Lesson 1.

1. Fundamentals of spectroscopy.
2. The electromagnetic radiation.
3. Wave properties of radiation.
4. Quantum mechanical aspects of radiation.
5. Atomic and molecular spectroscopy.

Introduction

Analytical chemistry is the science that identifies the components of a sample (qualitative analysis) and which determines the relative amounts of each of them (quantitative analysis). Usually, requires a prior separation of the analyte of interest.

Classical methods: wet chemistry (titration, gravimetry and systematic qualitative analysis)

Instrumental Methods: exploit the physical properties of the analyte to obtain both qualitative and quantitative information

Spectroscopy: studies the interaction of the electrical field component of electromagnetic radiation with matter by means of phenomena such as absorption, emission and scattering of light.

Some spectrometric modes

Electromagnetic spectrum and its regions

Interaction of radiant energy with matter

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Interaction of radiant energy with matter

Light: electromagnetic radiation

- Light has a dual nature:
  - Corpuscular (photons)
  - Wavelike (waves)

Light: discrete energy levels

The principle of energy quantization implies that only certain discrete values of energy are possible.

Electromagnetic wave

- Frequency: number of cycles per unit time. It depends on the emission source and remains invariant regardless of the medium it travels through.
- Period: time spent in a complete cycle. \( T = \frac{1}{\nu} \) s
- Wavelength, \( \lambda \): distance between two successive maxima or minima.
- Velocity of propagation: \( v = \frac{c}{\lambda} \), \( v \) and \( \lambda \) are a function of the medium they traverse.
- Wavenumber: \( K \) of waves per cm.

In a vacuum, the speed of radiation becomes independent of wavelength and reaches its maximum value: \( c = v \lambda = 3.00 \times 10^8 \text{ m/s} \)

Electromagnetic wave

- Power, \( P \), is the energy -expressed in watts- that reaches a given area per unit of time.
- Intensity, \( I \), is the radiation power per unit solid angle.

\[
E = h \nu = \frac{hc}{\lambda} \quad \text{or} \quad h = 6.63 \times 10^{-34} \text{ J s}
\]

In a beam, \( P \) is directly proportional to the \( \theta \) of photons per second.
Superposition of waves

The amplitude of the resulting wave depends on the phase out between the individual waves.

Constructive interference \( \Rightarrow \) waves add

Destructive interference \( \Rightarrow \) waves cancel

When the two waves are in phase, \( \theta = 0 \), a maximum constructive figure of interference is obtained.

When \( \theta = 180 \), a maximum destructive interference is produced.

Transmission of light through a dense medium

The shortening of the wave is a function of the nature and concentration of the matter.

Refractive index: \( n = \frac{c}{v} \)

Scattering of light

\( l_s = \frac{8 \pi^4 \alpha^2}{(1 + \cos^2 \theta)} l_i \)

\( \alpha \): polarizability

\( r \): distance from scattering center to detector

\( \theta \): angle between incident beam & scattered beam

\( l_i \): incident beam intensity

\( l_s \): scattered radiation emitted in all directions
Most of dispersed photons keep the frequency of the source (Rayleigh dispersion); the frequency of a very small fraction of photons (approx. 1 in 10^7) has changed (Raman dispersion).

Electromagnetic radiation is said to be depolarized when both the magnetic and electric vectors reach the same magnitude in all directions. Light becomes plane-polarized when it propagates in a single plane through space.

**Polarization**: E = E_0 \cos \omega t = E_0 \cos \omega t + E_0 \sin \omega t

**Plane-polarized light**: E = E_0 \cos \omega t

**Circular-polarized light**: E = E_0 \cos \omega t - E_0 \sin \omega t

**Refraction of radiation**: Snell’s law:

\[ \frac{\sin \theta_1}{\sin \theta_2} = \frac{c}{v_2} = \frac{\lambda_1}{\lambda_2} = \frac{\mu_1}{\mu_2} \]

When medium 1 is vacuum, \( n_1 = 1 \)

\[ n_1 \sin \theta_1 = n_2 \sin \theta_2 \]

**Abrupt change in direction**: radiation always travels the shortest way (shorter time).

Fermat’s rule: radiation always travels the shortest way (shorter time).

If the surface roughness is less than the wavelength, then an actual reflection takes place, for which the law of reflection is obeyed (angle of incidence = angle of reflection). Usually only a portion of the incident beam is reflected, because the other penetrates the medium and is refracted.

**Diffuse reflection**: If the roughness of the boundary surface is comparable to the wavelength of the incident beam, a diffusion is obtained (diffuse reflection).

If the beam angle exceeds a certain limit, the so-called total reflection takes place.
Reflection of radiation

For a beam that enters an interface at right angles, the fraction reflected (reflectance) is given by:

\[ \rho = \frac{(n_2 - n_1)^2}{(n_2 + n_1)^2} \]

And, in general, \( \rho = \frac{I_i}{I} \).

The total reflective loss will be the sum of the losses occurring at each of the interfaces. The more interfaces present, the higher the beam intensity loss.

For the air \((n=1)\) to glass \((n=1.5)\) interface a 4% loss is calculated \((\rho = 0.04; 4\%)\).

See Skoog, page 142
Example 6-2

Propagation of monochromatic radiation waves through slits: Diffractive

Diffractive: every parallel beam of radiation is bent as it passes by a sharp barrier or through a narrow opening.

Diffractive is a consequence of interference as shown by Young in 1800.

Coherent radiation

Radiation emitted by a source in which all elemental waves show a constant phase relationship along space and time. (IUPAC, 1997)

Laser light is:
- Monochromatic (unique wavelength)
- Coherent (in phase)
- Directional (very narrow diverging cone)

A tungsten filament lamp is:
- Chromatic
- Incoherent
- Non-directional

A monochromatic source:
- Coherent
- Non-directional

Simplified energy levels diagram

\[ \Delta E = \Delta E_{\text{rot}} + \Delta E_{\text{vibr}} + \Delta E_{\text{int}} \]

Atoms as absorbing species: atomic (line) spectrum

The two absorbing peaks surge from the 3s electron promotion to the 3p states.
Atoms as absorbing species: atomic (line) spectrum

The two absorbing peaks surge from the 3s electron promotion to the 3p states.

Molecules as absorbing species: band spectrum

Absorption bands

Absorption spectra and molecules

Radiative and non-radiative relaxation

Atomic and molecular spectra

Black body emission: continuum of radiation via discrete lines by XR source

\[ \lambda_{\text{max}} = \frac{1}{\pi} (\text{Wien}) \]

\[ W(B) = T^4 \text{ (Stefan)} \]

\[ W = V^4 \text{ (in VS)} \]
Lesson 2

1. Design and components of optical instruments.
2. Sources of radiation.
3. Cells.
4. Wavelength selectors.
5. Detectors.

General designs of optical instruments

Block diagram for absorption

Block diagram for fluorescence and phosphorescence

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Sources of radiation in Molecular Absorption Spectrometry (MAS)

**Relevant features:**

1. Spectral distribution: intensity at different \( \lambda \) (continuum sources vs. line sources)
2. Intensity
3. Stability
   - Noise (short-term fluctuations)
   - Drift (large-term fluctuations)
4. Cost
5. Life span
6. Geometry

**A) Thermic radiation (incandescence)**

Solids, when heated, emit "black-body" radiation, a continuum band, which properties depend upon equilibrium temperature.

Since \( W \sim T^4 \), in order to obtain a stable emission, a constant temperature is required for an incandescent source.

\[
\lambda_{\text{max}} = \frac{1}{T} \quad \text{(Wien)}
\]

\[
W_{\text{B. J}} = T^4 \quad \text{(Stefan)}
\]

\[
W = v^4 \quad \text{(en el VIS)}
\]

**VIS**

1. **Tungsten filament** encapsulated in a glass bulb
   - Operates at 3,000ºC
   - An inert atmosphere prevents the oxidation of the filament
   - Useful in the 350-2,200 nm (VIS- near IR) range
   - Glass absorbs most of radiation below 350 nm
2. **Halogen lamps** in a quartz bulb
   - Halogen generates volatile tungsten halides
   - When cooling down, W re-deposits on the wire
   - Longer performing life
   - They function up to ca. 3,500ºC
   - Provide higher illumination intensity
   - High temperatures demand quartz for the walls of the bulb
   - Quartz is a better transmitter in the UV spectrum.

**B) Gas discharge lamp**

Flow of current across two electrodes in a high-pressure gas-filled tube.

Electrons collide with the gas → excitation → emission

**Deuterium lamp**

\[
D_2 + E_{\text{electric}} \rightarrow D^+_2 \rightarrow D^+ + D^\text{''} + h\nu
\]

Interval of \( \lambda \): 160 - 380 nm
Most convenient for the **UV**

**H_2 lamp**: 3-5 times less intense than **D_2** lamp
**D_2** heavier than **H_2** → less collision-induced energy losses

Interval of \( \lambda \): 180 - 370 nm
Most convenient for the **UV**
B) Gas discharge lamp
Flow of current across two electrodes in a high-pressure gas-filled tube.
Electrons collide with the gas $\rightarrow$ excitation $\rightarrow$ emission

When a particularly intense source is needed:

**Xenon Arc Lamp**
- 10-20 atm Xe atmosphere
- (200-1000 nm)
- More collisions: continuum

**Drawbacks:**
- Relatively short life
- Stray arc
- Radiant power is time-dependent

B) Gas discharge lamp
Flow of current across two electrodes in a high-pressure gas-filled tube.
Electrons collide with the gas $\rightarrow$ excitation $\rightarrow$ emission

**Hg vapour Lamp**
Useful when a few discrete and very intense lines are needed (Fluorimetry)

Sources in UV-VIS MAS:

**UV-VIS:** either H$_2$ or D$_2$ in **UV** and W in **VIS** (change along scan)

- Scanning Espectrofluorimetry:
  - **Xe arc lamp**

- Fixed-wavelength fluorimeters:
  - **Low-pressure Hg lamp**

Light amplification by Stimulated Emission of Radiation

**Spontaneous**
- excitation & emission

**Stimulated**
- excitation & emission

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Light amplification by stimulated emission of radiation

The principle of the LASER. (a) Atoms in the ground state are pumped up to the energy level \( E_2 \) by increasing photons of energy \( h\nu_{12} \). (b) Atoms at \( E_1 \) rapidly decay to the metastable state at energy level \( E_1 \) by emitting photons or exciting lattice vibrations. \( \lambda_{abs} = \lambda_{stim} \). (c) As the states at \( E_2 \) are ionized, they quickly become populated, and there is a population inversion between \( E_2 \) and \( E_1 \).

