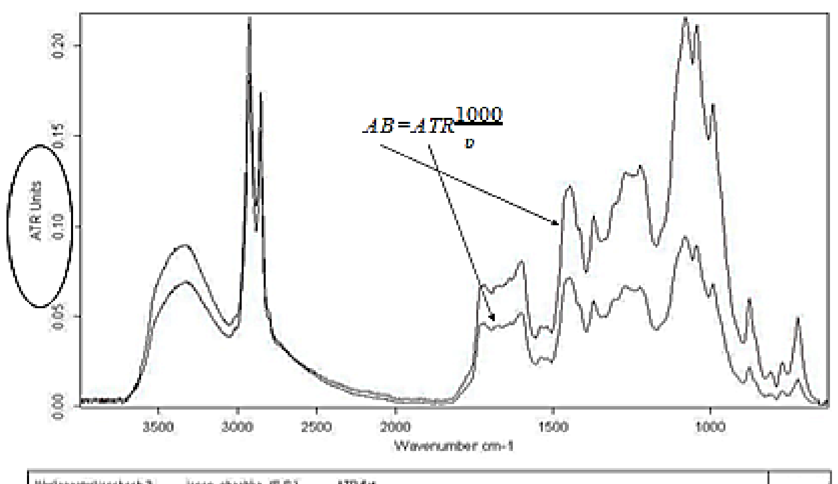
Notes:

Disturbed total internal reflection (continued)

Modes - multiple reflection
single reflection

Notes:

Disturbed total internal reflection spectra



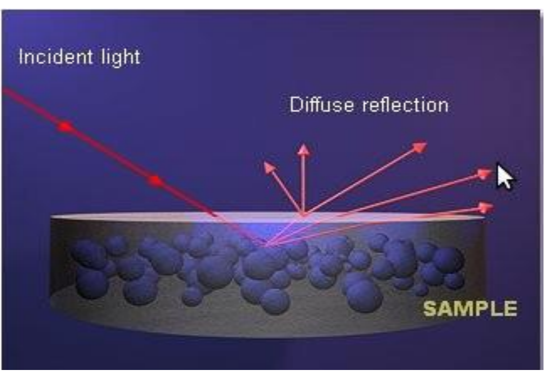
Notes:

Diffuse reflection

Notes:

It is used to record spectra of heterogeneous systems, powders or solids having rough surface.

The radiation diffusely reflected from the sample is collected at a wide angle and transmitted to the detector



Diffuse reflection (continued)

Notes:

The diffuse reflection spectrum is determined by the absorption and scattering power of the sample.

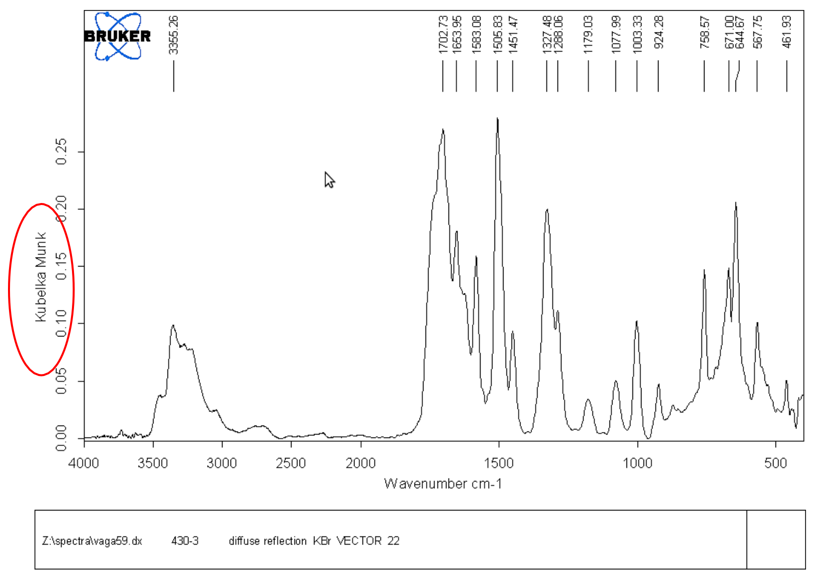
The relation between diffuse scattering and absorption of the sample is described by the equation of Kubelka-Munk

$$\frac{\beta}{s} = \frac{(1 - R_{\infty})^2}{2R_{\infty}}$$

Where R_{∞} - absolute diffuse reflection,
β — the coefficient of absorption,
S — the coefficient of scattering

Diffuse reflection: an example of spectrum

Notes:



Some applications

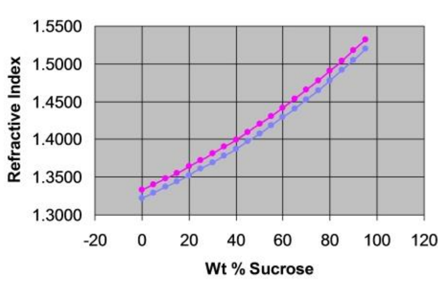
Notes:

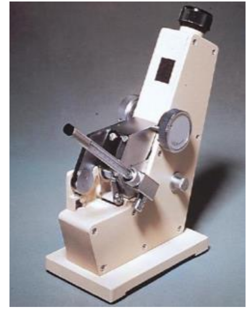
- **Sorting & differentiation:** plastic resins, paint, food products, recycling operations
- **Colour measurements:** textiles, industrial products, paint
- **Quality control:** organic fibres like wool, cotton and wood pulp; finished wood products, bales of hay, soil and manure
- **Material characterization:** plastics, metals, mineral powders, soil, core samples
- **Food testing:** moisture, protein, fat, carbohydrates, and sugar content in seeds, grains, fruits, vegetables, oils, flour, and dairy products
- **Noninvasive testing:** blood glucose monitoring through the skin, bilirubin in infants
- **Thin film thickness:** semiconductor materials, polymers, biological films, sensors
- **Forensics:** age of bloodstains

Notes:

Refractometry

(light refraction)





Definitions

Notes:

- Refractometers measure the refractive capabilities of liquid and pasty substances, and use this data to determine the concentration of a dissolved substance.
- Four main types of refractometers are known:
  - traditional handheld refractometers,
  - digital handheld refractometers,
  - laboratory or Abbe refractometers (named for the instrument's inventor and based on design of the 'critical angle') and
  - inline process refractometers.
- Automatic refractometers have also been developed. These are microprocessor-controlled electronic devices that can be combined with other measuring devices
- All devices use the effect of optical refraction, which occurs when an object passes from one medium to another, for example, from air into water.

Some theory

- The speed of light in a vacuum is always the same, but when light moves through any other medium it travels more slowly since it is constantly being absorbed and reemitted by the atoms in the material.
- The ratio of the speed of light in a vacuum to the speed of light in another substance is defined as the **index of refraction** (aka **refractive index** or ***n***) for the substance.

Light refraction is described by Shell's Law

By measuring the angles of Incidence and refraction, and knowing the index of refraction of the layer that is in contact with the sample, it is possible to determine the refractive index of the sample.

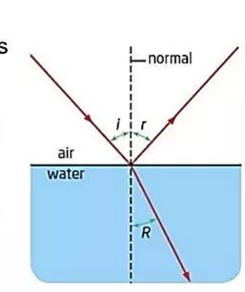
The index of refraction can also be calculated using sines of angles (trigonometry)

$$n_1 \, sine \angle i = n_2 \, sine \angle R$$

$\angle i$ is the angle of incidence
$\angle R$ is the angle of refraction

$$n_1 \sin\theta_1 = n_2 \sin\theta_2$$



Nearly all refractometers utilize this principle, but may differ in their optical design.

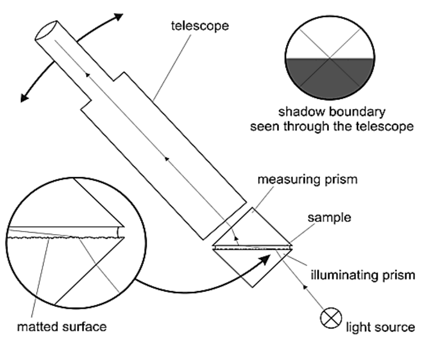
Notes:

Basics

- Refractometry is a method of testing physicochemical properties of a substance by measuring its refractive index.
- Refractive index is measured using a device called a refractometer.
- Refractive index can be used to asses substance purity or concentration, or to help identify the substance.
- While refractometry is most often used to measure the refractive index of solutions, it is also a very important method for testing solids and gases.
- Refractive index is always a function of temperature and wavelength, so they have to be precisely controlled during the test.
- There are many types of refractometers, the most popular model used in labs being Abbé refractometer.
- Refractometers measure the refractive capabilities of liquid and pasty process flows and uses this data to determine the concentration of a dissolved substance.
- To do so they use the effect of optical refraction, which occurs when an object passes from one medium to another, e.g., from air into water. From a certain angle of incidence, the light is reflected where the two media meet instead of refracting.

Notes:

Scheme of most known Abbe refractometer



Critical angle is the incident angle at which light - instead of getting to the other side of phase boundary - gets refracted in such a way that it becomes parallel to the phase boundary surface

Abbé refractometer working principle is based on critical angle.

Sample is put between two prisms – measuring and illuminating.

Light enters sample from the illuminating prism, gets refracted at critical angle at the bottom surface of measuring prism, and then the telescope is used to measure position of the border between bright and light areas.

Knowing the angle and refractive index of the measuring prism, one can calculate refractive index of the sample.

Surface of the illuminating prism is matted, so that the light enters the sample at all possible angles, including those almost parallel to the surface.

Notes:

Applications of refractometry

Typical applications	Analysed substances
PHARMACEUTICAL INDUSTRY	
Characterisation tests in research and development. Identity test, purity control and concentration determination of raw materials, semi-finished products and end products	Pharmaceuticals, infusion solutions, dialysis preparations, blood sera etc.
CHEMICAL INDUSTRY	
Characterisation tests in research and development. Identity test, purity control and concentration determination of raw materials, semi-finished products and end products. Tracking of chemical processes during production	Organic solvents, aliphatic or aromatic hydrocarbons, alcohols, salt solutions, acids, bases, stains, industrial oils, paints and varnishes, resins, glue components, tensides, extinguishing agents, polymer products, silicones, raw plastic materials etc.
FOOD INDUSTRY	
Quality and purity control of raw materials and end products. Determination of the sugar concentration.	Sugar, jams, honey, syrup, seasoning sauces, mustard and mayonnaise, convenience products, dairy products, baby food, egg products, oils, starch hydrolysis products etc.

Notes:

Typical applications	Analysed substances
SUGAR INDUSTRY	
Determination of the sugar concentration in semi-finished products and end products. Determination of the solids content in solutions. Determination of the purity in combination with a polarimeter.	Sugar cane, beet pulp, molasses, refined sugar, syrup, invert sugar etc.
MANUFACTURERS OF AROMAS, FRAGRANCES AND ESSENTIAL OILS	
Quality control of raw materials and auxiliary materials. Monitoring of the production of semi-finished products and end products	Essential oils (such as orange, lemon, lavender and peppermint oil), glyceric acid, Aromas and perfumes for the food, cosmetic and tobacco industry etc.
HOSPITALS AND PHARMACIES	
Incoming and outgoing goods inspection. Checking medicines for pharmacopeias. Analysis of body secretions.	Medicines, infusion solutions, blood sera, dialysis preparations, urine etc.

Notes:

Typical applications	Analysed substances
PETROCHEMICAL, AUTOMOTIVE AND AVIATION INDUSTRY, METAL PROCESSING AND BUILDING TECHNOLOGY	
Identity test and concentration determination. Outgoing goods inspection Stability test	Lubricating oils, fuels, gear oils, wax, lubricants, cooling lubricants, de-icing agents and anti-freeze agents, battery acid, AdBlue, tensides, cleaners, windshield wiper concentrate etc.
BEVERAGE INDUSTRY	
Routine analysis with high sample throughput. Quality and purity control of raw materials and end products. Determination of the sugar concentration in juices and alcohol-free beverages. Determination of the alcohol or extract content in beer, spices, wine or spirits. Quality control of dairy products. Sewage water check.	Fruit and vegetable juices, dietary beverages, beer, spices, wine, spirits, distillates, liquors, sugar concentrates, dairy products, aromas and colouring etc.

Notes:

Tasks to Section 14

1. Give definitions of these terms: scattering, refraction, polarisation, polarized light, Raman spectroscopy, IR Fourier, unpolarized and polarised light, diffuse reflection, nephelometry, turbidimetry, polarimetry, reflectometry, polarimeter.

2. List the factors that affect the intensity of scattered light and light absorbed by dispersed systems during nephelometric and turbidimetric determinations.

3. List the factors that affect the nature of light scattering by particles of dispersed systems.

4. What are radiation sources used in nephelometric (turbidimetric) determinations?

5. On what equipment and in compliance with what requirements do nephelometric and turbidimetric titration? List the advantages and disadvantages of these methods compared to direct nephelometry and turbidimetry.

6. A series of the external standard was prepared and analysed to evaluate the method of the turbidimetric determination of sulphate in the water. The results are shown in the following table.

mg SO_4^{2-}/L	0.00	10.00	20.00	30.00	40.00
transmittance	1.000	0.646	0.417	0.269	0.174

Analysis of a 100.0-mL sample of surface water gave a transmittance of 0.538. What is the concentration of sulphate in the sample?

7. To determine the content of chlorides in nitric acid, prepared a series of standard solutions containing 0.05; 0.1; 0.14; 0.19 and 0.24 mg/mL chloride ions. The optical density of these solutions was determined by the nephelometric method and the following data were obtained:

CCl^-, mg/mL	0,05	0,10	0,14	0,19	0,24
optical density	0,05	0,10	0,20	0,30	0,40

A sample of nitric acid with a volume of 100 ml (density 1.5 g/cm^3) was diluted in a volumetric flask with a volume of 250 ml. An aliquot of 5.0 ml of this solution was transferred to a 50 ml flask. After addition of Argentum nitrate and stabilizing solution, 50 ml of AgCl suspension was obtained. The optical density of the resulting solution was equal to Ax=0.35. Construct a calibration graph of the apparent absorption versus concentration (mol/L) and determine the content (in %) of chloride ions in nitric acid.

8. The content of chloride ions in water was determined by the turbidimetric method. 20.0 ml of KCl solution with the concentration of 1.051 mg/mL took to a 100 mL flask and added distilled water to the mark. To prepare standard solutions, the individual volumes of the resulting solution (Vst.) transferred into the 50 mL flasks, and added reagents to obtain a suspension of AgCl. After sediment formation, the contents of the flasks were diluted to 50 ml by distillate water. Received the following data:

Vst., mL	2,00	4,00	6,00	8,00
Optical density.	0,220	0,470	0,700	0,940

A 50.0 ml sample of the pool water was diluted in a 100 ml flask. An aliquot of 5.0 ml of this solution gave a suspension of AgCl in a 50 ml flask. Plot a calibration graph of the dependence of the optical density on the concentration of AgCl. Determine the content (in mg / l) of chloride ions in water, if the optical density of the solution is equal to Ax = 0,820.

9. 10 dm^3 of air of the production room containing hydrogen chloride was passed through 20 dm^3 of water. For nephelometric determination, 5 ml of this solution was added to a 10 ml volumetric flask and treated by HNO_3 and $AgNO_3$. 10 ml of AgCl suspension was obtained and mixed. At the same time, the standard solution from 3 ml of NaCl solution with a concentration of 0.100 mg/ml was prepared under the same conditions as the sample. After 10 min, the turbidity of the standard and analysed solutions was compared. Determine the concentration (in mg/m^3) of hydrogen chloride in the air if the turbidity intensities of the standard and analyte solutions were the same.

Section 15: Mass Spectroscopy

Contents:

- Introduction
- Basic principle and components
- Inlet systems
- Detectors
- Ionisation methods and ion sources
- Electron ionisation
- Chemical ionisation
- Fast atom bombardment
- Matrix-Assisted Laser Desorption/Ionisation (MALDI)
- Electrospray ionisation
- Inductively coupled plasma
- Types of mass-spectrometers
- Quadrupole
- Single and double-focusing magnetic deflection
- Time of flight spectrometers
- Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTIR MS)
- Applications of mass spectrometers
- Strengths and weaknesses of MS

Introduction

Mass spectrometry is an analytical technique for the determination of the elemental composition of a sample or molecule. The method has long been used to measure isotopes and decipher organic structures.

Mass spectrometry is a technique for studying the masses of atoms or molecules or fragments of molecules. To obtain a mass spectrum, gaseous species desorbed from condensed phases are ionized. The ions are accelerated by an electric field and then separated according to their mass-to-charge ratio, m/z. If all charges are +1, then m/z is numerically equal to the mass. If an ion has a charge of +2, for example, then m/z is 1/2 of the mass.

Francis W. Aston developed a “mass spectrograph” that could separate ions differing in mass by 1% and focus them onto a photographic plate. Aston immediately found that neon consists of two isotopes and went on to discover 212 of the 281 naturally occurring isotopes. Aston received the Nobel Prize for chemistry in 1922.

The modern mass spectrometer can create ions by ionisation of atoms/molecules of a neutral sample; separates the ions according to their mass/charge ratio; detects and measures the relative abundances of ions and their relative masses.

The information can be represented and interpreted by using a mass spectrum. The area of each peak of mass-spectra is proportional to the abundance of each isotope.

Mass spectrometry can identify the sequence of amino acids in a protein, the sequence of nucleic acids in DNA, the structure of a complex carbohydrate, and the types of lipids in a single organism. Mass spectrometry is the most powerful detector for chromatography. It offers both qualitative and quantitative information, provides high sensitivity, and distinguishes different substances with the same retention time.

Atomic mass is the weighted average of the masses of the isotopes of an element. For example, Bromine consists of 50.69% ^{79}Br with a mass of 78.918 34 Da (Daltons) and 49.31% ^{81}Br with a mass of 80.916 29 Da. Therefore, its atomic mass is 79.904 Da.

The unit of atomic mass is the Dalton (Da), defined as 1/12 of the mass of 12 C. Mass spectrometrists prefer “u” for “unified atomic mass unit”. Da and u are synonymous.

The molecular mass of a molecule or an ion is the sum of atomic masses listed in the periodic table. The nominal mass of a molecule or ion is the integer mass of the species with the most abundant isotope of each of the constituent atoms

History of mass spectrometry

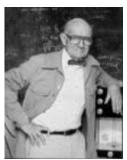
Notes:

Five Nobel Prize Winners in Mass-Spectroscopy Research:

Joseph John Thomson
Physics 1906
first mass spectrometer

Francis William Aston
Chemistry 1922
mass spectrometry of isotopes

Wolfgang Paul
Physics 1989
quadrupole and quadrupole ion trap MS



John B. Fenn
Chemistry 2002
electrospray ionization of biomolecules

Koichi Tanaka
Chemistry 2002
Matrix-assisted laser Desoprtion/ionization (MALDI)

A Long and Continuing History of Achievements

MS theory

Notes:

Mass analyzers use electric and magnetic fields.
Therefore, two force are applied to charged particles

The Newton second law force F = ma

where: F - force applied to the ion;
m - mass of the ion;
a – acceleration

The Lorentz law force F = e(E+ v x B)

where: e - ionic charge;
v x B - vector cross product of the ion velocity and the applied magnetic field;
E - electric field

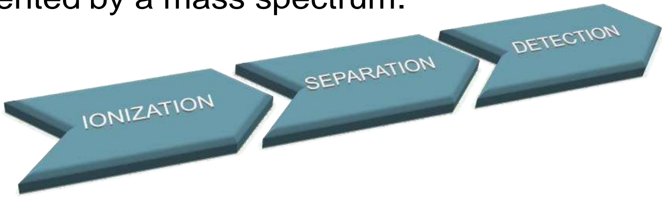
Therefore, force is dependent on both mass and charge and spectrometers separate ions according to their mass-to-charge ratio (m/z) - not by mass alone.

WHAT IS MASS SPECTROMETRY?

Notes:

It is an analytical technique for the determination of the elemental composition of a sample or molecule. A mass spectrometer executes three functions:

1. **Creates ions** by **ionisation** of atoms/molecules of a neutral sample.
2. **Separates** the ions according to their mass/charge ratio.
3. **Detects and measures** the relative abundances of ions and their relative masses; the information can be represented by a mass spectrum.



Notes:

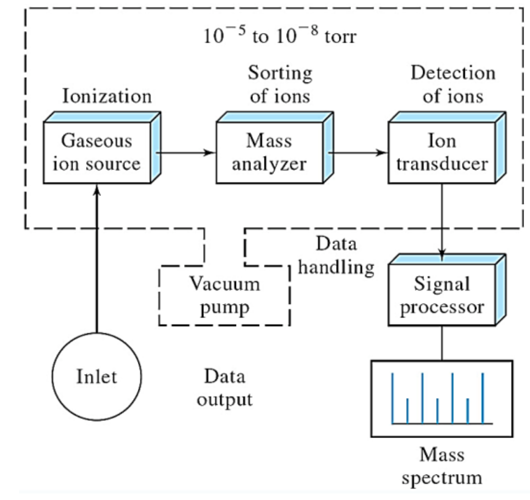
How the mass analyser works

- A sample is loaded onto the MS instrument;
- Components of the sample are ionised by one of a variety of methods, which results in the formation of charged particles (ions);
- The ions transit through electromagnetic fields; are accelerated by an electric field;
- The mass-to-charge ratio (*m/z*) of ions is computed using the details of their motion;
- The ions, which were sorted according to *m/z* in the previous step, are recorded and quantified.

Principal scheme of a mass-spectrometer

Notes:

1. Vacuum system to maintain low pressure (10^{-5} to 10^{-8} torr)
2. Inlet: to introduce sample into an ion source.
3. Ion source: sample converted into gaseous ion by bombardment with:
   - Electrons;
   - Photons;
   - Ions;
   - Molecules;
   - Thermal/electric energy.
4. Positive/negative ions accelerated into analyser.
5. Mass analyser: sort ions according *m/z*.
6. Detector system: detect (count) ions of for all m/z ratios.
7. Signal processor: convert beam of ions to electrical signal



Basic Components

Notes:

1. **Sample Introduction system:** Volatilizes the sample and introduces it to the ionization chamber under high vacuum
2. **Ion source:** Ionizes the sample and accelerates the particles into the mass analyzer
3. **Mass analyzer** (or Mass Separator): Separates ionized particles based on their mass-to-charge ratio (m/e^-)
4. **Detector - ion collector:** Monitors the number of ions reaching detector per unit time as a current flow
5. **Signal processor:** Amplifies the current signal and converts it to a DC Voltage
6. **Vacuum pump system:** A very high vacuum (10^{-5} to 10^{-8} torr) is required so that the generated ions are not deflected by collisions with internal gases. Since ions are highly reactive and short-lived, you must to perform any manipulations with ions in vacuum.

Vacuum pumps

$250\ m^3/h$
10^{-9} atm

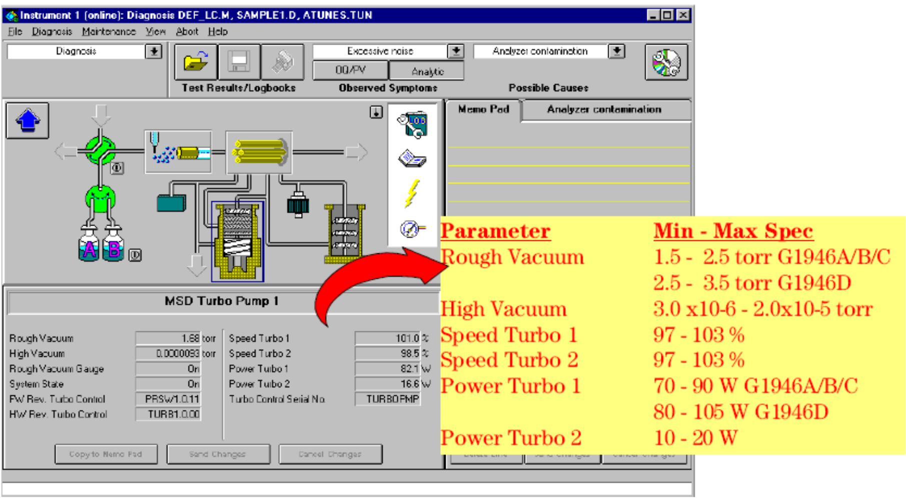
$250\ m^3/h$
10^{-9} atm

$12\text{-}30\ m^3/h$, 10^{-6} atm

$250\ m^3/h$, 10^{-13} atm

User interface to control an instrument

Notes:



All MS instruments **need a vacuum** to avoid unwanted interatomic collisions,
Also, the vacuum system is controlled by a PC-based interface

Inlet systems

Notes:

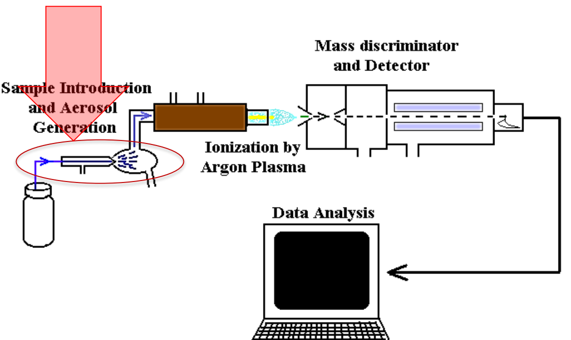
We will start consideration of an instrument with a unit which is used to introduce your sample into the system.

Two important requirements:

1) One need to Introduce a sample into ion source with minimum loss of vacuum;

2) One need to introduce different types of samples.

There are known four different types of inlet systems:

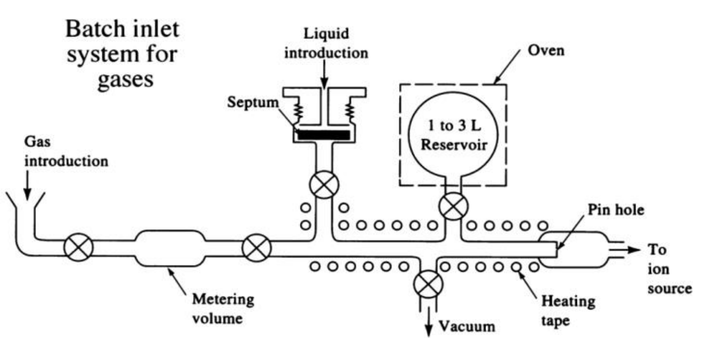


i) Batch Inlet;
ii) Direct Probe Inlet;
iii) Gas Chromatography;
iv) Liquid Chromatography

Batch (internal) Inlet

Notes:

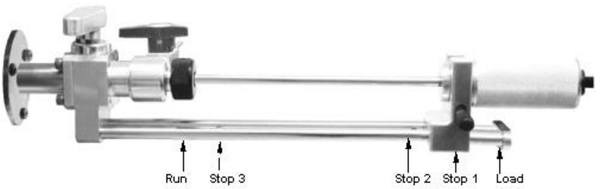
- It is the simplest system;
- Sample is volatilised externally and allowed to “leak” into the ion source;
- Good for gas and liquid samples with boiling points below 500°C;
- Sample heated (<400 °C) in small external oven;
- Sample pressure 10^{-4} to 10^{-5} torr;
- Vapour admitted to ioniser through a valve;
- Gas stream added to analyte.



Direct Probe

Notes:

- Good for non-volatile liquids, thermally unstable compounds & solids;
- Less sample is required & wasted (few ng);
- Sample held on the surface of glass or aluminium capillary tube, fine wire or small cup (= vials);
- Sample vial inserted through air-lock into ioniser chamber;
- Lock system minimizes amount of air that must be pumped from system;
- Vial heated to vaporize the sample.

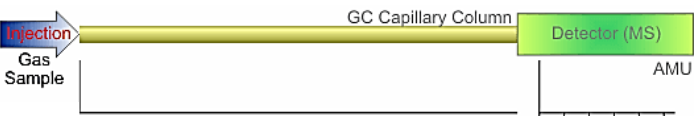


Probe moves through various lock system stages permits for a step-wise increase in the vacuum

The direct probe is only ¼" in diameter.

Chromatography Interface (GC-MS & LC-MS)

Notes:

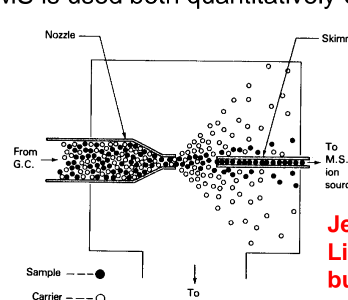


- Gas or liquid chromatography coupled to a MS;
- Requires specialised inlet systems;
- Permits separation & determination of components for complex mixtures;
- MS is used both quantitatively & qualitatively.

Sample

Argon gas

Major interface problem – carrier gas dilution



Jet separator (separates analyte from carrier gas): Lighter carrier gas are deflected by volume but heavier sample travels in straight line

Notes:

Inductively Coupled Plasma (ICP)

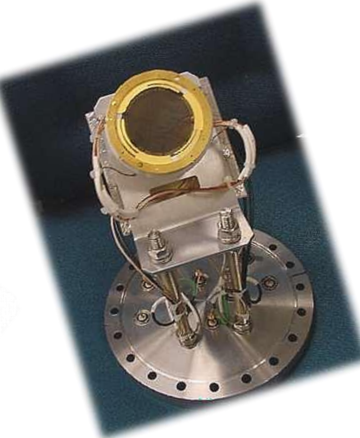
- Operates somewhat like a nebulizer in an AAS (atomic absorption spectroscopy);
- Also ionises the sample in argon stream (at very high temperatures, >6000 °C);
- Only a small amount of analyte is utilised (< 1%);
- Other details will be presented later this day.

Notes:

Detectors

Three key types of detectors in a MS:

- electron multipliers including various types of secondary electron multipliers;
- Faraday cups;
- microchannel plate detectors.



Notes:

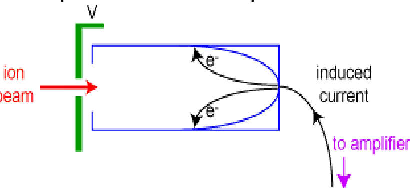
Electron multiplier

- An **electron multiplier** (continuous dynode electron multiplier) is a vacuum-tube structure that multiplies incident charges.
- In a process called secondary emission, a single electron can, when bombarded on secondary emissive material, induce emission of roughly 1 to 3 electrons.
- If an electric potential is applied between this metal plate and yet another, the emitted electrons will accelerate to the next metal plate and induce secondary emission of still more electrons.
- This can be repeated a number of times, resulting in a large shower of electrons all collected by a metal anode, all having been triggered by just one.

Faraday cup

Notes:

- A Faraday cup is a metal (conductive) hollow collector, open at one end and closed at the other, used to collect beams of ions in vacuum.
- Incident ion strikes the dynode surface which emits electrons and induces a current which is amplified and recorded. The resulting current can be measured and used to determine the number of ions or electrons hitting the cup.
- Faraday cup is surrounded by a cage which prevents the escape of reflected ions and ejected secondary electrons.



Advantages:
- Independent of the energy, mass or chemical nature of ion
- Inexpensive and simple mechanical and electronic device

Disadvantages:
- Need for a high-impedance amplifier
- Limits speed at which spectrum can be scanned
- Less sensitive than electron multipliers

Micro-channel plate (MCP)

Notes:

- It is a planar component used for detection of particles (electrons or ions) or radiation.
- It is closely related to an electron multiplier, as both intensify single particles or photons by the multiplication of electrons via secondary emission.
- Since a micro channel plate detector has many separate channels, it can additionally provide spatial resolution.

- A micro-channel plate is a slab made from highly resistive material of typically 2 mm thickness with a regular array of tiny tubes or slots (microchannels) leading from one face to the opposite, densely distributed over the whole surface.
- The microchannels are typically approximately 10 micrometers in diameter (6 micrometer in high resolution MCPs) and spaced apart by approximately 15 micrometers; they are parallel to each other and often enter the plate at a small angle to the surface (~8° from normal).

Hard and soft ionisation

Notes:

<u>Hard ionisation</u> techniques bring high quantities of residual energy in the subject molecule invoking large degrees of fragmentations (other than in the case of proton transfer and not including isotope peaks).

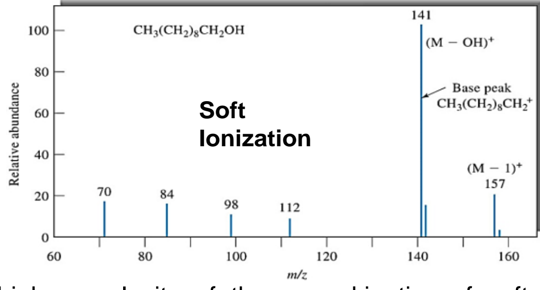
Hard sources:
- Sufficient energy, so analyte are in highly excited energy state.
- Relaxation involves rupture of bonds:
  - Produces fragment ions with *m/z* < molecular ion;
  - Kinds of functional groups → structural information.

<u>Disadvantage:</u> it is impossible to "lift" the molecular ions of peptides, sugars, nucleic acids and most other natural objects into the gas phase for mass analysis. Under severe exposure, they decompose

Soft ionization

Soft ionization refers to the processes which impart little residual energy onto the subject molecule and as such result in little fragmentation. Soft ionization is a useful technique when considering biological molecules of large molecular mass because this process does not fragment the macromolecules into smaller charged particles, rather it turns the macromolecule being ionized into small droplets.

Soft sources
- Cause little fragmentation
- Mass spectrum consists of molecular ion and only few, if any, other peaks
- Accurate mass



Disadvantage: due to the high complexity of the macrokinetics of soft ionization, the obtained mass spectra of the same sample, even on one device, may differ in the relative intensities of the ionic fractions; when analyzing the same sample by the same ionization method on another device, the mass spectra can be reproduced purely qualitatively. **Soft ionization is a semi-quantitative method**

Notes:

Ion source technologies

Hard methods:
- Electron ionization - EI
- Inductively Coupled Plasma Ionization - ICP
- Spark ionization and glow discharge ionization - SS & GD (for solid sample analysis)
- Secondary-ion mass spectrometry SIMS (direct ion sputtering of the solid surface)
- Direct laser desorption / ionization (laser surface spraying) - LDI
- Thermal / surface ionisation - TI / SI
- Photoionization (atmospheric pressure photoionization) - APPI

Soft methods:
- Chemical Ionization - CI
- Atmospheric pressure chemical ionization - APCI
- Fast atom bombardment - FAB
- Matrix-assisted laser desorption ionization - MALDI
- Electrospray (electrospray) – ESI
- Field ionization & field desorption - FI / FD

Notes:

Formation of gaseous analyte ions

Appearance of spectrum highly dependent on ionization technique

Gas-phase

Sample first vaporized then ionized
Thermally stable compounds boiling points < 500ºC
MW < 100 amu

Desorption

- Solid or liquid directly converted to gaseous ion
- MW as large as 10^5 daltons

Type	Name and Acronym	Ionizing Process
Gas Phase	Electron Impact (EI)	Exposure to electron stream
	Chemical Ionization (CI)	Reagent gaseous ions
	Field Ionization (FI)	High potential electrode
Desorption	Field Desorption (FD)	High potential electrode
	Electrospray Ionization (ESI)	High electric field
	Matrix-assisted desorption ionization (MALDI)	Laser beam
	Plasma Desorption (PD)	Fission fragments from ^{252}Cf
	Fast Atom Bombardment (FAB)	Energetic atomic beam
	Secondary Ion Mass Spectrometry (SIMS)	Energetic beam of ions
	Thermospray Ionization (TS)	

23

Notes:

Mass Analysers: some definitions

Notes:

Some general information to help electron impact (IE) mass spectra interpretation:

Molecular ion (M +): If the molecular ion appears, it will be the highest mass in an EI spectrum (except for isotope peaks discussed below). This peak will represent the molecular weight of the compound. Its appearance depends on the stability of the compound. Double bonds, cyclic structures and aromatic rings stabilize the molecular ion and increase the probability of its appearance.

Reference Spectra: Mass spectral patterns are reproducible. The mass spectra of many compounds have been published and may be used to identify unknowns. Instrument computers generally contain spectral libraries which can be searched for matches.

Fragmentation: General rules of fragmentation exist and are helpful to predict or interpret the fragmentation pattern produced by a compound. Functional groups and overall structure determine how some portions of molecules will resist fragmenting, while other portions will fragment easily. A detailed discussion of those rules is beyond the scope of this introduction and further information may be found in mass spectrometry reference books.

Isotopes: Isotopes occur in compounds analyzed by mass spectrometry in the same abundances that they occur in nature.

Electron ionisation

Notes:

- **Electron ionisation** (**EI**, formerly known as **electron impact**) is an ionization method in which energetic electrons interact with gas phase atoms or molecules to produce ions. This technique is widely used in mass spectrometry, particularly for gases and volatile organic molecules.
- It is a hard source: 50V is higher energy than chemical bond
- The following gas phase reaction describes the process : $M + e^- \rightarrow M^{\bullet+} + 2e^-$

where M is the analyte molecule being ionized, e^- is the electron and $M^{+\bullet}$ is the resulting ion

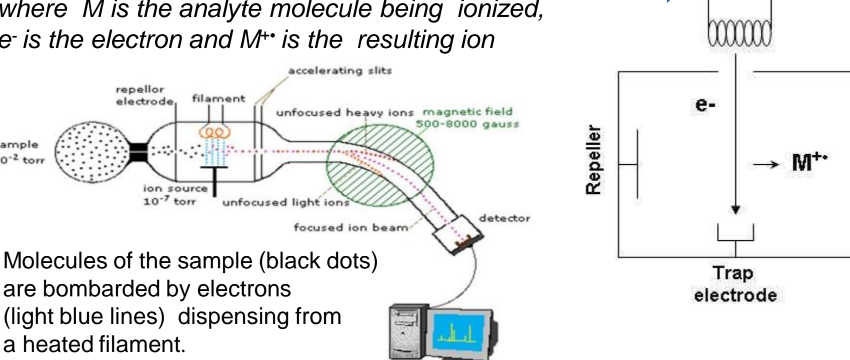
Filament

e-

Repeller

$M^{+\bullet}$

Trap electrode



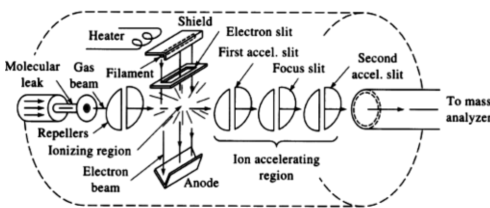
Molecules of the sample (black dots) are bombarded by electrons (light blue lines) dispensing from a heated filament.

Electron-Impact Source (EI)

Notes:

1) Sample heated to produce molecular vapour.
2) Bombard with a beam of electrons (energy 30-150 eV).
3) Electrons emitted from heated W or Re filament and accelerated at 50-70 V;
4) Electron beam expels electron (ionisation energy of molecules is usually 4-15 eV) and forms positive ions.
5) The source is not very efficient → one molecule in a million is ionized

6) Positive ions attracted to first slit by small potential 5V
7) High potential applied at accelerator plates 10^3 to 10^4 V and generates molecular ion velocity.



8) Relaxation results in extensive fragmentation:
   a) Large number of positive ions of various masses;
   b) Typically less mass than molecular ion;
   c) Lower mass ions called daughter ions
   d) Sometimes molecular ion is not present.

Electron-Impact Source (EI)

Notes:

1) Base peak → most intense peak:

Usually a daughter ion or fragment ion.

2) Peaks at MW *greater* than molecular ion:

Same chemical formula but different isotope composition;

Size of peak depends on relative natural abundance of isotopes.

3) Collision Product peak $(M+1)^+$:

Collision transfers a hydrogen atom to the ion to generate a protonated molecule;

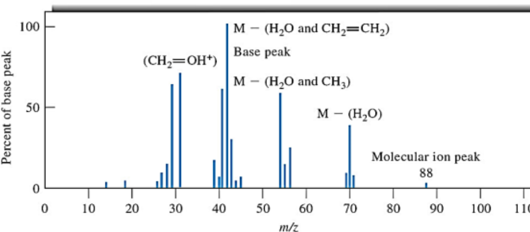
Second order reaction → depends on concentration;

Increases with increase in pressure.