A population inversion is essential in order to achieve stimulated emission:

\[ N_2 > N_1 \]

Laser mechanism

Energy pumping mechanism

Partially transmitting mirror

Conclusions:

A population inversion is essential in order to achieve stimulated emission:

\[ N_2 > N_1 \]

Three principal approaches:

1) Blocking of undesired radiation: FILTER
2) Selection of a narrow band of \( \lambda \) after radiation dispersion: MONOCHROMATOR
3) Modulation of \( \lambda \) at different frequencies: INTERFEROMETER
Wavelength selectors

1. Filters
   - Absorption (glass, film or dye suspended in gelatin: cheapest option)
   - Cutoff
   - Interference

2. Monochromators
   - Prism
   - Grating

Filter properties

Three parameters are associated with optical filters:
1. Effective bandwidth measured at half peak height.
2. %T
3. Nominal \( \lambda \) (450 & 500 nm, in these cases)

Absorption filters (band-pass filters)
- Very low transmittance
- Broad bandwidth
- Two or more filters may be combined
- Quartz, glass, plastic
- Relatively cheap

Cut-off filters
The combination of 2 cut-off filters works as a band-pass filter

Absorption and cut-off filters combined

Interference filter

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Interference filter

Path length between surfaces:
\[ \frac{1}{\cos \phi} \]

Re-inforcement:
\[ \phi \geq 0 \Rightarrow \cos \phi \geq 1 \]
\[ m \lambda' = 2t \]

In air:
\[ \lambda = \lambda' n \]

Transmitted wavelength:
\[ \Delta = \frac{2t n}{m} \]

Interference filter performance

Typically, %T decreases as bandwidth diminishes.

Interference filter vs. Absorption filter

Wedge filter

Useful for selecting different wavelengths by simply displacing the incident beam along the filter.

Typical bandwidths are \( \geq 20 \text{ nm} \)

Monochromator: Prism

Used from UV up to IR
Construction material depends on the spectrum zone
- Glass: VIS over the 350 nm region
- Quartz: UV
- Crystal salts (NaCl, KBr): IR

Monochromator: Reflection grating

In the Echellette grating several parallel and closely spaced grooves are engraved in a surface. Number of grooves per mm depend on the spectrum region.

<table>
<thead>
<tr>
<th>Spectrum Region</th>
<th>Grooves per mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV, VIS</td>
<td>500 to 5000</td>
</tr>
<tr>
<td>IR</td>
<td>50 to 200</td>
</tr>
</tbody>
</table>

\[ d = \text{constant of the grating} \]
An echellette grating that contains 1450 blazes/mm was irradiated with a polychromatic beam at an incident angle 48º to the grating normal. Calculate the wavelengths of radiation that would appear at an angle of reflection of +20º and -10º.

\[ m\lambda = (\overline{CD} - \overline{AB}) \]
\[ \overline{CD} = d \sin i \]
\[ \overline{AB} = -d \sin r \]
\[ m\lambda = d (\sin i + \sin r) \]

When \( r = 20º \):
\[ \lambda = \frac{689.7}{m} (\sin 48 + \sin 20) = 748.4/\text{mm} \]
(748, 474, 374, 249 nm, etc.)

When \( r = -10º \):
\[ \lambda = \frac{689.7}{m} [\sin 48 + \sin (-10)] = 397.9/\text{mm} \]
(397, 199, 133 nm, etc.)

Monochromator: Dispersion

Linear dispersion: \( D = \frac{dy}{d\lambda} = \frac{F}{d\lambda} \) (variation in \( \lambda \) as a function of \( y \) (distance in the focal plane)).

Reciprocal linear dispersion: \( D^{-1} = \frac{d\lambda}{dy} = \frac{1}{F} \cdot \frac{d\lambda}{dr} \) [=] nm/mm

Angular dispersion: change in the angle of reflection when \( \lambda \) varies.
\[ A = \frac{dr}{d\lambda} = \frac{m}{d \lambda} \frac{d\lambda}{dr} \]

When \( r < 20º \Rightarrow \cos r = 1 \)
\[ \frac{d\lambda}{dr} \text{ is constant} \]

\[ \frac{d\lambda}{dr} = \frac{m}{d \lambda} \]

The less \( d\lambda/dr \) the better the angular dispersion.

Grating: 10³ - 10⁶
Prism: 10³ - 10⁴

Example: if \( R = 10³ \) and \( \lambda = 500 \text{ nm} \), then \( \Delta \lambda = 0.05 \text{ nm} \)

Example: calculate the necessary resolution to observe absorption processes at 599.9 and 600.1 nm without interference. \( R = 600 / 0.2 = 3000 \)
The light-gathering power of a monochromator is a measure of the radiant power that reaches the detector. The f-number or speed is used to quantify this parameter:

\[ f = \frac{F}{d} \]

where:
- \( F \): focal distance
- \( d \): diameter of the lens

f/number: 1 - 10

To increase the signal-to-noise ratio, the radiant power reaching the detector should be as large as possible.

The light-gathering power is \( \sim f^{-2} \)

The lower the number, the larger the light-gathering power.

A monochromator with f/2 lens gathers 4 times more light than an f/4 lens.

### Echelle monochromator

**Comparison of the performance of different \( \lambda \) selectors**

- Higher dispersion
- Improved resolution

It uses two dispersing elements: an echelle grating and a low-dispersion prism.

#### Slit width and resolution

\[ \Delta \lambda = w D \]

\[ \Delta \lambda = \frac{D \Delta y}{S_i} \]
Slit width and resolution

When $w$ is adjusted so that $\Delta \lambda = \frac{1}{2} \Delta \lambda$, a complete spectral resolution is achieved.  

Lenses, prisms and cells need to be transparent to the used radiation.  
- In the VIS region glass may be employed from 350 nm onwards  
- In the UV, quartz is most adequate  
- For IR: NaCl, KBr...  
  The heavier the atom, the better transparency in the far IR (larger $\lambda$).  
  Drawback: the mineral salts tend to absorb humidity and thus becoming fogged.

Sample containers: cells or cuvettes

Common curves for visible and ultraviolet spectroscopy. (Source: Harris, Quantitative Chemical Analysis, p. 414)
Property of the ideal transducer

- High sensitivity
- High signal-to-noise (S/N) ratio
- Constant response over a considerable range of \( \lambda \)
- Fast response time
- Zero output signal in the absence of illumination (no dark current)
- Electrical signal directly proportional to the radiant power \( P \): \( S = kP \); \( S = kP + k_d \)

Radiant energy (hv) generates a current at the interface of a semiconductor layer and a metal, as a consequence of the cleavage of covalent bonds that generates electrons and holes.

Vacuum phototube

- Simple and rugged
- Lack of sensitivity at low levels of illumination
- Shows fatigue

Photomultiplier tube (PMT)

- Highly sensitive to UV-VIS radiation
- Fast response times
- Sensitivity limited by dark current (mainly of thermal type) that calls for cooling
- PMTs are limited to measuring low-power radiation

Silicon photodiode transducer

- More sensitive than vacuum phototubes
- Less sensitive than photomultiplier tubes.

- Consists of a reverse-biased pn junction on a silicon chip.
  - When radiation impinges on the chip, holes and electrons form in the depletion layer and a current flows that is proportional to the power of the radiation.
  - More sensitive than vacuum phototubes but less sensitive than photomultiplier tubes.

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A photodiode array (PDA) is a linear array of discrete photodiodes on an integrated circuit (IC) chip. For spectroscopy it is placed at the image plane of a spectrometer to allow a range of \( \lambda \) to be detected simultaneously.

PDAs have revolutionised the design of UV-VIS instrumentation making miniaturisation feasible.

A CCD is an integrated-circuit chip that contains an array of capacitors that store charge when light creates e-hole pairs. The charge accumulates and is read in a fixed time interval. CCDs are used in similar applications to other array detectors such as photodiode arrays, although the CCD is much more sensitive for measurement of low light levels. CCDs were first invented by Willard Boyle and George Smith back in 1969 at the Bell Laboratories.

Determining the brilliance distribution of an astronomical object (star, planet, galaxy,...) with the help of a CCD is pretty much similar to the measurements of the quantity of infalling rain on a farm. As soon as the rain stops, collecting buckets are displaced horizontally on conveyor belts. Then the water content of the buckets is collected in other buckets on a vertical conveyor belt. The overall content is sent onto a weighting system.

The way a CCD works is illustrated by means of a simplified CCD made of 9 pixels, an output register and an amplifier. Each pixel is divided in 3 regions (electrodes who serve to create a potential well). (a) when an exposure is made, the central electrode of each pixel is maintained at a higher potential (yellow) than the others (green) and the charges collecting process takes place. (b) At the end of the exposure, the electrodes potentials are changed and charges transferred from one electrode to the other.
(a) By changing in a synchronized way the potential of the electrodes, electrons are transferred from pixel to pixel. Charges on the right are guided to the output register.

(b) The horizontal transfer of charges is then stopped and charge packages at the output register are transferred vertically, one by one, to an output amplifier and then read one by one. The cycle starts again until all the charges have been read (reading time of about 1 minute for a large CCD).

Mosaic of 4 CCDs, containing each 2040 x 2048 pixels. This composite detector is about 6 cm large and contains a total of 16 millions pixels (Kitt Peak National Observatory, Arizona).

Quantum efficiency curves of different types of CCDs as a function of the wavelength compared to the one of other detectors. We can see on this plot the large domain of wavelengths for the spectral response of CCDs.

Every analytical measurement involves a signal (that carries information on the analyte).

Unfortunately, every measurement contains noise (unwanted extraneous information).

Sometimes noise is large

Sometimes noise is so small that goes unnoticed

In most measurements, the average strength of the noise (N) is constant and independent of the magnitude of the signal (S).

It is a useful figure of merit for describing the quality of an analytical method or the performance of an instrument.

$$\text{S/N ratio} = \frac{S}{N}$$

A low S/N ratio provides scarce certainty in the identification and measurement of analytical signals.

As a general rule, it becomes impossible to detect a signal when the S/N becomes less than 2 or 3.
Sources of noise

- **Chemical noise:** temperature, pressure, humidity, etc.
  Variations in these parameters provoke uncontrollable fluctuations

- **Instrumental noise:** due to the components of apparatus
  - **Thermal (Johnson noise):** thermal agitation of electrons in resistors
  - **Shot noise:** crossing of electrons through a junction
  - **Flicker noise:** whichever with a magnitude inversely proportional to the frequency of the signal: 1/f
  - **Environmental noise:** composite of different forms of noise that arise from the surroundings

Thermal noise (white noise)

\[ V_{rms} = \sqrt{4kTR\Delta f} \]

- **Vrms:** root mean square noise voltage lying in a...

\[ \Delta f: \text{frequency bandwidth in Hz} \]

k: Boltzmann’s constant (1.38·10^-23 J K^-1)
T: absolute temperature in K
R: resistance of the resistive element in \( \Omega \)

Rise time, \( t_r \), of an instrument is its response time in seconds to an abrupt change in input and normally is taken as the time required for the output to increase from 10% to 90% of its final value

\[ t_r = \frac{1}{3\Delta f} \]

Shot noise

\[ I_{rms} = \sqrt{2ie\Delta f} \]

- **Irms:** root mean square current fluctuation associated with...

i: the average direct current continua promedio
\( e \): electron charge (1.60·10^-19 C)
\( \Delta f \): bandwidth of frequencies being considered

It is also a white noise and, accordingly, is the same at any frequency

Environmental noise

Hardware & software

**Hardware:**
1. Grounding and shielding (Faraday’s cage)
2. Analog filtering
3. Modulation: DC signal converted to a high frequency AC signal and ulterior demodulation
4. “Chopping” of the signal: use of a rotating disk that helps discriminating continuous from transient signals
5. Lock-in amplifiers

**Software:**
- **Low-pass filter:**
  Allows low-frequencies pass-by, while effectively removing high-frequency components (thermal and shot noises) of the signal

Useful for instruments that measure low-frequency analytical signals
High-pass filter reduces the effects of drift and low-frequency flicker noise, while allowing the high-frequency signals to pass.

Filtered Data

Find considerable application in those analytical instruments where the analytical signal is at relatively high frequency.

Smart combination of low- and high-pass filters in order to just let go through the frequencies of interest.

**Modulation - demodulation**

- By means of a chopper, the low-frequency signal is transformed into a high-frequency signal (where the 1/f noise is easier to remove).
- Once amplified, the signal goes through a high-pass filter to remove the 1/f noise.
- Finally, the signal is demodulated and a low-pass filter is employed to yield an amplified DC signal.

**S/N enhancement**

**Hardware & software**

**Software:**

1. Ensemble averaging
2. Boxcar averaging
3. Digital filtering

**1. Ensemble averaging**

Successive sets of data stored in memory as arrays are collected and summed point by point for averaging (coaddition). Then, data are averaged by dividing the sum for each point by the sets of data summed.

- If signal at “x” has a value of $S_x$.
- When a series of $n$ data are summed, the sum will be: $nS_x$.
- Noise values, $N_x$, contained in $S_x$, will also be summed -but since noise signals are random signals- they accumulate as $\sqrt{n}$.
- Thus, the noise sum for a series of $n$ data is: $\sqrt{n} \cdot N_x$.

**Ensemble averaging**

\[
\frac{S}{N} = \frac{nS_x}{\sqrt{n} \cdot N_x} = \frac{\sqrt{n}}{N_x} \cdot S_x
\]

Successive spectra registered, point-by-point summed and averaged.

**Average of Noise Spectra**

\[
\frac{S}{N} = \sqrt{n} \cdot \frac{S_x}{N_x}
\]
Boxcar averaging

- Smooths high-frequency irregularities enhancing the S/N in a waveform
- It is performed by a computer in real time as data are collected
- Its utility is limited for complex signals that change rapidly as a function of time

Sensitivity

\[ S = mc + S_{bl} \]

- Calibration
- Analytical

\[ m = \text{calibration sensitivity (slope of the calibration curve; it fails to take into account the precision of individual measurements)} \]

LOD: minimum concentration or mass of an analyte that can be detected at a known confidence level (95%)

LOQ: lowest concentration at which quantitative measurements can be made

LOD

- Minimum distinguishable signal:
  \[ S_m = S_{\text{avg}, \text{bl}} + k \cdot S_d \]
  \( S_{\text{avg}, \text{bl}} \): average signal for a blank
  \( k \): whole number (3)
  \( S_d \): standard deviation for the blank

- Minimum distinguishable concentration:
  \[ c_m = \frac{S_m - S_{\text{avg}, \text{bl}}}{m} \]
  \[ c_m = \frac{k \cdot S_d}{m} \]

LOQ

- Minimum quantifiable signal:
  \[ S_q = S_{\text{avg}, \text{bl}} + 10 \cdot S_d \]

- Minimum quantifiable concentration:
  \[ c_q = \frac{10 \cdot S_d}{m} \]

The dynamic range extends from LOQ to LOL

LOQ

- Minimum quantifiable signal:
  \[ S_q = S_{\text{avg}, \text{bl}} + 10 \cdot S_d \]

- Minimum quantifiable concentration:
  \[ c_q = \frac{10 \cdot S_d}{m} \]
1. UV-VIS spectroscopy.
2. Absorbing species in the UV-VIS.
4. Instrumentation.
5. Methods and applications.

Beer’s law: basic prerequisites
1. Radiation must be monochromatic
2. Absorbing species do so in an independent way
3. Plain and uniform absorption section
4. Absorption and desorption processes of energy are to be fast
5. Degeneration of absorbed energy should follow a non-radiational pathway
6. Refraction index of solution must remain independent of concentration

Beer’s law: deduction
\[
\frac{dP}{P_0} = -K \cdot C \cdot b \cdot P
\]
\[
\frac{dP}{P_0} = -K \cdot C \cdot b \cdot \frac{dP}{P_0}
\]
\[
\log \frac{P}{P_0} = -K \cdot C \cdot b
\]
\[
A = -\log T = abc
\]

Beer’s law: Absorbance, Transmittance and Absorption
\[
T = \frac{P}{P_0}\%
\%
A = \frac{(P/P_0) \times 100}{\%}\%
\]

\[
A = a \cdot b \cdot C
\]
\[
\varepsilon = \frac{a \cdot \text{absorptivity}}{c \cdot \text{mol-L}^{-1} \cdot \text{cm}^{-1}}
\]
Beer’s law: losses of radiant power

In an absorbing medium, radiant power exponentially decreases with the optical pathlength

\[ P = P_0 \times 10^{-eb} \]

Experimental, only radiant power at the exit of the cell is measured

% Transmissions

Absorbance

Beer’s law: Correction of radiant power losses

\[ \text{Abs} = -\log T = \log \frac{P_0}{P_1} \]

In an absorbing medium, radiant power exponentially decreases with the concentration of the absorbing analyte

\[ P = P_0 \times 10^{-ebc} \]

Beer’s law: examples

- **Parsol 340** (10^{-4} M in MEOH)
  - **Beer-Lambert plot** for Parsol 340 in ethanol
    - \( y = 13245x + 0.0351 \)
    - \( R^2 = 0.9995 \)

- **T vs \[P340]\**
  - \( y = 0.9224e^{-30498x} \)
  - \( R^2 = 0.9995 \)

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Beer’s law: examples

\[ \text{Abs} = e \cdot b \cdot C \]

Values as high as \(10^5 \text{ L mol}^{-1} \text{ cm}^{-1}\) may be found for the proportionality constant.

Additivity of Beer’s law

\[ A = \sum e_i b \cdot C_i \]

Absorbances are always additive.

Deviations from Beer’s law

Intrinsic to the law
- Dependence of \(e\) on \(n\)
- Molecular interactions
- Non-absorbing processes

Chemical
- Solution equilibria
- Solvent
- Impurities in reagents
- Presence of absorbing interferents

Instrumental
- Polychromatic radiation
- Parasitic or stray light
- Instrumental noise
- Slit width

Reactions involving the absorbing species make Beer’s law untenable. Analyte associates, dissociates, or interacts with solvent to produce a product with different absorption spectrum than starting material.