Advantages:

a) Good sensitivity

b) Fragmentation provides unambiguous identification of analytes



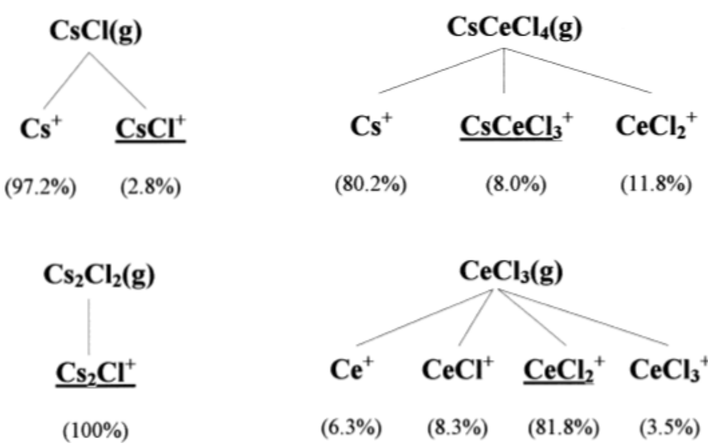
Disadvantages:

a) Need to volatize sample thermal decomposition before ionization

b) Fragmentation disappearance of molecular ion peak MW not determined

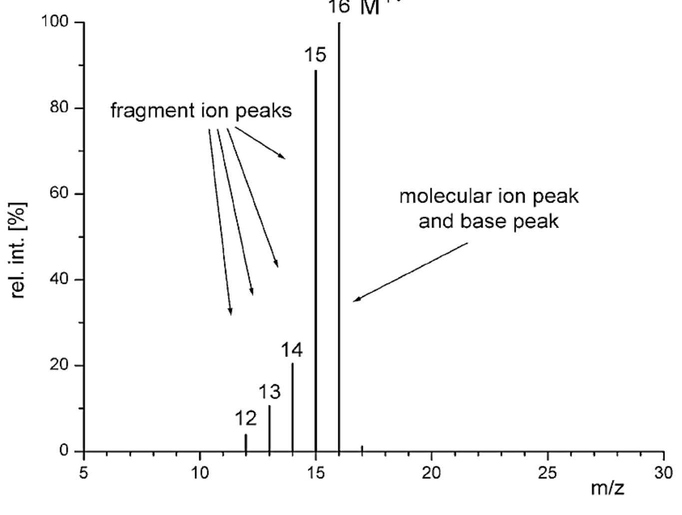
Mass-spectrum of Cs iodine compounds

Notes:



Methane mass-spectrum by electron impact

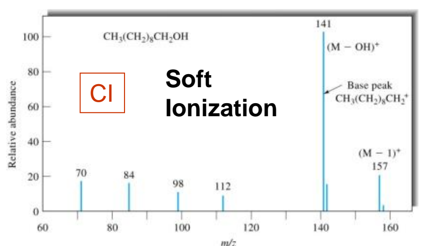
Notes:

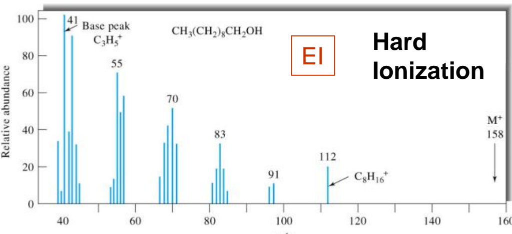


Chemical ionisation (CI) Ion Source

Notes:

- A modified form of EI
  - Higher gas pressure in ionization cavity (~1 torr)
  - Reagent gas (1000 to 10000-fold excess) added; usual choice is methane, CH_4
- Reagent gas is directly ionized instead of analyte
- Gentle; little fragmentation; even-electron ions produced more stable than odd-electron ions produced in EI
- Excess energy of excited ions removed by many ion-reagent gas collisions





Chemical ionisation reactions

Notes:

Methane is common reagent.
Also use propane, isobutane and ammonia.
Reacts with high-energy electron beam to generate several ions:
CH_4^+, CH_3^+ (~90% of product) and CH_2^+
React with other methane molecules.

Reagent gas ionization:

$CH_4 \rightarrow CH_4^+ + e^-$ (also CH_3^+, CH_2^+)

Secondary reactions:

$CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3$

$CH_3^+ + CH_4 \rightarrow H_2 + C_2H_5^+$ ($\rightarrow$ M+29)

Tertiary reactions

$CH_5^+ + MH \rightarrow CH_4 + MH_2^+$ (M+1) *proton exchange*

$CH_3^+ + MH \rightarrow CH_4 + M^+$ (M–1) *hydride exchange*

$CH_4^+ + MH \rightarrow CH_4 + MH^+$ (M) *charge exchange*

Chemical Ionisation Source (CI)

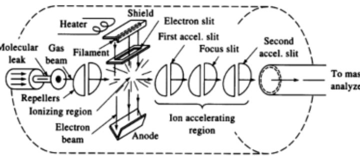
Notes:

1) EI and CI are Interchangeable in a spectrometer

2) Chemical ionisation is the 2nd most common procedure for generating ions

3) Gaseous atoms from the sample are:
- Heated from a probe
- Collide with ions produced reagent gas bombarded by electrons
- Usually positive ions are used

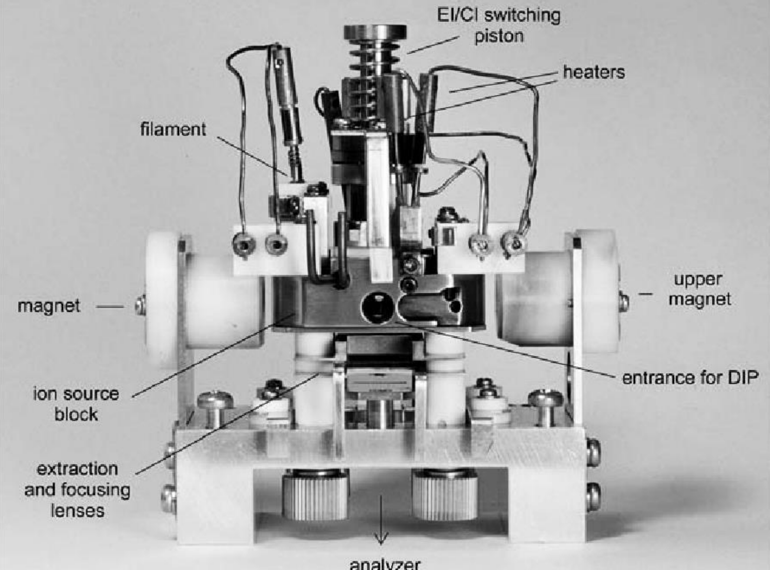
4) Need to modify electron beam
- Add vacuum pump capacity
- Reduce width of slit for mass analyzer
- Allow a reagent pressure of 1 torr in ionization area
- Keep pressure below 10^{-5} torr in analyzer



5) Concentration ratio of reagent to sample is 10^3 to 10^4
- Electron beam preferentially interacts with reagent instead of sample

Ion source for EI/CI of a Nier type

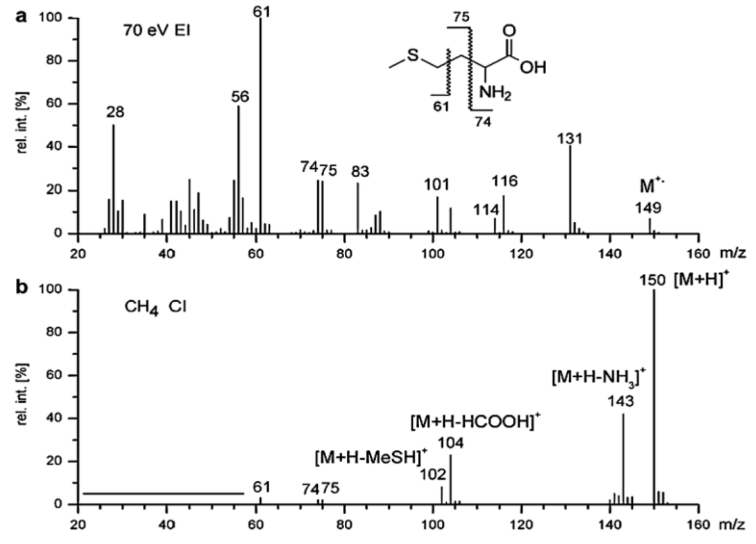
Notes:



An American physicist **Alfred Nier** (1911-1994) is a pioneer in the field of MS

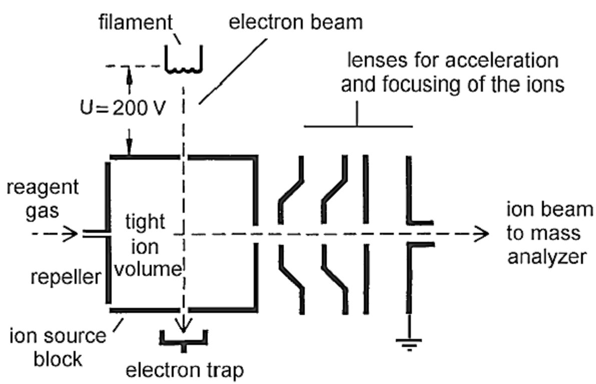
Compare mass-spectra of methionine by electron (top) and chemical (bottom) ionisation

Notes:



Scheme of ion source for EI and CI

Notes:



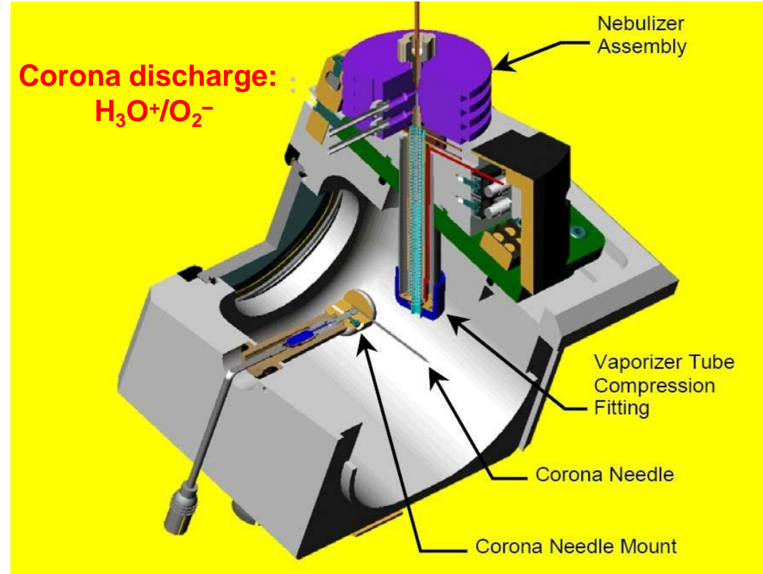
EI mode: P < 1 Па, E = 20-100 эВ

CI mode: P ~ 100 Па, E = 200-500 эВ, pumping speed >200l/s residence time 10^{-6} c, 30-40 collisions, sensitivity 10 times lower EI

Reagent gas: water, methane, ammonia, isobutane, argon, benzene, etc.

Atmospheric pressure chemical ionisation (APCI)

Notes:



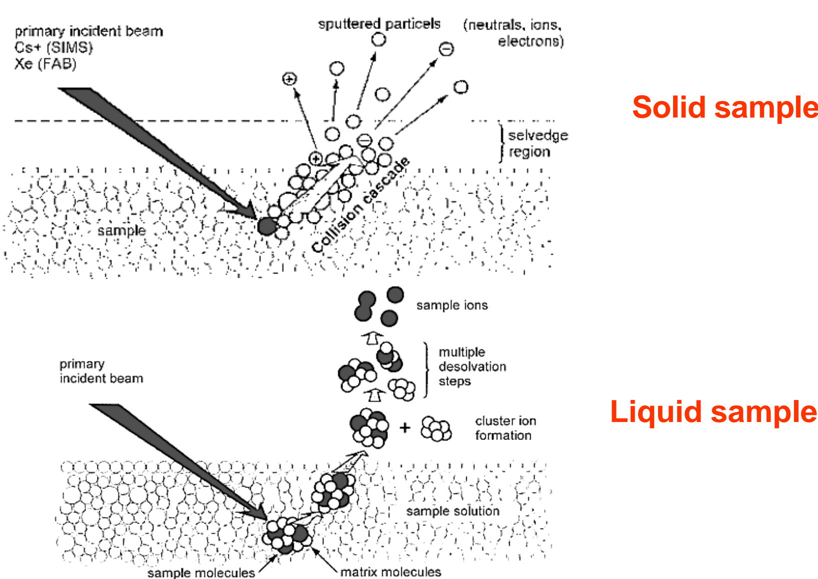
Notes:

Fast Atom Bombardment (FAB)

- In FAB, a high-energy beam of natural atoms, typically Xe or Ar, strikes a solid or low-vapor-pressure liquid sample causing desorption & ionization.
- It is used for large biological molecules that are difficult to get into the gas phase.
- FAB causes little fragmentation and usually gives a large molecular ion peak, making it useful for molecular weight determination.
- Atom-sample collisions produce ions as large as 25,000 Daltons.

Bombardment by fast atoms (scheme)

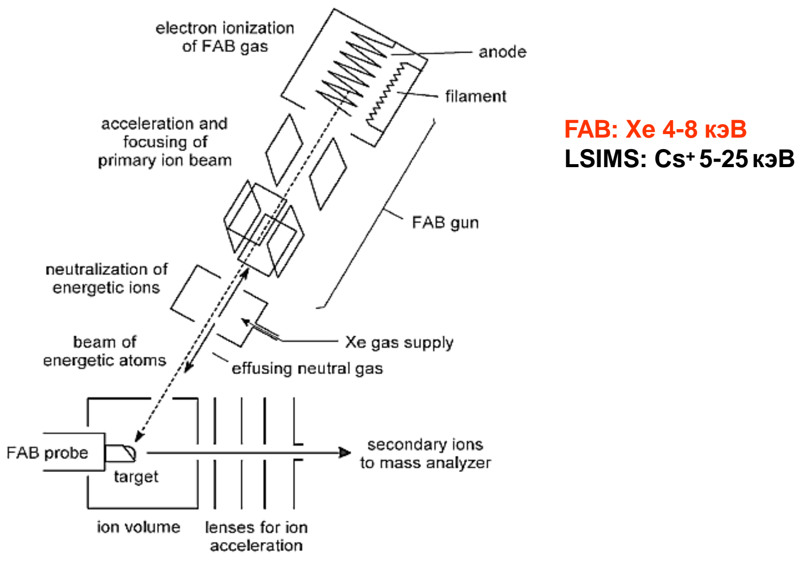
Notes:



Solid sample

Liquid sample

Ion source for FAB



FAB: Xe 4-8 кэВ
LSIMS: Cs^{+} 5-25 кэВ

Fast Atom Bombardment sources

Notes:

Beam of fast energetic atoms are generated by:
i) Passing accelerated Ar or Xe xenon ions from an ion source through a chamber
ii) Chamber contains Ar or Xe atoms at 10^{-5} torr
iii) High-velocity ions undergo a resonant electron-exchange reaction without substantial loss of translational energy.
iv) They pick up an electron in collisions with natural atoms to form a beam of high-energy neutral atoms.

Focusing
Atom beam
Extraction
Analyte ion beam (secondary ions)
Probe tip
Analyte metrix
© 1997 PAUL GATES

Scheme for production of "fast atoms"

$$Ar^{+*} + Ar^{0} \xrightarrow{\text{Charge transfer}} Ar^{+} + Ar^{0*}$$

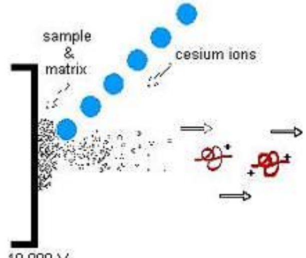
Accelerated argon ion from "ion gun" | Ground state argon atom | "slow ion" | "fast atom"

Fast Atom Bombardment sources

Notes:

Major role for MS studies of polar high molecular-weight species.
Soft Ionization technique:
- MW > 10,000;
- Structural information for MW ~3,000.

Samples are in a condensed state:
- Glycerol solution matrix;
- Liquid matrix helps reduce lattice energy.

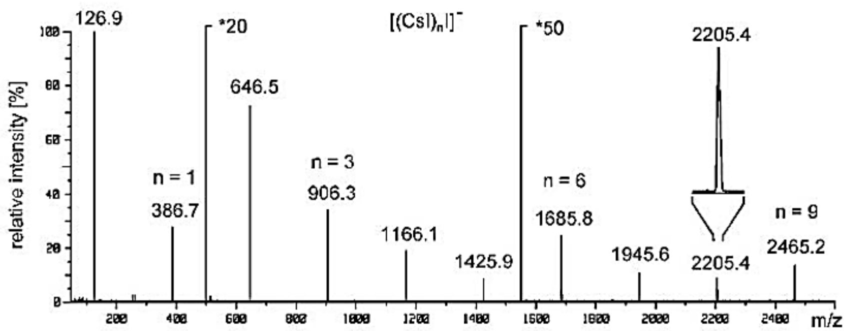


Ionized by bombardment with energetic (several keV) xenon or argon atoms:
- Very rapid sample heating;
- Reduces sample fragmentation.

Positive & negative analyte ions are sputtered from the surface.
Desorption process:
- Must overcome lattice energy to desorb an ion and condense a phase;
- "healing" the damage induced by bombardment.

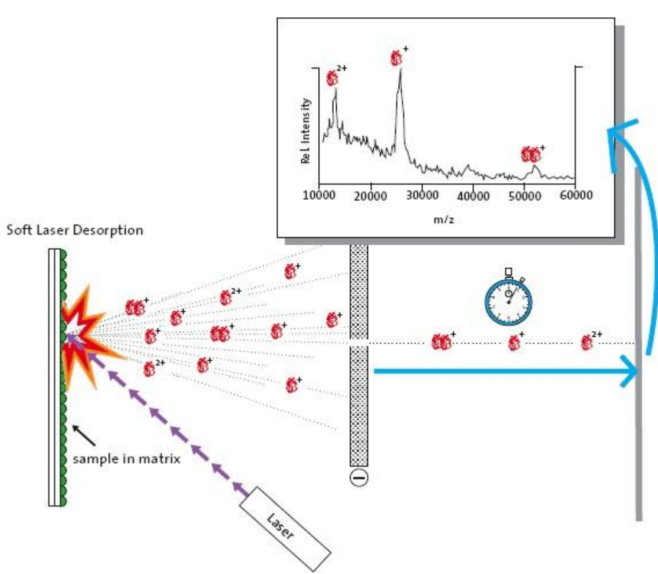
Notes:

FAB mass-spectrum for negative ions of CsI (hard ionisation)



Matrix-Assisted Laser Desorption/Ionisation (MALDI)

Notes:



Matrix-Assisted Laser Desorption/Ionisation (MALDI)

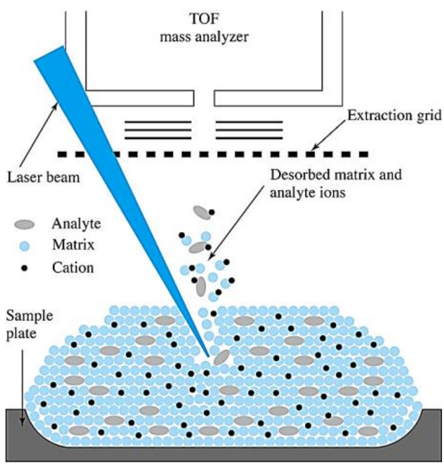
Notes:

- MALDI is a method of vaporizing and ionizing large biological molecules such as proteins or DNA fragments.
- The biological molecules mixed with radiation-absorbing material (such as nicotinic acid or dihydroxybenzoic acid).
- Solution is evaporated onto solid surface.
- Sample exposed to pulsed laser beam.
- A UV pulsed laser ablates the matrix with large molecules into the gas phase in an ionized form so they can be extracted into a mass spectrometer.
  - -Laser irradiation provides sublimation of analyte ions;
  - -MS spectra recorded between laser beam pulses.
- Matrix compound must absorb the laser radiation and be soluble enough in sample solvent to be present in large excess.
- Analyte should not absorb laser radiation.
- Often coupled to time-of-flight (TOF) detector
- Excellent for larger molecules, *e.g.* peptides, polymers

Matrix-Assisted Laser Desorption/Ionisation

Notes:

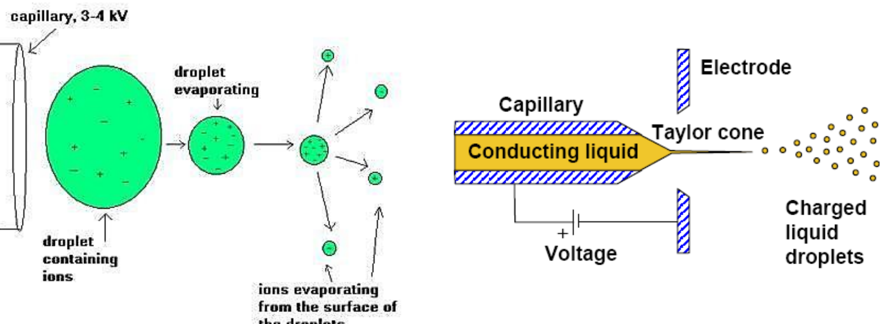
1. Accurate molecular weight (MW) for polar biopolymers:
DNA, RNA, Proteins;
Few thousands to several hundred thousand Da.
2. Low background noise.
3. Absence of fragmentation.
4. Multiple charged ions (+2, +3).
5. Observe dimers, trimers.
6. Mechanism is not completely understood.
7. MALDI spectra are greatly influenced by type of matrix, solvent and additive.



Electrospray Ionisation (ESI)

Notes:

- The ESI source consists of a very fine needle and a series of skimmers. A sample solution is sprayed into the source chamber to form droplets.
- Uses atmospheric pressure and temperature
- The droplets carry charge at the exit of the capillary.
- Since the solvent evaporates, the droplets disappear leaving highly charged analyte molecules.



Electrospray Ionisation (ESI)

Notes:

One of the most important techniques for analyzing biomolecules

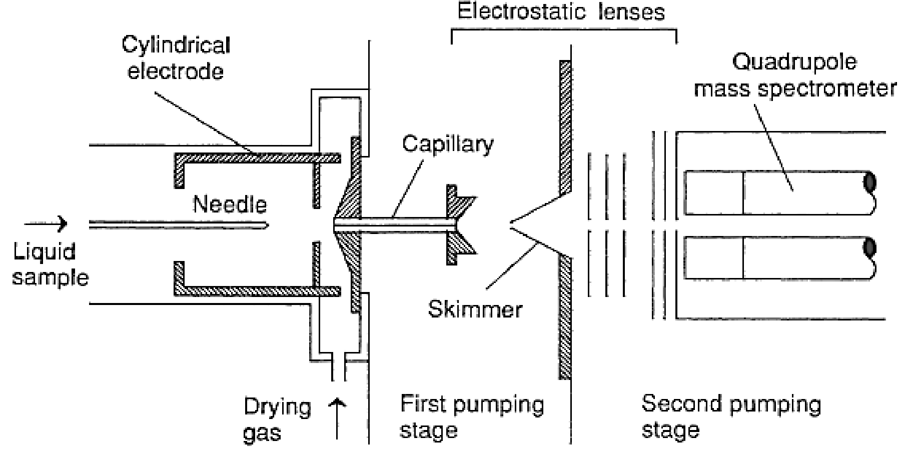
- Polypeptides, proteins and oligonucleotides
- Inorganic species synthetic polymers
- MW >100,000 Da

Sample pumped through a stainless steel capillary

- Rate of a few mls per minute
- Needle at several KVs potential

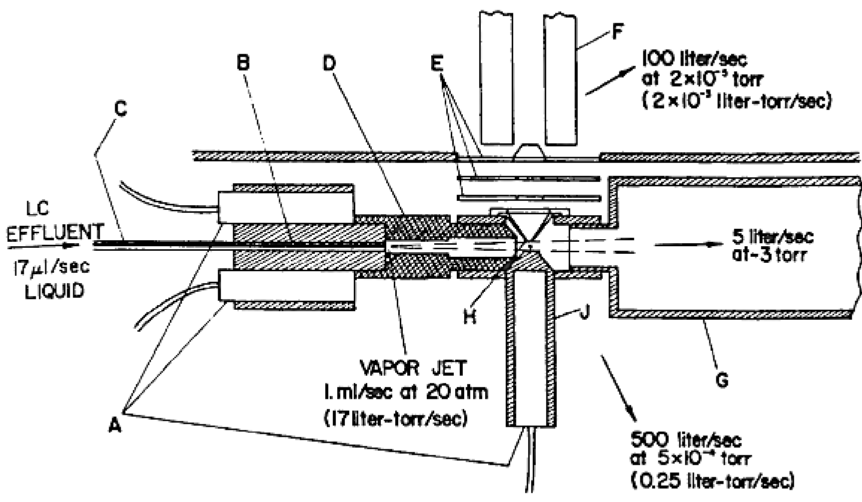
Creates charged spray of fine droplets

Ion source for electrospray



Notes:

Thermospray - similar to electrospray method



Electrospray ionisation spectra

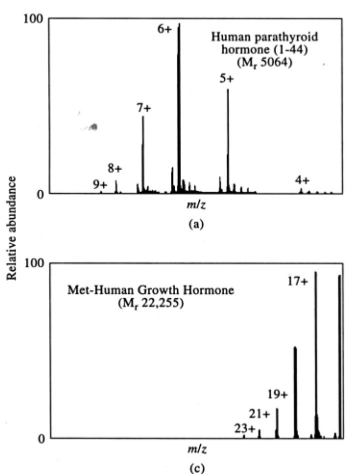
Notes:

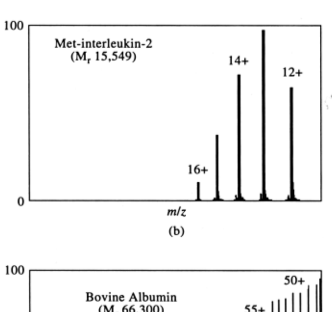
Little fragmentation of thermally fragile biomolecules ions are multiply charged

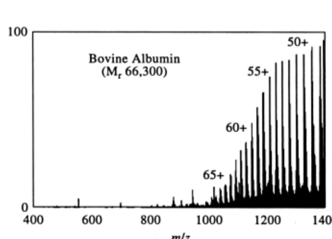
- *m*/*z* values are small
- Detectable with quadrupole with a mass range of 1500 or less

Average charge state increases ~linearly with MW

- MW determined from peak distribution







Other ionisation methods

Notes:

1) Laser ionization (LIMS)

A laser pulse ablates material from the surface of a sample and creates a microplasma that ionizes some of the sample constituents. The laser pulse accomplishes both vaporization and ionization of the sample.

2) Spark source

A spark source ionizes analytes in solid samples by pulsing an electric current across two electrodes. If the sample is a metal it can serve as one of the electrodes, otherwise, it can be mixed with graphite and placed in a cup-shaped electrode.

3) Thermal ionization (TIMS)

Thermal ionization is used for elemental or refractory materials. A sample is deposited on a metal ribbon, such as Pt or Re, and an electric current heats the metal to a high temperature. The ribbon is often coated with graphite to provide a reducing effect.

Other ionisation methods

Notes:

4) Plasma-desorption ionisation (PD)

He decay of ^{252}Cf produces two fission fragments that travel in opposite directions. One fragment strikes the sample knocking out 1-10 analyte ions. The other fragment strikes a detector and triggers the start of data acquisition. This ionization method is especially useful for large biological molecules.

5) Secondary ionisation (SIMS)

A primary ion beam; such as $^{3}He^{+}$,$^{16}O^{+}$, or $^{40}Ar^{+}$; is accelerated and focused onto the surface of a sample and sputters material into the gas phase. Approximately 1% of the sputtered material comes off as ions, which can then be analyzed by a mass spectrometer. SIMS has the advantage that material can be continually sputtered from a surface to determine analyte concentrations as a function of distance from the original surface (depth profiling).

Notes:

Other ionisation methods

6) Field Ionization

Molecules can lose an electron when placed in a very high electric field. High fields can be created in an ion source by applying a high voltage between a cathode and an anode called a field emitter. A field emitter consists of a wire covered with microscopic carbon dendrites, which greatly amplify the effective field at the carbon points.

Sample introduction/ionisation Method:

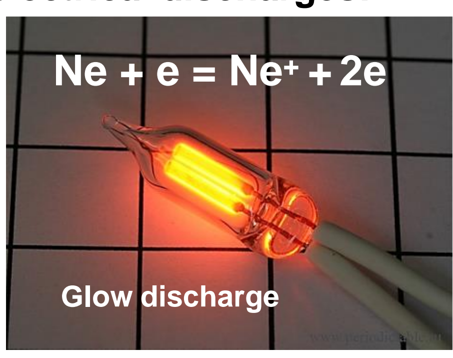
Notes:

Ionization method	Typical Analytes	Sample Introduction	Mass Range	Method Highlights
Electron Impact (EI)	Relatively small volatile	GC or liquid/solid probe	to 1,000 Daltons	Hard method versatile provides structure info
Chemical Ionization (CI)	Relatively small volatile	GC or liquid/solid probe	to 1,000 Daltons	Soft method molecular ion peak [M+H]+
Electrospray (ESI)	Peptides Proteins nonvolatile	Liquid Chromatography or syringe	to 200,000 Daltons	Soft method ions often multiply charged
Fast Atom Bombardment (FAB)	Carbohydrates Organometallics Peptides nonvolatile	Sample mixed in viscous matrix	to 6,000 Daltons	Soft method but harder than ESI or MALDI
Matrix Assisted Laser Desorption (MALDI)	Peptides Proteins Nucleotides	Sample mixed in solid matrix	to 500,000 Daltons	Soft method very high mass

The main types of electrical discharges:

Notes:

- glow discharge;
- corona discharge;
- spark;
- arc;
- high-frequency and microwave discharge.



All these discharge types can be used for analytical purposes.
The main application in analytical chemistry: elemental analysis of solid samples

Notes:

Types of Atomic and Molecular MS

- Thermal ionization & Spark source → first MS
- Inductively coupled plasma (ICP) → current common approach

Differ by types ion sources and mass analyzer

Types of Atomic Mass Spectrometry

Name	Acronym	Atomic Ion Sources	Typical Mass Analyzer
Inductively coupled plasma	ICPMS	High-temperature argon plasma	Quadrupole
Direct current plasma	DCPMS	High-temperature argon plasma	Quadrupole
Microwave-induced plasma	MIPMS	High-temperature argon plasma	Quadrupole
Spark source	SSMS	Radio-frequency electric spark	Double-focusing
Thermal ionization	TIMS	Electrically heated plasma	Double-focusing
Glow discharge	GDMS	Glow-discharge plasma	Double-focusing
Laser microprobe	LMMS	Focused laser beam	Time-of-flight
Secondary ion	SIMS	Accelerated ion bombardment	Double-focusing

Notes:

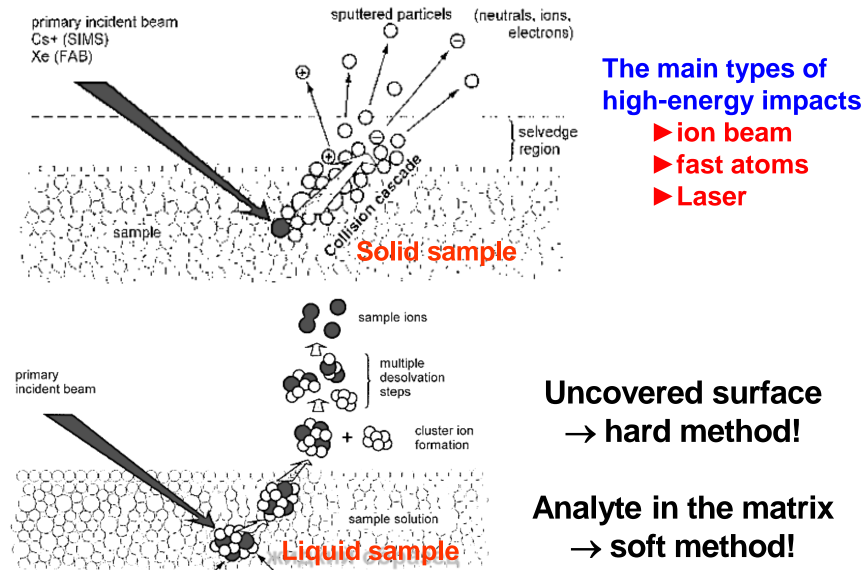
Types of Atomic and Molecular MS

Ion Sources for Molecular Spectrometry

Basic Type	Name and Acronym	Ionizing Agent
Gas phase	Electron impact (EI)	Energetic electrons
	Chemical ionization (CI)	Reagent gaseous ions
	Field ionization (FI)	High-potential electrode
Desorption	Field desorption (FD)	High-potential electrode
	Electrospray ionization (ESI)	High electrical field
	Matrix-assisted desorption-ionization (MALDI)	Laser beam
	Plasma desorption (PD)	Fission fragments from ^{252}Cf
	Fast atom bombardment (FAB)	Energetic atomic beam
	Secondary-ion mass spectrometry (SIMS)	Energetic beam of ions
	Thermospray ionization (TS)	High temperature

Other methods of surface sputtering

Notes:



The main types of high-energy impacts:

- ►**ion beam**
- ►**fast atoms**
- ►**Laser**

Uncovered surface → hard method!

Analyte in the matrix → soft method!

Notes:

Inductively Coupled Plasma Mass Spectrometry

ICP-MS combines two advantages:

1. Argon ICP as a highly efficient ion source
2. A mass spectrometer for fast scanning, high ion transmission and unit mass resolution

The main difference to ICP-OES is to analyze atomic ions. Most elements have the first ionization potential of 4 to10 eV, which are efficiently ionized in argon ICP.

Ions are passed into the high vacuum region for separation and detection. Photons and neutral species are rejected.

The mass spectrometer separates ions based on their mass-to-charge ratio (m/z).

Inductively Coupled Plasma Mass Spectrometry

Notes:

An electron multiplier detector generates a pulse for each ion reaching it.

Since the charge on a singly ionized element is 1, the *m/z* is equal to the mass, so ICP-MS measures the elements as a simple spectrum of characteristic atomic (isotopic) mass from ^{6}Li to ^{238}U.

ICP-MS

Advantages

- Most sensitive technique
- Multi-element analysis
- Isotopic information (IR, ID analysis)
- Wide dynamic range
- Tolerates complex matrices

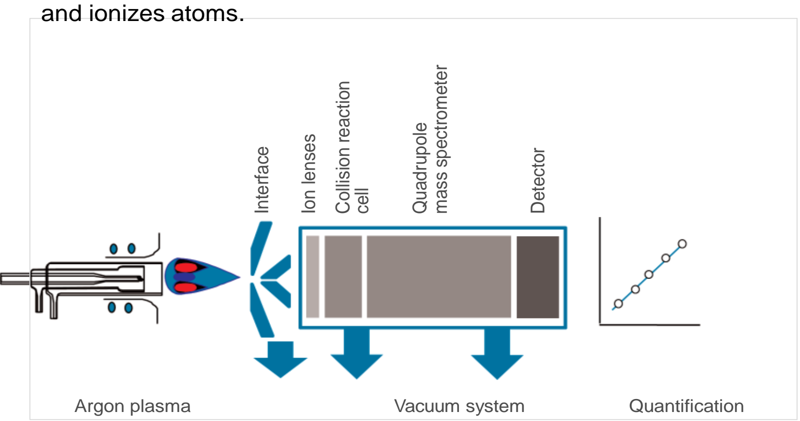
Limitations

- Less matrix tolerance than ICP-OES
- Most expensive technique (purchase and running costs)
- Subject to isobaric interferences

Inductively Coupled Plasma Mass Spectrometry

Notes:

Inductively coupled plasma (ICP) is a very high temperature (7000-8000 K) excitation source that efficiently dissolves, vaporizes, excites and ionizes atoms.

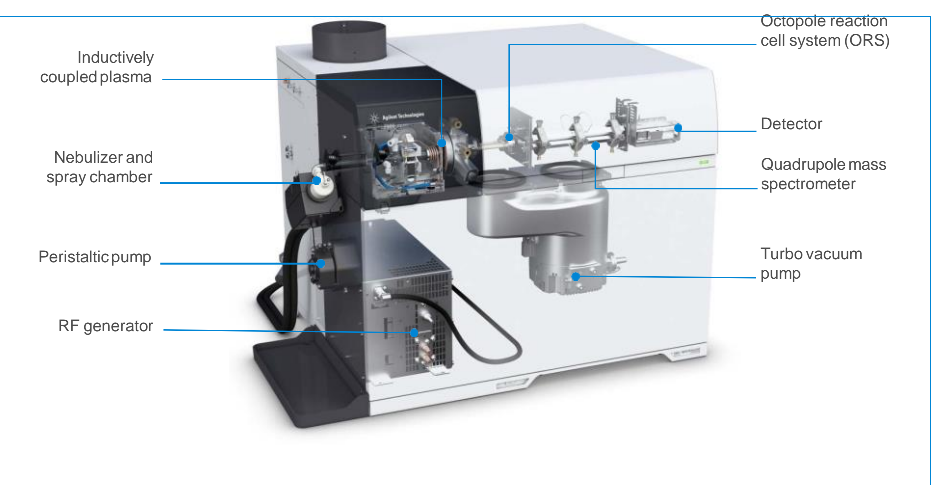


Plasma sources are used to ionize atoms for mass spectrometry.

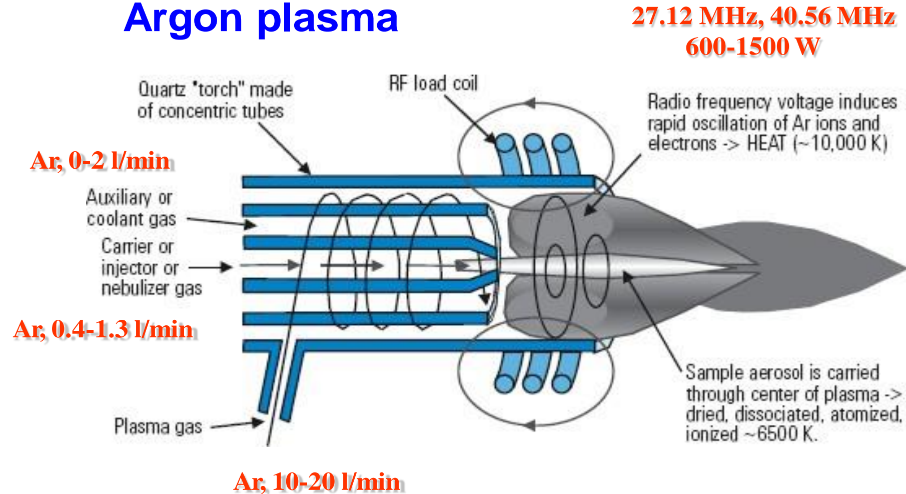
The sample is nebulized and entrained in the flow of plasma support gas (typically Ar)

Inductively Coupled Plasma Mass Spectrometry System

Notes:



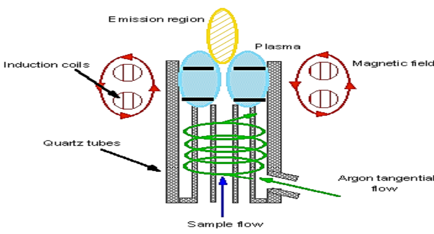
Inductive-coupled plasma (ICP) source



Inductively Coupled Plasma

Notes:

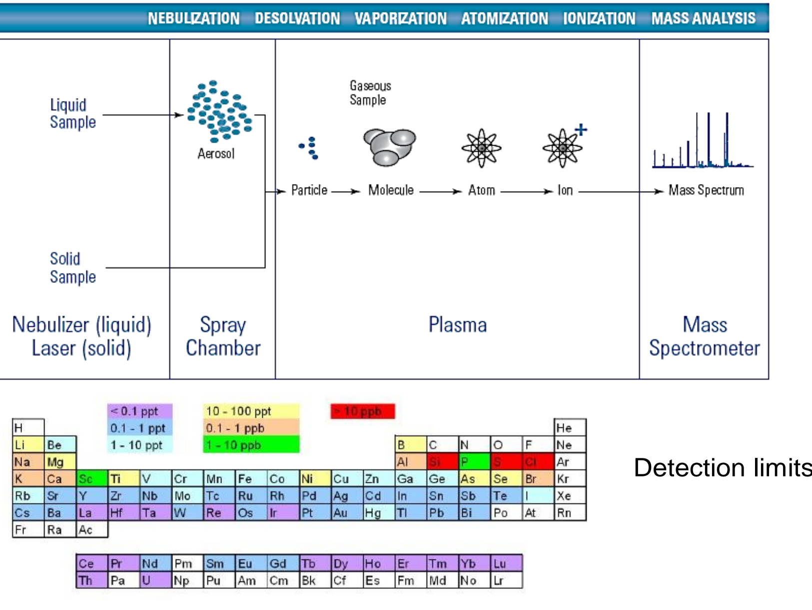
- The plasma torch consists of concentric quartz tubes, with the inner tube containing the sample aerosol and Ar support gas and the outer tube containing an Ar gas flow to cool the tubes (see schematic).
- A radiofrequency (RF) generator (typically 1-5 kW @ 27 MHz or 41 MHz) produces an oscillating current in an induction coil that wraps around the tubes.