Example: if \(K_a = 10^{-4}\)

<table>
<thead>
<tr>
<th>(C_{HIn})</th>
<th>(\text{H}^+) (\rightarrow) (\text{In}^-)</th>
<th>(\text{H}^+) (\rightarrow) (\text{HIn}^-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10^{-4})</td>
<td>8.5x10^{-5}</td>
<td>9.2x10^{-5}</td>
</tr>
<tr>
<td>(10^{-3})</td>
<td>3.8x10^{-4}</td>
<td>2.7x10^{-4}</td>
</tr>
</tbody>
</table>

Expected

Absorbance

Work out Ex. 13.1 in Skoog
An isosbestic point is observed whenever two absorbing species in equilibrium (X, Y) exhibit identical molar absorptivity at a given wavelength. Total absorbance at the wavelength of the isosbestic point is independent of relative concentrations of both species in equilibrium.

From an analytical point of view, an isosbestic point is very convenient since linear calibrations may be attained in an unbuffered solution when measurements are made at the wavelength of the isosbestic point.

\[ A = \varepsilon_b ([HIn] + [In^-]) \]

Deviations from Beer’s law

**Effect of polychromatic radiation**

In practice, monochromators select a band of radiation.

At \( \lambda \):

\[ A' = \log \frac{P_2}{P_1} = \varepsilon \lambda \]

\[ P_1 = P_2(\varepsilon_1 \lambda)^{-1} \]

The measured absorbance \( A_m \) is:

\[ A_m = \log \left( \frac{P_2}{P_1} \right) \]

\[ A_m = \varepsilon \lambda \]

Only when the molar absorptivity are the same at the wavelength \( \lambda \) and \( \lambda' \), the absorbance equation simplifies to:

\[ A_m = \varepsilon \lambda \]

Deviations from Beer’s law

**Effect of stray light**

Radiation from the instrument that is outside the nominal wavelength band chosen for the determination. It is the result of scattering and reflection off the surfaces of gratings, lenses or mirrors, filters and windows.

\[ A = \log \frac{P_2}{P_1 + P_3} \]

Stray light will cause more negative deviations at high concentrations and at longer path lengths, since both circumstances imply a smaller \( P \).

**Photometric error**

\[ A = -\log T + c \cdot C \]  
Equation (1)  
Uncertainty in measurement of \( T \) affects report on \( C \)

\[ \frac{dA}{dT} = -\frac{1}{T} + c \frac{dC}{dT} \]  
Differentiating:

\[ \frac{dA}{dT} \preceq \frac{dC}{dT} \]  
Equation (2)

Dividing (2) - (1):

\[ \frac{dA}{dT} = \frac{-0.434}{T} + \frac{c}{C} \]  
Writing it as finite increments:

\[ \frac{\Delta C}{C} = \frac{\Delta T \log T}{0.434} \]  
Derivative:

\[ \frac{\Delta T \log T}{0.434} \]  
Writing it as finite increments:

\[ \Delta C = \frac{\Delta T \log T}{0.434} \]  
Writing it as finite increments:

\[ \Delta C = \frac{\Delta T \log T}{0.434} \]

The relative error in the determination of \( C, \Delta C/C \), is a function of \( \Delta T \)

Error will be minimised when \( T \log T \) reaches a maximum:

\[ T \log T = 1 \]  
\[ \frac{d}{dT} (T \log T) = 1 + \log T = 0 \Rightarrow -\log T = 1 \]

\[ -2.303 \log T = 1 \Rightarrow 2.303 \]

\[ \Delta C = 100 \% \]  
\( \Delta C \) from 0.2 to 0.8

| \% T = 36.8% |

Uncertainty in measurement of \( T \) affects report on \( C \)

| \( A = -\log T + c \cdot C \) |

Deviations from Beer’s law

**Instrumental**

<table>
<thead>
<tr>
<th>Photometric error</th>
<th>Contribution of different types of noise</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Dark current (%T)</td>
<td>0.01%T</td>
</tr>
<tr>
<td>• Precision in reading (analog apparatus)</td>
<td>0.5%T (1-3% error in determination of ( C ))</td>
</tr>
<tr>
<td>• Shot noise</td>
<td>0.5%T (1-3% error in determination of ( C ))</td>
</tr>
<tr>
<td>• Flicker noise</td>
<td>(especially over 95%T)</td>
</tr>
<tr>
<td>• Uncertainty in the position of the cell</td>
<td>(at high transmittance)</td>
</tr>
<tr>
<td>• Uncertainty in the position of the cell</td>
<td>(is not a relevant factor)</td>
</tr>
</tbody>
</table>

**Types and Sources of Uncertainty in Transmittance Measurements**

<table>
<thead>
<tr>
<th>Component</th>
<th>Classification of Noise</th>
<th>Typical Sources</th>
<th>Likely To Be Important In</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case I</td>
<td>( \lambda = \lambda_0 )</td>
<td>Laser-induced noise</td>
<td>Laser-induced noise and spectrophotometers used in small samples or digital systems</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heat dissipation damages</td>
<td>Heat dissipation damages and spectrophotometers and photometers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark current and offset noise</td>
<td>Dark current and offset noise and spectrophotometers</td>
</tr>
<tr>
<td>Case II</td>
<td>( k_2 = k_1 )</td>
<td>Photoelectric shot noise</td>
<td>Photoelectric shot noise, detectors, and spectrophotometers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chain reaction in opportunity</td>
<td>Photoelectric shot noise and spectrophotometers</td>
</tr>
<tr>
<td>Case III</td>
<td>( i_0 = i_0' )</td>
<td>Cell producing unbalance</td>
<td>Cell producing unbalance and spectrophotometers</td>
</tr>
</tbody>
</table>

**From Dr. J.M. Fernández**
Absorbing species

Absorption of UV-VIS radiation generally results from excitation of bonding electrons ($\pi\rightarrow\pi^*$), so that the wavelengths of absorption can be correlated with the types of bonds in the species under study.

- $\pi\rightarrow\pi^*$: saturated hydrocarbons (for UV, <185 nm)
- $\pi\rightarrow\pi^*$: organic molecules with donor atoms such as O, S, N and Cl (180-230 nm)
- $n\rightarrow\pi^*$: aromatic compounds (200-700 nm)

Most relevant and useful in UV-VIS spectrophotometry:
- Molar absorptivities ($\varepsilon$):
  - $n\rightarrow\pi^*$: $10^2$–$10^3$ L cm$^{-1}$ mol$^{-1}$
  - $\pi\rightarrow\pi^*$: $10^2$–$10^3$ L cm$^{-1}$ mol$^{-1}$

### Absorbing species: chromophores

<table>
<thead>
<tr>
<th>Chromophore</th>
<th>Example</th>
<th>Excitation</th>
<th>$\lambda_{max}$ (nm)</th>
<th>$\varepsilon$</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=C</td>
<td>Ethene</td>
<td>$\pi\rightarrow\pi^*$</td>
<td>171</td>
<td>15,000</td>
<td>hexane</td>
</tr>
<tr>
<td>C=C</td>
<td>1-Hexene</td>
<td>$n\rightarrow\pi^*$</td>
<td>180</td>
<td>10,000</td>
<td>hexane</td>
</tr>
<tr>
<td>C=O</td>
<td>Ethanol</td>
<td>$\pi\rightarrow\pi^*$</td>
<td>290</td>
<td>15</td>
<td>hexane</td>
</tr>
<tr>
<td>C=O</td>
<td>Ethanol</td>
<td>$n\rightarrow\pi^*$</td>
<td>180</td>
<td>10,000</td>
<td>hexane</td>
</tr>
<tr>
<td>N=O</td>
<td>Nitromethane</td>
<td>$n\rightarrow\pi^*$</td>
<td>275</td>
<td>17</td>
<td>ethanol</td>
</tr>
<tr>
<td>C-X</td>
<td>X=Br</td>
<td>$n\rightarrow\sigma^*$</td>
<td>205</td>
<td>200</td>
<td>hexane</td>
</tr>
<tr>
<td>C-X</td>
<td>X=I</td>
<td>$n\rightarrow\sigma^*$</td>
<td>235</td>
<td>300</td>
<td>hexane</td>
</tr>
</tbody>
</table>

### Absorbing species: chromophores

**It is worth to emphasize:**

Intense absorption bands ($\varepsilon >10^4$) are obtained for transitions that are more likely to occur (bands $n\rightarrow\pi^*$), whereas less intense absorption processes belong to less probable transitions ($n\rightarrow\pi^*$).

Every functional group has typical absorption bands with characteristic intensities (given by the values of $\varepsilon$).

UV-VIS radiation absorption is associated with the chromophore rather than with the molecule as a whole. This fact enables the identification of functional groups based on both $\lambda_{max}$ and $\varepsilon$.

Conjugation of chromophores gives rise to an increase in intensity ($\varepsilon$) and dramatic changes in the $\lambda_{max}$.

Most spectra of organic compounds are usually complex:

- Superposition of vibrational transitions on the electronic transitions leads to overlapping lines
- Broad absorption bands
- Detailed theoretical analysis and unequivocal identification becomes difficult or even impossible.
- Semi-quantitative and qualitative statements can be made concerning the types of electronic transitions involved.
- Solvent and substituents effects may blur the situation but -at the same- may provide useful information to elucidate the type of transition.
Chromophores and auxochromes

Chromophores: functional groups able to absorb in either the VIS or in the near UV (λ > 200 nm) when bonded to a non-absorbent saturated organic moiety.

Auxochromes: functional groups with non-bonding valence electrons, n, that are unable to absorb at λ > 220 nm, but rather strongly absorb in the far UV (λ: 180-200 nm), due to n → σ* transitions. Examples: -OH; -NH2; -Cl, etc.

- When an auxochrome group get associated with the chain of a chromophore,
  - the intensity increases (hyperchromic effect)
  - and the absorption band is shifted to larger wavelengths (bathochromic effect or red shift).
- Positions of absorption maxima are influenced by the nature of the solvent. In the presence of a more polar solvent, λmax becomes shorter (hypsochromic effect or blue shift).
- The decrease in the intensity of an absorption band is called hypochromic effect.
- Conjugation of chromophores produces a red shift.

Positions of absorption maxima are influenced by the nature of the solvent. In the presence of a more polar solvent, λmax becomes shorter (hypsochromic effect or blue shift).

The presence of multiple chromophores has scarce influence on λmax, but notably increases ε.

Combination of chromophores

Some chromophores and auxochromes

- UV spectra of aromatic hydrocarbons are characterized by three sets of bands that originate from π → π* transitions.

- Polar solvents such as water, alcohols, esters and ketones tend to obliterate spectral fine structure arising from vibrational effects.

An increase in the polarity of the solvent used, causes:

- n→σ*: hypsochromic (blue) shift
- n→π*: hypsochromic (blue) shift
- π→π*: bathochromic (red) shift

Substituents in aromatic compounds

<table>
<thead>
<tr>
<th>Component</th>
<th>E1 band</th>
<th>E2 band</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetone</td>
<td>293</td>
<td>254</td>
</tr>
<tr>
<td>benzoic</td>
<td>240</td>
<td>240</td>
</tr>
<tr>
<td>benzene</td>
<td>256</td>
<td>256</td>
</tr>
<tr>
<td>benzyl</td>
<td>254</td>
<td>254</td>
</tr>
<tr>
<td>chloroacetone</td>
<td>254</td>
<td>254</td>
</tr>
<tr>
<td>phenol</td>
<td>254</td>
<td>254</td>
</tr>
<tr>
<td>pyridine</td>
<td>254</td>
<td>254</td>
</tr>
<tr>
<td>toluene</td>
<td>254</td>
<td>254</td>
</tr>
</tbody>
</table>

Spectra are given in nm.
Common solvents, influence of polarity

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Lower Wavelength Limit, nm</th>
<th>Lower Wavelength Limit, cm</th>
<th>Non-polar solvents tend to keep the gas-phase like fine spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>180</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>220</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Hexane</td>
<td>280</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>380</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>260</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Metals belonging to series 4f and 5f absorb radiation which energy matches transitions involving electrons f.

The effect of either solvent or ligand is unnoticeable, since electrons are in internal shielded levels.

Absorption spectra show well-defined and sharp signals.

It involves the presence of an acceptor (metallic ion) and a donor (ligand) of e⁻. A charge-transfer complex consist of an electron-donor group bonded to an electron acceptor.

The radiation absorption makes possible the transfer of one electron from the orbital in the donor to an orbital in the acceptor.

A kind of red-ox reaction takes place in the excited state.

High intensities (ε > 10,000 L mol⁻¹ cm⁻¹)

Ex.: Fe(SCN)³⁻ + H₂O → Fe²⁺ + SCN⁻
Four basic types of instrumental designs

- Single beam
- Space-resolved double beam
- Time-resolved double beam
- Multichannel

**Single beam**

**Space-resolved double beam**

Signal outputs from the sample and reference (blank) cuvettes are **simultaneously** measured and subtracted.

Two detectors are necessary ⇒ high cost

**Time-resolved double beam**

This is the mostly used commercial design

Advantages of the double beam vs. the single beam:
- Source’s power fluctuations are corrected
- Compensates the drift in the detector and in the amplifier (time-resolved)
- Compensates the variations of the radiant power at different wavelengths

**Multichannel**

- A complete spectrum (scan) can be recorded in 0.1 s
- Signal is averaged over 1 s or more to enhance the S/N ratio
- Scarce # of optical components: minimal loss of beam’s power
- D2 lamp and resolution (band width) of 2 nm

A deuterium lamp and its spectrum

\[ D_2 + E_\text{e} \rightarrow D^*_2 \rightarrow D^+ + D + h\nu \]

Excited deuterium ions emit light, a heated oxide-coated filament provides electrons.

Tungsten filament lamp and its spectrum
Instrumentation: common cells for UV-VIS

- Volume ($\mu$L): 160, 100 or 50

b = 10 mm

Instrumentation: detectors

Instrumentation: typical Instruments

**Photometers**
- Simple instrument that use filters to isolate the $\lambda$
- They can only carry out sequential measurements at every given $\lambda$
- Very little loss of light power (scarce # of optical devices) ⇒ good S/N
- Cheap and affordable

**Spectrophotometers**
- They use a monochromator or a dispersive device in order to select the $\lambda$
- The scan of $\lambda$ is feasible ⇒ record of spectra
- More complex optic ⇒ strongly affected S/N
- Expensive

Instrumentation: photometer (VIS)

Read through Skoog’s section 13D-3

Instrumentation: photometer (UV)

- Commonly used in HPLC
- It uses a Hg lamp and a filter to select a line at 254 nm
- Practical to quantitate organic compounds absorbing at 254 nm.

**Probe photometer**
- It conveys light through the sample by means of optic fiber and a mirror.
- It is appropriate for field instruments
- Eliminates the need for a cell.

Instrumentation: spectrophotometer (VIS)

- Low cost: 450 – 2500 €
- Range: 380 – 800 nm
- Resolution: 8 – 20 nm
Instrumentation: single and double beam UV-VIS spectrophotometers

- Normally, they use interchangeable W and D₂ lamps
- Range: 200 – 900 nm
- Cost: 3000 – 8000 € (single beam); 4000 – 15000 € (double beam)
- Resolution: 0.5 – 8 nm (single beam); 0.1 – 3 nm (double beam)

Instrumentation: double beam UV-VIS spectrophotometers

- Light traverses twice through the dispersing device, largely improving the resolution
- Expensive: > 10,000 €
- Bandwidth: 0.07 nm
- Stray light: 0.0008%

Instrumentation: photodiode array UV-VIS spectrophotometers

- They may become very compact in size
- Fast record of spectra: 0.1 s
- Range: 200 – 820 nm
- Bandwidth: 2 nm
- Cost: 3000 - 20000 €

Elucidation of stoichiometry: method of continuous variations (Ordinate of Job)

Iso-molar solutions of both cation and ligand are mixed in different volume ratios so that the total volume of every sample is the same.

\[
M_0 + C_L = \text{const.}
\]

<table>
<thead>
<tr>
<th>M</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>10</td>
<td>9</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>ml</td>
</tr>
</tbody>
</table>

At a beforehand selected \( \lambda \), the absorbances are measured for every resulting solution and a correction is made in order to subtract the absorbance of the mixture had it not reacted.

\[
X_M + X_L = 1
\]
Elucidation of stoichiometry: method of continuous variations (Ordenate of Job)

\[ AJ = \epsilon_{MLn} [MLn] + \epsilon_{M} [M] + \epsilon_{L} [L] \]  
uncorrected.  
Subtracting the correction:

\[ AJ = \epsilon_{MLn} [MLn] + \epsilon_{M} [M] + \epsilon_{L} [L] - \left( \epsilon_{M} \cdot C_M + \epsilon_{L} \cdot C_L \right) = \]

\[ AJ = \epsilon_{MLn} [MLn] + \epsilon_{M} [M] + \epsilon_{L} [L] - \epsilon_{M} [MLn] - \epsilon_{L} \cdot n [MLn] \]

\[ AJ = (\epsilon_{MLn} - \epsilon_{M} - n \cdot \epsilon_{L}) [MLn] = K [MLn] \]

In the maximum:

\[ \frac{\partial AJ}{\partial x} = 0 \quad \Rightarrow \quad \frac{[MLn]}{[M]} = \frac{C_M}{C_L} = \frac{C_{MLn}}{n[C_{MLn}]} \]

\[ \frac{[MLn]}{[M]} = K_{fs} \]

Determination of acid-base equilibrium constant

\[ K_f = [H^+] \frac{[\text{In}]}{[\text{HIn}]} \quad \text{pK}_f = \text{pH} + \log \frac{[\text{In}]}{[\text{HIn}]} \]

Absorptions of solutions with a known identical analytical concentration (C_i) are measured at three different pHs.

1. Acidic pH (\text{HIn})
   \[ A_i = \varepsilon_{\text{HIn}} C_i \Rightarrow \varepsilon_{\text{HIn}} \]

2. Alkaline pH (\text{In}⁻)
   \[ A_i = \varepsilon_{\text{In}⁻} C_i \Rightarrow \varepsilon_{\text{In}⁻} \]

3. Intermediate pH (\text{HIn} + \text{In}⁻)
   \[ C_i = [\text{HIn} + \text{In}⁻] \]
   \[ A_i = \varepsilon_{\text{HIn} \cdot C_i \cdot [\text{In}⁻]} \Rightarrow \varepsilon_{\text{HIn} \cdot C_i \cdot [\text{In}⁻]} \]

By experimentally measuring the pH of the third solution and substituting, \( K_f \) is obtained.
### Determination of the degree of dissociation

\[
\text{HIn} \rightleftharpoons \text{In}^- + \text{H}^+ \quad K = \frac{c \times (1 - \alpha)^2}{c^2 \times \alpha} \quad \frac{c \times (1 - \alpha)^2}{c^2 \times \alpha}
\]

\[
A_3 = \varepsilon_{\text{HIn}}[c(1-\alpha)] + \varepsilon_{\text{In}^-} c \alpha = \varepsilon_{\text{HIn}} c - \varepsilon_{\text{H}} c (1-\alpha)
\]

\[
A_2 = A_1 + \alpha (\varepsilon_{\text{HIn}} c - \varepsilon_{\text{H}} c) = A_1 + \alpha (A_2 - A_1)
\]

\[
\alpha = \frac{A_3 - A_1}{A_2 - A_1}
\]