- The induction coil creates an oscillating magnetic field, which produces an oscillating magnetic field.
- The magnetic field in turn sets up an oscillating current in the ions and electrons of the support gas.
- These ions and electrons transfer energy to other atoms in the support gas by collisions to create a very high temperature plasma.

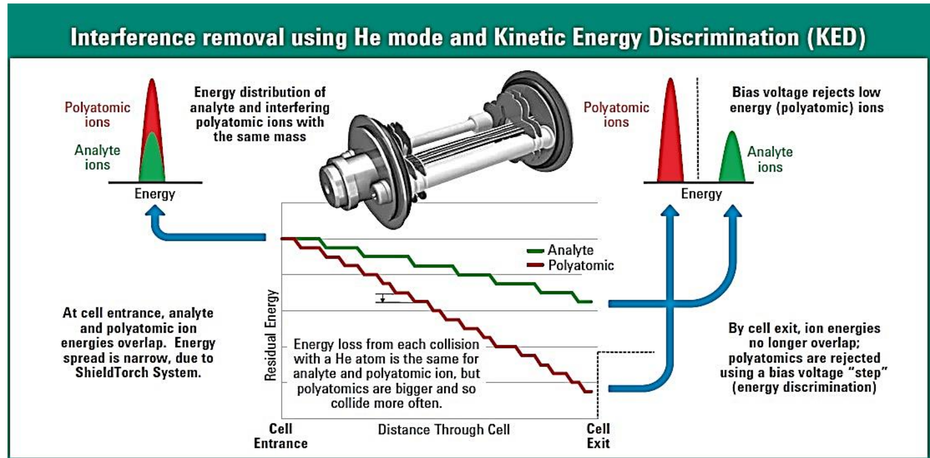
Ionisation in Inductively Coupled Plasma

Notes:



How Helium Collision Cell Mode Removes Spectra Interference in ICP-MS

Notes:



Notes:

Some actual mass analyser technologies:

1. Quadrupole spectrometers;
2. Magnetic deflectors;
3. Time-of-flight spectrometers;
4. Quadrupole ion traps;
5. Fourier transformation ion cyclotron resonance;
6. Orbitraps.

1. Quadrupole Mass Spectrometer

Notes:

- A quadrupole (simple in construction and lightweight) mass filter consists of four parallel metal rods arranged as in the figure below.
- Two opposite rods have an applied potential of $(U+V_0\cos(\omega t))$ and the other two rods have a potential of $-(U+V_0\cos(\omega t))$, where U is a DC voltage and $V_0\cos(\omega t)$ is an AC voltage.
- The applied voltages affect the trajectory of ions travelling down the flight path centred between the four rods.
- For given DC and AC voltages, only ions of a certain mass-to-charge ratio pass-through the quadrupole filter.
- A mass spectrum is recorded as the number of ions passing through the quadrupole filter as a function of the voltages on the rods.
- There are two methods: varying ω and holding U and V_0 constant, or varying U and V_0 (U/V) fixed for a constant ω.

Detector

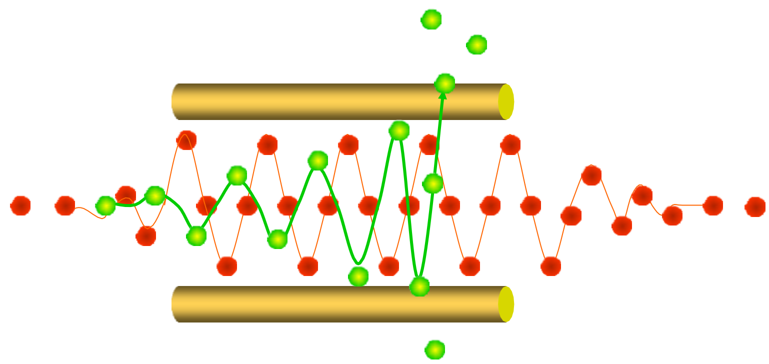
resonant ion

nonresonant ion

Source

Ppriniciple of quadrupole operation

Notes:

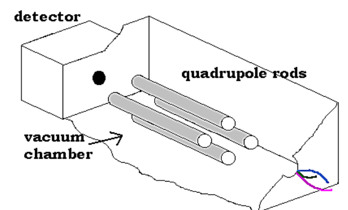


For each combination of radiofrequency (RF) and Direct Current (DC) potentials, only ions with a specific m/z (resonant ions) pass through the quadrupole

Quadrupole mass analyser

Notes:

More compact, less expensive, rugged
High scan rate → spectrum in < 100ms



Four parallel cylindrical rods serve as electrodes:

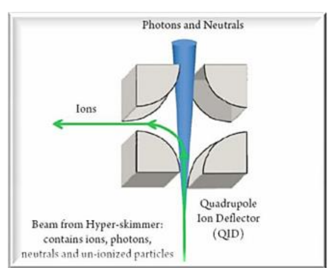
- Opposite rods are connected electrically:
  - One pair attached to positive side of variable DC source;
  - One pair attached to negative side of variable DC source;
- Variable radio-frequency AC potential (180º out of phase) applied to each pair of rods.

Ions accelerated through space between rods:

- Potential of 5 to 10 V;
- AC and DC voltages increased simultaneously with ratio being constant;
- All ions without specific *m/z* strike rods and become neutral:
  - only ions having a limited range of *m/z* reach transducer (detector).

Quadrupole ICP-MS

Notes:



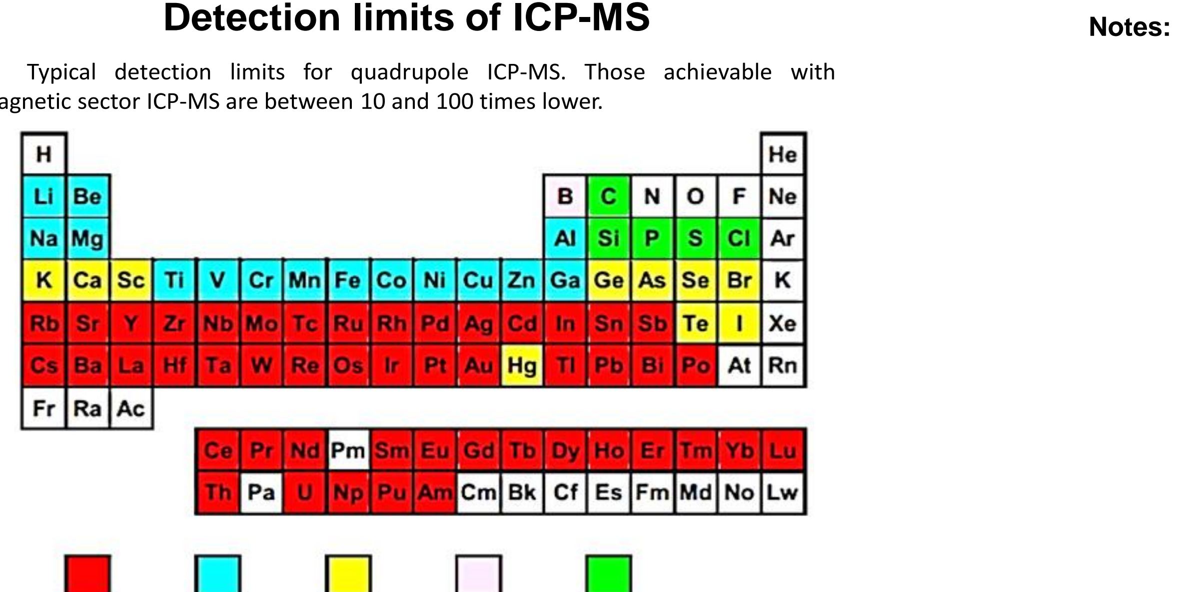
Quadrupole:

- is easily controlled by computer;
- has a good dynamic range (10^5);
- fits with all input systems;
- is able to separate both positive and negative ions without modification;
- has a high scan speed (full spectrum in 5 seconds);
- is of small size;
- is cheap;
- has ability to work at elevated (up to 5×10^{-5} mm Hg) pressure.

Quadrupole Ion Deflector (QID)
Detector
Quadrupole Mass Filter
Universal Cell
Triple Cone Interface

Detection limits of ICP-MS

Typical detection limits for quadrupole ICP-MS. Those achievable with magnetic sector ICP-MS are between 10 and 100 times lower.

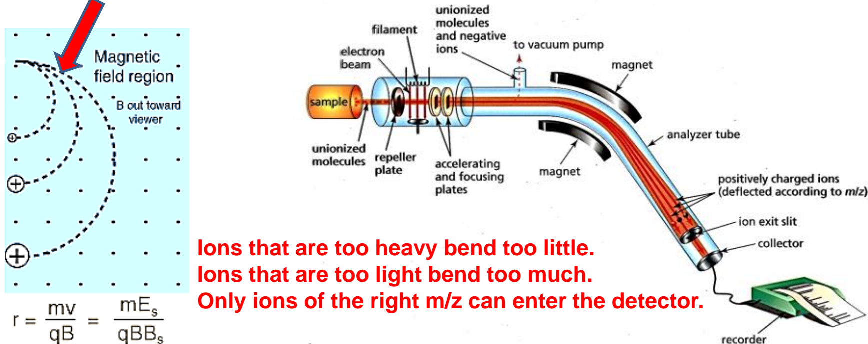


~0.1ppt 1–10 ppt 10–100 ppt 0.1–1 ppb >10–ppb

Notes:

Magnetic Deflection Mass Spectrometer

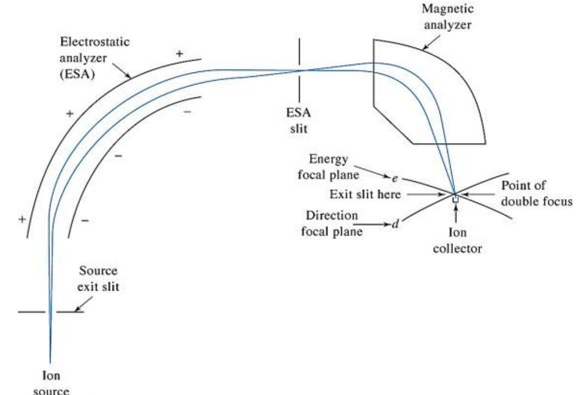
- The heated tungsten filament produces an electron beam, which passes between the plates.
- A difference in electric potential between the plates pulls ions out of the beam so that they pass through the slit.
- **Single Focusing analysers:** A circular beam path of 180, 90 or 60 degrees can be used.
- The various forces influencing the particle separate ions with different mass-to-charge ratios.



Notes:

Double-Focusing analyser

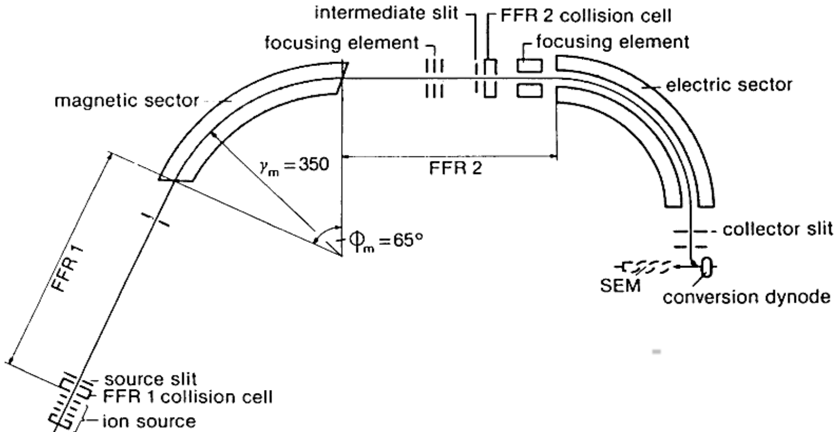
- Two devices are used for focusing an ion beam: the first is electrostatic analyser and the second - magnetic sector analyser.
- An electrostatic analyser is added to separate particles with difference in kinetic energies.
- Ions accelerated through slit into curved electrostatic field;
- Focus beam of ions with narrow band of kinetic energies into slit;
- Ions enter curved magnetic field;
- Lighter ions deflected more than heavier ions.



Notes:

Double focusing analysers with Nier-Johnson reverse geometry

Notes:



In reverse geometry, magnetic sector is installed first, and electrostatic analyzer is added as a second part of the instrument.

Notes:

Single focusing	Double focusing
Magnetic field	Magnetic field & electric field
Ions of same mass number are separated	Ions of same mass no & same velocity are separated
Less sensitivity(10^{-1})	Highly sensitive(10^{-12})

Time-of-Flight (TOF) Mass Analysers

Notes:

Ions generated by bombardment of the sample with a brief pulse of:

- Electrons, secondary ions, laser-generated photons;
- Frequency of pulse 10 to 50 kHz, duration of pulse 0.25 ms.

Ions accelerated by electric field pulse 10^3 to 10^4 V:

- Same frequency of ionization pulse, but lags behind.

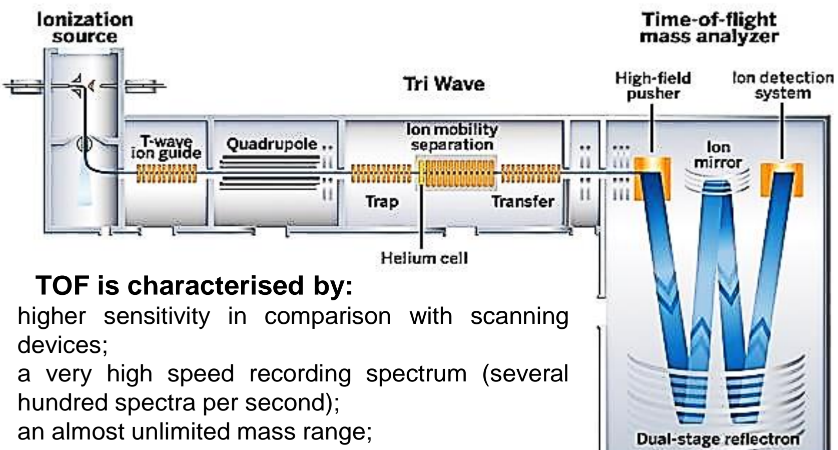
Laser
Detector
Time of flight tube
Matrix
Electric field generator

Accelerated particle enter *field-free* drift tube:

- Ions enter tube with *same* kinetic energy;
- Ion velocity vary inversely with mass;
- Lighter particles arrive at detector before heavier particles:
  Flight times are 1 to 30 ms so that fast electronics is required;
- Peak broadening due to variability in ion energies and initial position:
  Limits resolution compared to magnets and quadrupole;
  Less widely used than quadrupole;
- Advantages: unlimited mass range, rapid data acquisition, simplicity, ruggedness, ease of access to ion source

Time Of Flight Mass Spectrometer

Notes:



TOF is characterised by:

- higher sensitivity in comparison with scanning devices;
- a very high speed recording spectrum (several hundred spectra per second);
- an almost unlimited mass range;
- a resolution of more than 10,000;
- relatively small sizes
- compatible with a wide variety of ion sources.
- It is an ideal second analyser for tandem mass spectrometry.

Time Of Flight Mass Spectrometer

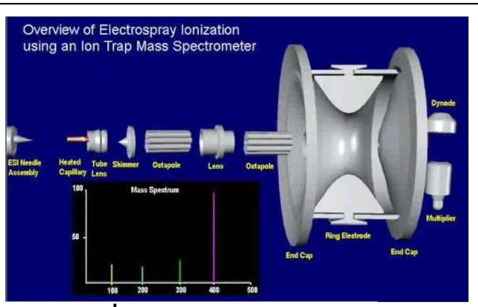
Notes:

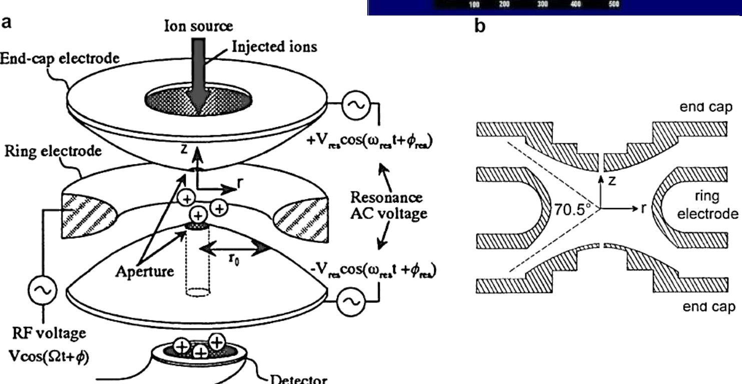
- This schematic shows ablation of ions from a solid sample with a pulsed laser.
- The reflectron is a series of rings or grids that act as an ion mirror.
- The ions leaving the ion source of a ToF MS have neither exactly the same starting time nor exactly the same kinetic energies.
- Ions spread due to different velocities; The velocity is inversely proportional to mass. Ions arrive to the detector one after another
- The ion mirror compensates for the spread in kinetic energies of the ions as they enter the drift region and improves the resolution of the instrument.
- The output of an ion detector is displayed on an oscilloscope as a function of time to produce the mass spectrum.
- **The main advantages** of a ToF include its speed and ability to record entire mass spectrum at one time.
- **The disadvantage** is its poor resolution.

Quadrupole Ion Trap

Notes:

- Ions follow complex trajectories between two pairs of electrodes that switch polarity rapidly.
- Ions can be ejected from trap by *m/z* value by varying the frequency of end cap electrodes





The advantages of quadrupole ion traps:

Notes:

- Works in tandem mass spectrometry mode.
- The ability to achieve a resolution of 25,000.
- Small size.
- The lowest cost of the device.
- The possibility of expanding the range of recorded masses in the mode of resonant extraction of ions to tens of thousands (~>10,000).

- **The disadvantages** include the occurrence of ion-molecular reactions in the trap, which leads to distortions of the standard mass spectrum.

Fourier Transform Ion Cyclotron Resonance Mass Spectrometer

Notes:

- Fourier transform ion cyclotron resonance mass spectrometry is a type of mass analyser (or mass spectrometer) for determining the mass-to-charge ratio *(m/z*) of ions based on the cyclotron frequency of the ions in a fixed magnetic field.
- The ions are trapped in a Penning trap (a magnetic field with electric trapping plates) where they are excited (at their resonant cyclotron frequencies) to a larger cyclotron radius by an oscillating electric field orthogonal to the magnetic field.
- After the excitation field is removed, the ions are rotating at their cyclotron frequency in phase (as a "packet" of ions).

Fourier Transform Ion Cyclotron Resonance Mass Spectrometer

Notes:

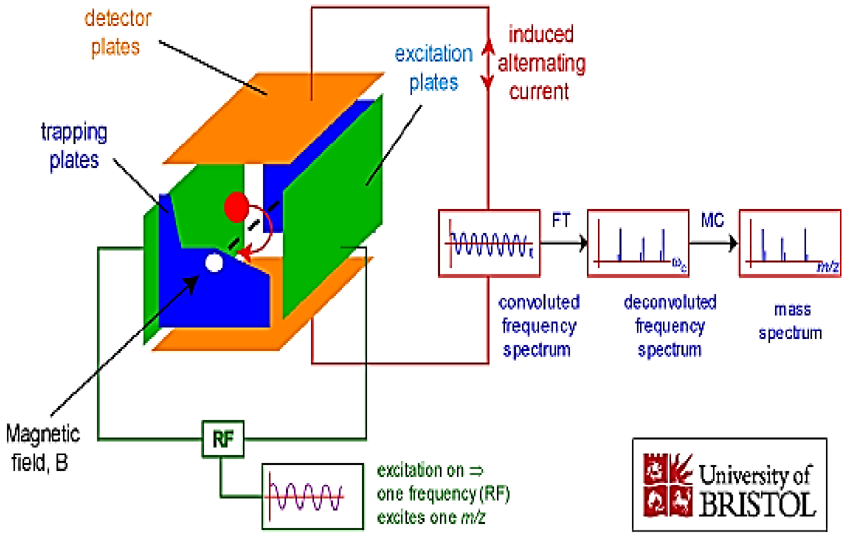
- These ions induce a charge (detected as an image current) on a pair of electrodes as the packets of ions pass close to them.
- The resulting signal is called a free induction decay (FID), transient or interferogram that consists of a superposition of sine (=*sinus)* waves.
- The useful signal is extracted from this data by performing a Fourier transform to give a mass spectrum.
- In the simplest form (idealised), the relationship between the cyclotron frequency and the mass to charge ratio is given by:

$$f = \frac{qB}{2\pi m}$$

where f = cyclotron frequency, q = ion charge, B = magnetic field strength and m = ion mass.

Scheme of Fourier Transform Ion Cyclotron Resonance Mass Spectrometer

Notes:



Ion Cyclotron Resonance Analyzer

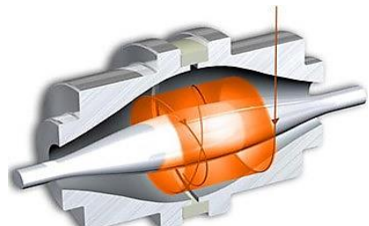
Notes:

- High resolution m / Δm = 10^6.
- The ability to determine the masses accurately (up to 10^{-4}).
- High sensitivity (up to 14 ions can be recorded).
- Possibility of tandem mass spectrometry.
- The possibility of studying the kinetics of reactions.
- **The disadvantage** is a very high cost of the instrument.

Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR)

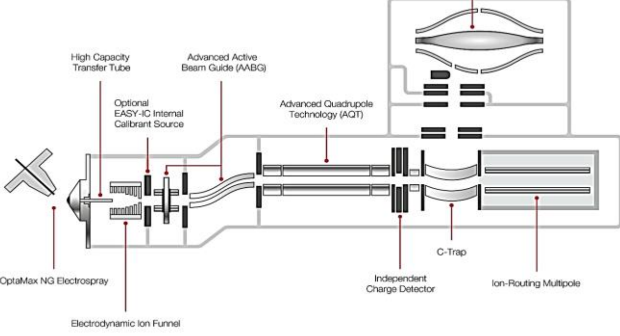
Notes:

Orbitrap



It is a special kind of ion trap.
The difference from other types of ion traps is the presence of a central electrode of a special shape, to which a negative potential is applied, as well as the absence of magnetic fields (as in double-focusing MS and ion-cyclotron resonance) and radio frequencies (as in a quadrupole or quadrupole ion traps).

The method is based on a symmetric static electric field between the external and internal electrodes.



Notes:

Comparison of characteristics of some mass analysers

Analyser	System Highlights
Quadrupole	Unit mass resolution, fast scan, low cost
Sector (Magnetic and/or Electrostatic)	High resolution, exact mass
Time-of-Flight (TOF)	Theoretically, no limitation for m/z maximum, high throughput
Ion Cyclotron Resonance (ICR)	Very high resolution, exact mass, perform ion chemistry

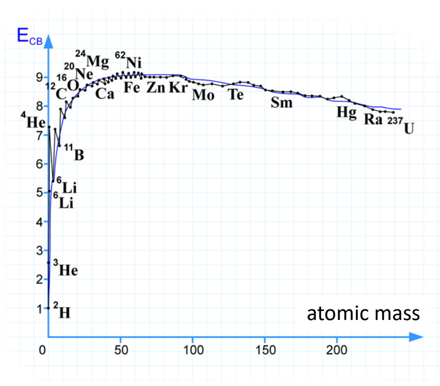
Notes:

Why do we need a high-resolution mass-spectrometer?

To resolve peaks with close masses.
Examples:

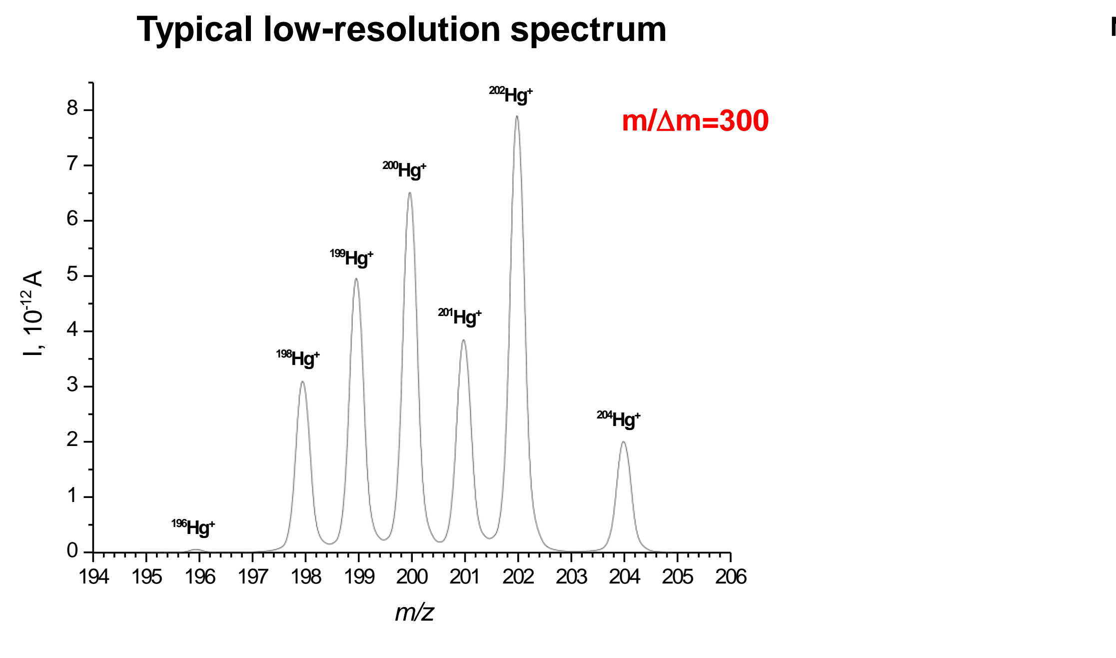
- M =28:
- N_2 28.0062
- CHO 27.9949
- CH_2N 28.0181
- C_2H_4 28.0313
- Si 27.9769

- M =92:
- N_2O_4 91.9858
- CH_2NO_4 91.9983
- C_7H_8 92.0626
- Mo 91.9068



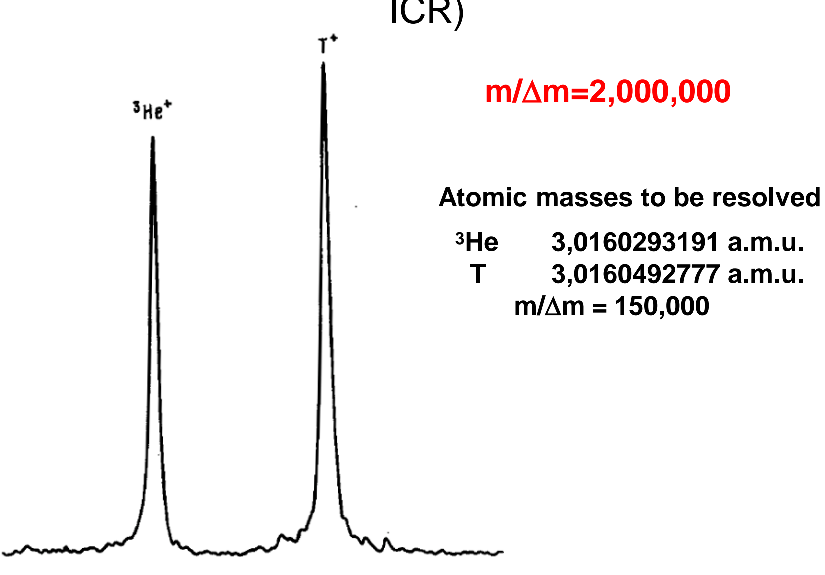
Notes:

Typical low-resolution spectrum



Notes:

Mass-spectrum of a Tritium-Helium-3 doublet (FT-ICR)



m/Δm=2,000,000

Atomic masses to be resolved

^{3}He 3,0160293191 a.m.u.
T 3,0160492777 a.m.u.
m/Δm = 150,000

Notes:

High Resolution Mass Spectrometry:

- Distinguishing between formulas shown in the table below, which have the same nominal masses (m=84), is possible, if you use a MS capable of determining m/z values accurately to four decimal places.
- The table below shows such a scenario and a double-focusing high-resolution mass spectrometer can easily distinguish ions having these compositions.
- Mass spectrometry therefore can now determine specific molecular mass values and also the molecular formulas of unknown compounds.

Formula	C_6H_{12}	C_5H_8O	$C_4H_8N_2$
Mass	84.0939	84.0575	84.0688

Notes:

Strength & weakness of mass-spectrometry methods

Notes:

MS is a powerful analytical technique which allows to:

(a) identify unknown compounds; (b) quantify known materials, and (C) clarify structural and chemical properties.

Advantaged over other analytical methods:

- Requires minimal quantities of matter (< picograms).
- Identification of analyte molecules at very low concentrations in complex matrices.
- Increased sensitivity over most other analytical techniques because the analyser, as a mass-charge filter, reduces background interference.
- Excellent specificity from characteristic fragmentation patterns to identify unknowns or confirm the presence of suspected compounds.
- Information about molecular weight.
- Information about the isotopic abundance of elements.
- Timeline of chemical data.

Weaknesses of the method:

- Often fails to distinguish between optical and geometrical isomers
- The positions of substituent in different positions in an aromatic ring.
- Its scope is limited in identifying hydrocarbons that produce similar fragmented ions.

Atomic mass spectra and interferences

Notes:

Spectroscopic interference - an ionic species in the plasma has the same *m/z* values as an analyte

Isobaric interference

Two elements have isotopes with nearly the same mass (differ less than 1 amu):

$^{113}In^+$ overlaps with $^{113}Cd^+$ and $^{115}In^+$ overlaps with $^{115}Sn^+$

Isobaric interference occurs with the most abundant and most sensitive isotope

$^{40}Ar^+$ overlaps with $^{40}Ca^+$ (97%) need to use $^{44}Ca^+$ (2.1%);

$^{58}Ni^+$ overlaps with $^{56}Fe^+$ need to use $^{56}Fe^+$.

Isobaric interference exactly predictable from abundance tables.

Polyatomic ion interference

- **Various interactions between species in plasma, matrix or atmosphere form polyatomic species.**
- **They are typically observed for *m/z* < 82 amu.**
- **Serious interference:** $^{14}N_2^+$ with $^{28}Si^+$, NOH^+ with $^{31}P^+$, $^{16}O_2^+$ with $^{32}S^+$, $^{40}ArO^+$ with $^{56}Fe^+$, $^{40}Ar_2^+$ with $^{80}Se^+$

Correct with blank samples

Calcium Oxide and Hydroxide Species and Other Potential Interferences in the Mass Region for Ni Determination

m/z	Element[a]	Interferences
56	Fe(91.66)	^{40}ArO, ^{40}CaO
57	Fe(2.19)	$^{40}ArOH$, $^{40}CaOH$
58	Ni(67.77), Fe(0.33)	^{42}CaO, NaCl
59	Co(100)	^{43}CaO, $^{42}CaOH$
60	Ni(26.16)	$^{43}CaOH$, ^{44}CaO
61	Ni(1.25)	$^{44}CaOH$
62	Ni(3.66)	^{46}CaO, Na_2O, NaK
63	Cu(69.1)	$^{46}CaOH$, $^{40}ArNa$
64	Ni(1.16), Zn(48.89)	$^{32}SO_2$, $^{32}S_2$, ^{48}CaO
65	Cu(30.9)	$^{33}S^{32}S$, $^{33}SO_2$, $^{48}CaOH$

Fields of application of MS:

Notes:

Chemical & structural analysis:

- Biochemistry
- Proteomics
- Clinical chemistry
- Cosmetics, perfumes
- Pharmaceuticals, doping, drugs
- Environmental control
- Food products
- Forensics
- Oil products
- Polymers

Elemental analysis:

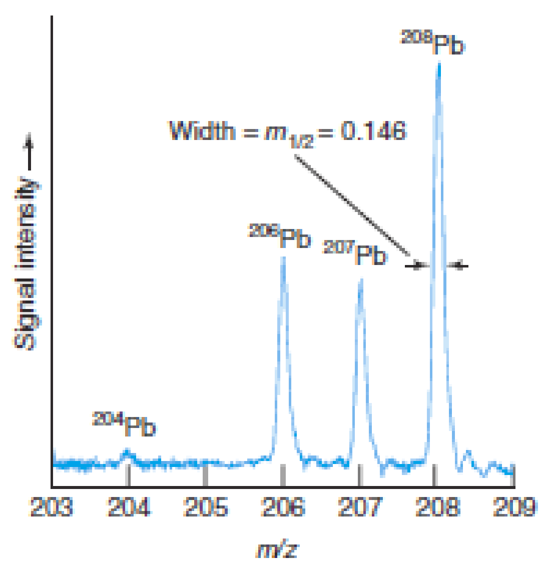
- Archeology
- Geochemistry
- Ecology
- Nuclear energy
- Medicine and Toxicology
- Pharmaceuticals, cosmetics
- Food
- Metallurgy
- Semiconductor industry
- Oil and oil products
- Forensics

Isotope analysis:

- Nuclear industry
- Agriculture, Food
- Medical diagnostics, doping control
- Geochronology (radiocarbon method)
- Geology, hydrology, oil development
- Environmental control
- Climate research
- Forensics

Tasks to Section 15

1. Give definitions of these terms: mass spectroscopy, mass spectrometer, ionization,
2. Briefly describe how a magnetic sector mass spectrometer works.
3. How are ions created for each of the mass spectra?
4. Define the unit Dalton. From this definition, compute the mass of 1 Da in grams.
5. Explain how a double-focusing mass spectrometer achieves high resolution.
6. What are the ionization methods used in mass spectroscopy? Why are the different ionization methods used?
7. What types of ions are observed in the mass spectrometer? Under what conditions and for what type of molecules will the probability of molecular ion formation below?
8. Give the concept of the term ionization cross-section. Will it depend on the energy of the ionizing electrons?
9. Explain the schematic diagram of the mass spectrometer.
10. Name the features of static mass spectrometers. Are there any restrictions on the mass of the ion?
11. Name the types of dynamic mass spectrometers.
12. Explain the focusing effect of the magnetic field of the analyzer of the mass spectrometer.
13. Define a mass spectrometer resolution. What factors determine it?
14. Define a mass spectrometer sensitivity? What factors determine it?
15. What is the basis for the identification of ions in the mass spectrometer?
16. How can the gross formula of a substance be established?
17. Give examples of patterns of dissociative ionization of organic compounds.
18. How can the ionization potentials of molecules be determined? Why the accuracy of determining ionization potentials is the highest in photoionization?
19. How can the energy of breaking chemical bonds be determined? What data is needed to determine?
20. Name the conditions for the mass-spectroscopic thermodynamic experiment.
21. Name the methods of studying ion-molecular reactions.
22. Using the ^{208}Pb peak in the figure, find the resolving power from the expression m/m 1/2.
23. A limitation on how many spectra can a time-of-flight mass spectrometer record per second is the time, which the slowest ions take to go from the source to the detector. Suppose we want to scan up to m/z 500. Calculate the speed of this heaviest ion if it is accelerated through 5.00 kV in the source. How long would it take to drift 2.00 m through a spectrometer? At what frequency could you record spectra if a new extraction cycle were begun each time this heaviest ion reached the detector? What would be the frequency if you wanted to scan up to m/z 1 000?
24. What is the purpose of the reflectron in a time-of-flight mass spectrometer?
25. You should detect the drug ibuprofen by liquid chromatography/mass spectrometry. Would you choose the positive or negative ion mode for the spectrometer? State your reasons.



Mass spectrum showing natural isotopes of Pb observed as an impurity in brass

Section 16: Potentiometric Methods

Contents:

- Introduction
- Important concepts
- Controlling and measuring current and potential
- Potentiometric measurements
- Reference electrodes
- Metallic indicator electrodes
- Membrane electrodes
- Quantitative applications

Introduction

Electrochemistry is a major branch of analytical chemistry that uses electrical measurements of chemical systems for analytical purposes. The term "electrochemistry" is also used to describe chemical processes that occur under the action of current or vice versa during which electricity is produced.

Electroanalytical chemistry unites a group of analytical methods based upon electrical properties of analytes when they are parts of an electrochemical cell.

Electrochemical techniques are divided into bulk and interfacial ones. For bulk techniques, we measure a property of the solution in the electrochemical cell. For interfacial techniques, the potential, current or charge depend on the species present at the interface between an electrode and the solution. The measurement of a solution's conductivity, which is proportional to the total concentration of dissolved ions, is one example of a bulk electrochemical technique. A determination of pH with the use of a pH electrode is an example of an interfacial electrochemical technique. Only interfacial electrochemical methods receive further consideration in Section 16.

A redox reaction involves the transfer of electrons from one species to another. A species is said to be oxidized when it loses electrons. It is reduced when it gains electrons. An oxidizing agent also called an oxidant takes electrons from another substance and becomes reduced.

A reducing agent, also called a reductant, gives electrons to another substance and is oxidized in the process.

When electrons from a redox reaction flow through an electric circuit, we can learn something about the reaction by measuring current and voltage. Electric current is proportional to the rate of reaction, and the cell voltage is proportional to the free energy change for the electrochemical reaction. In techniques such as voltammetry, the voltage can be used to identify reactants.

Electrochemical measurements are made in an electrochemical cell consisting of two or more electrodes and the electronic circuitry for controlling and measuring the current and the potential.

The simplest electrochemical cell uses two electrodes.

The potential of one electrode is sensitive to the analyte's concentration. It is called the working electrode or the indicator electrode.

The second electrode, which we call the counter electrode, completes the electrical circuit and provides a reference potential against which we measure the working electrode's potential.

Ideally, the counter electrode's potential remains constant so that we can assign to the working electrode any change in the overall cell potential.

The Nernst equation provides a mathematical relationship between the electrode's potential and the concentrations of an analyte's oxidized and reduced forms in solution.

Potentiometry is an analytical method in which an electric potential difference (a voltage) of a cell is measured. The potentiometric method of analysis is based on the measurement of electrode potentials and electromotive force (EMF) in electrolyte solutions.

Notes:

General Advantages of Electrochemistry:

a) selective for particular redox state of a species e.g. Ce^{III} vs. Ce^{IV}

b) cost - $4,000 - $25,000 for a good instrument compared to $10,000 - $50,000 - $250,000 for a good spectrophotometer

c) measures activity (not concentration), activity usually of more physiological importance

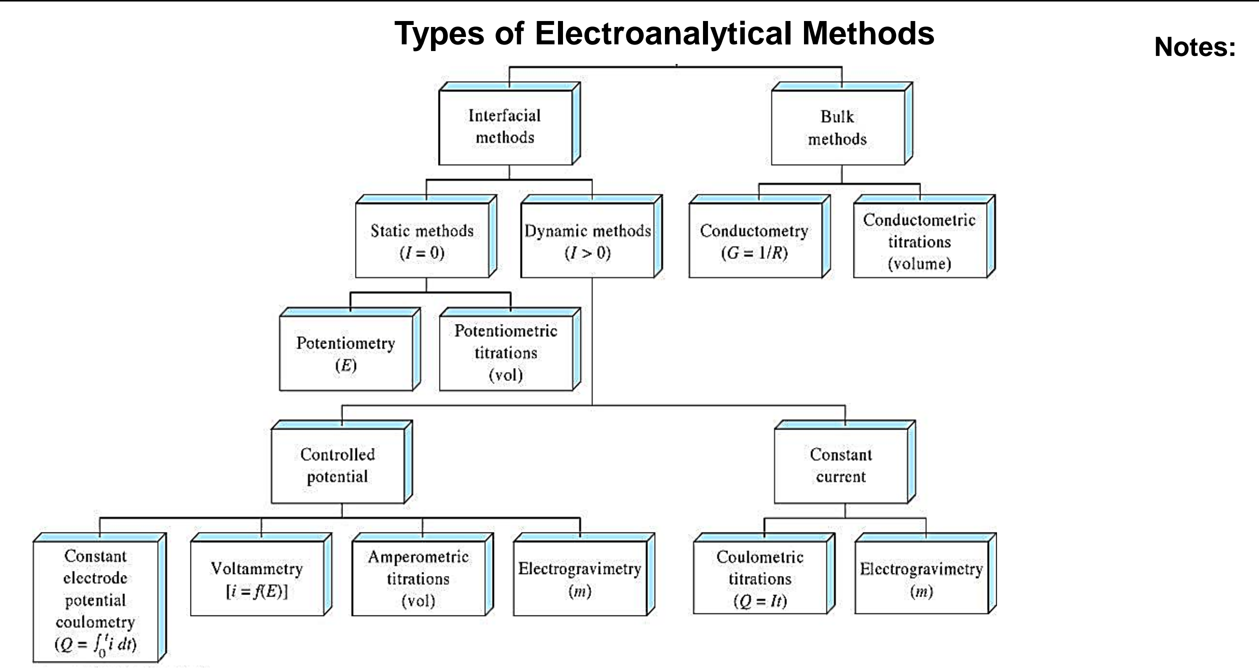
d) fast

e) in situ

f) information about: oxidation states, stoichiometry, rates, charge transfer, equilibrium constants

Types of Electroanalytical Methods

Notes:



Electrochemical Cell

Notes:

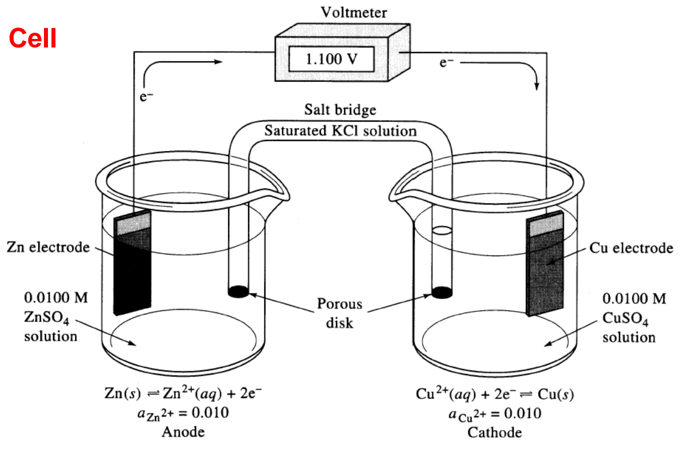
Basic Set-up:

a) Two electrodes (electrode is an electrical conductor through which electro flow into or out of chemical species involved in a redox reaction).

b) electrolytes solution

c) external connection between electrodes (wire)

d) internal connection via contact with a common solution or by different solutions connected by a salt bridge.