### Applications: qualitative analysis

- **Analysis of functional groups** (complement to IR and RMN)
- **Quality control**: Certain products must be transparent to UV-VIS radiation
  - Ex.: Ethyl alcohol 96º may contain benzene and thus it is mandatory to undergo purification until it does not absorb (184, 204, 256 nm).
- **Elucidation of structural isomers**: Ex.: cis-trans biphenyls
  - Ex.: keto-enol tautomerism of the ethyl acetocacetate
    \[
    \text{H}_2\text{C} = \text{C} \text{O} \text{C}_2\text{H}_5 \quad \lambda_{\text{max}} = 275 \text{ nm} \quad \varepsilon = 20 \text{ Lmol}^{-1}\text{cm}^{-1}
    \]
    \[
    \text{H}_2\text{C} = \text{C} \text{O} \text{C}_2\text{H}_5 \quad \lambda_{\text{max}} = 240 \text{ nm} \quad \text{This form exhibits conjugated double bonds} \quad \varepsilon = 18000 \text{ Lmol}^{-1}\text{cm}^{-1}
    \]

### Applications: quantitative analysis by means of photometric titrimetry

\[
\text{A}^+ (\text{analyte}) + \text{T}^- (\text{titrant}) \rightarrow \text{P}^- (\text{product})
\]

<table>
<thead>
<tr>
<th>Substance</th>
<th>pH</th>
<th>(\lambda_{\text{max}}) (nm)</th>
<th>(\varepsilon(\text{Lmol}^{-1}\text{cm}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>7</td>
<td>296</td>
<td>1500</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>6</td>
<td>275</td>
<td>3.000</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>6</td>
<td>280</td>
<td>5.000</td>
</tr>
<tr>
<td>Adenosine-5'-phosphate</td>
<td>7</td>
<td>259</td>
<td>15.000</td>
</tr>
<tr>
<td>Cytidine-5'-phosphate</td>
<td>7</td>
<td>271</td>
<td>9.000</td>
</tr>
<tr>
<td>Uridine-5'-phosphate</td>
<td>7</td>
<td>262</td>
<td>10.000</td>
</tr>
<tr>
<td>Guanosine-5'-phosphate</td>
<td>7</td>
<td>252</td>
<td>14.000</td>
</tr>
</tbody>
</table>

### Applications: determination of amino acids and nucleotides

- A class of biological molecules containing a ring of seven, fifteen, or any arbitrarily large number of atoms
- Important class is porphyrins which include the heme group and Chlorophyll a
- Heme has a \(n-n^*\) transition in the blue region at 400 nm. Chlorophyll a \(n-n^*\) transition in the 650 nm region to give it its green color
Applications: conformational studies

Useful to characterise the conformation of nucleic acids and proteins

- DNA melting: Hypochromism: Decrease in $\varepsilon$ upon formation of double helix (H-bond)

External standards are used to calibrate instruments and procedures when there are no interference effects from matrix components in the analyte solution.

Standard-addition method

Standard-addition methods are particularly useful for analyzing complex samples in which the likelihood of matrix effects is substantial.

1. Prepare several identical aliquots, of volume $V_x$, of unknown sample.
2. Add (spike) to each sample aliquot an increasing volume, $V_s$, of a standard solution of known concentration, $C_s$, of the analyte to be determined in the sample.
3. Dilute each solution to a fixed volume, $V_t$.
4. Carry out the experimental measurement in each solution.
5. Plot the calibration curve.
6. Calculate the analyte concentration, $C_x$, in the sample.

Where:

- $S$: instrument response or analytical signal
- $k$: proportionality constant
- $V_x$: variable volume of standard solution added
- $C_s$: concentration of the analyte in the standard solution
- $V_s$: volume of the unknown solution in each aliquot
- $C_x$: concentration of analyte in the unknown solution
- $V_t$: total volume of the flasks after diluting

Standard-addition method: example

Ex.: As present in a biological sample was determined by the standard-addition method. Several 10.00 mL aliquots of the unknown sample were transferred to 100.00 mL flasks. Afterwards, increasing volumes of a 22.1 ppm As standard were added to 4 out of the 5 volumetric flasks, and then the 5 flasks were diluted to the mark with distilled water. From the absorbance readings provided, calculate the concentration of As in the unknown sample.

<table>
<thead>
<tr>
<th>Standard (mL)</th>
<th>Sample (mL)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>10.00</td>
<td>0.298</td>
</tr>
<tr>
<td>2.50</td>
<td>10.00</td>
<td>0.395</td>
</tr>
<tr>
<td>5.00</td>
<td>10.00</td>
<td>0.399</td>
</tr>
<tr>
<td>7.50</td>
<td>10.00</td>
<td>0.374</td>
</tr>
<tr>
<td>10.00</td>
<td>10.00</td>
<td>0.353</td>
</tr>
</tbody>
</table>

Arsenic Standard Addition

$C_x = \frac{b C_s}{mV_s}$
Determining the concentration by standard-addition method

1. Get ready two identical aliquots of the unknown sample.
2. Make a standard spike in just one of the aliquots.
3. Dilute both aliquots to the mark and do the instrumental measurement.

\[
C_a = \frac{S \cdot C_v \cdot V_v}{S_v - S_1 \cdot V_v}
\]

where:
- \( C_a \): concentration of analyte
- \( S \): signal arising from the internal standard
- \( C_v \): concentration of the standard
- \( V_v \): volume of the standard
- \( S_1 \): signal arising from the analyte
- \( S_v \): total signal

The quinine spike amounts to:

\[
\text{V}_{\text{standard}} = \frac{(\text{final absorbance} - \text{initial absorbance}) \times \text{initial volume}}{\text{final absorbance}}
\]

Taking into account the 10:100 dilution, concentration in the sample is:

\[
\frac{\text{C} \times \text{vol}_{\text{final}}}{\text{vol}_{\text{initial}}} = \text{C}_{\text{sample}}
\]

Example: a 25.00 mL aliquot of an aqueous solution that contains quinine was diluted to 50.00 mL and its absorbance at 348 nm was seen to be 0.416 when a 1.00 cm path length cell was used. A 10.00 mL spike from a 23.4 ppm stock solution of quinine was done and the final volume was made up to 50.00 mL with deionised H2O. The absorbance of this latter solution was measured to be 0.610. Calculate the quinine concentration in the sample.

\[
\frac{23.4 \times 10 \times 0.416}{100 \times 50} = 0.0468 \text{ mg mL}^{-1}
\]

Sanalyte/Sint'l std

\[
\text{Sanalyte} = \frac{\text{C}_{\text{analyte}}}{\text{C}_{\text{standard}}}
\]

For A = 0:

\[
A = \frac{I(422.7) - I(460.7)}{I(460.7)}
\]

Note: This method assumes a linear relationship between instrumental response and sample concentration.

S1 S2 V1 V2

\[
\frac{S_{\text{standard}} - \text{vol}_{\text{standard}}}{\text{vol}_{\text{initial}}} = \text{C}_{\text{unknown}}
\]

Standard-addition method: example

Ex: As present in a biological sample was determined by the standard-addition method. Several 10.00 mL aliquots of the unknown sample were transferred to 100.00 mL flasks. Afterwards, increasing volumes of a 22.1 ppm As standard were added to 4 out of the 5 volumetric flasks, and then the 5 flasks were diluted to the mark with distilled water.

From the absorbance readings provided, calculate the concentration of As in the unknown sample.

Concentration of analyte

\[
S_{\text{signal}} (\text{at} N \text{ mg mL}^{-1})
\]

\[
\text{Concentration of analyte} = \frac{S_{\text{signal}}}{S_{\text{signal}} (\text{at} N \text{ mg mL}^{-1})}
\]

Emission intensities corresponding to Ca and Sr were measured at 422.7 and 460.7 nm, respectively.

Emission intensities corresponding to Ca and Sr were measured at 422.7 and 460.7 nm, respectively.

\[
\text{Sanalyte/Sint'l std} = \frac{\text{C}_{\text{analyte}}}{\text{C}_{\text{standard}}}
\]

Instrument response and sample concentration.

\[
\text{Sanalyte/Sint'l std} = \frac{\text{C}_{\text{analyte}}}{\text{C}_{\text{standard}}}
\]

A typical series of solutions containing a known and increasing concentration of the analyte is prepared. A fixed concentration of an internal standard (diverse from the analyzed) is added to all these solutions as well as to the unknown sample (problem).

The choice of the substance acting as internal standard should be made considering that:
- Its behaviour should resemble that of the analyte, and –at the same time–
- Its instrumental signal should not interfere with that of the analyte.
- The signal arising from the internal standard should remain constant and any possible deviations are assumed to be the suffered as well by the analyte.
- The quotient of the analyte's and the internal standard's signals is plotted vs. the concentration of the analyte in the usual way.

This method tries to conceal both operational and instrumental fluctuations in the measurements.

\[
S_{\text{signal}} (\text{at} N \text{ mg mL}^{-1})
\]

Concentration of analyte

\[
\text{Sanalyte/Sint'l std} = \frac{\text{C}_{\text{analyte}}}{\text{C}_{\text{standard}}}
\]

Note: This method assumes a linear relationship between instrumental response and sample concentration.