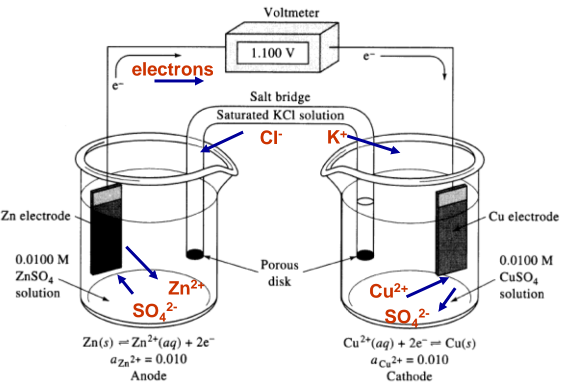


Salt bridge – acts to isolate two halves of electrochemical cell while allowing migration of ions and current flow:

- usually consists of a tube filled with potassium chloride;
- separate species to prevent direct chemical reactions

Flow of current (charge) in cell:

a) electrons (e^-) within wires between two electrodes
b) ions within solution of each ½ cell (anions & cations) and through salt bridge
c) electrochemical reactions at electrode



At Cu electrode: $Cu^{2+} + 2e^- \leftrightarrow Cu(s)$ **reduction** – gain of e^- net decrease in charge of species

At Zn electrode: $Zn(s) \leftrightarrow Zn^{2+} + 2e^-$ **oxidation** – loss of e^- net increase in charge of species

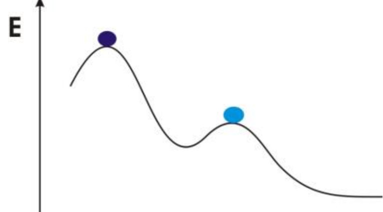
Notes:

Net Reaction in Cell – sum of reactions occurring in the two ½ cells

$$Zn(s) \leftrightarrow Zn^{2+} + 2e^-$$
$$\underline{Cu^{2+} + 2e^- \leftrightarrow Cu(s)}$$
$$Cu^{2+} + Zn(s) \leftrightarrow Zn^{2+} + Cu(s)$$

The potential of overall cell = measure of the tendency of this reaction to proceed to equilibrium. **At equilibrium** the potential (E_{cell}) = 0

Larger the potential, the further the reaction is from equilibrium and the greater the driving force that exists

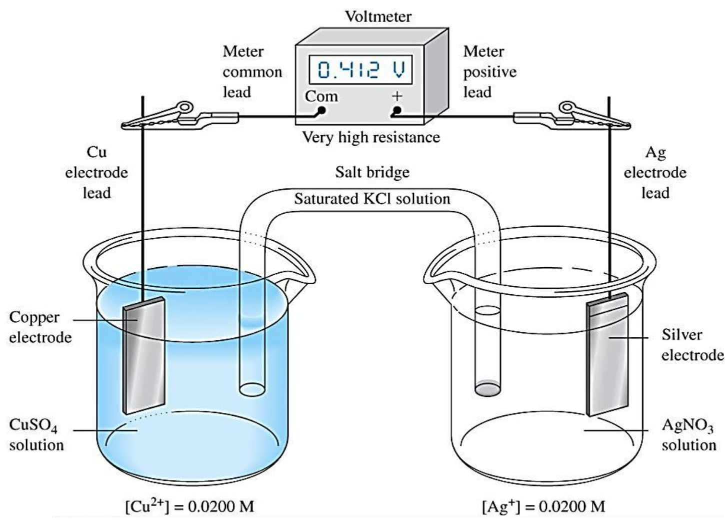


Similar in concept to balls sitting at different heights along a hill

Notes:

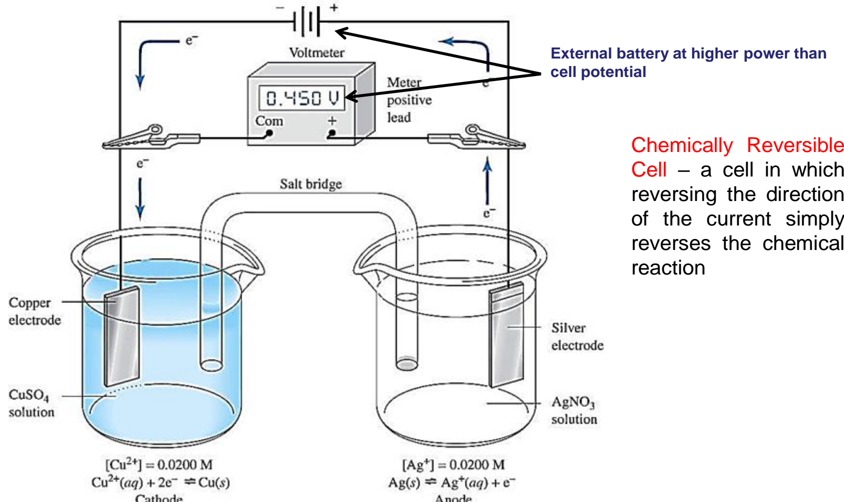
Types of Cells: Galvanic Cells – reaction occurs naturally

- positive potential (E_{cell} = +)
- exothermic → produces energy



Notes:

Types of Cells: **Electrolytic Cells** – reaction does not occur naturally, requires external stimulus (energy) to occur
- negative potential (E_{cell} = -)
- endothermic → requires energy



Chemically Reversible Cell – a cell in which reversing the direction of the current simply reverses the chemical reaction

Notes:

Electrodes:

Cathode – electrode where *reduction* occurs

Anode – electrode where *oxidation* occurs

Examples of cathode half-reactions:

$Cu^{2+} + 2e^- \leftrightarrow Cu(s)$

$Fe^{3+} + e^- \leftrightarrow Fe^{2+}$

$AgCl(s) + e^- \leftrightarrow Ag(s) + Cl^-$

- e^- supplied by electrical current via electrode
- species (products/reactants) can both be in solution (Fe^{3+}/Fe^{2+}) solids or coated on electrodes (AgCl(s)/Ag(s) or combination (Cu^{2+}/Cu(s))

e⁻
Cu electrode
0.0100 M $CuSO_4$ solution
$Cu^{2+}(aq) + 2e^- \rightleftharpoons Cu(s)$
$a_{Cu^{2+}} = 0.010$
Cathode

Notes:

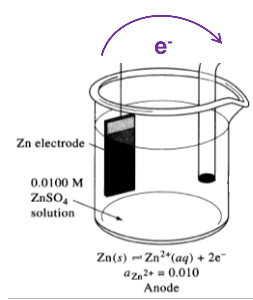
Examples of anode ½ reactions:

$Cu(s) \leftrightarrow Cu^{2+} + 2e^-$

$Fe^{2+} \leftrightarrow Fe^{2+} + e^-$

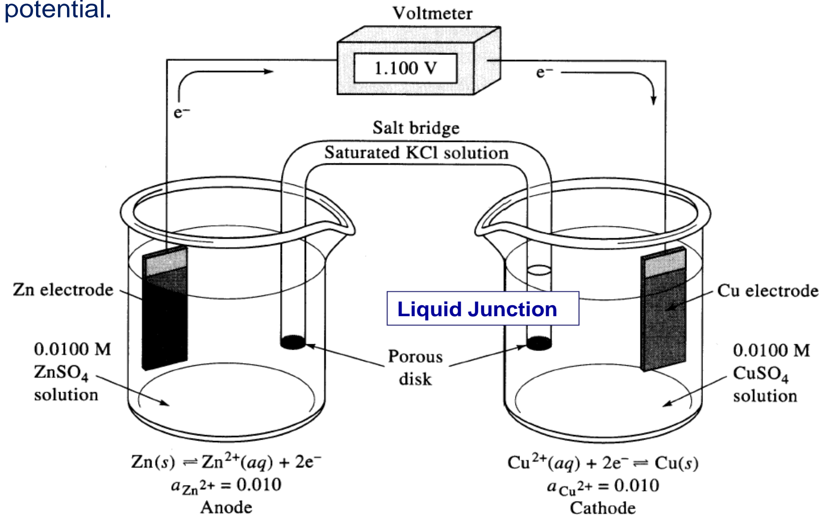
$Ag(s) + Cl^- \leftrightarrow AgCl(s) + e^-$

- e^- is taken up by electrode into electrical circuit



Liquid junctions – interface between two solutions with different components or concentrations.

Small potentials may develop at junction that affect overall cell potential.

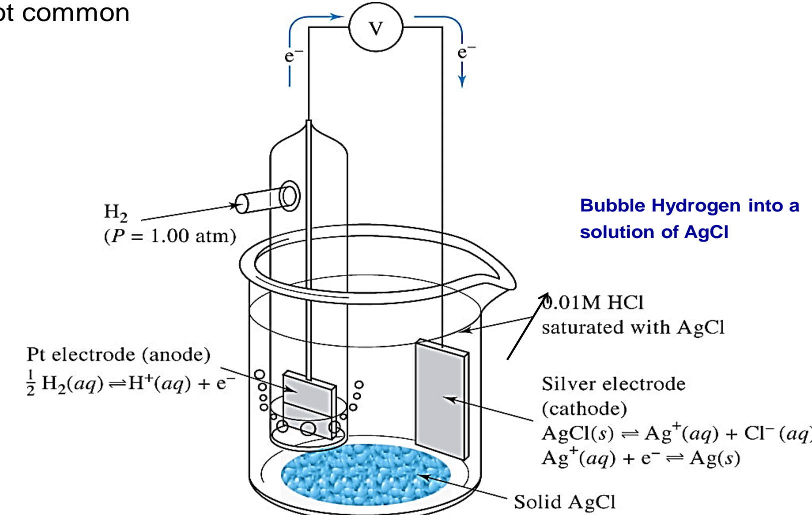


Notes:

Galvanic cell without liquid junction

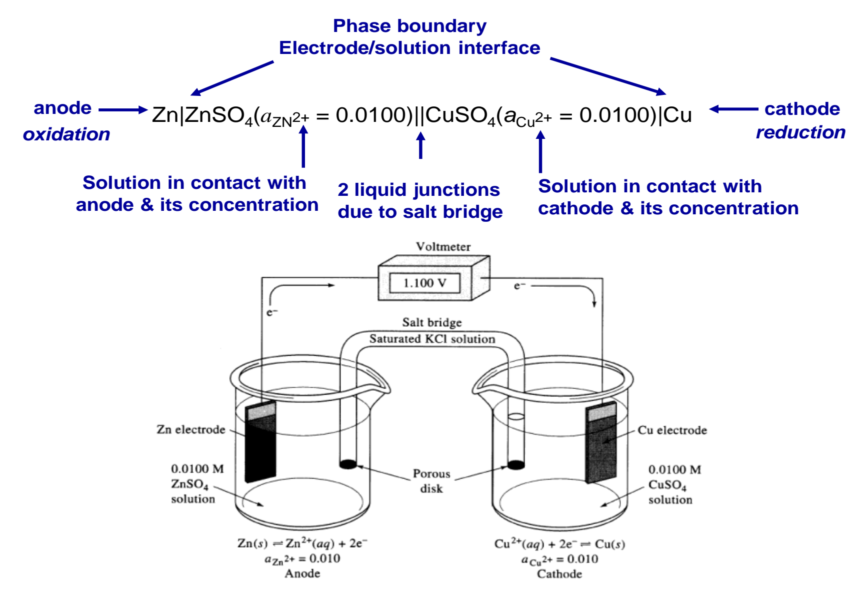
- two species have high potential for reaction, but the reaction is slow
- mix two species directly into common solution
- not common

Notes:



Representation of Cells: by convention start with anode on left

Notes:



Electrode Potentials

Notes:

For convenience, represent overall reaction in cell as two ½ reactions

i. one at anode & other at cathode

ii. each half-reaction has certain potential associated with it

iii. by convention, write both half-reactions as reduction:

$Cu^{2+} + 2e^- \leftrightarrow$ Cu(s) ($E_{cathode}$)

$Zn^{2+} + 2e^- \leftrightarrow$ Zn(s) ($-E_{anode}$)

iv. potential of cell is then defined as:

$$E_{cell} = E_{cathode} - E_{anode}$$

Standard Electrode Potentials

Reaction	E^0 at 25°C, V
$Cl_2(g) + 2e^- \rightleftharpoons 2Cl_2$	+1.359
$O_2(g) + 4H^+ + 4e^- \rightleftharpoons 2H_2O$	+1.229
$Br_2(aq) + 2e^- \rightleftharpoons 2Br_2$	+1.087
$Br_2(l) + 2e^- \rightleftharpoons 2Br_2$	+1.065
$Ag^+ + e^- \rightleftharpoons Ag(s)$	+0.799
$Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$	+0.771
$I_3^- + 2e^- \rightleftharpoons 3I^-$	+0.536
$Cu^{2+} + 2e^- \rightleftharpoons Cu(s)$	+0.337
$Hg_2Cl_2(s) + 2e^- \rightleftharpoons 2Hg(l) + 2Cl_2$	+0.268
$AgCl(s) + e^- \rightleftharpoons Ag(s) + Cl_2$	+0.222
$Ag(S_2O_3)_2^{3-} + e^- \rightleftharpoons Ag(s) + 2S_2O_3^{2-}$	+0.010
$2H^+ + 2e^- \rightleftharpoons H_2(g)$	0.000
$AgI(s) + e^- \rightleftharpoons Ag(s) + I_2$	−0.151
$PbSO_4(s) + 2e^- \rightleftharpoons Pb(s) + SO_4^{2-}$	−0.350
$Cd^{2+} + 2e^- \rightleftharpoons Cd(s)$	−0.403
$Zn^{2+} + 2e^- \rightleftharpoons Zn(s)$	−0.763

Electrode Potentials

Problem – can not measure potential of just one electrode.

i. need to compare to another electrode

ii. determine potential of all half-cell reactions vs. a common reference electrode

iii. reference electrode – standard hydrogen electrode (SHE)

$Pt, H_2(p\ atm)\ |\ H^+(a_{H^+} = x)$

$2H^+ + 2e^- \leftrightarrow H_2(g)$

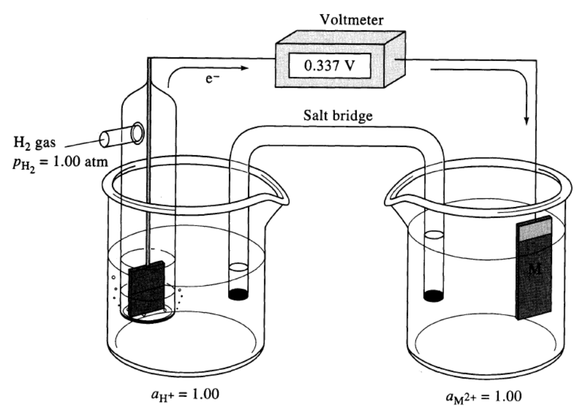
stream of H_2 keeps surface at electrode saturated w/$H_2(g)$

Note: potential affected by pH, $[H^+]$, used as an early pH indicator, also dependent on P_{H2}

V
e−
e−
H_2 (P = 1.00 atm)
0.01M HCl saturated with AgCl
Pt electrode (anode) $\frac{1}{2} H_2(aq) \rightleftharpoons H^+(aq) + e^-$
Silver electrode (cathode) $AgCl(s) \rightleftharpoons Ag^+(aq) + Cl^-(aq)$ $Ag^+(aq) + e^- \rightleftharpoons Ag(s)$
Solid AgCl

Notes:

By convention, E_{SHE} = 0V at $[H^+]$ = 1M, P_{H2} = 1 atm and at all temperatures



Potentials of other electrodes are compared to SHE using electrode in question as cathode and SHE as anode:

$M^{n+} + ne^- \leftrightarrow M(s)$

$E_{cell} = E_{cathode} - E_{anode}$

$E_{cell} = E_{cathode} - E_{SHE}$

By definition:

$E_{cell} = E_{cathode} - 0$

$E_{cell} = E_{cathode}$

Notes:

Standard Electrode Potential (E°) – measured E_{cell} when all species in solution or gas has an activity of 1.00

Activity (a) – proportional to molar concentration.

If E° is "+", it indicates that the reaction:

$M^{n+} + n/2H_2(g) \leftrightarrow M(s) + nH^+$

is favored or spontaneous.

M^{n+} is readily reduced by $H_2(g)$ \ M^{n+} is better e^- acceptor or oxidizing agent.

If E° is "-", it indicates that the reaction is not favoured or spontaneous and requires energy to proceed

M(s) is readily oxidized by H^+ \ M(s) is better e^- donor or reducing agent.

Notes:

As E° increases → oxidizing ability of half-cell reaction increases

Notes:

Easily reduced, Better Oxidizing Agent (↓)

Reaction at Interface	Half-cell Potential (E°)
$Al^{3+} + 3e^- \rightarrow Al$	-1.706 V
$Zn^{2+} + 2e^- \rightarrow Zn$	-0.763 V
$Cr^{3+} + 3e^- \rightarrow Cr$	-0.744
$Fe^{2+} + 2e^- \rightarrow Fe$	-0.409V
$Cd^{2+} + 2e^- \rightarrow Cd$	-0.401 V
$Ni^{2+} + 2e^- \rightarrow Ni$	-0.230 V
$Pb^{2+} + 2e^- \rightarrow Pb$	-0.126 V
$2H^+ + 2e^- \rightarrow H_2$	0.00 V
$AgCl + e^- \rightarrow Ag + Cl^-$	+0.223 V
$Hg_2Cl_2 + 2e^- \rightarrow 2Hg + 2Cl^-$	+0.268 V
$Cu^{2+} + 2e^- \rightarrow Cu$	+0.340 V
$Ag^+ + e^- \rightarrow Ag$	+0.799 V
$Au^+ + e^- \rightarrow Au$	+1.680 V

Easily oxidized, Better Reducing Agent (↑)

Nernst Equation

Notes:

Values of $E_{electrodes}$ can also be calculated at other concentrations (activities) of species

For ½ reaction:

$$pP + qQ + \ldots + ne^- \leftrightarrow rR + sS + \ldots$$

$$E_{electrode} = E^0 - \frac{RT}{nF} \ln \frac{(a_R)^r (a_R)^s \ldots}{(a_P)^p (a_Q)^q \ldots}$$

products (numerator)

reactants (denominator)

where:

R = ideal gas law constant (8.316 J mol^{-1} K^{-1})

T = absolute temperature (K)

n = number of electrons in process

F = Faraday's constant (96487 C mol^{-1})

a = activities of each species (γ[X])

- in solution at time of measurement
- not necessarily at equilibrium

At room Temperature:

Notes:

$$\frac{RT}{nF} = \frac{2.5693\times10^{-2}}{n} \text{ V}$$

Also, using $\log_{10}$:

$$E_{electrode} = E^0 - \frac{0.0592}{n} \lg \frac{(a_R)^r (a_R)^s \ldots}{(a_P)^p (a_Q)^q \ldots}$$

Note: Calculation has to be done Twice!!

- Once for the anode electrode
- Once for the cathode electrode

A very common mistake is to simply do the calculation once and report the $E_{electrode}$ as the E_{cell}

If know E°, allows $E_{electrode}$ to be calculated under non-standard conditions

Note: If all activity values = 1, $E_{electrode}$ = E°

Once have $E_{cathode}$ & $-E_{anode}$ by above procedure, can also get E_{cell}:

$$E_{cell} = E_{cathode} - E_{anode}$$

may need to also include junction potential, etc., but good first approximation

Activity Coefficients

- experimental determination of individual activity coefficients appears to be impossible
- can determine mean activity coefficient (γ)

For electrolyte A_mB_n $\gamma = (\gamma_A^m \gamma_B^n)^{1/(m+n)}$

Debye-Huckel Equation:
$$-\log \gamma_A = \frac{0.509 Z_A^2 \sqrt{\mu}}{1 + 3.28\alpha_A \sqrt{\mu}}$$

where:

Z_A = charge on the species A
μ = ionic strength of solution
α_A = the effective diameter of the hydrated ion

		Activity Coefficients at Indicated Ionic Strength				
Ion	**a_x nm**	**0.001**	**0.005**	**0.01**	**0.05**	**0.1**
H_3O^+	0.9	0.967	0.933	0.914	0.86	0.83
Li^+	0.6	0.965	0.929	0.907	0.84	0.80
Na^+	0.4-0.45	0.964	0.928	0.902	0.82	0.78
Cl^-	0.3	0.964	0.925	0.899	0.80	0.76

Note: At ionic strengths > 0.1, Debye-Huckle Equation fails

Notes:

Example: Calculate E_{cell} for the Cell:

Pt | H_2(1.00 atm) | HCl ($3.215\cdot10^{-3}$M), AgCl (satd.) | Ag

Half-cell reactions:

$AgCl(s) + e^- \leftrightarrow Ag(s) + Cl^-$ $E^o = 0.222$ V
$H^+ + e^- \leftrightarrow \frac{1}{2} H_2(g)$ $E^o = 0.00$ V

$E^o_{AgCl/Ag} > E^o_{H^+/H_2}$, so net reaction is spontaneous:
$AgCl(s) + \frac{1}{2}H_2 \leftrightarrow Ag(s) + H^+ + Cl^-$

Actual Potentials:

Cathode

$E_{cathode} = E^0_{AgCl} - (0.0592/1) \log a_{cl^-}$ **since satd. solids, activity of AgCl and Ag = 1**

$E_{cathode} = E^0_{AgCl} - 0.0592 \log \gamma_{cl^-}[Cl^-]$

$E_{cathode} = 0.222 \text{ V} - 0.0592 \log(0.939)(3.215\cdot10^{-3})$ M
0.939 from Debye-Huckle equation, where $\mu = 3.215\cdot10^{-3}$ Cl^-

$E_{cathode} = 0.371$ V

Notes:

Half-cell reactions:

$AgCl(s) + e^- \leftrightarrow Ag(s) + Cl^-$ $E^o = 0.222$ V
$H^+ + e^- \leftrightarrow \frac{1}{2} H_2(g)$ $E^o = 0.00$ V

$AgCl(s) + \frac{1}{2}H_2 \leftrightarrow Ag(s) + H^+ + Cl^-$

Actual Potentials:

Anode

$E_{anode} = E^0_{H^+/H_2} - (0.0592/1) \lg (a_{H^+})/(P^{1/2}_{H_2})$

$E_{anode} = E^0_{H^+/H_2} - 0.0592 \lg (\gamma_{H^+}[HCl])/(P^{1/2}_{H_2})$

$E_{anode} = 0.00 \text{ V} - 0.0592 \lg (0.945)(3.215\cdot10^{-3}\text{M})/(1\text{atm})^{1/2}$
0.945 from Debye-Huckle equation for $\mu = 3.215\cdot10^{-3}$ H^+

$E_{anode} = 0.149$ V

$\mathbf{E_{cell} = E_{cathode} - E_{anode}} = 0.371 \text{ V} - 0.149 \text{ V} = 0.222$ V

Notes:

Formal Potential (E^f or $E^{o'}$):

- used to compensate for problems with E° in using activity and with side- reactions

- based on conditions of 1M concentration with all species being specified e.g. HCl vs. $HClO_4$ as acid

- gives better agreement than E° with experimental data and Nernst Equation conditions need to be similar to conditions where $E^{o'}$ was measured

Reaction Rates:

Some E° half-reactions listed in tables have been determined by calculations from *equilibrium* measurements rather than actual measurements of the ½ cell in an electrode system.

e.g. $2CO_2 + 2H^+ + 2e^- \leftrightarrow H_2C_2O_4$ $E^0 = -0.49$ V

Problem: reaction is slow and difficult to see in practice;

thermodynamics vs. kinetics;

no suitable electrode.

Potentially useful for computational purposes

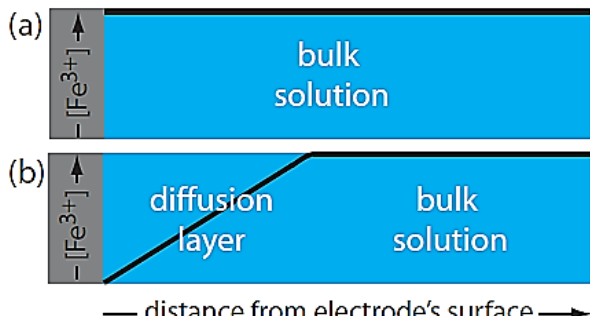
Notes:

To understand electrochemistry we need to appreciate five important and interrelated concepts:

(1) the electrode's potential determines the analyte's form at the electrode's surface;
(2) the concentration of analyte at the electrode's surface may not be the same as its concentration in bulk solution;
(3) in addition to an oxidation–reduction reaction, the analyte may participate in other reactions;
(4) current is a measure of the rate of the analyte's oxidation or reduction;
(5) we cannot simultaneously control current and potential.

Notes:

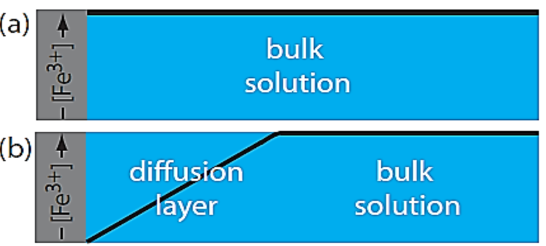
The concentration of analyte at the electrode's surface may not be the same as its concentration in bulk solution



Concentration of Fe^{3+} as a function of distance from the electrode's surface at (a) E = +1.00 V and (b) E = +0.500 V.
The electrode is shown in gray and the solution in blue

Interfacial concentrations may not equal bulk concentrations

Notes:



Notes:

Suppose we place an electrode in a solution of Fe^{3+} and fix its potential at 1.00 V. From the ladder diagram, we know that Fe^{3+} is stable at this potential and the concentration of Fe^{3+} remains the same at all distances from the electrode's surface.

If we change the electrode's potential to +0.500 V, the concentration of Fe^{3+} at the electrode's surface decreases to approximately zero.

As shown in Figure, the concentration of Fe^{3+} increases as we move away from the electrode's surface until it equals the concentration of Fe^{3+} in bulk solution. The resulting concentration gradient causes additional Fe^{3+} from the bulk solution to diffuse to the electrode's surface.

Notes:

The Nernst equation provides a mathematical relationship between the electrode's potential and the concentrations of an analyte's oxidized and reduced forms in solution.

Because it is the potential of the electrode that determines the analyte's form at the electrode's surface, the concentration terms in equation are those at the electrode's surface, not the concentrations in bulk solution.

This distinction between surface concentrations and bulk concentrations is important.

Notes:

The analyte may participate in other reactions

The reduction of Fe^{3+} to Fe^{2+}, may not be the only reaction affecting the concentration of Fe^{3+} in bulk solution or at the electrode's surface.

The adsorption of Fe^{3+} at the electrode's surface or the formation of a metal-ligand complex in bulk solution, such as $Fe(OH)^{2+}$, also affects the concentration of Fe^{3+}.

Electrochemical measurements are made in an electrochemical cell consisting of two or more electrodes and the electronic circuitry for controlling and measuring the current and the potential.

The simplest electrochemical cell uses two electrodes.

The potential of one electrode is sensitive to the analyte's concentration and is called the working electrode or the indicator electrode.

The second electrode, which we call the counter electrode, completes the electrical circuit and provides a reference potential against which we measure the working electrode's potential.

Ideally, the counter electrode's potential remains constant so that we can assign to the working electrode any change in the overall cell potential.

Notes:

Potentiometry is an analytical method in which an electric potential difference (a voltage) of a cell is measured.

The potentiometric method of analysis is based on the measurement of electrode potentials and electromotive force (EMF) in electrolyte solutions.

Direct potentiometry or **ionometry**
Potentiometric titration

Notes:

Direct potentiometry is the direct determination of the activity (a) of ions in solution under conditions of reversibility of the electrode process (the course of the process on the electrode surface).

If the individual activity coefficients of the components (f) are known, then the concentration (C) of the component can be determined directly:

$$C = \frac{a}{f}$$

The electrode potential at the interface of the electrode-solution phase is related to the establishment of equilibrium in the system:

$$Me^0 - ne^- \leftrightarrow Me^{n+}$$

Notes:

The double electric layer, which is formed at the boundary of the section "electrode solution", causes the jump potential.

The magnitude of the electrode potential is described by the Nernst equation:

$$E = E^0 + \frac{RT}{nF} \ln \frac{aOx}{a\text{Re}d}$$

where E is the potential at a certain temperature and concentration
E^0 – standard electrode potential (given in a series of voltages)
a^+ i a^0 – are the activities of the Me^{n+} ion and the molecules (atoms) of the electrode in solution
R – universal gas steel (8.316 J mol^{-1} K^{-1})
T – absolute temperature (K)
F – Faraday steel (96487 C mol^{-1} or ≈ 96 500 K)
n – is the charge of the ion (the number of electrons involved in the process)

At 25°C

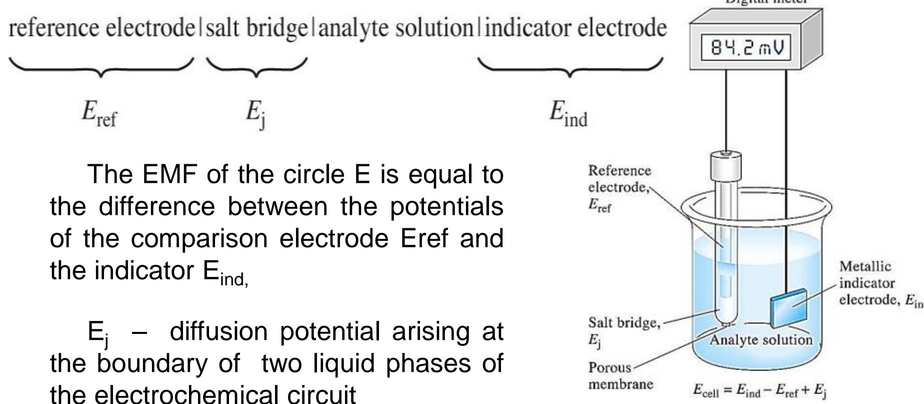
$$E = E^0 + \frac{0{,}059}{n} \lg \frac{aOx}{a\text{Re}d}$$

$$\frac{2{,}3RT}{F} = 0{,}059$$

Notes:

Notes:

Basic Components:
a) reference electrode: gives reference for potential measurement
b) indicator electrode: where species of interest is measured
c) potential measuring device



The EMF of the circle E is equal to the difference between the potentials of the comparison electrode Eref and the indicator E_{ind},

E_j – diffusion potential arising at the boundary of two liquid phases of the electrochemical circuit

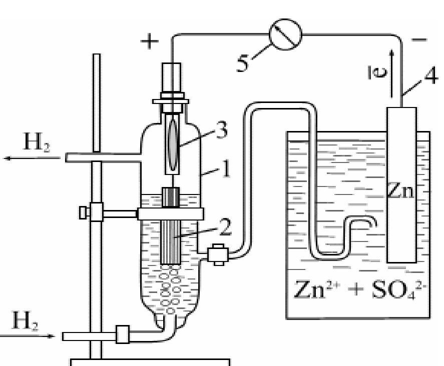
Notes:

Electrochemical circuits consisting of two electrodes are used in potentiometric methods: the indicator and the reference electrode

Indicator electrode - one that develops a potential whose magnitude depends on the activity of one or more species in contact with the electrode.

Reference electrode - called an electrode whose potential is of constant importance,

one that maintains a constant potential against which the potential of another half-cell may be measured.



Scheme of electrode potential determination:

1 is a hydrogen electrode;
2 - platinum electrode;
3 - glass tube;
4 - indicator electrode;
5 - potentiometer

The voltage reduction in the solution consists of two separate potential's jumps - at the anode and at the cathode.

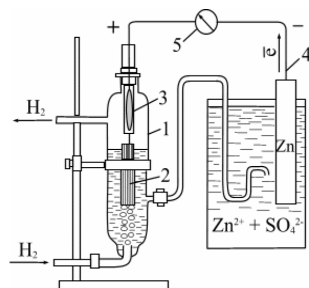
For their measurement, use a **reference electrode** - a hydrogen electrode, as "zero".

The design of the hydrogen electrode is shown on the picture.

The platinum plate (or wire) is covered with a sponge platinum to increase the surface of the electrode and to better adsorb hydrogen on its surface.

The plate is immersed in a solution of sulfuric acid, through which hydrogen is passed under 101 kPa pressure.

Notes:



Scheme of electrode potential determination:

1 is a hydrogen electrode;
2 - platinum electrode;
3 - glass tube;
4 - indicator electrode;
5 - potentiometer

To determine the potential jump between different electrodes in solutions, the hydrogen electrode is connected to the indicator electrode in a galvanic element

The standard electrode potential is most often taken as the potential of a certain electrode relative to a hydrogen electrode immersed in a solution with a concentration of ions H^+ 1 mol/L

Notes:

There are a few kinds of reference electrode used in analyses to construct electrochemical cells. **Reference Electrodes:**

Desired Characteristics:

a) known or fixed potential
b) constant response
c) insensitive to composition of solution under study
d) obeys Nernst Equation
e) reversible

Potencial of Reference Electrodes in Aqueous Solutions

Temperature, °C	Electrode Potential vs. SHE, V				
	0.1 M[c] Calomel[a]	3.5 M[c] Calomel[b]	Saturated[c] Calomel[a]	3.5 M[b,c] Ag-AgCl	Saturated[b,c] Ag-AgCl
10	–	0.256	–	0.215	0.214
12	0.3362	–	0.2528	–	–
15	0.3362	0.254	0.2511	0.212	0.209
20	0.3359	0.252	0.2479	0.208	0.204
25	0.3356	0.250	0.2444	0.205	0.199
30	0.3351	0.248	0.2411	0.201	0.194
35	0.3344	0.246	0.2376	0.197	0.189
38	0.3338	–	0.2355	–	0.184
40	–	0.244	–	0.193	–

Notes:

Notes:

Reference electrodes

Notes:

Reference Electrodes:

Common Reference Electrodes used in Potentiometry

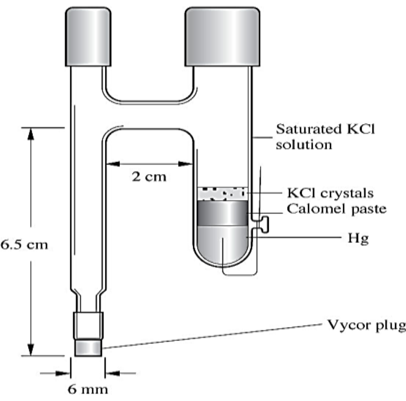
a) Calomel Electrode (Hg in contact with Hg_2Cl_2 & KCl)

Saturated Calomel Electrode (SCE) very widely used

½ cell: Hg/Hg_2Cl_2 (satd), KCl (xM)||

½ reaction: $Hg_2Cl_2 (s) + 2e^- \leftrightarrow 2Hg + 2Cl^-$

SCE



Note: response is dependent on $[Cl^-]$

Notes:

b) Silver/Silver Chloride Electrode

- most widely used reference electrode system
- Ag electrode in KCl solution saturated with AgCl

½ cell: Ag/AgCl (satd), KCl (xM)||

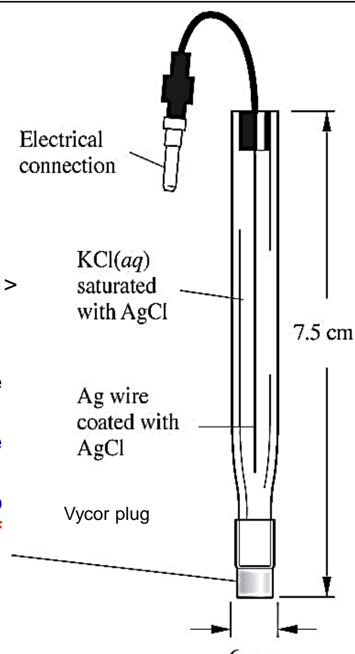
½ reaction: $AgCl (s) + e^- \leftrightarrow Ag(s) + Cl^-$

Advantage – one advantage over SCE is that Ag/AgCl electrode can be used at temperatures > 60°C

Disadvantage – Ag reacts with more ions

c) Precautions in the Use of Reference Electrodes

- need to keep level of solution in reference electrode above level in analyte solution
- need to prevent flow of analyte solution into reference electrode **can result in plugging of electrode at junction → erratic behavior**



Notes:

Indicator Electrodes:

Detects or Responds to Presence of Analyte

Three Common Types:

a) Metallic Indicator Electrodes
b) Membrane Indicator Electrodes
c) Molecular Selective Electrode

reference electrode | salt bridge | analyte solution | indicator electrode

E_{ind}

Notes:

There are many types of indicator electrodes used in analyses to construct electrochemical cells.
They may be classified as shown in the Table

Class	Description	Example
Class 1	Metal/metal ion	Ag/Ag^+ (cation reversible)
Class 2	Metal/saturated metal salt/anion	$Ag/AgCl/Cl^-$ (anion reversible
Redox	Inert metal/redox couple	$Pt/Ce^{4+}, Ce^{3+}$ $Pt/H^+, H_2$
Membrane	Inner electrode/solution/ ion selective membrane	Glass electrode Fluoride electrode
ISFET	Coated field-effect transistor	pH-sensitive
Gas-sensing electrodes	pH-electrode + membrane	For CO_2, NH_3

Notes:

Electrodes of the first kind - the jump of the electrode potential is a function of the concentration of the cation in solution (for example, silver and mercury electrodes).

Electrodes of the second kind - the jump of the electrode potential depend on the concentration of anions (chloride silver and calomel).

Electrodes of the second kind are the most common and are made according to one principle - the metal (Ag, Hg) is immersed in a solution, saturated with respect to its low soluble salt (AgCl, Hg_2Cl_2).
Most often these salts are applied directly to the metal.
The electrodes thus obtained are immersed in a buffer solution (saturated KCl solution).

Metallic Indicator Electrode (Four Main Types)

Notes:

a) Metallic Electrodes of the **First Kind**

Involves single reaction

Detection of cathode derived from the metal used in the electrode

Example: use of a copper electrode to detect Cu^{2+} in solution

½ reaction: $Cu^{2+} + 2e^- \leftrightarrow Cu(s)$

E_{ind} gives a direct measure of Cu^{2+}:

$$E_{ind} = E^o_{Cu} - (0.0592/2) \lg a_{Cu(s)}/a_{Cu^{2+}}$$

since $a_{Cu(s)} = 1$: $E_{ind} = E^o_{Cu} - (0.0592/2) \lg 1/a_{Cu^{2+}}$

or using $pCu = -\lg a_{Cu^{2+}}$: $E_{ind} = E^o_{Cu} - (0.0592/2)\, pCu$

Problems:

- not very selective
- many can only be used at neutral pH → metals dissolve in acids
- some metals readily oxidize
- certain hard metals (Fe, Cr, Co, Ni) do not yield reproducible results
- pX versus activity differ significantly and irregularly from theory

b) Metallic Electrodes of the **Second Kind**

Notes:

Detection of anion derived from the interaction with a metal ion (M^{n+}) from the electrode anion forms precipitate or stable complex with a metal ion (M^{n+})

Example: Detection of Cl^- with Ag electrode

½ reaction: $AgCl(s) + e^- \leftrightarrow Ag(s) + Cl^-$ $E^O = 0.222$ V

E_{ind} gives direct measure of Cl^-:

$$E_{ind} = E^o - (0.0592/1) \lg a_{Ag(s)}\, a_{Cl^-}/a_{AgCl(s)}$$

since $a_{Ag(s)}$, $a_{AgCl(s)} = 1$ and $E^o = 0.222$ V:

$$E_{ind} = 0.222 - (0.0592/1) \log a_{Cl^-}$$

Example: Detection of EDTA ion (Y^{4-}) with Hg Electrode

½ reaction: $HgY^{2-} + 2e^- \leftrightarrow Hg(l) + Y^{4-}$ $E^o = 0.21$ V

E_{ind} responds to $a_{Y^{4-}}$:

$$E_{ind} = E^o - (0.0592/2) \lg a_{Hg(l)}\, a_{Y^{4-}}/a_{HgY^{2-}}$$

since $a_{Hg(l)} = 1$ and $E^o = 0.21$ V:

$$E_{ind} = 0.21 - (0.0592/1) \lg a_{Y^{4-}}/a_{HgY^{2-}}$$

c) Metallic Electrodes of the **Third Kind**

Notes:

Metal electrodes responds to a different cation

Linked to cation by an intermediate reaction

Already saw detection of EDTA by Hg electrode (2nd Kind)

Can be made to detect other cations that bind to EDTA → affecting $a_{Y^{4-}}$

Example: Detect Ca by complex with EDTA

Equilibrium reaction: $CaY^{2-} \leftrightarrow Ca^{2+} + Y^{4-}$

Where: $K_f = \dfrac{a_{ca^{2+}} \cdot a_{Y^{4-}}}{a_{CaY^{2-}}}$ $a_{y^{4-}} = \dfrac{a_{CaY^{2-}}}{K_f \cdot a_{ca^{2+}}}$

$$E_{ind} = 0.21 - (0.0592/1) \lg a_{Y^{4-}}/a_{HgY^{2-}}$$

Note: $a_{Y^{4-}}$ and E_{ind} now also changes with $a_{Ca^{2+}}$

d) Metallic Redox Indicators

Notes:

Electrodes made from inert metals (Pt, Au, Pd)

Used to detect oxidation/reduction in solution

Electrode acts as e^- source/sink

Example: Detection of Ce^{3+} with Pt electrode

½ reaction: $Ce^{4+} + e^- \leftrightarrow Ce^{3+}$

E_{ind} responds to Ce^{4+}:

$$E_{ind} = E^o - (0.0592/1) \lg a_{Ce^{3+}}/a_{Ce^{4+}}$$

Problems:

- electron-transfer processes at inert electrodes are frequently not reversible
- do not respond predictably to ½ reactions in tables

Membrane Indicator Electrodes

Notes:

a) General

Electrodes based on determination of cations or anions by the selective adsorption of these ions to a membrane surface.

Often called Ion Selective Electrodes (ISE)

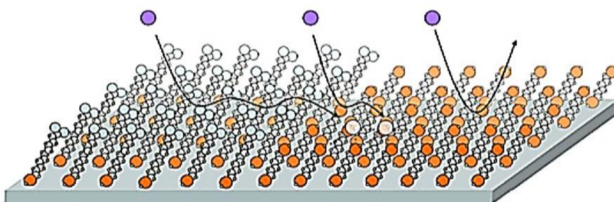
Desired properties of ISE's

minimal solubility – membrane will not dissolve in solution during measurement

silica, polymers, low solubility inorganic compounds (AgX) can be use

Need some electrical conductivity

Selectively binds ion of interest



Membrane Indicator Electrodes

b) pH Electrode

Notes:

Most common example of an ISE, based on use of glass membrane that preferentially binds H^+

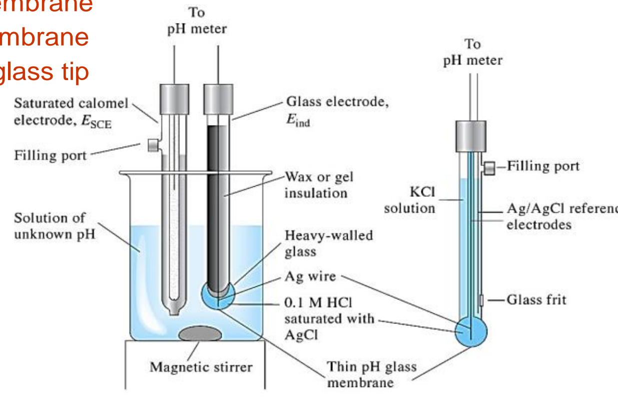
Typical pH electrode system is shown

Two reference electrodes here

One SCE outside of membrane

One Ag/AgCl inside membrane

pH sensing element is glass tip of Ag/AgCl electrode



To
pH meter
Glass electrode, E_{ind}
Wax or gel insulation
KCl solution
Heavy-walled glass
Ag wire
0.1 M HCl saturated with AgCl
Thin pH glass membrane
Filling port
Ag/AgCl reference electrodes
Glass frit

Glass electrodes consisting of a special glass ball containing a buffer solution and a chloride silver electrode are very common.

Glass has a certain potential due to the ion exchange reaction

$$(Na^+)_{glass} + H^+ \leftrightarrow (H^+)_{glass} + Na^+$$

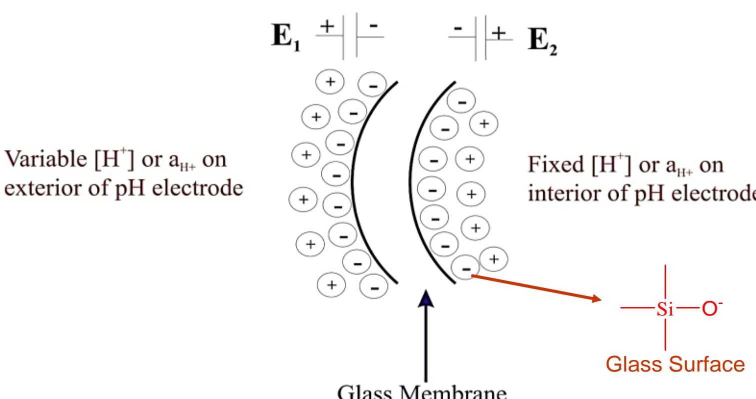
The difference ΔE of the chloride (internal) electrode does not depend on the test compound, so the jump in potential depends only on the equilibrium of the above reaction,

The difference ΔE of the chloride (internal) electrode does not depend on the test compound, so the jump in potential depends only on the equilibrium of the above reaction and the concentration of H+ cations in solution

Notes:

pH is determined by formation of boundary potential across glass membrane

Notes:



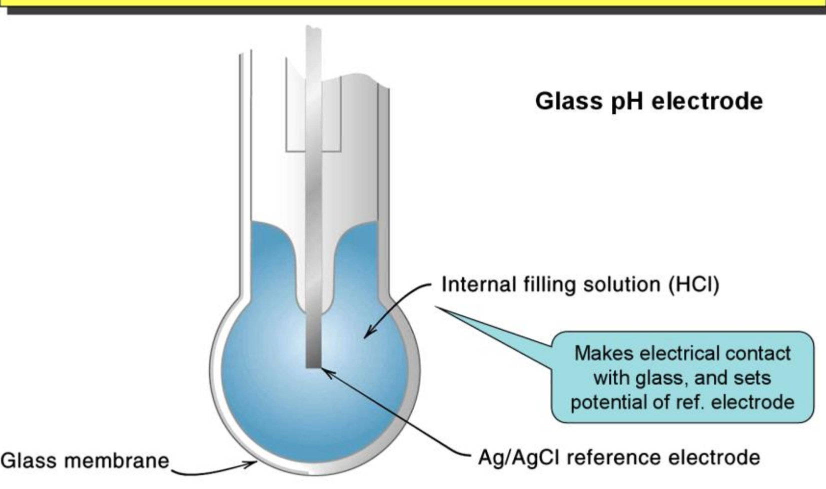
At each membrane-solvent interface, a small local potential develops due to the preferential adsorption of H^+ onto the glass surface

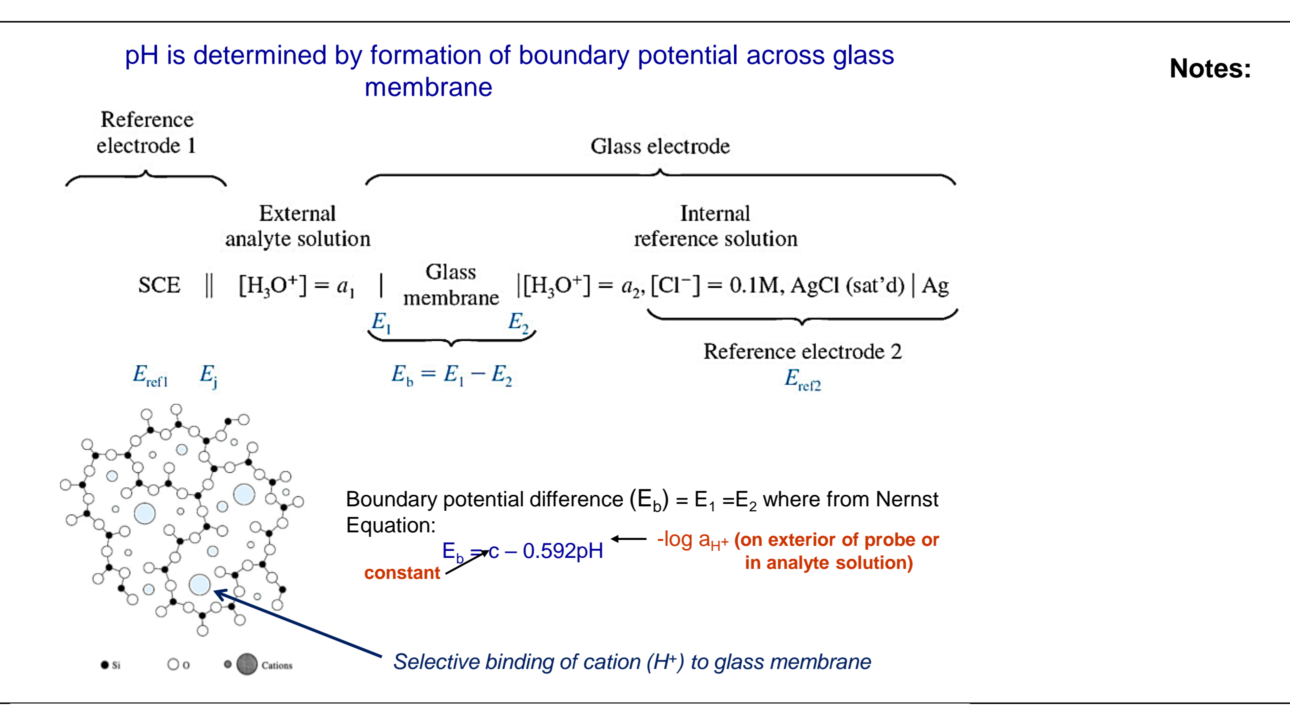
Notes:

E_{glass} = constant – 2.303RT/F log ($a_{H+ \; int}$/ $a_{H+ \; ext}$)

The hydrated glass responds to a_{H+}.

The asymmetry potential of the glass membrane is unknown, so the electrode must be calibrated with a standard buffer.

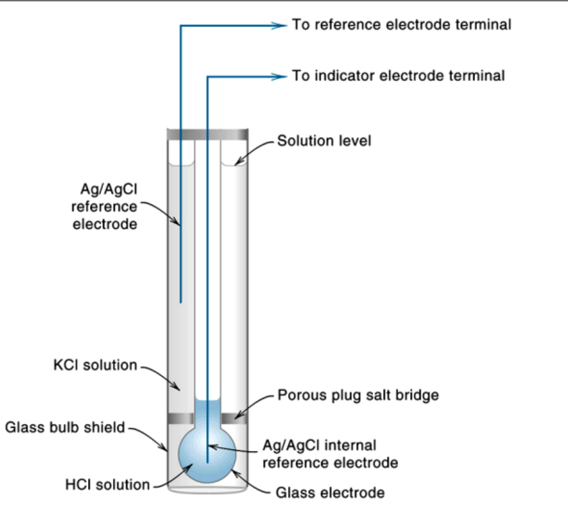




Notes:

This is two electrodes in one.

The porous plug salt bridge must be immersed in the solution



Combination pH-reference electrode

Notes:

The potential scale is calibrated in pH units (59.16 mV/pH at 25° C)

A temperature adjustment feature changes the slope by 2.303RT/F

Typical pH meter

Notes:

Indicator electrodes

Notes:

- **electrodes at the interfacial boundaries of which electron reactions occur.**
  Such electrodes are called electron-exchange or redox.
  In analytical practice, the platinum dot electrode was most widely used

- **electrodes at the interfacial boundaries of which ion-exchange reactions occur.**
  Such electrodes are called ion-exchange, membrane or ion-selective

Ion-selective electrodes

Notes:

In the direct determination of cations and anions, ion-selective electrodes are used.

Ion-selective electrodes consist of membranes that separate electrodes of the first or second kind from solution and are permeable to only one type of ions.

Diffusing through the membrane, selective ions cause a potential-jump on the electrode surface.

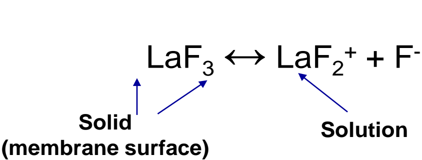
Ion selective electrodes are designed to determine ions: Cl^-; Br^-; I^-; CN^-; CNS^-; ClO_4^-; $H_2PO_4^-$; NO_3^-; Na^+; K^+; Li^+; NH_4^+; Ca^{2+}; Ag^+, Cs^+; Cd^{2+}; Cu^{2+}; Fe^{2+}; Pb^{2+} etc.

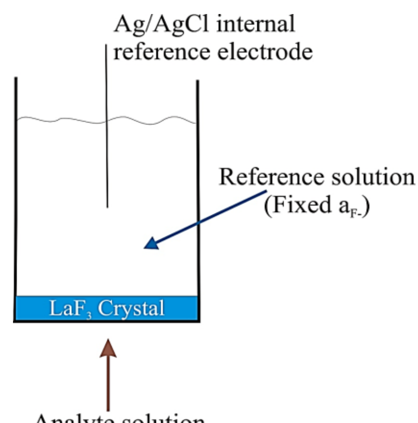
Crystalline Membrane Electrode

Notes:

Fluoride Electrode

LaF_3 crystal doped with EuF_2 mechanism similar to pH electrode with potential developing at two interfaces of the membrane from the reaction:

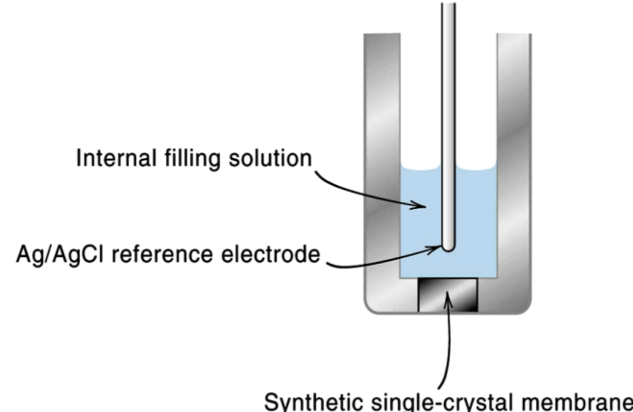




The side of the membrane with the lower a_{F^-} becomes positive relative to the other surface:

$$E_{ind} = c - 0.0592\ pF$$

The most common ion-selective electrode of this type is the fluoride electrode: LaF_3 crystal doped with Eu(II) to increase conductivity. Plastic membrane-ionophore electrodes have a similar design, with a soft plastic PVC membrane containing a neutral lipophilic ionophore that selectively complexes with the test ion.



Crystal membrane electrode

Notes:

Liquid Membrane Electrode

Membrane usually consists of an organic liquid (not soluble in the sample) held by porous disk between aqueous reference solution and aqueous sample solution.

The membrane has the ability to selectively bind ions of interest

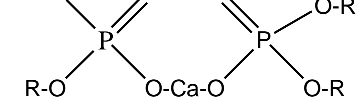
Ag/AgCl electrode

electrode body

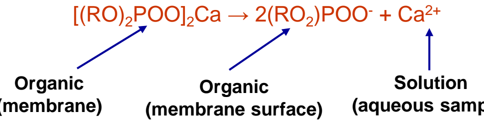
internal electrolyte

ion-selective membrane

Example: Calcium dialkyl phosphate Liquid membrane electrodes



At solution/membrane interfaces:

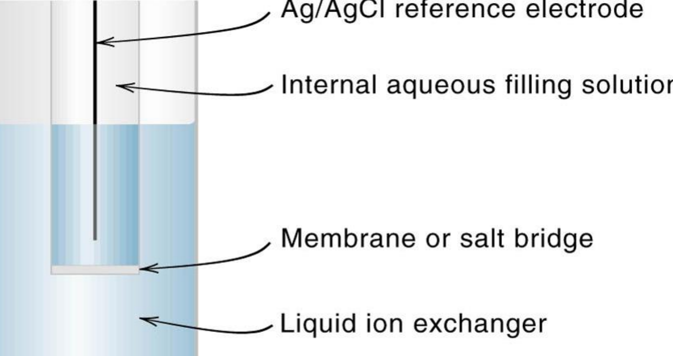


The side of the membrane with the lower $a_{Ca^{2+}}$ becomes negative relative to the other surface:

$$E_{ind} = c - 0.0592/2 \text{ pCa}$$

Notes:

The potential determining "membrane" is a layer of water-immiscible liquid ion exchanger at the porous membrane face.



Liquid-membrane electrode

Notes:

Liquid Membrane Electrode

Can design Liquid Membrane Electrodes for either cations or anions

- cations → use cation exchangers in membrane
- anions → use anion exchangers in membrane

Analyte Ion	Concentration Range, M	Interferences[c]
Ca^{2+}	10^0 to 5×10^{-7}	10^{-5} Pb^{2+}; 4×10^{-3} Hg^{2+}, H^+, 6×10^{-3} Sr^{2+}; 2×10^{-2} Fe^{2+}; 4×10^{-2} Cu^{2+}; 5×10^{-2} Ni^{2+}; 0.2 NH_3; 0.2 Na^+; 0.3 $Tris^+$; 0.3 Li^+; 0.4 K^+; 0.7 Ba^{2+}; 1.0 Zn^{2+}; 1.0 Mg^{2+}
BF_4^-	10^0 to 7×10^{-6}	5×10^{-7} ClO_4^-; 5×10^{-6} I^-; 5×10^{-5} ClO_3^-; 5×10^{-4} CN^-; 10^{-3} Br^-; 10^{-3} NO_2^-; 5×10^{-3} NO_3^-; 3×10^{-3} HCO_3^-; 5×10^{-2} Cl^-; 8×10^{-2} $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}; 0.2 OAc^-; 0.6 F^-; 1.0 SO_4^{2-}
NO_3^-	10^0 to 7×10^{-6}	10^{-7} ClO_4^-; 5×10^{-6} I^-; 5×10^{-5} ClO_3^-; 10^{-4} CN^-; 7×10^{-4} Br^-; 10^{-3} HS^-; 10^{-2} HCO_3^-; 2×10^{-2} CO_3^{2-}; 3×10^{-2} Cl^-; 5×10^{-2} $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}; 0.2 OAc^-; 0.6 F^-; 1.0 SO_4^{2-}
ClO_4^-	10^0 to 7×10^{-6}	2×10^{-3} I^-; 2×10^{-2} ClO_3^-; 4×10^{-2} CN^-, Br^-; 5×10^{-2} NO_2^-, NO_3^-; 2 HCO_3^-, CO_3^{2-}, Cl^-, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, OAc^-, F^-, SO_4^{2-}
K^+	10^0 to 10^{-6}	3×10^{-4} Cs^+; 6×10^{-3} NH_4^+, Tl^+; 10^{-2} H^+; 1.0 Ag^+, $Tris^+$; 2.0 Li^+, Na^+
Water Hardness ($Ca^{2+} + Mg^{2+}$)	10^{-3} to 6×10^{-6}	3×10^{-5} Cu^{2+}, Zn^{2+}; 10^{-4} Ni^{2+}; 4×10^{-4} Sr^{2+}; 6×10^{-5} Fe^{2+}; 6×10^{-4} Ba^{2+}; 3×10^{-2} Na^+; 0.1 K^+

Notes:

Molecular Selective Electrodes

Electrodes designed for the detection of molecules instead of ions

Gas sensing electrodes (or gas-sensing probes)

Typically based on ISE surrounded by electrolyte solution

-activity of ion measured is affected by dissolved gas

-gas enters interior solution from sample by passing through a gas permeable membrane

Gas effuses through membrane:

CO_2 (aq) ↔ CO_2 (g) ↔ CO_2 (aq)

external solution — **membrane pores** — **internal solution**

In internal solution, pH changes:

CO_2 (aq) + H_2O ↔ HCO_3^- + H^+

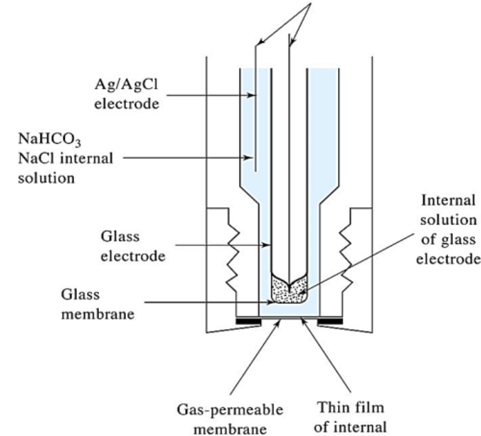
which is detected by ISE probe

Overall reaction:

CO_2 (aq) + H_2O ↔ H^+ + HCO_3^-

external solution — **internal solution**

$E_{ind} = c + 0.0592 \log [CO_2]_{ext}$

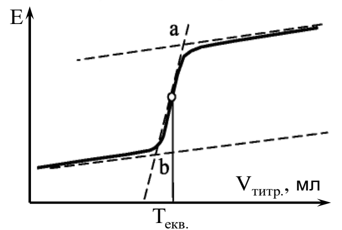


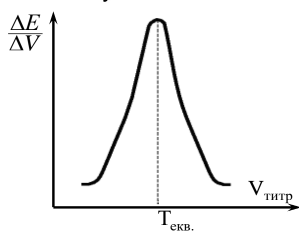
Notes:

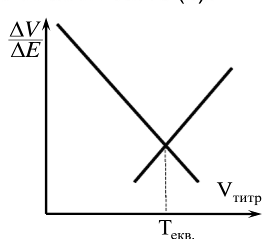
Potentiometric titration is based on the determination of the equivalence point by changing the EMF of the potentiometric cell during the titration.

The following types of titration curves are most commonly used:

- integral titration curve; it is built in the coordinates: E-Vtyr; the equivalence point is defined as the middle of the titration jump by constructing tangents (a);
- differential titration curve; it is built in the coordinates: ΔE / ΔV – V, where ΔE is the change in EMF when the volume of the titrant ΔV (b) is changed; the equivalence point is determined by the maximum of the titration curve;
- curve Gran; it is constructed in the coordinates ΔV / ΔE – V; the equivalence point is determined by the minimum of the titration curve (c).







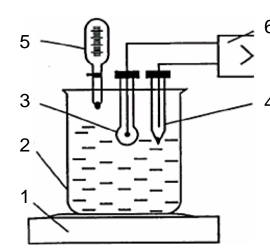
Notes:

Equipment for potentiometric measurements

Notes:

The device for potentiometric measurements consists of a beaker with a test solution and electrodes.

If necessary, this electrode cell can be thermostatically controlled and supplemented with other elements, such as a burette, magnetic stirrer, etc.



1 - magnetic stirrer;
2 - a chemical glass with test solution;
3 - reference electrode;
4 - indicator electrode;
5 - burette;
6 - device for measuring EMF.

Laboratory pH-meter with auto-calibration

Notes:

Measurement range **– pH** **– mV**	 **-2…..16** **± 999,9 mV; ± 2000 mV**
Sensitivity **– pH** **– mV**	**pH ±0,01 pH ±0,01;** **0,002 pH** **±0,2 mV; ±1 mV**
Operating temperature range	**-20°C…+120°C**
Feeding	**220 B / 50 Hz**
Overall dimensions, mm, not more than	**390 x 238 x 105**

Application: in laboratories of food, pharmaceutical, chemical and printing industries and medical institutions:
to determine the EMF of the system (pH, voltage, redox potential, redox potential) and temperature.

Notes:

These are ion-selective field-effect transistor electrodes.

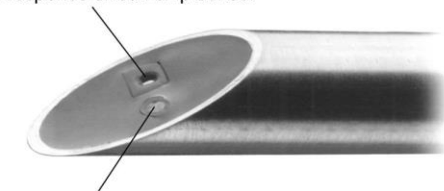
A semiconductor transistor serves as the base for electrical contact.

It is coated with insulating layers of SiO_2 and Si_3N_4, and then an ion-sensitive membrane.

This one is a non-glass ISFET pH electrode.

Fast response silicon chip sensor

Solid-state ISFET electrode



Built-in reference and medical grade temperature sensor

Tasks to Section 16

1. Give definitions of these terms: electrochemistry, electrochemical cell, salt bridge, electrode potential, standard electrode potential, formal potential, potentiometric measurements, cathode, anode, reference electrodes, indicator electrodes, membrane electrode, potentiometric analyses, coulometry method.

2. Write the reactions occurring at the anode and the cathode for the potentiometric electrochemical cell with the following shorthand notation

$$Pt(s) \mid H_2(g), H^+(aq) \parallel Cu^{2+}(aq) \mid Cu(s)$$

What is the potential of the electrochemical cell?

3. What is the potential for the electrochemical cell from previous task 2 if the activity of H+ in the anodic half-cell is 0.100, the fugacity of H_2 in the anodic half-cell is 0.500, and the activity of Cu^{2+} in the cathodic half-cell is 0.0500?

4. What is the activity of Cu^{2+} in the electrochemical cell from task 2, if the activity of H^+ in the anodic half-cell is 1.00 with a fugacity of 1.00 for H_2, and an E_{cell} is equal to +0.257 V?

5. The potential for a Fe^{3+}/Fe^{2+} half-cell is +0.750 V relative to the standard hydrogen electrode. What is its potential if we use a saturated calomel electrode or a saturated silver/silver chloride electrode?

6. The potential of a UO_2^{+}/U^{4+} half-cell is –0.0190 V relative to a saturated calomel electrode. What is its potential when using a saturated silver/silver chloride electrode or a standard hydrogen electrode?

7. The concentration of Ca^{2+} in a water sample is determined using the method of external standards. The ionic strength of the samples and the standards is maintained at a nearly constant level by making each solution 0.5 M in KNO_3. The measured cell potentials for the external standards are shown in the following table.

$[Ca^{2+}]$ (M)	1.00×10^{-5}	5.00×10^{-5}	1.00×10^{-4}	5.00×10^{-4}	1.00×10^{-3}	5.00×10^{-3}	1.00×10^{-2}
E_{cell} (V)	–0.125	–0.103	–0.093	–0.072	–0.065	–0.043	–0.033

What is the concentration of Ca2+ in a water sample if its cell potential is found to be –0.084 V?

8. The concentration of Ca^{2+} in a sample of seawater is determined using a Ca ion-selective electrode and a one-point standard addition. A 10.00-mL sample is transferred to a 100-mL volumetric flask and diluted to volume. A 50.00-mL aliquot of the sample is placed in a beaker with the Ca ISE and a reference electrode. The potential is measured as –0.05290 V. After adding a 1.00-mL aliquot of a 5.00×10^{-2} M standard solution of Ca^{2+} the potential is –0.04417 V. What is the concentration of Ca^{2+} in the sample of seawater?

9. You are responsible for determining the amount of KI in iodized salt and decide to use an I^- ion-selective electrode. Describe how you would perform this analysis using external standards and how you would perform this analysis using the method of standard additions.

10. Analytic describes a new membrane electrode for the determination of cocaine, a weak base alkaloid with a pKa of 8.64. The electrode's response for a fixed concentration of cocaine is independent of pH in the range of 1–8. However, it decreases sharply above a pH of 8. Explain this pH dependency.

11. There is a membrane electrode for the quantitative analysis of penicillin. In this membrane, the enzyme penicillinase is immobilized in a polyacrylamide gel coated on the glass membrane of a pH electrode. The following data were collected using a set of penicillin standards.

[penicillin] (M)	1.0×10^{-2}	2.0×10^{-3}	1.0×10^{-3}	2.0×10^{-4}	1.0×10^{-4}	1.0×10^{-5}	1.0×10^{-6}
potential (mV)	220	204	190	153	135	96	80

(a) Over what range of concentrations is there a linear response?
(b) What is the calibration curve's equation for this concentration range?
(c) What is the concentration of penicillin in a sample that yields a potential of 142 mV?

Section 17: Voltammetry, Amperometry and Other Electrochemical Methods

Contents:

- Introduction
- Voltammetry methods
- Shape of voltammograms
- Quantitative and qualitative aspects of voltammetry
- Other electrochemical methods

Introduction

Voltammetry is a group of electrochemical methods of analysis in which the processes of polarization of a microelectrode are used, and the polarization (voltammetric) curves of the dependence of the current on voltage are obtained.

The analysis determines the potential of the indicator electrode, which changes over time rather slowly. The measured value is the current flowing through the indicator electrode.

The voltammogram may be compared with the spectrum in spectroscopy. It is the electrochemical equivalent of the spectrum. In Section 17, we consider how we can extract quantitative and qualitative information from a voltammogram.

In voltammetry, there are three critical experimental parameters under our control: how we change the potential applied to the working electrode, when we choose to measure the current, and whether we choose to stir the solution. Not surprisingly, there are many different voltammetric techniques. In this section, we consider several important examples.

The first important voltammetric technique to be developed — polarography, in which a liquid metal electrode in the form of a drop (usually mercury, DME), which flows from the capillary, is used as an indicator electrode.

The polarographic method was proposed in 1922 by Czech scientist J. Geyrovsky. He observed phenomena occurring on a drip mercury electrode.

The name of the method is related to the processes of polarization that occur when passing an electric current through electrolyte solutions.

Most often, the polarographic method is used to determine metal ions that are electrolytically reduced on a mercury cathode.

In polarography, we obtain a limiting current because each drop of mercury mixes the solution. It is because drops fall to the bottom of the electrochemical cell. If we replace the DME with a solid electrode, we can still obtain a limiting current, if we mechanically stir the solution during the analysis, using either a stir bar or by rotating the electrode. We call this approach hydrodynamic voltammetry.

Hydrodynamic voltammetry uses the same potential profiles as in polarography, such as a linear scan or a differential pulse. The resulting voltammograms are identical to those for polarography, except for the lack of current oscillations from the growth of the mercury drops. Since hydrodynamic voltammetry is not limited to Hg electrodes, it is useful for analytes that undergo oxidation or reduction at more positive potentials.

Another important voltammetric technique is stripping voltammetry. It consists of three related techniques: anodic stripping voltammetry, cathodic stripping voltammetry, and adsorptive stripping voltammetry.

In the voltammetric techniques, we scan the potential in one direction, either to more positive potentials or more negative potentials. In cyclic voltammetry, we complete a scan in both directions.

In the voltammetric techniques, we scan the potential in one direction, either to more positive potentials or more negative potentials. In cyclic voltammetry, we complete a scan in both directions.

Scanning the potential in both directions provides an opportunity to explore the electrochemical behaviour of species generated at the electrode. It is a distinct advantage of cyclic voltammetry over other voltammetric techniques.

The final voltammetric technique we will consider is amperometry. We apply a constant potential to the working electrode and measure current as a function of time. Since we do not vary the potential, amperometry does not result in a voltammogram. A critical application of amperometry is in the construction of chemical sensors. One of the first amperometric sensors was developed by L. C. Clark to measure dissolved O_2 in blood.

To obtain polarization curves, an electrochemical circle of two electrodes is made:

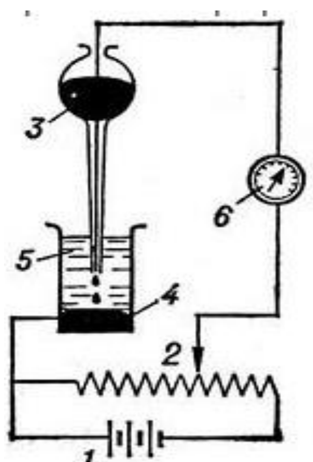
Indicator or polarized (cathode) electrode has a small surface.

The current density on this electrode is large, so it is polarized, its equilibrium potential is permanently changing.

Most often it is mercury that flows from the droplets of a very thin capillary.

Reference electrode or unpolarized electrode (anode) is most often a mercury layer at the bottom of an electrolytic vessel having a relatively large surface.

The current density is low, so the reference electrode is not polarized.

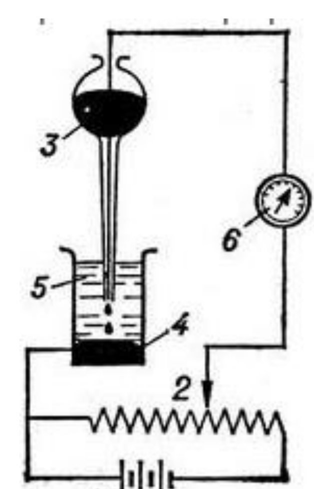


Notes:

The electrodes are connected to the DC source and gradually increase the voltage, observing the change in current depending on the applied voltage.

This dependence is uneven and is expressed by a curve with inflections - waves.

The dependence of the current strength on the applied voltage reflects the electrochemical process carried out on a polarized electrode and is called a polarization curve or a polarogram.



Notes:

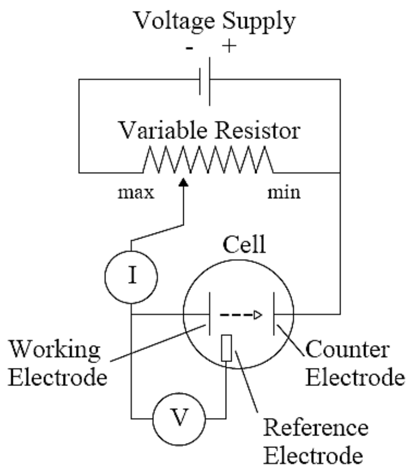
Instrumentation – Three electrodes in solution containing analyte.

Working electrode: microelectrode whose potential is varied with time.

Reference electrode: potential remains constant (Ag/AgCl electrode or calomel).

Counter electrode: Hg or Pt that completes circuit, conducts e^- from signal source through solution to the working electrode.

Supporting electrolyte: excess of nonreactive electrolyte (alkali metal) to conduct current.

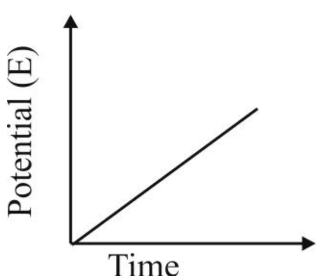


Notes:

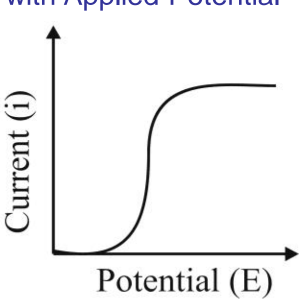
The location and height of these waves can be concluded about the composition and concentration of the electrolyte, that is, to conduct a qualitative and quantitative analysis of the solution.

Notes:

Apply Linear Potential with Time



Observe Current Changes with Applied Potential

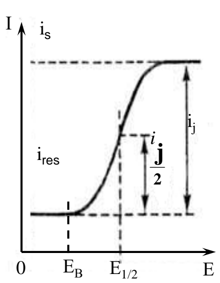


When passing current through the electrolytic cell, the ratio is:

Notes:

$$E = E_a - E_c + I \cdot R,$$

where E is the applied voltage, V;
I is the current passing through the cell, A;
R is the resistance of the electrolytic cell, Ohm;
E_a is the potential of the anode, V;
E_c is the cathode potential, V.



i_s - sustaining current
I_r – limited current

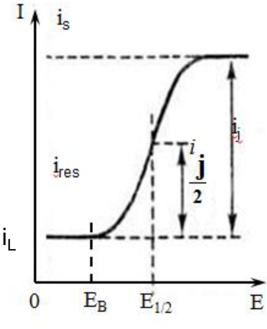
To reduce the impedance electrolytic cell to the test solution add indifferent (background) great concentration electrolyte to improve conductivity of the solution. If R≈1000 Ohms and the current does not exceed 10^{-5} A, then the value of I·R can be neglected.

Notes:

The reference electrode is not polarized, so its potential remains constant.

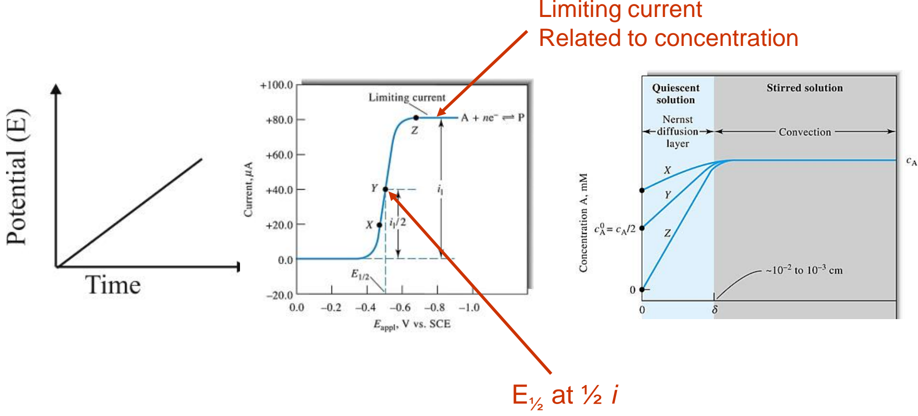
Taking this potential conditionally equal to zero, we obtain that $E = - E_c$,

that is, the potential of the working electrode is determined by the amount of applied voltage.



i_S - sustaining current
i_L – limiting current

Combining Potential and Current Together



Half-wave potential: $E_{1/2} = -0.5 \approx \quad E^0 - E_{ref}$

$E^0 = -0.5 + SCE \qquad \text{for } M^{n+} + me^- \leftrightarrow M^{(n-m)+}$

Notes:

If there are ions in the solution that are able to recover electrochemically (rarely - to be oxidized), then a very weak current (residual) passes through the cell with a small voltage value.

It marginally changes with increasing voltage.

When the applied potential values are insufficient for the discharge of the conditioned ions, the current strength increases with the voltage.

Residual current consists of:

- Faraday current due to the discharge of impurities present in the solution;
- The capacitive current caused by the capacity of a double electric layer on the newly formed surface of a mercury droplet

Notes:

Residual current limits the sensitivity of the method.

As soon as the discharge potential for a particular ion is reached, the electrochemical reaction begins, and the current increases sharply:

- a decrease in the concentration of the electrochemically active substance is observed in the pre-electrode layer.

With a further increase in voltage, the current reaches its limit value because the concentration in the electrode layer becomes almost zero.

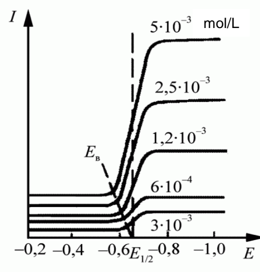
Notes:

The half-wave potential $E_{1/2}$ corresponds to the potential at a current equal to half the diffusion current Id.

Unlike the recovery potential, $E_{1/2}$ does not depend on the concentration of electrically recovered ions in solution.

The concentration of the substance in solution is determined by the value of the maximum diffusion current Id, and the nature of the ion by the half-wave potential $E_{1/2}$.

The value of $E_{1/2}$ can be estimated by lowering the perpendicular from the midpoint of the polarographic wave to the potential axis.