Determining the concentration by internal-standard method

The quinine spike amounts to:

\[
\text{V}_{\text{standard}} = \frac{(\text{final absorbance} - \text{initial absorbance}) \times \text{initial volume}}{\text{final absorbance}}
\]

Taking into account the 10:100 dilution, concentration in the sample is:

\[
\frac{\text{C} \times \text{vol}_{\text{final}}}{\text{vol}_{\text{initial}}} = \text{C}_{\text{sample}}
\]
Lesson 4

1. Molecular luminiscence spectroscopy
2. Fluorescence and phosphorescence
3. Chemiluminiscence
4. Instrumentation
5. Applications

Incandescence: light emitted by a high temperature heated body

Luminescence

Photoluminescence: previous absorption of IR or UV-VIS light

Chemiluminescence: \[\text{NO} + \text{O}_3 \rightarrow \text{NO}_2^* + \text{O}_2\]
\[\text{NO}_2^* \rightarrow \text{NO}_2 + \text{h}\nu\ (\lambda = 600 - 2800 \text{ nm})\]

Bioluminescence

Electroluminescence: electrical discharge by a ionized gas

Energy-level diagram for a photoluminescent system

Transitions involved in fluorescence

Pathways and time scale

Jablonski Energy Diagram

Absorption and emission bands
Fluorescence transitions and spectrum

**Stokes Shift**

It is the difference in energy between the lowest-energy absorption band (highest \( \lambda_a \)) and the highest-energy emission peak (lowest \( \lambda_e \)).

**Thumb rules for fluorescence spectra**

- The fluorescence spectrum of a substance in a unique chemical form is unvariant and independent of the excitation source.
- Fluorescence spectrum is always recorded at longer wavelengths than the corresponding absorption spectrum.
- Fluorescence spectrum is kind of mirror-image of the lowest energy absorption (especially if the molecule has a rigid structure and does not undergo dissociation equilibria in the excited state).

**Transition types in fluorescence**

- \( \pi \rightarrow \pi^* \): most likely and frequently being deactivated through fluorescence. The likelihood of an intersystem crossing \( S^1 \rightarrow T^1 \) is not high due to considerable difference of energy between levels involved.
- \( n \rightarrow \pi^* \): transitions may also return to ground state by fluorescent emission.

**Quantum yield**

Quantum yield or quantum efficiency is just the ratio of the number of molecules that fluoresce to the total number of excited molecules (the same applies to phosphorescence)

\[
\Phi = \frac{\text{# of emitted photons}}{\text{# of absorbed photons}} = \frac{k_f}{k_r + k_{ir}}
\]

The fluorescence quantum yield for a compound is determined by the relative rate constants \( k_r \) for the processes by which the lowest excited singlet state is deactivated.

\[
\Phi = \frac{k_f}{k_r + k_{ir} + k_{ic} + k_{ip} + k_{di}}
\]

Lifetime of fluorescence (s)

\[
\tau = \frac{1}{k_r + k_{ir}}
\]

**Table 4.2 Quantum yields for fluorescence \((\phi_f = \phi_{bc})\) and intersystem crossing \((\phi_{isc} = \phi_{bc})\) for some aromatic hydrocarbons in ethanol solution (Hars, from Birks, J. B. (ed.) (1975). Organic molecular photophysics, Vol. 2, Tables 2.6 and 3.4. Wiley, London)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \phi_f )</th>
<th>( \phi_{bc} )</th>
<th>( \phi_f + \phi_{bc} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>0.04</td>
<td>0.15</td>
<td>0.19</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.00</td>
<td>0.21</td>
<td>1.01</td>
</tr>
<tr>
<td>Fluorene</td>
<td>0.32</td>
<td>0.68</td>
<td>1.00</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.72</td>
<td>0.32</td>
<td>1.02</td>
</tr>
<tr>
<td>Tetracene</td>
<td>0.60</td>
<td>0.16</td>
<td>0.82</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>0.85</td>
<td>0.13</td>
<td>0.98</td>
</tr>
<tr>
<td>Pyrene</td>
<td>0.38</td>
<td>0.65</td>
<td>1.03</td>
</tr>
<tr>
<td>Chrysene</td>
<td>0.85</td>
<td>0.17</td>
<td>1.03</td>
</tr>
</tbody>
</table>

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Since radiation absorption is a mandatory previous step for luminiscence, a good luminiscence process calls for an efficient absorption process (high molar absorptivity) in the first place. A good fluorophore or a good phosphorophore should be itself a good chromophore.

**Fluorescence**
- Considerable energy differences between the excited singlet and triplet states (hampers intersystem crossing and, accordingly, phosphorescence).
- Ground singlet and first excited singlet should differ significantly in energy as to prevent non-radiational deactivations to take place.

**Phosphorescence**
- It is favoured by energetically close excited singlet and triplet states.
- Low probability of a non-radiational transition from the excited triplet to the ground state singlet.

**Factors affecting luminiscence**

### Fluorescence
- Considerable energy differences between the excited singlet and triplet states (hampers intersystem crossing and, accordingly, phosphorescence).
- Ground singlet and first excited singlet should differ significantly in energy as to prevent non-radiational deactivations to take place.

### Phosphorescence
- It is favoured by energetically close excited singlet and triplet states.
- Low probability of a non-radiational transition from the excited triplet to the ground state singlet.

### Structural rigidity
Structural rigidity avoids energy loss through vibrational and rotational deactivations (internal conversion), thus enhancing the fluorescence.

### Fluorescence: heteroatoms and substituents
Substituents that delocalize \( \pi \) electrons make \( \Phi \) to increase.
- First order substituents (direct to \( \pi \) or to the ring) donate \( \pi \) electrons: \( \text{H} \), \( \text{OH} \), \( \text{OCH}_3 \), \( \text{CH}_3 \)
- Second order substituents (direct to \( \pi \) or withdraw \( \pi \) electrons: \( \text{NO}_2 \), \( \text{COOH} \), \( \text{CONH}_2 \))

### Fluorescence: Intensifiers and substituents

### Compuesto | Fórmula | \( \lambda \) (nm) | \( I \) relativa |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimero de etileno</td>
<td>( \text{C}_2\text{H}_4 )</td>
<td>270-310</td>
<td>10</td>
</tr>
<tr>
<td>Toluene</td>
<td>( \text{C}<em>8\text{H}</em>{10} )</td>
<td>270-320</td>
<td>17</td>
</tr>
<tr>
<td>Propilbenceno</td>
<td>( \text{C}<em>9\text{H}</em>{14} )</td>
<td>270-330</td>
<td>10</td>
</tr>
<tr>
<td>Fluorobenceno</td>
<td>( \text{C}_7\text{H}_5\text{F} )</td>
<td>270-320</td>
<td>10</td>
</tr>
<tr>
<td>Chlorobenceno</td>
<td>( \text{C}_7\text{H}_5\text{Cl} )</td>
<td>275-345</td>
<td>7</td>
</tr>
<tr>
<td>Bromobenceno</td>
<td>( \text{C}_7\text{H}_5\text{Br} )</td>
<td>290-380</td>
<td>5</td>
</tr>
<tr>
<td>Iodobenceno</td>
<td>( \text{C}_7\text{H}_5\text{I} )</td>
<td>---</td>
<td>0</td>
</tr>
<tr>
<td>Phenol</td>
<td>( \text{C}_8\text{H}_7\text{OH} )</td>
<td>285-365</td>
<td>18</td>
</tr>
<tr>
<td>Phenolato ion</td>
<td>( \text{C}_8\text{H}_7\text{O}^- )</td>
<td>310-400</td>
<td>10</td>
</tr>
<tr>
<td>Anilina</td>
<td>( \text{C}_8\text{H}_7\text{NH}_2 )</td>
<td>310-400</td>
<td>20</td>
</tr>
<tr>
<td>Anilinato ion</td>
<td>( \text{C}_8\text{H}_7\text{NH}_3^+ )</td>
<td>---</td>
<td>0</td>
</tr>
<tr>
<td>Benzoico acid</td>
<td>( \text{C}_8\text{H}_7\text{COOH} )</td>
<td>300-350</td>
<td>3</td>
</tr>
<tr>
<td>Anilinobenceno</td>
<td>( \text{C}_8\text{H}_7\text{NH}_2 )</td>
<td>280-360</td>
<td>20</td>
</tr>
<tr>
<td>Nitrobenzeno</td>
<td>( \text{C}_8\text{H}_7\text{NO}_2 )</td>
<td>---</td>
<td>0</td>
</tr>
</tbody>
</table>

### Probability of predissociation
- Substituents that delocalize \( \pi \) electrons make \( \Phi \) to increase.
- First order substituents (direct to \( \pi \) or to the ring) donate \( \pi \) electrons: \( \text{H} \), \( \text{OH} \), \( \text{OCH}_3 \), \( \text{CH}_3 \)
- Second order substituents (direct to \( \pi \) or withdraw \( \pi \) electrons: \( \text{NO}_2 \), \( \text{COOH} \), \( \text{CONH}_2 \))
Effect of heavy atom

Heavy metal effect
Any process enhancing intersystem crossing or external conversion makes fluorescence to quench.

Heavy atoms favour a strong spin-orbital coupling and—as a consequence—a change in the spin.

Paramagnetic species
Dissolved O₂ behaves as a strong inhibitor of fluorescence because (as any other paramagnetic substance) facilitates intersystem crossing.

Fluorescence quenching

\[ M^* \rightarrow M + h_\nu \quad \text{Fluorescence} \]
\[ M^* + Q \rightarrow M + Q + \text{Heat} \]

"Quenching"

Stern-Volmer equation considers the influence of the "quencher" on the fluorescence intensity.

\[ F_0 - F = 1 + K_{SV}[Q] \]

Effect of increasing concentrations of NaI on the fluorescence of 0.1 mM triptophan.

Effects of T and solvent (nature and polarity)

- An increase in T causes a drop in \( I_F \)
- A less viscous (more fluidic) solvent makes \( I_F \) to diminish
- In both instances, the likelihood of an external conversion is accentuated
- CBr₄, C₂H₅I: these solvents hamper fluorescence and enhance phosphorescence

Aniline: three resonant forms
\( \pi \rightarrow \pi^* \) lower, less \( \Delta E \), longer \( \lambda \): fluorescence is observable in the VIS.