A more accurate way of determining the half-wave potential is a graphical method of constructing dependencies

$$\frac{\lg i}{i_j - i} = f(E)$$

Notes:

If the composition of solution, pH and temperature are constant, then the value of $E_{1/2}$ is determined by the nature of a depolarizer.

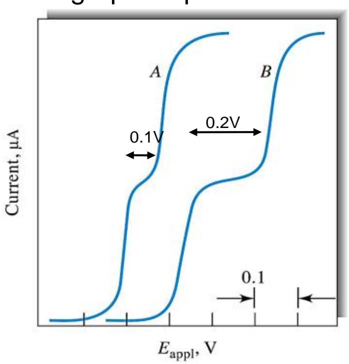
Therefore, it is possible to determine what ion is contained in the test solution by measuring the values of $E_{1/2}$

Notes:

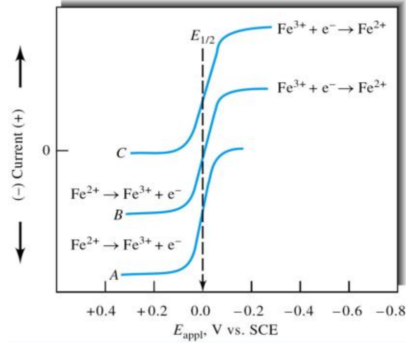
Voltammograms for Mixtures of Reactants

The determination of $E_{1/2}$ is the basis of qualitative polarographic analysis.

If the potentials of half-waves of substances differ by more than 0.1-0.2 V, then the expressed waves of several substances can be obtained on the same polarograph. This curve is called the polarographic spectrum.



Two or more species are observed in voltammogram if the difference in separate half-wave potentials are sufficient



Different concentrations result in different currents, but the same potential

Notes:

Compared to other electrochemical methods of analysis, polarography has the advantage. This method involves both qualitative and quantitative analysis.

However, irrespective of the type of analysis, it is mandatory to obtain a polarograph of the solution to be analyzed.

One of the parameters of a polarograph is the half-wave potential depends on the nature of the ion being oxidized or reduced by the microelectrode. Therefore, this behaviour can be used to identify it.

The value of the limiting diffusion current is a function of the concentration of the recovered or oxidized ion on the dropping mercury electrode. This dependence is described by the equation.

Notes:

$$i_j = 708{,}1 \cdot n \cdot D^{1/2} \cdot m^{2/3} \cdot t^{1/6} \cdot C$$

where

n is the number of electrons involved in the oxidation or reduction reaction

D is the diffusion coefficient (cm^2/s)

m is the mass of drop of mercury formed in 1 s (mg)

t is the life time drops of mercury with (s)

C is the concentration of a recovering or oxidizing ion (mol/L)

If the imaging is performed according to the same parameters of a dropping mercury microelectrode, then all input values in the equation will be constant and the equation will look like

$$i_j = k \times C$$

Notes:

Ilkovich's equation is derived with the following assumptions:

The rate of mercury leakage remains constant throughout the life of the droplet - this is not entirely true, especially during the initial period of the droplet's existence;

Mercury drops have a spherical shape - photographs show that the shape of the droplets deviates from strictly spherical; however, this assumption is acceptable for small drops;

The center of symmetry of each drop does not change its position - in fact, with the increase of the drop, it gradually decreases;

The shielding action of the lower slice of the dropping electrode is disregarded;

The concentration of the recovered substances decreases to zero at the electrode surface and remains constant in the depth of the solution: the decrease in the bulk concentration due to the electrode reaction is considered to be insignificant, which is quite acceptable for small electrodes;

The mixing is assumed to be completely absent;

The theory of linear rather than spherical diffusion is applied.

Notes:

Amperometric titration is based on the determination of the equivalence point by a sharp change in the diffusion current during the titration process.

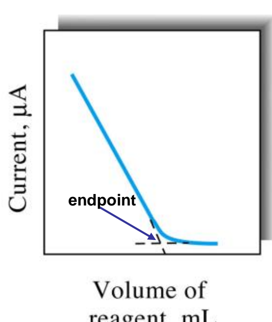
It is carried out at a potential corresponding to the half-wave potential of a substance involved in the electrode process.

Record the diffusion current passing through the electrochemical cell, and plot the dependence of the diffusion current on the volume of added titrant.

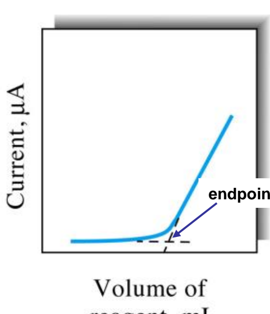
Notes:

Amperometric Titrations

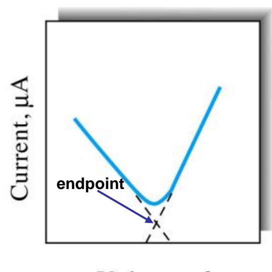
Measure equivalence point if analyte or reagent are oxidized or reduced at working electrode. Current is measured at fixed potential as a function of reagent volume. The endpoint is intersection of both lines



Only analyte is reduced



Only reagent is reduced



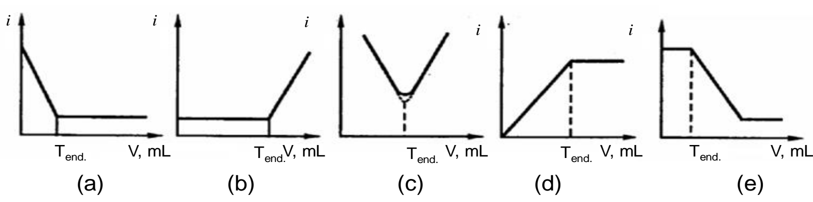
Both analyte and reagent are reduced

Notes:

Unlike polarography, amperometric titration does not necessarily require an ion to be involved in an electrochemical reaction.

It is sufficient that one of the two reagents or the reaction product is involved in the electrode reaction.

The shape of the amperometric titration curves depends on which of the components of the chemical reaction is polarographically active.



(a) a polarographically active substance;
(b) is a polarographically active titrant;
(c) the polarographically active substance to be titrated and the titrant;
(d) a polarographically active reaction product;
(e) titration with a polarographic indicator.

Notes:

Before performing the amperometric titration, it is necessary to select a titration potential that would correspond to the diffusion current of the ion involved in the electrochemical reaction.

To do this, remove the polarograph of this ion under the same conditions under which the amperometric titration will be carried out.

Most often, the potential of the indicator electrode is set to 0.1-0.2 V more negative than the half-wave potential.

Amperometric titration is widely used in analytical practice.

Advantages of the method:

- high selectivity;
- determination can be made in dilute solutions (up to 10^{-6} mol/L);
- ability to identify most elements of the periodic table and many organic substances (thiols, amino acids);
- high accuracy, easy to perform analysis.

Notes:

Amperometric titration is performed using polarographs.

Mercury droplets (in the potential range from +0.2 to –1.86V) or rotating solid electrodes (platinum, graphite) are used as indicator electrodes.

The reference electrodes are calomel, chlorine-silver electrodes or a layer of mercury at the bottom of the electrochemical cell.

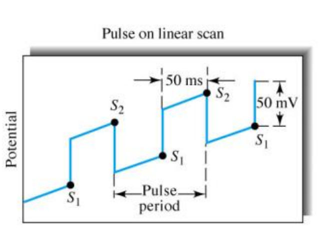
Notes:

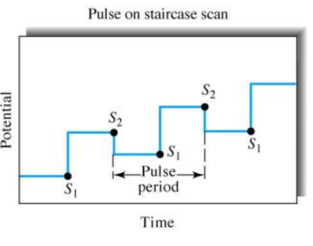
Pulse Voltammetry

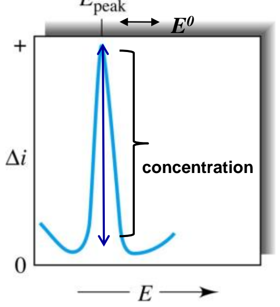
a) Instead of linear change in E_{appl} with time use step changes (pulses in E_{appl}) with time

b) Measure two currents at each cycle: S_1 before pulse & S_2 at end of pulse; plot Δi vs. E ($\Delta i = E_{S2} - E_{S1}$); peak height ~ concentration; for reversible reaction, peak potential → standard potential for ½ reaction.

c) differential-pulse voltammetry







d) Advantages:

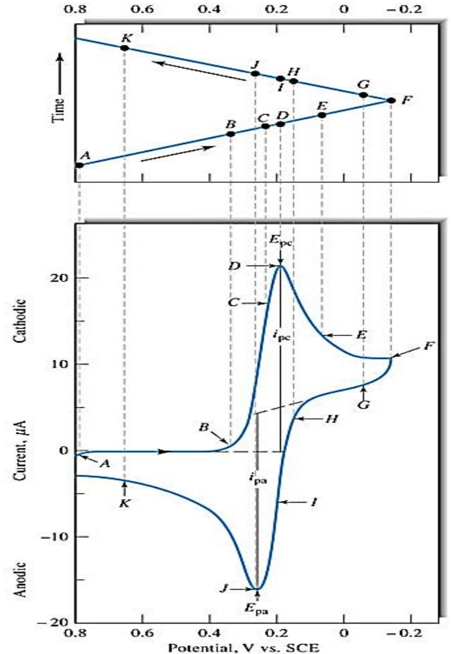
- can detect peak maxima differing by as little as 0.04 – 0.05 V 0.2V peak separation for normal voltammetry
- decrease limits of detection by 100-1000x compared to normal voltammetry 10^{-7} to 10^{-8} M

Notes:

Cyclic Voltammetry

1) Method used to look at mechanisms of redox reactions in solution.

2) Looks at *i* vs. *E* response of small, stationary electrode in unstirred solution using triangular waveform for excitation



Cyclic voltammogram

Notes:

6 mM $K_3Fe(CN)_6$ & 1 M KNO_3

Working Electrode is Pt & Reference electrode is SCE

A. Initial negative current due to oxidation of H_2O to give O_2

No current between A & B (+0.7 to +0.4V) no reducible or oxidizable species present in this potential range

B. At 0.4V, current begins because of the following reduction at the cathode:

$$Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-}$$

B.-D. Rapid increase in current as the surface concentration of $Fe(CN)_6^{3-}$ decreases

D. Cathodic peak potential (E_{pc}) and peak current (i_{pc})

D.-F. Current decays rapidly as the diffusion layer is extended further from electrode surface

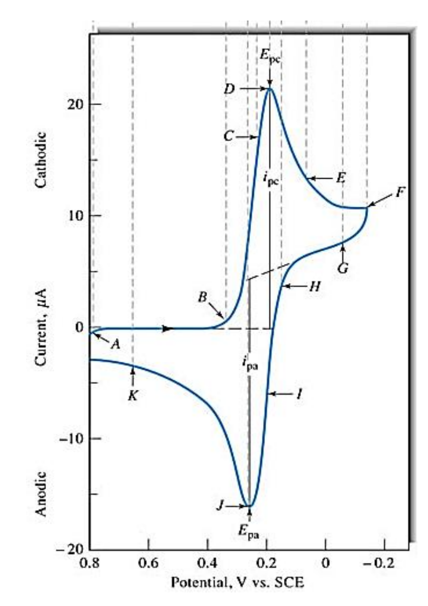
F. Scan direction switched (-0.15V), potential still negative enough to cause reduction of $Fe(CN)_6^{3-}$

F.-J. Eventually reduction of $Fe(CN)_6^{3-}$ no longer occurs and anodic current results from the reoxidation of $Fe(CN)_6^{4-}$

J. Anodic peak potential (E_{pa}) and peak current (i_{pa})

K. Anodic current decreases as the accumulated $Fe(CN)_6^{4-}$ is used up at the anodic reaction

Notes:



Important Quantitative Information

i_{pc} i_{pa}

$\Delta E_p = (E_{pa} - E_{pc}) = 0.0592/n$,
where n = number of electrons in reaction

E^0 = midpoint of $E_{pa} \rightarrow E_{pc}$

$i_p = 2.686 \times 10^5 n^{3/2} A c D^{1/2} v^{1/2}$

- ***A*: electrode area**
- ***c*: concentration**
- **v: scan rate**
- ***D*: diffusion coefficient**

Thus,
can calculate standard potential for half-reaction
number of electrons involved in half-reaction
diffusion coefficients
if reaction is reversible

Notes:

Volvamprometric analyzer IVA-5

Notes:

Measuring range, μg / L: - in the analysis of water - for mercury - in the analysis of food products	 0,1…10000 0,01…500 0,02…500
Working electrode Comparison electrode	Graphite chlorine - silver
Measurement error	±2,5%
Operating temperature range	+0 ...+50°C
Feeding	220V
Overall dimensions, mm, not more than	250x175x75

Application: to determine the concentration of ions of copper, lead, cadmium, iron, cobalt, chromium, zinc, nickel, molybdenum, manganese, arsenic, tin, mercury in natural, drinking and waste water, in food and in raw materials, in biological fluids (blood fractions, urine).

Differences from Other Electrochemical Methods

Notes:

a) Potentiometry: measure potential of sample or system at or near zero current.
voltammetry – measure current as a change in potential

b) Coulometry: use up all of analyte in process of measurement at fixed current or potential
voltammetry – use only small amount of analyte while vary potential

Notes:

Coulometry - an electrochemical method of quantitative analysis is based on measuring the amount of electrical current spent on the electrochemical oxidation or reduction of ions or elements that determine the process of electrolysis. The results of the analysis are calculated according to Faraday's law:

$$m = \frac{M \cdot I \cdot t}{n \cdot F} = \frac{E \cdot Q}{F}$$

where
m is the mass of the recovered (or oxidized) substance, g;
F is the Faraday constant (96 500 coulomb, C);
M is the molar mass of the substance, g/mol;
n is the number of electrons involved in the electrochemical oxidation-reduction process;
I is the electric current, A;
t is the time of electrolysis, s;
Q is the amount of electric current, coulomb, C
E is the equivalent of a substance.

The coulometric method makes it relatively easy to determine ultra-micro quantities (0.01-0.001 μg).

When carrying out any coulometric determinations, the current should be consumed only for the required electrochemical reaction.

All by-processes that occur with the consumption of electric current should be excluded.

Therefore, it is very important to ensure the following conditions for electrolysis:

- strictly regulate the external voltage:
• it must provide the electrolysis of the substance being determined, and
• at the same time, be insufficient for adverse electrochemical reactions;
- electrochemical decomposition of water should be completely prevented.

Notes:

Methods for establishing the termination of electrolysis

The point at which the oxidation or reduction of the test substance is almost complete should be determined precisely.

The termination of electrolysis is established in various ways:

1) adding to the test solution a reagent that gives a coloured compound with a detectable rhubarb (end of electrolysis is determined by the disappearance of the characteristic colour of the solution);
2) measuring the potential of an electrode that responds to changes in the concentration of detectable ions.

At the end point there is a sharp change in the potential of this indicator electrode, which indicates that the electrolysis should be stopped;

3) the concentration of the determined ions is controlled amperometrically.

Notes:

Classification of coulometry methods by reaction type:

1) Recovery of metals and release of the latter in the free state.

Reactions are based on:

$$Me^{n+} + n\bar{e} = Me^{0}$$

In this way, one can determine copper, bismuth, cadmium and some other metals.

Metallic mercury is used as the cathode because the formation of amalgam facilitates the electrolytic separation of many metals.

In addition, hydrogen is released on the metallic mercury with high overvoltage, so it is easy to eliminate the side reaction of water decomposition under the influence of electric current.

Notes:

Notes:

2) Oxidation of metals recovered by electrolysis from the test solution. Reactions are based on:

$$Me^0 = Me^{n+} + n\bar{e}$$

In this way, submicrograms of argentum (10^{-8}-10^{-10} g) and some other heavy metals are determined.

3) Electrolytic oxidation or reduction of ions in solution. Reactions are based on:

$$Me^{n+} + a\bar{e} = Me^{(n-a)+}$$

Direct coulometric methods are rarely used in practice.

Notes:

Much more common is the method of coulometric titration.

In this method, in parallel with the electrochemical reaction that occurs when passing an electric current, the solution also has a chemical reaction between the substance being determined and the product of the electrochemical reaction.

The current is spent mainly on the electrochemical oxidation-reduction of extraneous ions, which are specially added to the solution in large excess.

The products of oxidation-reduction react further with the substance that is determined.

This method eliminates unwanted side processes, the main of which is the decomposition of water.

Notes:

Measurement of the amount of electric current by coulometers

Coulometers - devices that measure the amount of electrical current included in the circuit in series with the cell.

They are divided into gas and titration coulometers.

A gas coulometer is a water coulometer, in which the passage of current is the electrolysis of water and released a gaseous mixture of hydrogen and oxygen.

The volume of the gas mixture, proportional to the amount of electrical current passing through the solution, is measured with a calibrated burette.

Notes:

The **titration coulometer** is vanadium.

Its action is based on the oxidation of vanadil in solution of its sulfate:

$$VO^{2+} + 2H_2O = VO_3^{-} + 4H^{+} + \bar{e}.$$

The amount of vanadate formed during electrochemical oxidation is determined by titrating the working solution $FeSO_4$ in the presence of an indicator of phenylanthranilic acid (96 500 C is equal 1000 mL 1 N $FeSO_{4\ solv.}$, so 1 C equals 0,104 mL 0,1 N $FeSO_{4\ solv.}$).

Notes:

Coulomb at constant current (chronometric method for determining the amount of electric current, Q).

The method is that during electrolysis, the current strength is kept constant, and the duration of the electrolysis is determined by a stopwatch.

The amount of electric current Q is calculated by the formula:

$$Q = I \cdot t$$

To maintain a constant current, a high-ohmic resistance is included in series with the cell.

The voltage on the electrodes during electrolysis is slightly increased due to changes in the concentration of ions in solution (several tenths of a volt).

Such fluctuations are insignificant in comparison with the magnitude of the voltage of the current source and therefore have almost no effect on the current, which remains almost constant in the process of all electrolysis.

According to this method, coulometric titration is carried out.

Notes:

Coulomb at a constant potential.

The method is based on determining the value of the potential of the working electrode while maintaining a constant value of this potential throughout the electrolysis.

The amperage gradually decreases as the concentration of the substance being determined decreases continuously.

The experimental dependence of the current strength on the time of electrolysis is expressed by an exponential curve.

The total amount of electric current, consumed for the complete oxidation or reduction of the ions that is determined, is expressed by the area bounded by the curvature of the current - time and the coordinate axes.

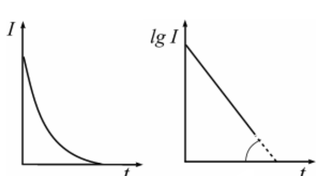
It is more convenient to use the logarithmic dependence lgI = f (t), which is expressed by a straight line.

The value of Q is calculated by the equation:

$$Q = \frac{I_0}{\mathrm{tg}\alpha}.$$

where
I_0 is the initial amperage;
tgα is the tangent of the angle of inclination of the line corresponding to the dependence of lg I = f (t).

The electrolysis is carried out until the current decreases to almost zero.



Dependence of current (a) and logarithmic (b) from the time of electrolysis

Notes:

Coulometric titrator

Modes of operation	galvanostatic with decreasing magnitude of current when approaching equivalence point - automatic with drift
- potentiometric - conductometric - for optical signal (color change)	- potentiometric - conductometric for optical signal (color change)
Measurement error	± 2,0%
Coulometer feed	220V
Overall dimensions, mm	220x210x70

Application: determination of the content of substances in solution in the form of ions, complex compounds, neutral molecules and other electroactive compounds.

In the pharmaceutical analysis, the quantitative content of barbituric acid derivatives, antibiotics, atropine, codeine, papaverine, sulfonamides, cysteine and the like is determined by the coulometric titration method.

Notes:

Tasks to Section 17.

1. Give definitions of these terms: polarography, voltammetry, amperometry, working electrode, reference electrode, counter electrode, supporting electrode, half-wave potential, diffusion current, amperometric titration, pulse voltammetry, cyclic voltammetry, coulometry.

2. Explain why each of the following decreases the analysis time in controlled-potential coulometry: a larger surface area for the working electrode; a smaller volume of the solution; and a faster stirring rate.

3. The concentration of As(III) in water is determined by differential pulse polarography in 1 M HCl. The initial potential is set to –0.1 V versus the SCE and is scanned toward more negative potentials at a rate of 5 mV/s. Reduction of As(III) to As(0) occurs at a potential of approximately –0.44 V versus the SCE. The peak currents for a set of standard solutions, corrected for the residual current, are shown in the following table.

[As(III)] (μM)	1.00	3.00	6.00	9.00
ip (μA)	0.298	0.947	1.83	2.72

What is the concentration of As(III) in a sample of water if its peak current is 1.37 μA?

4. The concentration of copper in a sample of seawater is determined by anodic stripping voltammetry using the method of standard additions. The analysis of a 50.0-mL sample gives a peak current of 0.886 μA. After adding a 5.00-μL spike of 10.0 mg/L Cu^{2+}, the peak current increases to 2.52 μA. Calculate the μg/L copper in the sample of seawater.

Section 18: Analytical Separations. Chromatography

Contents:

- Introduction
- Overview of analytical separations techniques
- General theory of separation efficiency
- Classifying separation
- Chromatographic methods
- General theory of column chromatography
- Gas chromatography
- Liquid chromatography
- Other forms of liquid chromatography. Combined techniques

Introduction

Many chemical analyses are not specific for one compound. It is often necessary to purify first the compound of interest. It requires a separation step.

So the analytical procedures often include a step to separate the analyte from potential interferents. Although effective, each additional step in an analytical procedure increases the analysis time and the cost of the analysis and introduces uncertainty. In Section 18, we consider the analytical technique that avoids these limitations by combining separation and analysis.

There are several methods for separating an analyte from potential interferents. For example, in liquid-liquid extraction, the analyte and interferent initially are present in a single liquid phase. We add a second, immiscible liquid phase and thoroughly mix them by shaking. During this process, the analyte and interferents partition between the two phases to different extents, effecting their separation. After allowing the phases to separate, we draw off the phase enriched in the analyte.

Despite the power of liquid-liquid extractions, there are significant limitations. The problem with a liquid-liquid extraction is that the separation occurs in one direction only: from the sample to the extracting phase.

In chromatography, we pass a sample-free phase, which we call the mobile phase, over a second sample-free stationary phase that remains fixed in space. We inject or place the sample into the mobile phase.

The mobile phase is a liquid or a gas, and the stationary phase is a solid or a liquid film coated on a solid substrate. We often name chromatographic techniques by listing the type of mobile phase followed by the type of stationary phase. In gas-liquid chromatography, for example, the mobile phase is a gas, and the stationary phase is a liquid film coated on a solid substrate. If a technique's name includes only one phase, as in gas chromatography, it is the mobile phase.

As the sample moves with the mobile phase, its components divide between the mobile phase and the stationary phase. A component whose distribution ratio favours the stationary phase requires more time to pass through the system. Given sufficient time and sufficient stationary and mobile phase, we can separate solutes even if they have similar distribution ratios.

There are many ways in which we can identify a chromatographic separation: by describing the physical state of the mobile phase and the stationary phase; by describing how we bring the stationary phase and the mobile phase into contact with each other; or by describing the chemical or physical interactions between the solute and the stationary phase. In Section 18, we will consider how we might use each of these classifications.

Sample Purity.

Many chemical analysis are not specific for one compound. Actually respond to many potential interferences in the sample. Often it is necessary to first purify the compound of interest.

Remove interfering substances before a selective analysis is possible. This requires a separation step.

Techniques available for Chemical Separations:

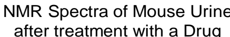
- Extraction
- Distillation
- Precipitation
- Chromatography
- Many others (centrifugation, filtration, etc)

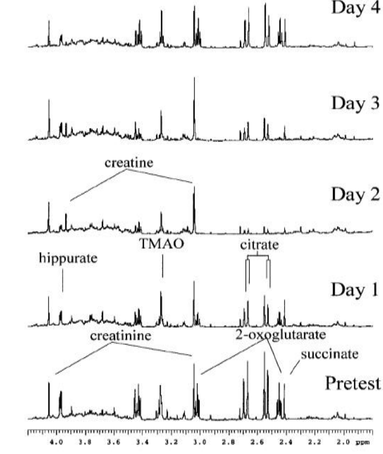
Extractions and Chromatography are especially useful in analytical methods

Notes:

Biological samples are composed of complex mixtures

Analysis of composition and changes help in understanding disease and the development of treatments





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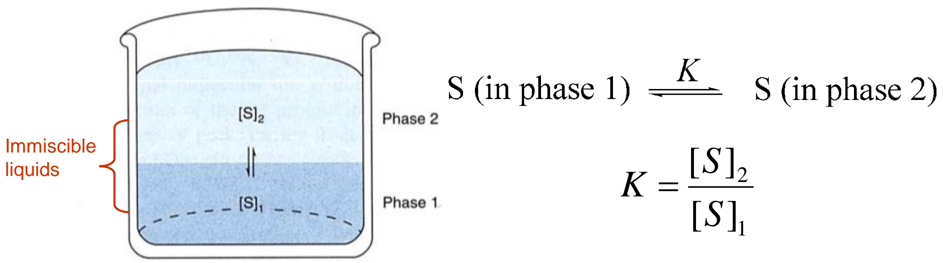
Notes:

Introduction to Analytical Separations

Extractions is the transfer of a compound from one chemical phase to another

The two phases used can be liquid-liquid, liquid-solid, gas-solid

Liquid-liquid is the most common type of extraction



$$\text{S (in phase 1)} \overset{K}{\rightleftharpoons} \text{S (in phase 2)}$$

$$K = \frac{[S]_2}{[S]_1}$$

The partitioning of solute s between two chemical phases (1 and 2) is described by the equilibrium constant *K*

K is called the partition coefficient

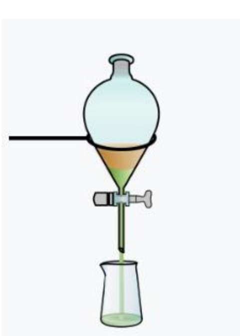
Notes:

Notes:

Extraction Efficiency

The fraction of moles of **S** remaining in phase 1 after <u>one</u> extraction can be determined.

The value of *K* and the volumes of phases 1 and 2 need to be known.



$$q = \frac{V_1}{(V_1 + KV_2)}$$

where: q = fraction of moles of S remaining in phase 1
V_1 = volume of phase 1
V_2 = volume of phase 2
K = partition coefficient

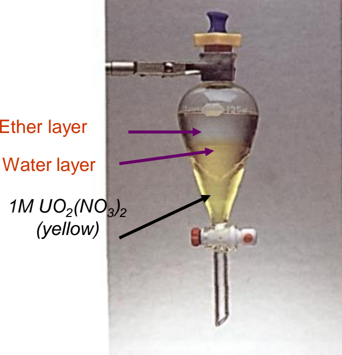
The fraction of **S** remaining in phase 1 after <u>*n*</u> extractions is

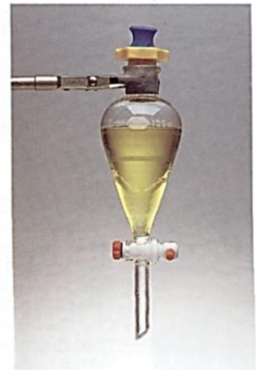
Assumes V_2 is constant

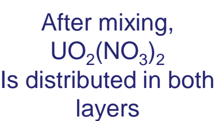
$$q_n = \left[\frac{V_1}{(V_1 + KV_2)}\right]^n$$

Notes:

Extraction Efficiency







After mixing, $UO_2(NO_3)_2$ Is distributed in both layers

After 8 extractions, $UO_2(NO_3)_2$ has been removed from water

Notes:

Example 1:

Solute A has a *K* = 3 for an extraction between water (phase 1) and benzene (phase 2).

If 100 mL of a 0.01M solution of A in water is extracted one time with 500 mL benzene, what fraction will be extracted?

Solution: First determines fraction not extracted (fraction still in phase 1, q):

$$q_n = \left[\frac{V_1}{(V_1 + KV_2)}\right]^n = \left[\frac{100mL}{100mL + (3)\times(500mL)}\right]^1 = 0.062 = 6.2\%$$

The fraction of S extracted (p) is simply:

$$p = 1 - q = 1 - 0.062 = 0.938 = 93.8\%$$

Example 2: For the same example, what fraction will be extracted if 5 extractions with 100 mL benzene each are used (instead of one 500 mL extraction)?

Solution: Determine fraction not extracted (fraction still in phase 1, q):

$$q_n = \left[\frac{V_1}{(V_1 + KV_2)}\right]^n = \left[\frac{100mL}{100mL + (3)\times(100mL)}\right]^5 = 0.00098 = 0.98\%$$

The fraction of S extracted (p) is:

$$p = 1 - q = 1 - 0.00098 = 0.99902 = 99.902\%$$

Note: For the same total volume of benzene (500 mL), more *A* is extracted if several small portions of benzene are used rather than one large portion

Notes:

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Notes:

The distribution of a weak base or weak acid is <u>pH dependent</u>

For a weak base (B) where BH^+ only exists in phase 1:

$$D = \frac{Total\ Concentration\ of\ Base\ in\ Phase\ 2}{Total\ Concentration\ of\ Base\ in\ Phase\ 1}$$

$$K_{BH^+} = \frac{0}{[BH^+]_1} = 0$$

$$D = \frac{[B]_2}{[B]_1 + [BH^+]_1}$$

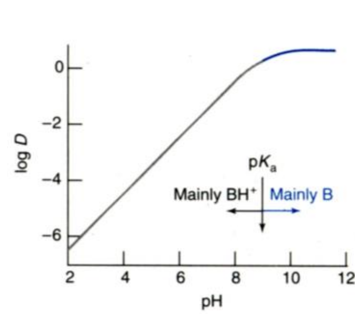
Notes:

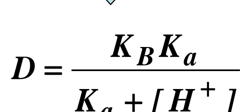
Notes:

Substitute definition of K_B and K_a into D:

$$D = \frac{[B]_2}{[B]_1 + [BH^+]_1} \qquad K_B = \frac{[B]_2}{[B]_1} \qquad K_a = K_w K_b \frac{[H^+][B]}{[BH^+]}$$

partition coefficient — equilibrium constant





$$D = \frac{K_B K_a}{K_a + [H^+]}$$

D is directly related to [H+]

Notes:

A similar expression can be written for a weak acid (HA)

$$D = \frac{K_{HA}[H^+]}{K_a + [H^+]}$$

where: $K_{HA} = \frac{[HA]_2}{[HA]_1}$

The ability to change the distribution ratio of a weak acid or weak base with pH is useful in selecting conditions that will extract some compounds but not others.

- Use low pH to extract HA but not BH^+ (weak acid extractions).
- Use high pH to extract B but not A^- (weak base extractions).

Notes:

Chromatography

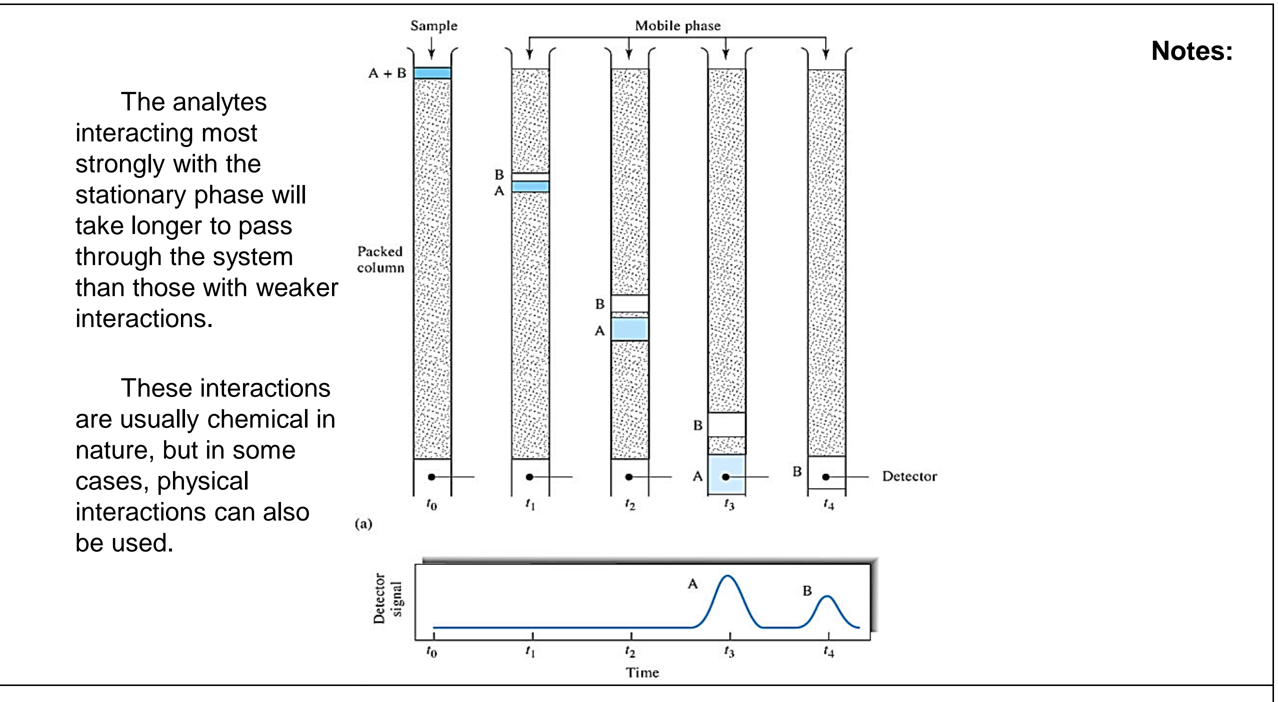
Chromatography is a separation technique based on the different interactions of compounds with two phases, a mobile phase and a stationary phase, as the compounds travel through a supporting medium.

Components:

- the mobile phase is a solvent that flows through the supporting medium;
- the stationary phase is a layer or coating on the supporting medium that interacts with the analytes;
- supporting medium is a solid surface on which the stationary phase is bound or coated.

The analytes interacting most strongly with the stationary phase will take longer to pass through the system than those with weaker interactions.

These interactions are usually chemical in nature, but in some cases, physical interactions can also be used.



Notes:

Types of Chromatography

1. The primary division of chromatographic techniques is based on the type of mobile phase used in the system:

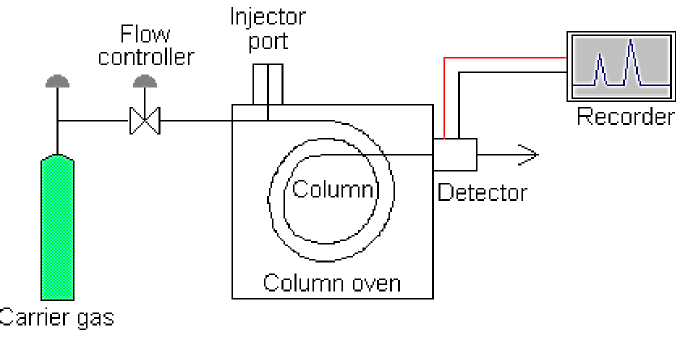
Type of Chromatography	Type of Mobile Phase
Gas chromatography (GC)	gas
Liquid chromatograph (LC)	liquid

2. Further divisions can be made based on the type of stationary phase used in the system.

Notes:

Gas Chromatography

Name of GC Method	Type of Stationary Phase
Gas-solid chromatography	solid, underivatized support
Gas-liquid chromatography	liquid-coated support
Bonded-phase gas chromatography	chemically-derivatized support

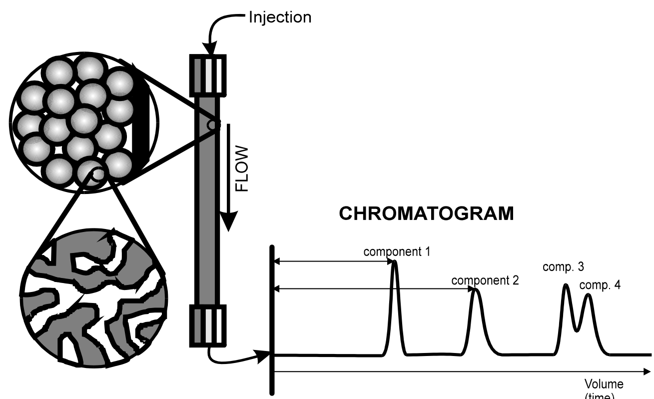


Notes:

Liquid Chromatography

Notes:

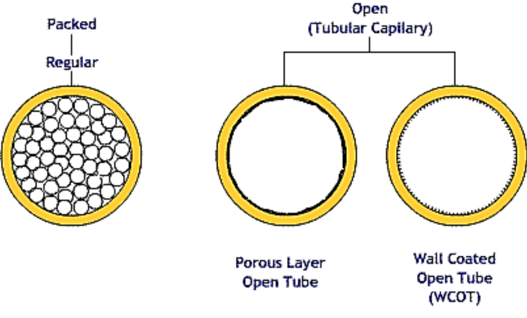
Name of LC Method	Type of Stationary Phase
Adsorption chromatography	solid, underivatized support
Partition chromatography	liquid-coated or derivatized support
Ion-exchange chromatography	support containing fixed charges
Size exclusion chromatography	porous support
Affinity chromatography	support with immobilized ligand



Notes:

Chromatographic techniques may also be classified based on the type of support material used in the system:

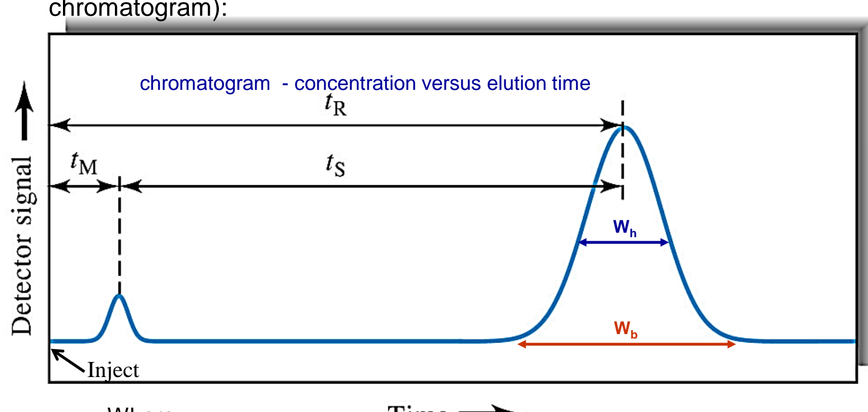
Packed bed (column) chromatography
Open tubular (capillary) chromatography
Open bed (planar) chromatography



Theory of Chromatography

Notes:

Typical response obtained by chromatography (i.e., a chromatogram):



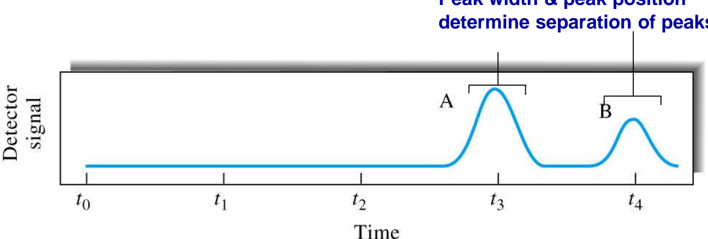
Where:

t_R = retention time
t_M = void time
W_b = baseline width of the peak in time units
W_h = half-height width of the peak in time units

Note: The separation of solutes in chromatography depends on two factors:

a) a difference in the retention of solutes (i.e., a difference in their time or volume of elution

b) a sufficiently narrow width of the solute peaks (i.e, good efficiency for the separation system)



A similar plot can be made in terms of elution volume instead of elution time. If volumes are used, the volume of the mobile phase that it takes to elute a peak off of the column is referred to as the retention volume (V_R) and the amount of mobile phase that it takes to elute a non-retained component is referred to as the void volume (V_M).

Notes:

Solute Retention: A solute's retention time or retention volume in chromatography is directly related to the strength of the solute's interaction with the mobile and stationary phases.

Retention on a given column pertains to the particulars of that system: - the size of the column

- the flow rate of the mobile phase

Capacity factor (k') is the more universal measure of retention, determined from t_R or V_R.

$$k' = (t_R - t_M)/t_M$$

or

$$k' = (V_R - V_M)/V_M$$

Average migration rate $\bar{v} = \frac{L}{t_R}$ column length

Capacity factor is useful for comparing results obtained on different systems since it is independent on column length and flow-rate.

Notes:

The value of the capacity factor is useful in understanding the retention mechanisms for a solute, since the fundamental definition of k' is:

$$k' = \frac{\text{moles } A_{\text{stationary phase}}}{\text{moles } A_{\text{mobile phase}}}$$

k' is directly related to the strength of the interaction between a solute with the stationary and mobile phases.

Moles $A_{\text{stationary phase}}$ and moles $A_{\text{mobile phase}}$ represents the amount of solute present in each phase at equilibrium.

Equilibrium is achieved or approached at the center of a chromatographic peak.

When k' is ≤ 1.0, separation is poor
When k' is > 30, separation is slow
When k' is = 2-10, separation is optimum

Notes:

A simple example relating k' to the interactions of a solute in a column is illustrated for partition chromatography:

Notes:

$$\text{A (mobile phase)} \xrightarrow{K_D} \text{A (stationary phase)}$$

where: K_D = equilibrium constant for the distribution of A between the mobile phase and stationary phase

Assuming local equilibrium at the center of the chromatographic peak:

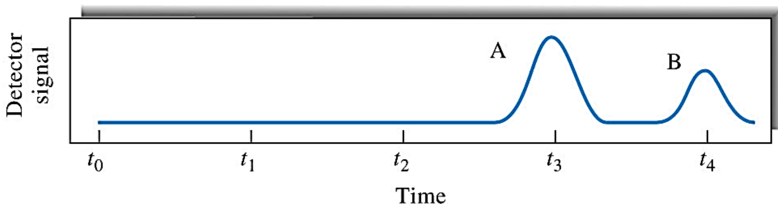
$$k' = \frac{[A]_{\text{stationary phase}} \text{Volume}_{\text{stationary phase}}}{[A]_{\text{mobile phase}} \text{Volume}_{\text{mobile phase}}}$$

$$k' = K_D \frac{\text{Volume}_{\text{stationary phase}}}{\text{Volume}_{\text{mobile phase}}}$$

As K_D increases, interaction of the solute with the stationary phase becomes more favorable and the solute's retention (k') increases

Notes:

$$k' = K_D \frac{\text{Volume}_{\text{stationary phase}}}{\text{Volume}_{\text{mobile phase}}}$$



The separation between two solutes requires different K_D's for their interactions with the mobile and stationary phases since $\Delta G = -RT \ln K_D$

peak separation also represents different changes in free energy

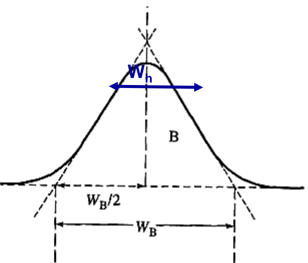
Notes:

Efficiency is related <u>experimentally</u> to a solute's peak width.

- an efficient system will produce narrow peaks
- narrow peaks → smaller difference in interactions in order to separate two solutes

Efficiency is related <u>theoretically</u> to the various kinetic processes that are involved in solute retention and transport in the column

- determine the width or standard deviation (s) of peaks



Estimate s from peak widths, assuming Gaussian shaped peak:

$$W_b = 4\sigma$$

$$W_h = 2.354\sigma$$

Dependent on the amount of time that a solute spends in the column (k' or t_R)

Number of theoretical plates (N): compare efficiencies of a system for solutes that have different retention times

$$N = (t_R/s)^2$$

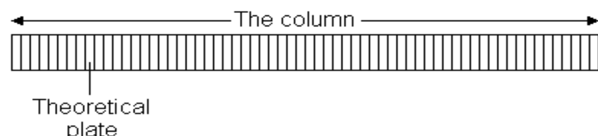
or for a Gaussian shaped peak

$$N = 16\ (t_R/W_b)^2$$

$$N = 5.54\ (t_R/W_h)^2$$

The larger the value of N is for a column, the better the column will be able to separate two compounds.

- the better the ability to resolve solutes that have small differences in retention
- N is independent of solute retention
- N is dependent on the length of the column



Notes:

Plate height or height equivalent of a theoretical plate (H or HETP): compare efficiencies of columns with different lengths:

$$H = L/N$$

where: L = column length;

N = number of theoretical plates for the column

Note: H simply gives the length of the column that corresponds to one theoretical plate

H can be also used to relate various chromatographic parameters (e.g., flow rate, particle size, etc.) to the kinetic processes that give rise to peak broadening: Why Do Bands Spread?

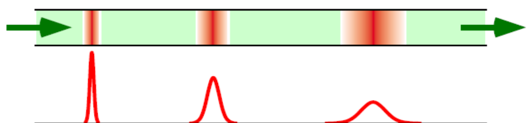
a. Eddy diffusion

b. Mobile phase mass transfer

c. Stagnant mobile phase mass transfer

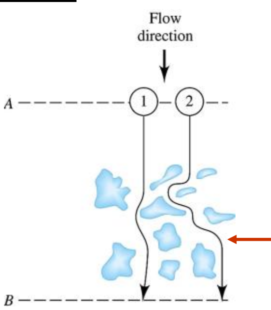
d. Stationary phase mass transfer

e. Longitudinal diffusion



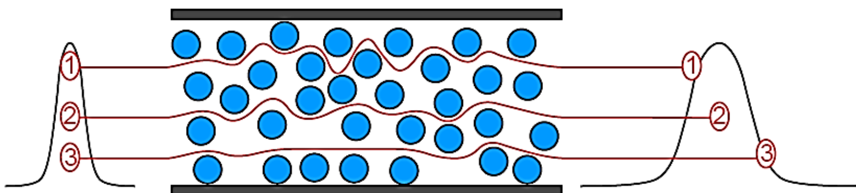
Notes:

Eddy diffusion – a process that leads to peak (band) broadening due to the presence of multiple flow paths through a packed column.



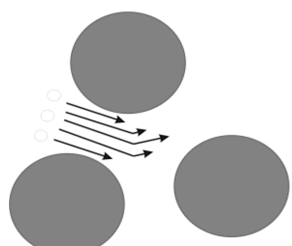
As solute molecules travel through the column, some arrive at the end sooner then others simply due to the different path traveled around the support particles in the column that result in different travel distances.

Longer path arrives at end of column after (1).



Notes:

Mobile phase mass transfer – a process of peak broadening caused by the presence of different flow profile within channels or between particles of the support in the column.



A solute in the center of the channel moves more quickly than solute at the edges, it will tend to reach the end of the channel first leading to band-broadening

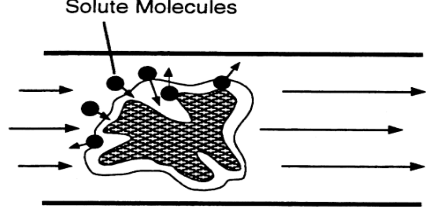
The degree of band-broadening due to eddy diffusion and mobile phase mass transfer depends mainly on:

1) the size of the packing material
2) the diffusion rate of the solute

Notes:

Stagnant mobile phase mass transfer – band-broadening due to differences in the rate of diffusion of the solute molecules between the mobile phase outside the pores of the support (flowing mobile phase) to the mobile phase within the pores of the support (stagnant mobile phase).

Since a solute does not travel down the column when it is in the stagnant mobile phase, it spends a longer time in the column than solute that remains in the flowing mobile phase.

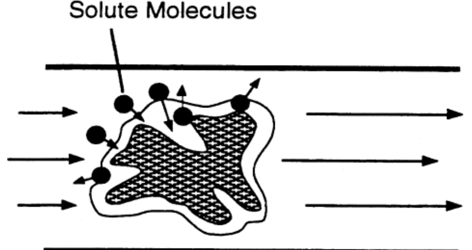


The degree of band-broadening due to stagnant mobile phase mass transfer depends on:

1) the size, shape and pore structure of the packing material
2) the diffusion and retention of the solute
3) the flow-rate of the solute through the column

Notes:

Stationary phase mass transfer – band-broadening due to the movement of solute between the stagnant phase and the stationary phase.



Since different solute molecules spend different lengths of time in the stationary phase, they also spend different amounts of time on the column, giving rise to band-broadening.

The degree of band-broadening due to stationary phase mass transfer depends on:

1) the retention and diffusion of the solute
2) the flow-rate of the solute through the column
3) the kinetics of interaction between the solute and the stationary phase

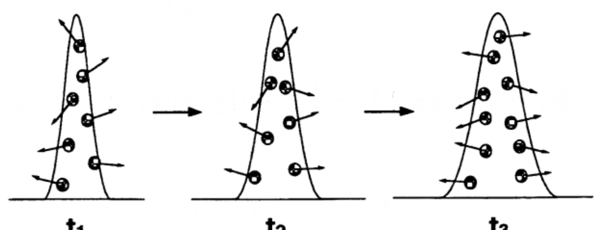
Notes:

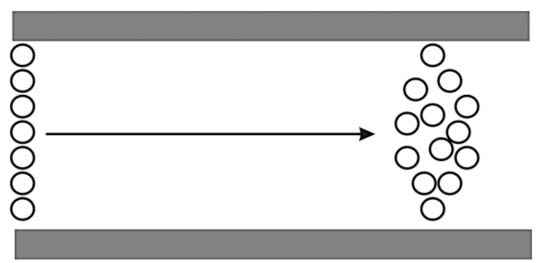
Longitudinal diffusion – band-broadening due to the diffusion of the solute along the length of the column in the flowing mobile phase.

Notes:

The degree of band-broadening due to longitudinal diffusion depends on:

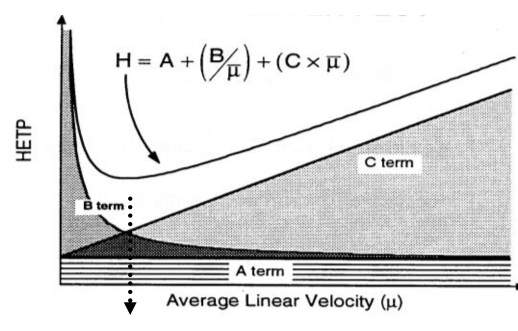
1) the diffusion of the solute
2) the flow-rate of the solute through the column





Plot of van Deemter equation shows how H changes with the linear velocity (flow-rate) of the mobile phase

Notes:



μ optimum

Optimum linear velocity (m_{opt}) - where H has a minimum value and the point of maximum column efficiency:

$$\mu_{opt} = \sqrt{B/C}$$

μ_{opt} is easy to achieve for gas chromatography, but is usually too small for liquid chromatography requiring flow-rates higher than optimal to separate compounds

Measures of Solute Separation:

Notes:

separation factor (a) – parameter used to describe how well two solutes are separated by a chromatographic system:

a = k'_2/k'_1 $k' = (t_R - t_M)/t_M$

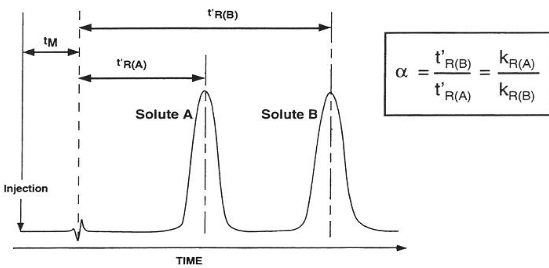
where:

k'_1 = the capacity factor of the first solute

k'_2 = the capacity factor of the second solute,

with k'_2 ³ k'_1

A value of a $1.1 is usually indicative of a good separation



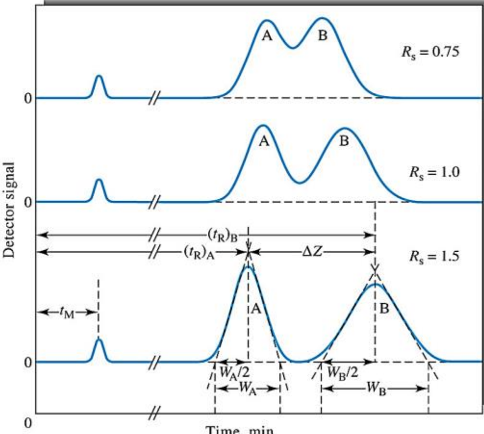
Does not consider the effect of column efficiency or peak widths, only retention.

Resolution (R_S) – resolution between two peaks is a second measure of how well two peaks are separated:

Notes:

$$R_S = \frac{t_{r2} - t_{r1}}{(W_{b2} + W_{b1})/2}$$

where: t_{r1}, W_{b1} = retention time and baseline width for the first eluting peak
t_{r2}, W_{b2} = retention time and baseline width for the second eluting peak



R_s is preferred over α since both retention (t_r) and column efficiency (W_b) are considered in defining peak separation.

R_s 1.5 represents *baseline resolution*, or complete separation of two neighboring solutes → ideal case.

R_s 1.0 considered adequate for most separations.

Gas Chromatography

Notes:

Gas Chromatography (GC) – a chromatographic technique where the mobile phase is a gas.

GC is currently one of the most popular methods for separating and analyzing compounds. This is due to its high resolution, low limits of detection, speed, accuracy and reproducibility.

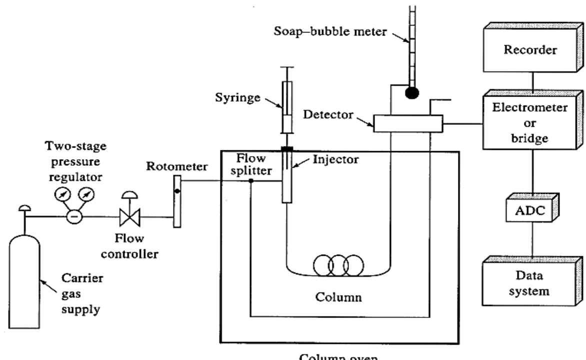
GC can be applied to the separation of any compound that is either naturally volatile (i.e., readily goes into the gas phase) or can be converted to a volatile derivative. This makes GC useful in the separation of a number of small organic and inorganic compounds.

A simple GC system consists of:

1. Gas source (with pressure and flow regulators)
2. Injector or sample application system
3. Chromatographic column (with oven for temperature control)
4. Detector & computer or recorder

A typical GC system used is shown below (a gas chromatograph)

Notes:



Carrier gas:	He (common), N_2, H_2
	P_{inlet} 10-50 psig
	Flow = 25-150 mL/min packed column
	Flow = 1-25 mL/min open tubular column
Column:	2-50 m coiled stainless steel/glass/Teflon
Oven:	0-400 °C ~ average boiling point of sample
	Accurate to <1 °C
Detectors:	FID, TCD, ECD, (MS)

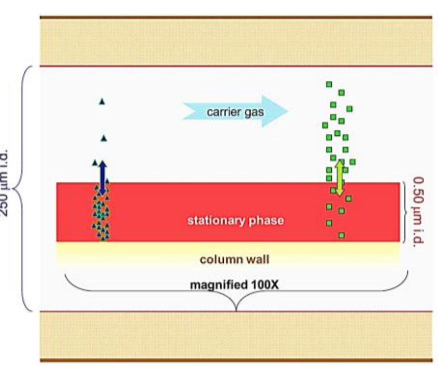
Notes:

Mobile Phase:

GC separates solutes based on their different interactions with the mobile and stationary phases.

Solute's retention is determined mostly by its vapour pressure and volatility.

Solute's retention is controlled by its interaction with the stationary phase.

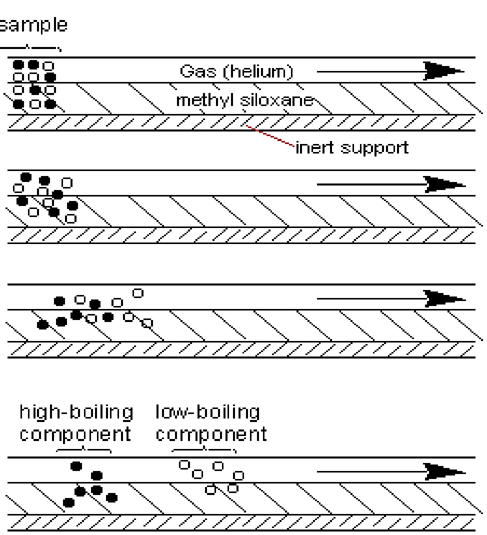


The gas mobile phase has a much lower density:
- decreased chance for interacting with solute;
- increased chance that solid or liquid stationary phase interacts with solute.

Notes:

Carrier gas – the main purpose of the gas in GC is to move the solutes along the column, the mobile phase is often referred to as carrier gas.

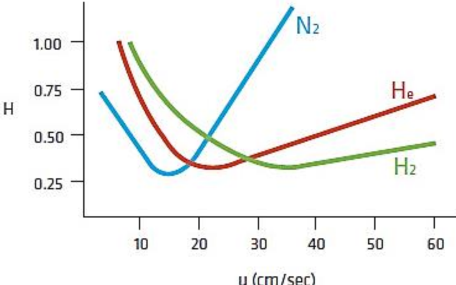
Common carrier gas: include **He, Ar, H_2, N_2**



Notes:

Mobile phase does not affect solute retention, but does affect:

Desired efficiency for the GC System:
- low molecular weight gases (He, H_2) so larger diffusion coefficients;
- low molecular weight gases, therefore, faster, more efficient separation.



Stability of column and solutes: H_2 or O_2 can react with functional groups on solutes and stationary or with surfaces of the injector, connections and detector.

Response of the detector:
- thermal conductor requires H2 or He;
- other detectors require specific carrier gas.

Notes:

Stationary Phases:

Stationary phase in GC is the main factor determining the selectivity and retention of solutes.

There are three types of stationary phases used in GC:

Solid adsorbents
Liquids coated on solid supports
Bonded-phase supports

Notes:

Gas-solid chromatography (GSC)

- same material is used as both the stationary phase and support material;
- common adsorbents include: alumina; molecular sieves crystalline aluminosilicates [zeolites] and clay, silica, active carbon

Advantages:

- long column lifetimes;
- ability to retain and separate some compounds not easily resolved by other GC methods, such as geometrical isomers.

Disadvantage:

- very strong retention of low volatility or polar solutes;
- catalytic changes that can occur on GSC supports;
- GSC supports have a range of chemical and physical environments different strength retention sites, non-symmetrical peaks, variable retention times

Notes:

Gas-liquid chromatography (GLC)

The stationary phase is some liquid coated on a solid support.

Over 400 liquid stationary phases available for GLC, many stationary phases are very similar in terms of their retention properties.

Material range from polymers (polysiloxanes, polyesters, polyethylene glycols) to fluorocarbons, molten salts and liquid crystals.

Based on polarity, of the 400 phases available only 6-12 are needed for most separations.

The routinely recommended phases are listed below:

Notes:

Name	Chemical nature of polysiloxane	Max. temp.	McReynolds' constants x'	y'	z'	m'	s'
SE-30	Dimethyl	350	14	53	44	64	41
Dexsil300	Carborane-dimethyl	450	43	64	111	151	101
OV-17	50% Phenyl methyl	375	119	158	162	243	202
OV-210	50% Trifluoropropyl	270	146	238	358	468	310
OV-225	25% Cyanopropyl-25% phenyl	250	238	369	338	492	386
Silar-SCP	50% Cyanopropyl-50% phenyl	275	319	495	446	637	531
SP-2340	75% Cyanopropyl	275	520	757	659	942	804
OV-275	Dicyanoallyl	250	629	872	763	1106	849

Higher the number the higher the absorption

/lcReynolds' constants based on retention of 5 standard "probe" analytes –
Benzene, n-butanol, 2-pentanone, nitropropanone, pyridine

Preparing a stationary phase for GLC:

Notes:

Slurry of the desired liquid phase and solvent is made with a solid support, solid support is usually diatomaceous earth (fossilized shells of ancient aquatic algae (diatoms), silica-based material)

Solvent is evaporated off, coating the liquid stationary phase on the support

Partition Chromatography

The resulting material is then packed into the column

Disadvantage:

- liquid may slowly bleed off with time:
- especially if high temperatures are used;
- contribute to background;
- change characteristics of the column with time

Bonded-Phase Gas chromatography

Notes:

Bonded-Phase Gas chromatography

Covalently attach stationary phase to the solid support material.

Avoids column bleeding in GLC.

Bonded phases are prepared by reacting the desired phase with the surface of silica-based support reactions.

Form a Si-O-Si bond between the stationary phase and support or form a Si-C-C-Si bond between the stationary phase and support.

Many bonded phases exist, but most separations can be formed with the following commonly recommended bonded-phases:

Dimethylpolysiloxane
Methyl(phenyl)polysiloxane
Polyethylene glycol (Carbowax 20M)
Trifluoropropylpolysiloxane
Cyanopropylpolysiloxane

$$\left[-O-Si(CH_3)_2-\right]_n\left[-O-Si(C_6H_5)_2-\right]_m$$

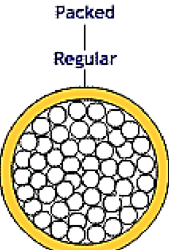
$$\left[-O-Si(CH_3)_2-\right]_n$$

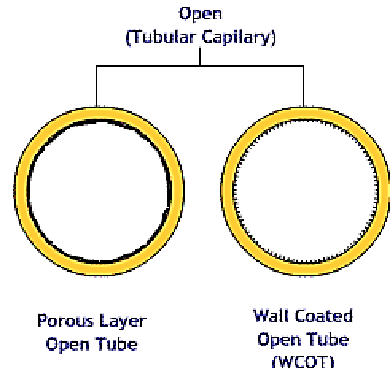
Advantages:

- more stable than coated liquid phases
- can be placed on support with thinner and more uniform thickness than liquid phases

There are two main types of supports used in GC:

Packed columns	**Capillary (open-tubular) columns**
- large sample capacity	- higher efficiency
- preparative work	- smaller sample size
	- analytical applications





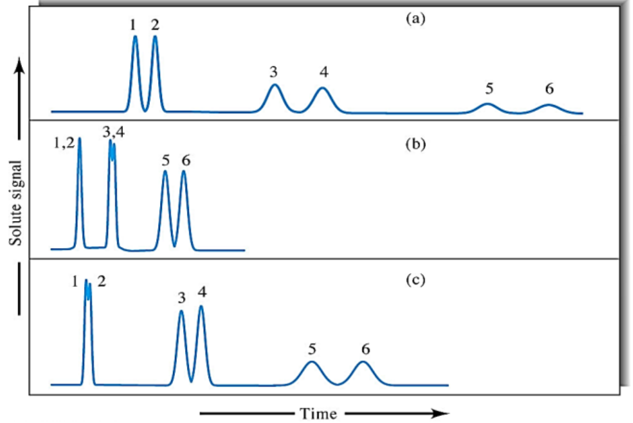
Notes:

Recommended stationary phases for various sample types

Compound to be separated	Types of stationary phases used	Recommended stationary phases
gases	alumina, silica gel, zeolites (molecular sieves) porous polymers	Gas: solid
nonpolar liquids	methylsiloxanes	Gas: liq
polychlorinated biphenyl (PCB), petrochemical samples	phenylmethylsiloxanes, polysiloxane carbones	
herbicides/pesticides,	phenyl polysilphenylene siloxanes	
sugars	cyanopropylphenyl methylsiloxanes	
free fatty acids, alcohols	polyethylene glycols	
alcohols, amines	Phenylmethylsiloxanes (>50% phenyl)	

Notes:

Elution Methods: A common problem to all chromatographic techniques is that in any one sample there may be many solutes present, each retained by the column to a different degree:



Best separation and limits of detection are usually obtained with solutes with k' values of 2-10

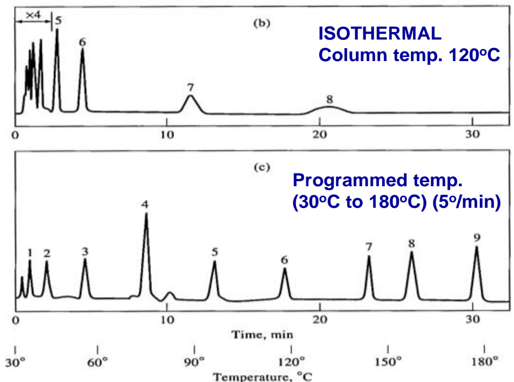
Difficult to find one condition that elutes all solutes in this k' range → general elution problem

Gradient elution - change column condition with time which changes retention of solutes to overcome general elution problem

Notes:

Temperature Programming – changing the temperature on the column with time to simulate gradient elution in GC since a solute's retention in GC is related to its volatility.

Comparison of a GC separation using isothermal conditions and temperature programming:



Temperature programming is usually done either in a stepwise change, a linear change or a combination of several linear changes. A single linear change or ramp is the most common

Notes:

GC Detectors:

The following devices are common types of GC detectors:

1. Thermal Conductivity Detector (TCD)
2. Flame Ionization Detector (FID)
3. Nitrogen-phosphorus Detector (NPD)
4. Electron Capture Detector (ECD)
5. Mass Spectrometers (discussed later in the course)

The choice of detector will depend on the analyte and how the GC method is being used (i.e., analytical or preparative scale)

Detector	Application	Approx. Cost	Sensitivity	Notes
TCD	everything	$3-5	10's of nanograms	Not very sensitive, easy to operate, only one gas required
FID	hydrocarbons	$4-6	Sub-nanogram	Very linear, relatively easy to operate, required fuel gasses, not sensitive to all
NPD	Nitrogen/sulfur	$10-12	Low-picograms	Very selective, hard to operate, required fuel gases
ECD	Halogenated, nitro	$6-9	Low-picograms	Very sensitive, radiation source, not very linear, selective, two gases
MS	Almost everything	$40	Depends on operation	Sensitive, requires pump system, failry complicated requires cleaning

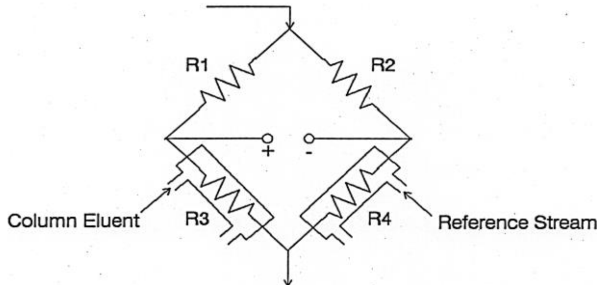
Notes:

Thermal Conductivity Detector (TCD):

- katherometer or hot-wire detector;
- first universal detector developed for GC.

Process

- measures a bulk property of the mobile phase leaving the column;
- measures ability to conduct heat away from a hot-wire (i.e., thermal conductivity);
- thermal conductivity changes with presence of other components in the mobile phase.

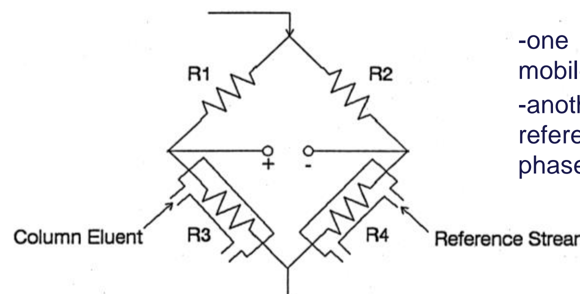


Notes:

Notes:

Design is based on an electronic circuit known as a Wheatstone bridge. The circuit consists of an arrangement of four resistors with a fixed current applied to them. Thermal conductivity changes with the presence of other components in the mobile phase.

The voltage between points (+) and (-) will be zero as long as the resistances in the different arms of the circuit are properly balanced



-one resistor in contact with mobile phase leaving column

-another in contact with reference stream of pure mobile phase

As solute emerge from column: change in thermal conductivity → change in amount of heat removed from resistor → change in resistor's temperature and resistance → change in voltage difference between points (+) and (-).

Notes:

Considerations:

- mobile phase must have very different thermal conductivity then solutes being separated.
- most compounds separated in GC have thermal conductivity of about 1-4X10^{-5}.
- H_2 and He are carrier gases with significantly different thermal conductivity values.
- H_2 reacts with metal oxides present on the resistors, so not used

Advantages:	Disadvantage:
Truly universal detector - applicable to the detection of any compound in GC Non-destructive – useful for detecting compounds from preparative-scale columns; useful in combination with other types of GC detectors	Detect mobile phase impurities Sensitive to changes in flow-rates Limit of detection ~ 10^{-7} M much higher then other GC detectors

Notes:

Flame Ionization Detector (FID) is the most common type of GC detector.

It is the "universal" detector capable of measuring the presence of almost any organic and many inorganic compounds.

Process:

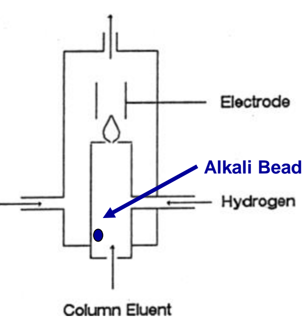
- ❑ measures the production of ions when a solute is burned in a flame;
- ❑ ions are collected at an electrode to create a current.

Advantages:	Disadvantage:
- universal detector for organics, doesn't respond to common inorganic compounds - mobile phase impurities not detected - carrier gases not detected - limit of detection: FID is 1000x better than TCD - linear and dynamic range better than TCD	- destructive detector

Nitrogen-Phosphorus Detector (NPD) is used for detecting nitrogen- or phosphorus-containing compounds. It also is known as alkali flame ionization detector or thermionic detector

Process:

- the same basic principal as FID;
- measures production of ions when a solute is burned in a flame;
- ions are collected at an electrode to create a current;
- contains a small amount of alkali metal vapor in the flame;
- enhances the formation of ions from nitrogen- and phosphorus- containing compounds.



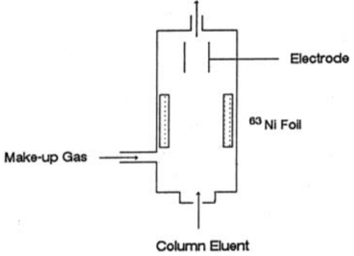
Advantages:	Disadvantage:
- useful for environmental testing and detection of organophosphate pesticides, for drug analysis, determination of amine-containing or basic drugs; - like FID, does not detect common mobile phase impurities or carrier gases; - limit of detection: NPD is 500x better than FID in detecting nitrogen- and phosphorus-containing compounds; - NPD more sensitive to other hetero compounds, such as sulfur-, halogen-and arsenic-containing molecules	- destructive detector - NPD is less sensitive to organic compounds compared to FID

Notes:

Electron Capture Detector (ECD) is the radiation-based detector. It is selective for compounds containing electronegative atoms, such as halogens

Process:

- based on the capture of electrons by electronegative atoms in a molecule
- electrons are produced by ionization of the carrier gas with a radioactive source, ^{3}H or ^{63}Ni
- in absence of solute, steady stream of these electrons is produced
- electrons go to collector electrode where they produce a current
- compounds with electronegative atoms capture electrons, reducing current



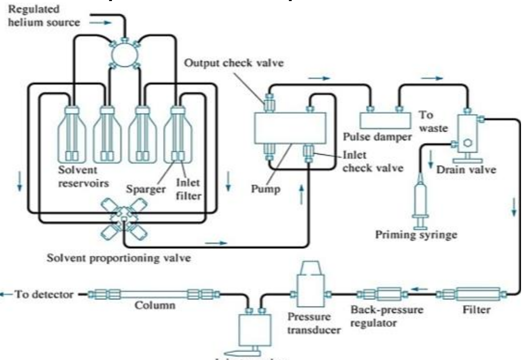
Advantages:
- useful for environmental testing, detection of chlorinated pesticides or herbicides, detection of polynuclear aromatic carcinogens, detection of organometallic compounds; - selective for halogen- (I, Br, Cl, F), nitro-, and sulfur-containing compounds; - detects polynuclear aromatic compounds, anhydrides and conjugated carbonyl compounds.

Notes:

Liquid Chromatography

Liquid Chromatography (LC) is a chromatographic technique in which the mobile phase is a liquid.

LC is a much older technique than GC, but was overshadowed by the rapid development of GC in the 1950's and 1960's.



LC is currently the dominate type of chromatography and is even replacing GC in its more traditional applications.

Notes:

Advantages of LC compared to GC:

- LC can be applied to the separation of any compound that is soluble in a liquid phase. LC more useful in the separation of biological compounds, synthetic or natural polymers, and inorganic compounds
- Liquid mobile phase allows LC to be used at lower temperatures than required by GC. LC better suited than GC for separating compounds that may be thermally labile
- Retention of solutes in LC depends on their interaction with both the mobile phase and stationary phase. GC retention based on volatility and interaction with the stationary phase. LC is more flexible in optimizing separations → change either the stationary or mobile phase
- Most LC detectors are non-destructive, most GC detectors are destructive, LC is better suited for preparative or process-scale separations

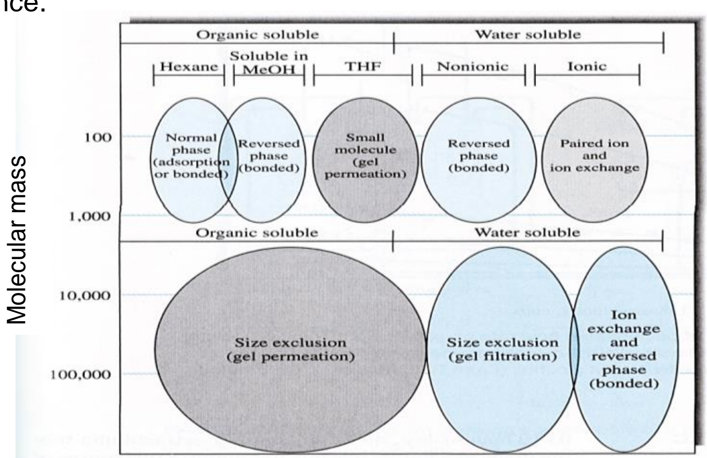
The disadvantage of LC compared to GC:

LC is subject to greater peak or band-broadening. Much larger diffusion coefficients of solutes in gases vs. liquids

Notes:

Low- and High-performance Liquid Chromatography:

Many types of liquid chromatography are available, based on different stationary phase and mobile phase combinations. Each type may be characterized based on its overall efficiency or performance.



Notes:

Low-performance liquid chromatography

LC methods that use large, non-rigid support material with particles > 40 mm in diameter. Method has poor system efficiencies and large plate heights. Such systems have the following characteristics: broad peaks; poor limits of detection; long separation times; columns can only tolerate low operating pressures

< gravity flow or peristaltic pump to apply mobile phase to colum

High Performance

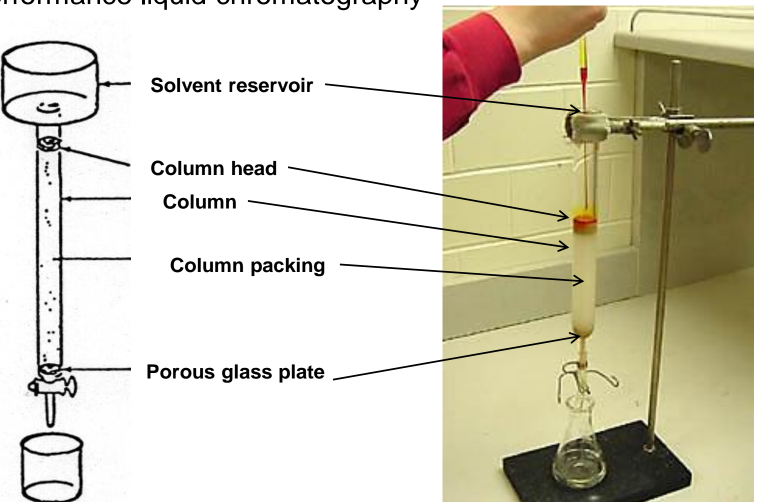
Low Performance

Normalized concentration

Elution volume (mL)

Notes:

Column chromatography is an example of the equipment used in low-performance liquid chromatography



Sample is usually applied directly to the top of the column.
Detection is by fraction collection with later analysis of each fraction

Notes:

Notes:

Low-performance liquid chromatography

Advantages:	Disadvantage:
simple system requirements low cost popular in sample purification used in the removal of interferences from samples used in some analytical applications, not common due to low efficiency	long analysis times and poor limits of detection

High-performance liquid chromatography (HPLC)

Notes:

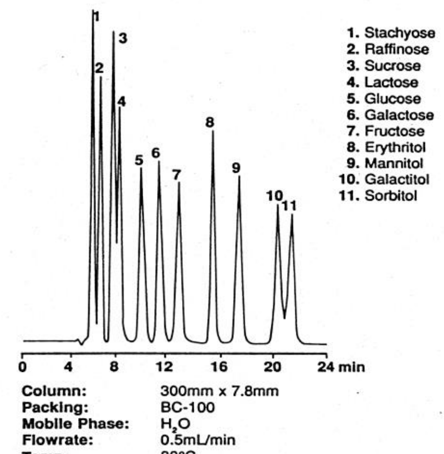
LC methods that use small, uniform, rigid support material with particles < 40 mm in diameter, usually 3-10 mm in practice.

The method has good system efficiencies and small plate heights.

Such systems have the following characteristics:

- narrow peaks;
- low limits of detection;
- short separation times.

Columns can only tolerate high operating pressures and faster flow-rates

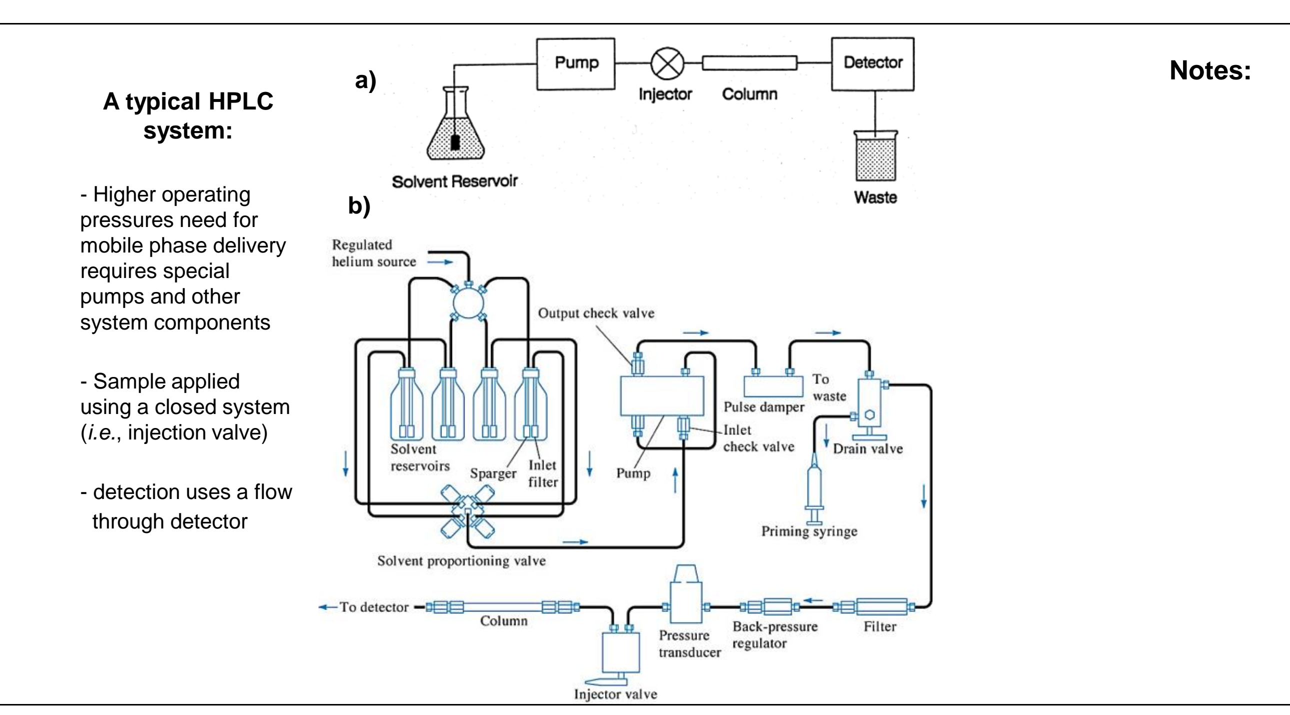


A typical HPLC system:

- Higher operating pressures need for mobile phase delivery requires special pumps and other system components

- Sample applied using a closed system (*i.e.*, injection valve)

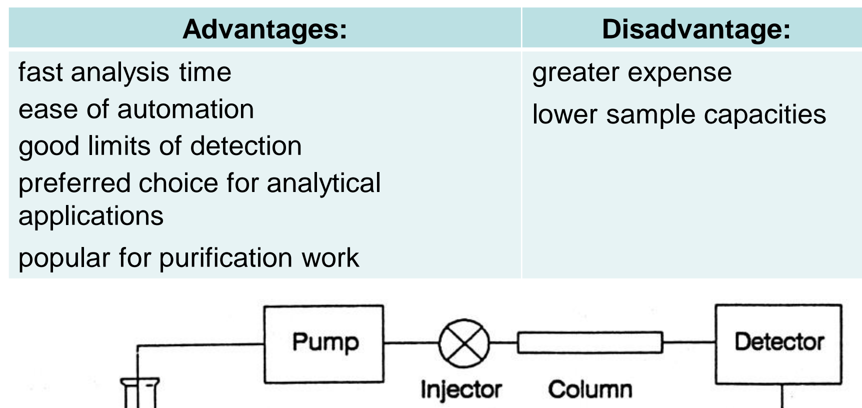
- detection uses a flow through detector



Notes:

High-performance liquid chromatography

Advantages:	Disadvantage:
fast analysis time ease of automation good limits of detection preferred choice for analytical applications popular for purification work	greater expense lower sample capacities



Notes:

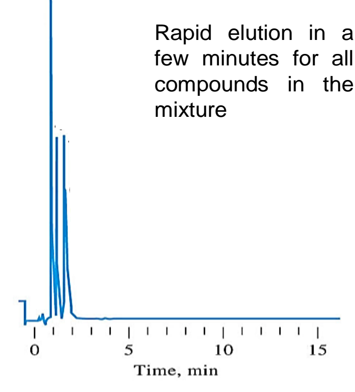
Elution: Retention and elution of solutes in LC depends on the interactions of solutes with <u>both</u> the mobile and stationary phases:

- to describe how well solutes are retained on a column with different solvents, the terms weak;
- mobile phase and strong mobile phase are used.

Strong mobile phase is a solvent that quickly elutes solutes from the column (*i.e.*, small k')

This occurs if the mobile phase is very similar to the stationary phase in its intermolecular interactions with the solutes.

The polar solvent would be a strong mobile phase for a column containing a polar stationary phase.



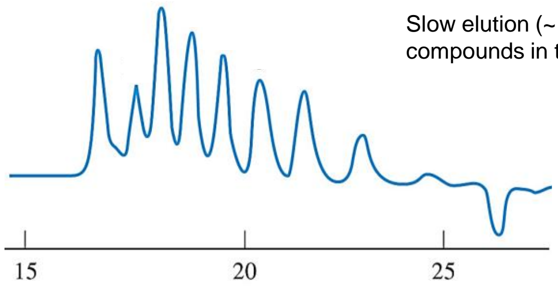
Rapid elution in a few minutes for all compounds in the mixture

Notes:

Weak mobile phase: a solvent that slowly elutes solutes from the column (*i.e.*, high solute retention or large k'). This occurs if the mobile phase is very different from the stationary phase in its intermolecular interactions with the solutes.

A non-polar solvent would be a weak mobile phase for a column containing a polar stationary phase

Notes:



Slow elution (~ 20 minutes) for all compounds in the mixture

Note: whether a solvent is a weak or strong mobile phase depends on the stationary phase being used. Hexane is a weak mobile phase on a polar stationary phase, but a strong mobile phase on a non-polar stationary phase.

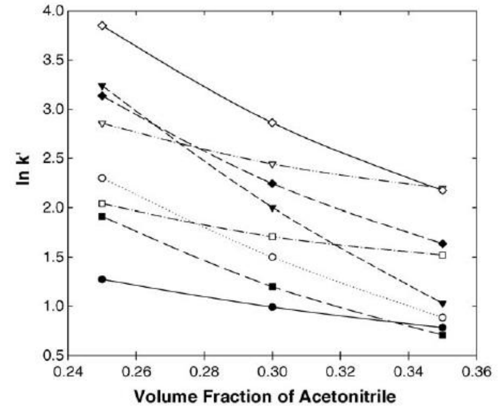
Similar to GC, solutes can be eluted from a column by using either constant column conditions or gradient elution

Notes:

Isocratic elution: use of a constant mobile phase composition to elute solutes. That method is simple, inexpensive.

There is the general elution problem: it is difficult to elute all solutes with good resolution in a reasonable amount of time.

Need to identify solvent composition to obtain optimal separation



phenol (●), alprenolol (○), perphenazine (▼), methylbenzoate (▽), 1-(diphenylmethyl)-4-methylpiperazine (■), acetophenone (□), promethazine (♦) and amitriptyline (◊).

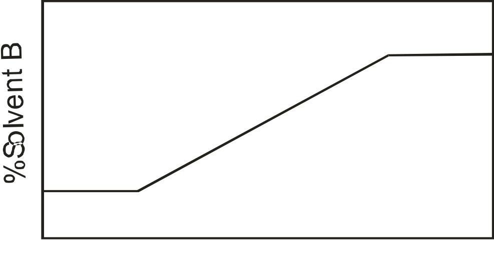
Notes:

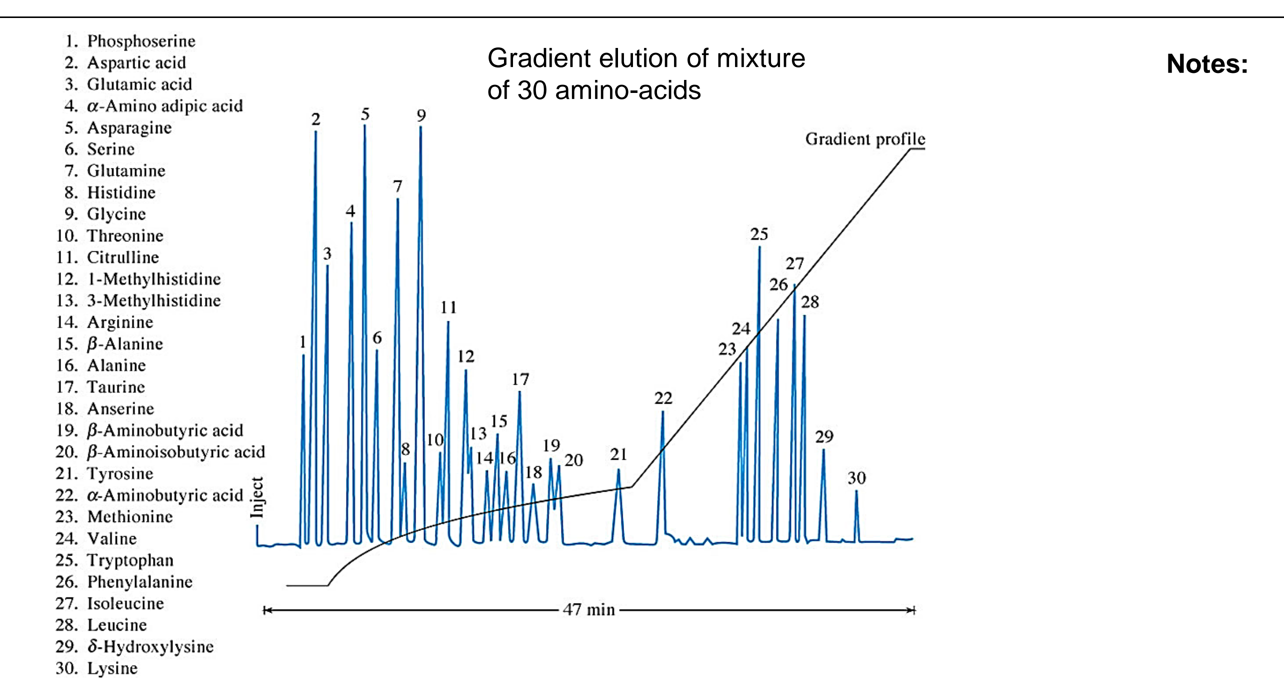
Similar to GC, solutes can be eluted from a column by using either constant column conditions or gradient elution.

Gradient elution: changing composition of mobile phase with time - solvent programming, going from a weak mobile phase to a strong one.

Weak mobile phase - solvent A
Strong mobile phase - solvent B

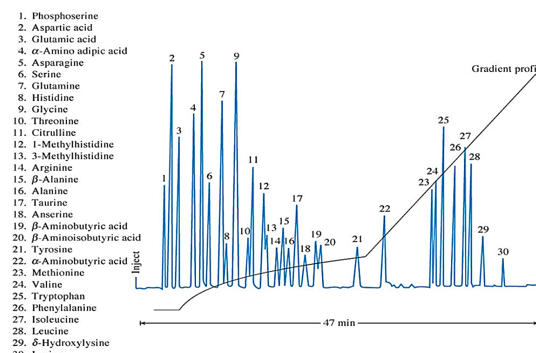
Solvent change can be stepwise, linear or non-linear





Gradient elution of mixture of 30 amino-acids

Notes:



In choosing a mobile phase for LC, several factors need to be considered:

– type of stationary phase used, determines what will be a strong or weak mobile phase;
– solubility of the solutes;
– viscosity of the mobile phase;
– type of detector used and solvent's background signal;
– purity of the solvents;
– miscibility of the solvents (for gradient elution).

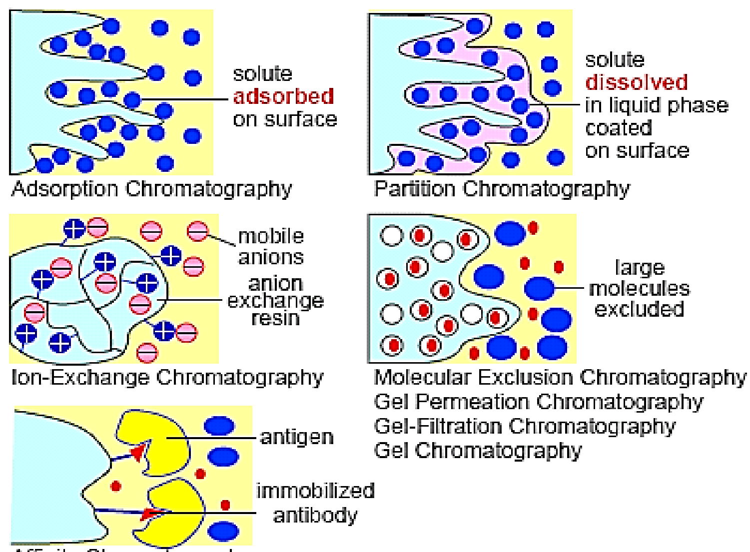
Notes:

Selection of a mobile phase for a particular LC application can be done by using various tables that summarize properties for common LC solvents:

Solvent	Refractive Index	Viscosity (cP)	Boiling Point (°C)	Polarity Index (P)	Eluent Strength (e°)
Fluoroalkanes	1.27-1.29	0.4-2.6	50-174	<-2	-0.25
cyclohexane	1.423	0.90	81	0.04	-0.2
N-hexane	1.327	0.30	69	0.1	0.01
1-chlorobutane	1.400	0.42	78	1.0	0.26
Carbon tetrachloride	1.457	0.90	77	1.6	0.18
i-propyl ether	1.365	0.38	68	2.4	0.28
toluene	1.494	0.55	110	2.4	0.29
Diethyl ether	1.350	0.24	35	2.8	0.38
tetrahydrofuran	1.405	0.46	66	4.0	0.57
chloroform	1.443	0.53	61	4.1	0.40
ethanol	1.359	1.08	78	4.3	0.88
Ethyl acetate	1.370	0.43	77	4.4	0.58
dioxane	1.420	1.2	101	4.8	0.56
methanol	1.326	0.54	65	5.1	0.95
acetonitrile	1.341	0.34	82	5.8	0.65
nitromethane	1.380	0.61	101	6.0	0.64
Ethylene glycol	1.431	16.5	182	6.9	1.11
water	1.333	0.89	100	10.2	large

Types of Liquid Chromatography

Notes:



Adsorption Chromatography is the separates solutes based on their adsorption to underivatized solid particles.

The similar to gas-solid chromatography in that the same material is used as both the stationary phase and support material.

Notes:

Advantages:	Disadvantage:
retain and separate some compounds that can not be separated by other methods separation of geometrical isomers	very strong retention of some solutes may cause catalytic changes in solutes solid support may have a range of chemical and physical environments → non-symmetrical peaks and variable retention times

Common applications of Adsorption LC:
- purification of synthetic organic compounds from reaction mixtures
- separation of geometrical isomers (ortho/meta/para, cis/trans, etc)

Adsorption chromatography stationary phase (or solid support) may be either polar or non-polar

Notes:

Adsorbent	Surface Type	Application
Silica	Slightly acidic	General Purpose – Basic compounds
Alumina	Slightly basic	General Purpose – Acidic Compounds
Charcoal	Non-polar	Non-polar Compounds
Florisil	Strongly acidic	General purpose – Basic Compounds
Polyamides	Basic	Phenols and Aromatic Nitro Compounds
Others (clay, Kieselguhr, diatomaceous earth, celite, etc.)	Relatively Non-polar	Polar Compounds

For polar supports (silica/alumina), the weak mobile phase is a non-polar solvent (hexane, benzene, etc.) and the strong mobile phase is a polar solvent (water, methanol, etc.)

For non-polar supports (charcoal), the weak mobile phase is a polar solvent and the strong mobile phase is a non-polar solvent.

Notes:

Partition Chromatography separates solutes based on their partitioning between a liquid mobile phase and a liquid stationary phase coated on a solid support.

Support Material – is usually silica, originally involved coating this support with some liquid stationary phase that was not readily soluble in the mobile phase.

Two main types of partition chromatography based on the type of stationary phase:

normal-phase liquid chromatography
reversed-phase liquid chromatography

Notes:

Normal Phase liquid Chromatography (NPLC) is the partition chromatography where the stationary phase is polar.

NPLC column strongly retains polar compounds.

- weak mobile phase is a non-polar liquid: organic solvent
- strong mobile phase is a polar liquid: water or methanol
- stationary phase must have a low miscibility with the mobile phase so the stationary phase is not dissolved from the column

Examples of liquid NPLC stationary phases:

< Dimethylsulfoxide	< Water
< Ethylene glycol	< Ethylene diamine

These liquid stationary phases slowly bleed from the column, changing the properties and solute retention time. Use stationary phases chemically attached to the support

CN	Cyanopropyl
NH_2	Aminopropyl
PSA	N-propylethylenediamine

—Si—$CH_2CH_2CH_2CN$

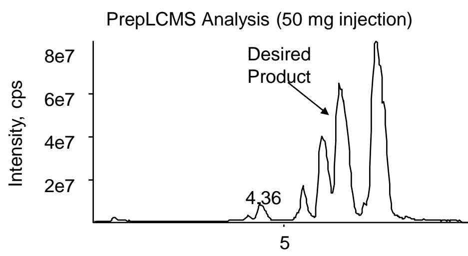
—Si—$CH_2CH_2CH_2NH_2$

—Si—$CH_2CH_2CH_2NHCH_2CH_2NH_2$

Notes:

Common applications of NPLC:

- purification of synthetic organic and inorganic compounds from reaction mixtures;
- general purpose separation of polar/non-polar compounds when the sample is in a non-polar solvent.



Automated chromatography purification of designed drug combinatorial libraries

Reverse Phase liquid Chromatography (RPLC) is the partition chromatography where the stationary phase is non-polar:

- reverse polarity of normal phase LC;
- retains non-polar compounds most strongly.

Weak mobile phase is a polar liquid: water

Strong mobile phase is more non-polar liquid: methanol or acetonitrile

Stationary phase must have a low miscibility with the mobile phase so the stationary phase is not dissolved from the column.

Examples of liquid RPLC stationary phases:

heptane	squalene
hydrocarbon polymers	dimethylpolysiloxane

	Comparison of RPLC & NPLC		
Type	**Stationary phase**	**Weak mobile phase**	**Strong Mobile phase**
RPLC	Non-polar	Polar liquid	More non-polar
NPLC	polar	Non-polar liquid	Polar liquid

Notes:

Like NPLC, these liquid stationary phases slowly bleed from the column, changing the properties and solute retention time.

Use stationary phases chemically attached to the support, C_8 and C_{18} are most common

C_{18}	Octadecyl
C_8	Octyl
C_2	Ethyl
CH	Cyclohexyl
PH	Phenyl

—Si—$C_{18}H_{37}$

—Si—C_8H_{17}

—Si—C_2H_5

Notes:

RPLC is the most popular type of liquid chromatography that may be used for separation of a wide variety of non-polar and polar solutes.

The most popularity system involves a polar solvent (e.g., water) as a weak mobile phase and is ideal for the separation of solutes in aqueous-based samples, such as biological compounds

Normal-phase chromatography

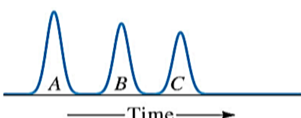
Low-polarity mobile phase

C B A

Time

Reversed-phase chromatography

High-polarity mobile phase

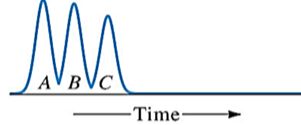


Medium-polarity mobile phase

C B A

Time

Medium-polarity mobile phase



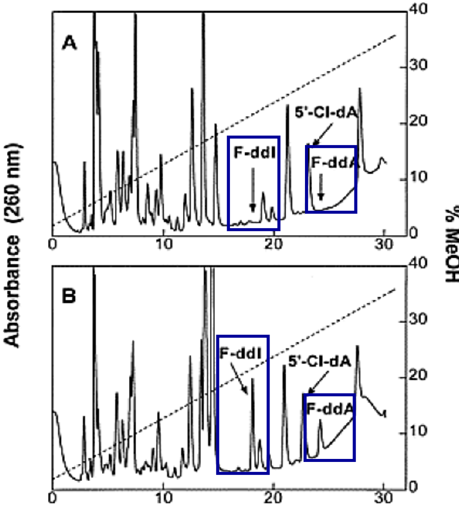
Solute polarities: $A > B > C$

Notes:

Common applications of RPLC (continued):

- purification of biological and organic compounds present in aqueous solutions

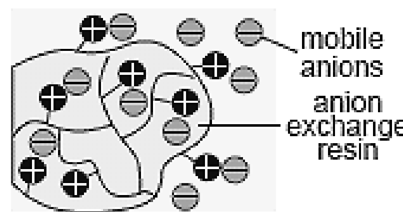
- pharmaceutical analysis (drug quantitation and quality control)
- protein & peptide mapping
- analysis of soil and water samples
- clinical analysis of blood and urine samples



RPLC Analysis of Patient blood serum for the presence of drug during the clinical trial

Notes:

Ion-exchange Chromatography (IEC) separates solutes by their adsorption onto a support containing fixed charges on its surface. A high concentration of a competing ion is often added to the mobile phase to elute the analytes from the column.



Ion-Exchange Chromatography

$$xRSO_3^-H^+ + M^{x+} \leftrightarrow (RSO_3^-)_xM^{x+} + xH^+$$

$$xRN(CH_3)_3OH^- + A^{x-} \leftrightarrow [RH(CH_3)_3^+]_xA^{x-} + xOH^-$$

Notes:

Two General Types of Stationary Phases Can be Used in IEC:

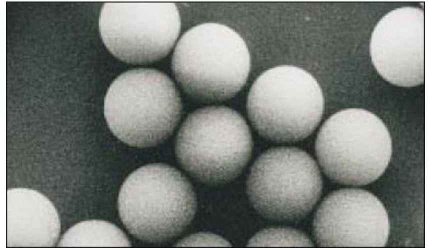
Cation-exchangers: have fixed negatively charged groups, used to separate positively-charged ions

Anion-exchangers: have fixed positively-charged groups, used to separate negatively-charged ions

Chemical Structure	Functional Group	Chemical Nature	Type of Exchange
$-SO^-H^+$	Sulfonic acid	Strong acid	Cation
$-COO^-H^+$	Carboxylic acid	Weak acid	Cation
$-CH_2COO^-H^+$	Carboxymethyl	Weak acid	Cation
$-CH_2N^+(CH_3)_3Cl^-$	Quaternary ammonium	Strong base	Anion
$-CH_2N^+(CH_3)(CH_3)-CH_2CH_2CH(Cl^-)$	Quaternary ammonium	Strong base	Anion
$-CH_2NH^+(CH_3)(CH_3)\ OH^-$	Tertiary ammonium	Weak base	Anion
$-CH_2CH_2NH^+(CH_2CH_3)(CH_2CH_3)\ OH^-$	Diethylaminoethyl (DEAE)	Weak base	Anion

Notes:

The charged groups that make up the stationary phase can be placed on several different types of support materials:


Rigid polystyrene/divinyl benzene beads

Notes:

Cross-linked polystyrene resins: for use with the separation of inorganic ions and small organic ions.

Carbohydrate-based resins: for low-performance separations of biological molecules (dextran, agarose, cellulose).

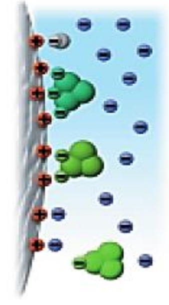
Silica-based supports: for high-performance separations of biological molecules.

A strong mobile phase in IEC:

- contains a high concentration of a competing ion for displacement of the sample ion from the stationary phase

Notes:

Cation exchange resin (K_{ex}):


Ion exchange

$Tl^+ > Ag^+ > Cs^+ > Rb^+ > K^+ > NH_4^+ > Na^+ > H^+ > Li^+$

$Ba^{2+} > Pb^{2+} > Sr^{2+} > Ca^{2+} > Ni^{2+} > Cd^{2+} > Cu^{2+} > Co^{2+} > Zn^{2+} > Mg^{2+} > UO_2^{2+}$

Anion exchange resin (Kex):

$SO_4^{2-} > C_2O_4^{2-} > I^- > NO_3^- > Br^- > Cl^- > HCO_2^- > CH_3CO_2^- > OH^- > F^-$

or

a solvent that has a pH which decreases ionization of the analyte or stationary phase

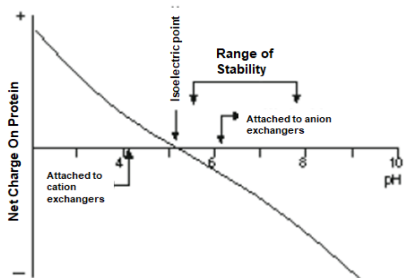
---

Factors That Affect Mobile Phase Strength Are:

Notes:

- Mobile phase pH, **especially for weak acid or base analytes and weak acid or base stationary phases;**
- Mobile phase concentration of competing ion;
- Type of competing ion.

The net charge of protein as a function of pH

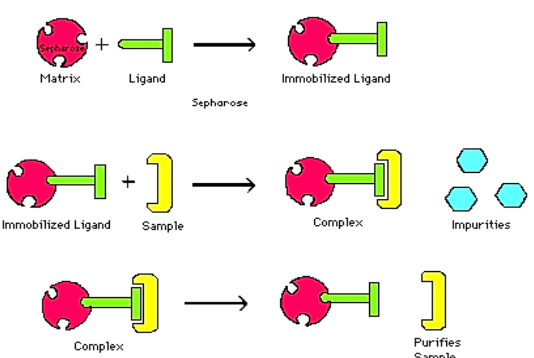


Common applications of IEC:

- Removal or replacement of ionic compounds in samples (sample pretreatment);
- Separation of inorganic ions and organic ions;
- Analysis/purification of charged biological compounds, amino acids, proteins, peptides, nucleic acids.

Affinity Chromatography (AC)

Separates based on the use of immobilized biological molecules (and related compounds) as the stationary phase



Based on the selective, reversible interactions that characterize most biological systems:

- binding of an enzyme with its substrate or a hormone with its receptor
- immobilize one of a pair of interacting molecules onto a solid support
- immobilized molecule on column is referred to as the *affinity ligand*

Notes:

Two Main Types of Affinity Ligands Used in AC:

High-specificity ligands – compounds which bind to only one or a few very closely related molecules

Affinity Ligand	Retained Compounds
Antibodies	Antigens
Antigens	Antibodies
Inhibitors/Substrates	Enzymes
Nucleic Acids	Complimentary Nucleic acids

General or group specific ligands – molecules which bind to a family or class of related molecules

Affinity Ligand	Retained Compounds
Lectins	Glycoproteins, carbohydrates, membrane proteins
Triazine dyes	NADH- or NADPH Dependent Enzymes
Phenylboronic acid	*Cis*-Diol Containing Compounds
Protein A/Protein G	Antibodies
Metal Chelates	Metal-Binding Proteins & Peptides

Note: the affinity ligand does not necessarily have to be of biological origin

Notes:

Due to the very selective nature of most biological interactions, there is interference from other components of the sample.

A weak mobile phase is usually a solvent that mimics the pH, ionic strength and polarity of the solute and ligand in their natural binding environment.

A strong mobile phase is a solvent that produces low retention for the solute-ligand interaction:

- by decreasing its binding constant

or

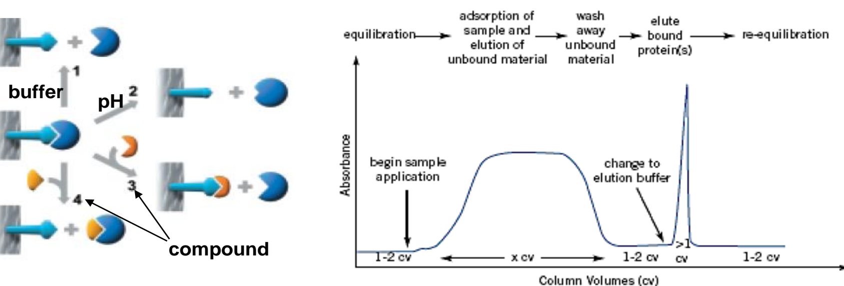
- displaces solute by the addition of an agent with competes for solute sites on the column

Notes:

Two Approaches to Elution Used in Affinity Chromatography:

Biospecific Elution - solutes are eluted by a mobile phase that contains a compound which competes with sample solutes for the ligand's active sites: very gentle and useful in the purification of active biological molecules; produces slow elution with broad solute peaks

Non-specific elution - change condition in the column to disrupt the interactions between the sample solutes and immobilized ligand: done by changing pH or ionic strength; harsher than biospecific elution; gives narrow peaks and faster run times; commonly used in analytical applications of AC

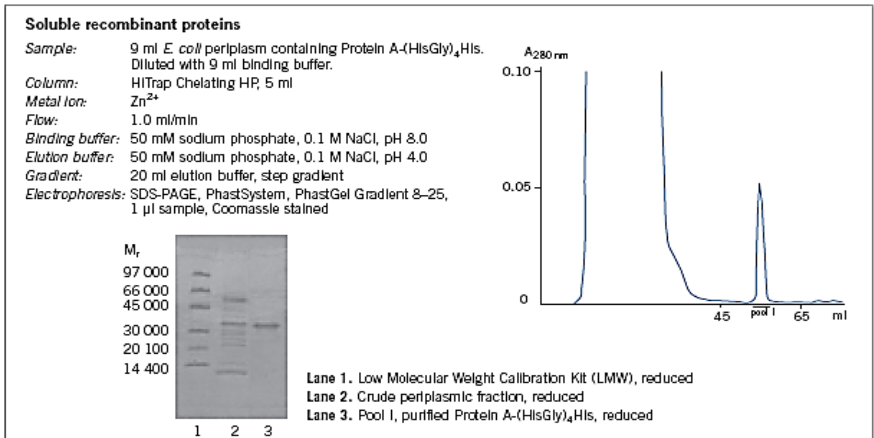


Notes:

Common applications of AC:

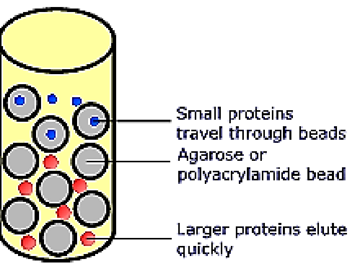
- Purification of enzymes, proteins and peptides
- Isolation of cells and viruses
- Purification of nucleic acids
- Specific analysis of components in clinical and biological samples
- Study of biomolecular interactions

Purification of His-Tag Protein Using a pH Change



Notes:

Size Exclusion Chromatography (SEC) separates molecules according to differences in their size



SEC is based on the use of a support material that has a certain range of pore sizes:

- separation based on size or molecular weight;
- as solute travels through the support, small molecules can enter the pores while large molecules can not
- since the larger molecules sample a smaller volume of the column, they elute before the smaller molecules.

SEC is based on the different interactions of solutes with the flowing mobile phase and the stagnant mobile phase:

- no true stationary phase is present in this system;
- stagnant mobile phase acts as the "stationary phase".

Notes:

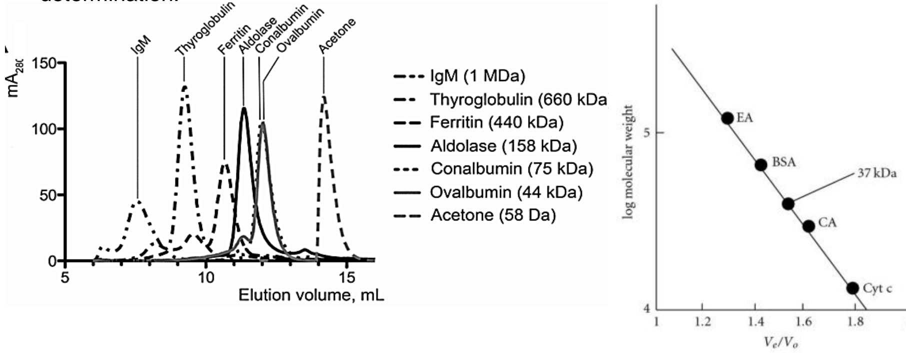
SEC does not have a “weak” or “strong” mobile phase since retention is based only on the size/shape of the analyte and the pore distribution of the support.

There are two types SEC:

gel-filtration chromatography - an aqueous mobile phase is used;

gel permeation chromatography - an organic mobile phase is used (usually tetrahydrofuran)

Common applications of SEC: separation of biological molecules (e.g., proteins from peptides); separation/analysis of organic polymers; molecular-weight determination.



Notes:

Common types of LC Detectors

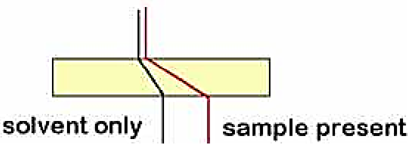
Refractive Index Detector
UV/Vis Absorbance Detector
Conductivity Detector
Electrochemical Detector
Fluorescence Detector

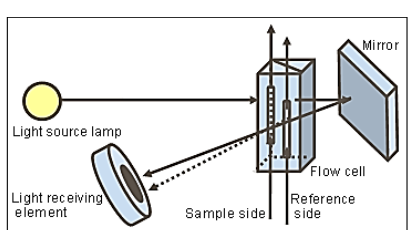
As in GC, the choice of detector will depend on the analyte and how the LC method is being used (*i.e.,* analytical or preparative scale)

Detector	Selectivity	Sensitivity	Notes
Refractive Index	Poor	Poor	Any component that differs in refractive index from the eluate can be detected, despite its low sensitivity. Cannot be used to perform gradient analysis.
UV/Vis	Moderate	Good	A wide variety of substances can be detected that absorb light from 190 to 900 nm. Sensitivity depends strongly on the component.
Fluorescence	Good	Excellent	Components emitting fluorescence can be detected selectively with high sensitivity. This is often used for pre-column and post-column derivatization.
Conductivity	Moderate	Good	Ionized components are detected. This detector is used mainly for ion chromatography.
Electrochemical	Good	Excellent	Electric currents are detected that are generated by electric oxidation-reduction reactions. Electrically active components are detected with high sensitivity.

Notes:

Refractive Index Detector (RI) Measures the overall ability of the mobile phase and its solutes to refract or bend light. One of the few universal detectors available for LC

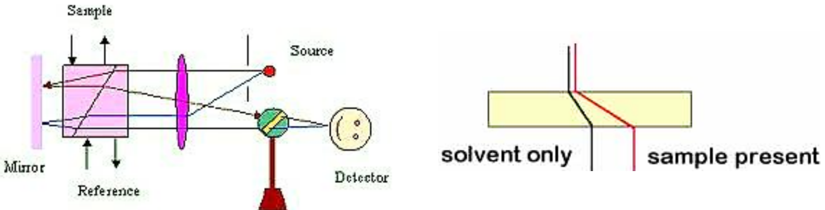




Advantages:	Disadvantage:
non-destructive and universal detector, applicable to the detection of any solute in LC applicable to preliminary LC work where the nature and properties of the solute are unknown, provided concentration is high enough for detection	high limits of detection (10^{-6} to 10^{-5} M) difficult to use with gradient elution

Refractive Index Detector (RI)

Notes:



Light from the source passes through flow-cells containing either sample stream or a reference stream

When the refractive index is the same between the two cells, no bending of light occurs at the interface between the flow-cells, maximum amount of light reaches the detector

As solute elutes, refractive index changes between reference and sample cell.

Light is bent as it passes through flow cell interface, amount of light reaching detector is decreased

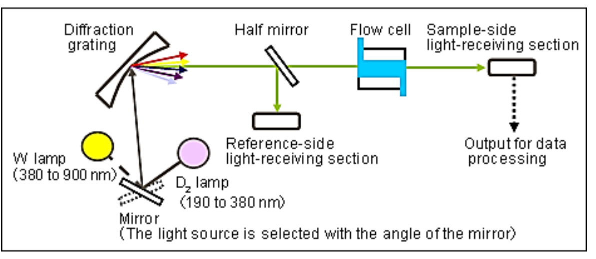
Notes:

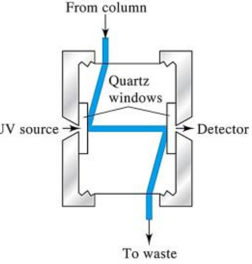
UV/Vis Absorbance Detector

Measures the ability of solutes to absorb light at a particular wavelength(s) in the ultraviolet (UV) or visible (Vis) wavelength range. It is the most common type of LC detector.

Three common types of UV/Vis Absorbance Detectors

- Fixed wavelength detectors
- Variable wavelength detectors
- Photodiode array detectors





Notes:

UV/Vis Absorbance Detector

Fixed Wavelength Detector absorbance of only one given wavelength is monitored by the system at all times (usually 254 nm).

It is the simplest and cheapest of the UV/Vis detector with low flexibility and limited in the types of compounds tested.

A single wavelength is monitored by Variable Wavelength Detector but at any given time but any wavelength in a wide spectral range can be selected. Wavelengths vary from 190-900 nm. The detector is more expensive because it requires more advanced optics, versatile, and applicable for a wider range of compounds.

Photo Diode Array Detector operates by simultaneously monitoring the absorbance of solutes at several different wavelengths:

- uses a series or an array of several detector cells within the instrument, with each responding to changes in absorbance at different wavelengths;

- the entire spectrum of a compound can be taken in a minimum amount of time;

- useful in detecting the presence of poorly resolved peaks or peak contaminants

Notes:

Applications:

UV/Vis absorbance detectors can be used to detect any compound that absorbs at the wavelength being monitored

Common wavelengths:

254 nm for unsaturated organic compounds

260 nm for nucleic acids

280 or 215 nm for proteins or peptides

Absorbance detectors can be used with gradient elution. Wavelength being monitored is above the cutoff range of the solvents being used in the mobile phase

- limits of detection for fixed and variable UV/Vis absorbance detectors are ~ 10^{-8} M
- limits of detection for photodiode array detectors are ~ 10^{-7} M

Fluorescence Detector

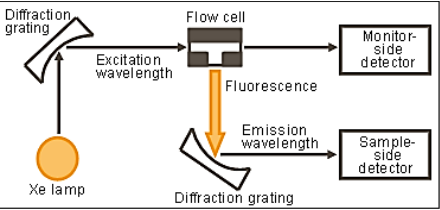
Notes:

A selective LC detector that measures the ability of eluting solutes to fluoresce at a given set of excitation and emission wavelengths.

Fluorescence can be used to selectively detect any compound that absorbs and emits light at the chosen set of excitation and emission wavelengths.

Relatively few compounds undergo fluorescence.

The method is highly selective and has a low background signal.



Limits of detection for a fluorescence detector are ~ 10^{-10} M

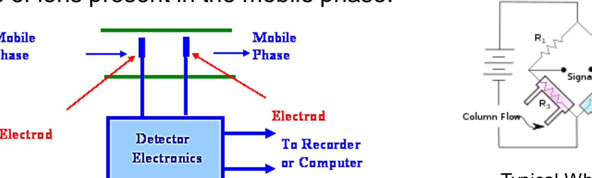
Typical applications: drugs, food additives, environmental pollutants, any compound that can be converted to a fluorescent derivative (alcohols, amines, amino acids and proteins)

Can be used with gradient elution, requires extremely pure mobile phases, trace impurities can affect background signal or quench the fluorescence of solutes.

Conductivity Detector

Notes:

Used in analytical applications of ion-exchange chromatography for the detection of ionic compounds. The detector measures the ability of the mobile phase to conduct a current when placed in a flow-cell between two electrodes. Current conducted within the cell will depend on the number and types of ions present in the mobile phase.



Two electrodes placed in mobile phase each corresponding to one arm of a Wheatstone Bridge

Typical Wheatstone Bridge

When ions flow into the sensor cell, the impedance between the electrodes changes producing an “out of balance” signal

Detector can be used: to detect any compound that is ionic or weakly ionic (high selectivity, low background signal); with gradient elution (constant ionic strength and pH of mobile phase; background conductance of the mobile phase is sufficiently low).

Typical applications: food components, industrial samples, environmental samples.

Limits of detection for a conductivity detector are ~ 10^{-6} M

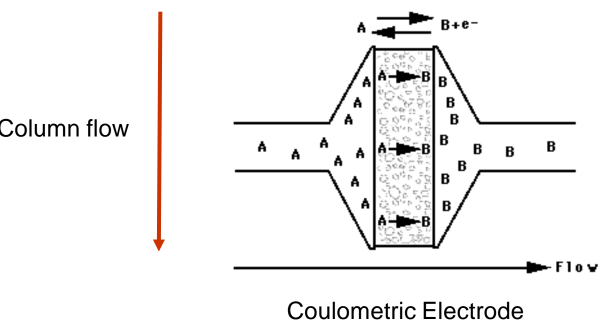
Electrochemical Detector

Notes:

Used to monitor any compound in the mobile phase that can undergo oxidation or reduction. The electrochemical detection in liquid chromatography is sometimes referred to as LC/EC.

Detector generally includes two or more electrodes which monitor the current that is produced by the oxidation or reduction of eluting compounds at a fixed potential

Generally, electrical output is an electron flow generated by a reaction that takes place at the surface of the electrodes.



Coulometric Electrode

Notes:

The electrochemical detector can be used to detect any solute that can undergo oxidation or reduction. Detectors can be made specifically for a given compound or class of compounds by properly choosing the conditions at the electrodes. Detectors are high selectivity, have low background signal.

Limits of detection for an electrochemical detector are ~ 10^{-11} M due to extreme accuracy

Compounds that can be detected by a reduction: aldehydes, ketones, esters, unsaturated compounds

Compounds that can be detected by oxidation: phenols, mercaptans (RSH), aromatic amines, dihydroxy compounds

Tasks to Section 18

1. Give definitions of these terms: chromatography, chromatogram, mobile phase, elution, ion suppressor column, chromatography column, baseline width bleed bonded stationary phase, electrochromatography, gas chromatography, gas-liquid chromatography, gas-solid chromatography.
2. A mixture of n-heptane, tetrahydrofuran, 2-butanone, and n-propanol elutes in this order when using a polar stationary phase such as Carbowax. The elution order is precisely the opposite when using a nonpolar stationary phase such as polydimethylsiloxane. Explain the order of elution in each case.
3. In a chromatographic analysis of lemon oil, a peak for limonene has a retention time of 8.36 min with a baseline width of 0.96 min. γ-Terpinene elutes at 9.54 min with a baseline width of 0.64 min. What is the resolution between the two peaks?
4. In a chromatographic analysis of low molecular weight acids, butyric acid elutes with a retention time of 7.63 min. The column's void time is 0.31 min. Calculate the retention factor for butyric acid.
5. The adjusted retention times for octane, toluene, and nonane on a particular GC column are 15.98 min, 17.73 min, and 20.42 min, respectively. Determine the retention factor for each solute, assuming the sample was injected at time t=0.

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У книзі представлені основні розділи аналітичної хімії, такі як якісний та кількісний хімічний аналіз, відбір проб та підготовка проб, обробка статистичних даних, методи розділення. Розглянуто сучасні фізико-хімічні методи аналізу. Викладено теоретичні основи методів, визначено умови та галузі їх практичного застосування. Контрольні запитання та завдання, подані в кінці кожного розділу, допоможуть користувачам закріпити вивчений матеріал.

Книга призначена для студентів спеціальностей: хімічна технологія та інженерія, біотехнологія та біоінженерія, фармація та промислова фармація. Конспект лекції складається з двох частин. Перша частина включає розділи 1-9 та охоплює загальні питання аналітичної хімії, рівняння та рівноваги, класичні методи хімічного аналізу. Друга частина включає розділи 10-18 і охоплює інструментальні методи хімічного аналізу.

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Т. М. Деркач

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