Anilinium ion: just the one resonant form
Fluorescence is not seen in the VIS.

Stock solutions and unknown sample must be buffered

Influence of pH on fluorescence

<table>
<thead>
<tr>
<th>pH</th>
<th>( \lambda_{max} )</th>
<th>( \Phi_F )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>285-365 nm</td>
<td>0.18</td>
</tr>
<tr>
<td>7</td>
<td>350-400 nm</td>
<td>0.09</td>
</tr>
</tbody>
</table>

In biological systems

Observing single DNA molecules with molecular beacons

Stock solutions and unknown sample must be buffered

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Dye ANS (8-anilinonaphthalene sulfonate)

\[ \Phi_f = 0 \text{ in aqueous solution} \]

\[ \Phi_f = 0.98 \text{ when bound to hydrophobic moieties of proteins and membranes} \]

\[ \tau_f = 16 \text{ ns, } \lambda_{	ext{max}} = 454 \text{ nm} \]

Ethidium bromide

\[ \Phi_f = 0 \text{ in aqueous solution} \]

\[ \Phi_f = 1 \text{ when bound to nucleic acids} \]

\[ \tau_f = 26.5 \text{ ns} \]

**Self-quenching and inner cell effect**

**Self-quenching**
Collisions among excited molecules.

**Non-radiational deactivation augmented**

**Inner cell effect** (inner filter effect)
Overlapping of absorption and emission bands: there is some fluorescent radiation that does not abandon the cell.

**Trick:** work with diluted samples (maximum absorption: \( \approx 0.1 \))

**Extinction of luminescence**

Fluorescence lifetime, \( \tau_f \), can be defined as the time that has to elapse from the moment the source lamp is shut off to make the fluorescence become \( 1/e \) (0.368) of the maximum fluorescence observed when the excitation lamp was on.

\[ F = F_0 e^{- \frac{t}{\tau_f}} \]

Fluorescence decay follows a first-order kinetics (in absence of other processes)

Fluorescence lifetime, \( \tau_f \), can be defined as the time that has to elapse from the moment the source lamp is shut off to make the fluorescence become \( 1/e \) (0.368) of the maximum fluorescence observed when the excitation lamp was on.

\[ F = F_0 e^{- \frac{t}{\tau_f}} \]

\[ \tau_f = \frac{F_0}{F} e^{\frac{1}{c} - \frac{1}{\tau_f}} \]

\[ \ln c = k \tau_f \Rightarrow \tau_f = \frac{1}{k_f} \]
• Generally, fluorescent measurements are limited by the blank emissions
• Solvents and impurities often produce fluorescence that dies out in < 2 ns
• The signal corresponding to the analyte may be discerned when measuring the fluorescent emission at longer times.

Time-resolved fluorimetry

• High lifetime >500 μs
• Large Stokes shift
• Finely shaped emission peaks
• Fluorescence is based on charge transfer from the ligand to the central atom.

Fluorescent behaviour of lanthanide coordination complexes

Applications of lanthanide coordination complexes

Green Fluorescent Protein (GFP), from jellyfish, is most often used to follow the expression of genes.
Molecules, placed near one to another, may transfer energy between themselves. This happens when the fluorescence emission spectrum of one molecule overlaps with the absorption spectrum of another molecule.

$D^* + A \rightarrow D + A^*$

(Fluorescence Resonant Energy Transfer (FRET))

- FRET is a distance-dependent interaction between the electronic excited states of two dye molecules in which excitation is transferred from a donor molecule to an acceptor molecule without emission of a photon.
- The efficiency of FRET is dependent on the inverse sixth power of the intermolecular separation, making it useful over distances comparable to the dimensions of biological macromolecules.
- Thus, FRET is an important technique for investigating a variety of biological phenomena that produce changes in molecular proximity.
- FRET takes place when donor and acceptor molecules are at a distance 10-100 Å from one another.
- FRET may be employed to shift the fluorescence emission spectrum of a given molecular combination.
- The absorption spectrum of the acceptor must overlap the fluorescence emission spectrum of the donor.

Critical point: to find the adequate matching donor-acceptor couple

Dansyl group is most appropriate for FRET studies of tryptophan-containing proteins. It can be linked to the protein through a covalent bond.
Peptidic cleavage assay

The peptide cleavage implies the loss of FRET, so that acceptor fluorescence disappears.

Fluorescence Resonant Energy Transfer (FRET)

Tryptophan - $\lambda_{\text{exc}} = 350$ nm
dansyl - $\lambda_{\text{exc}} = 340$ nm
$\lambda_{\text{em}} = 510$ nm

FRET: Example of application TaqMan

- Bi-labeled fluorophor (FRET)
- The primer and the label hybridize with target DNA
- During the Taq polymerase extension step, the label is destroyed; FRET disappears
- Real-time quantitative PCR amplification follow-up

Bioluminescence Resonance Energy Transfer (BRET)

BRET: similar to FRET

Bioluminescence Resonance Energy Transfer

Luciferase

Green Fluorescent Protein

Coelenterazine

Coelenteramide

Luminescence in micellar media

Sodium dodecyl sulfate, SDS

$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{O}^-\text{SO}_3^-$

$\text{Na}^+$

Luminescence in micellar media
Luminescence in micellar media

When surface gets saturated...

No water in the interstices = exclusion zone

Aggregates are formed

**MICELLES**

Surfactant concn. (mol/L)

Freezing-point depression (°C)

Critical micellar concentration (CMC)

Phosphorescence

Intersystem crossing and phosphorescence

Absorption and luminescence spectra

Room temperature phosphorescence (RTP)

Figure 1. Transition from the excited singlet state (S1) to the triplet state (intersystem crossing)

Figure 1. Axial (A) and equatorial (B) inclusion of phenanthrene within the β-cyclodextrin cavity
Chemiluminescence has a number of important applications. A dramatic example is the use of luminol in forensics. Luminol reacts with tiny amounts of iron to generate luminescence. Detectives use this property at crime scenes, by spraying luminol on surfaces to detect the presence of blood, which is rich in iron.

Light sticks (also known as glow sticks) utilize chemiluminescence, and are used by everyone from emergency workers and military personnel to rave dancers and Halloween trick-or-treaters. While they do not provide bright illumination, they are perfect for highlighting someone’s position in the dark. They are particularly useful in situations (such as the aftermath of an earthquake or nighttime scuba diving) where it is dangerous or impractical to use electricity. The sticks are turned on by “snapping” the central separator to mix two sets of chemicals. The reaction emits light in a variety of bright colors. A typical light stick contains hydrogen peroxide (H₂O₂) and Cyalume, a phenyl oxalate ester. These are kept separate until the stick is snapped. A fluorescent dye glows when excited by energy from this chemical reaction. Peroxides are ideal reactants for chemiluminescence, since the bond between the two oxygen atoms is easily cleaved, and releases a significant quantity of energy when broken.

Chemiluminescence

Determination of oxidizing agents

Cl₂, HOCl, OCl⁻, H₂O₂, NO₂

Emerging applications

- Determination of gaseous reagents in reactors
- Analysis of air pollutants
- Atmospheric studies
- Endpoint indication in gas phase titrations
- Determination of trace metals
- Determination of biochemicals and organic reagents

Bioluminescence

Luciferase + O₂ → Spontaneous → CO₂ + H₂O + Light

510–670 nm

“Emitting” plants

Luciferase gene cloned in plants

Intracellulareolum: fibers-equipped fluorimeter

Mercury arc lamp
Excitation filter
Emission filter
Fluorescence beam

Arc Discharge Fluorescence Lamps

Figure 2

Mercury HBO
Reveve HBO

Reactor device
• Good stability
• Beam spatially diffuse
• Low intensity

• Good stability
• Continuous from 300 – 1300 nm
• High intensity
Lesson 5

1. An introduction to optical atomic spectroscopy
2. Optical atomic spectra
3. Spectral characteristics
4. Atomic Line widths
5. Atomization methods: flame and electrothermal
6. Interferences in atomic absorption spectroscopy
7. Emission spectroscopy based on plasma sources
8. Emission spectroscopy based on arc and spark sources
9. Instrumentation
10. Applications

Atomic Emission (AE)
Kirchhoff and Bunsen were the pioneers in XIX century

Atomic Absorption (AA)
60's: Walsh (Australia) published first papers on AA backed by Alkemade and Milatz in Netherlands

Atomic Fluorescence (AF)
1964: West and Winefordner started to work on FA to determine Zn, Hg and Cd

Atomic vs. Molecular energy diagrams

Energy level diagrams for atomic sodium magnesium (I) ion Mg+

Similar energy patterns for atoms but differently spaced energy levels
- The spectrum of an ion is significantly different from that of its parent atom
- Energy differences are measured in eV

\[ E = 1.602 \times 10^{-19} J = 96.484 \text{ kJ} \times \text{mol}^{-1} \]
- The higher the number of electrons, the higher the number of energy levels and spectra become more complex.

Li 30 lines
Cs 645 lines
Cr 2277 lines

Emission spectrum of atomic Na
Line spectra: purely electronic transitions

Line width of effective line width, $\Delta \lambda_{\text{eff}}$, of an atomic absorption or emission line is defined as its width in wavelength units when measured at one half the maximum signal.

Typical values: hundredths of Å

Examples:
- Carbon
- Oxygen
- Nitrogen

• Lines positions are well established and are proper for every element
• It is possible to carry out qualitative analysis by atomic spectroscopy

Line broadening from the Uncertainty Effect (natural broadening)

The lifetimes of one or both transition states are finite, which leads to uncertainties in the transition times and to line broadening as a consequence of the uncertainty principle.

The more precisely the position is determined, the less precisely the momentum is known in this instant, and vice versa. (Heisenberg, uncertainty paper, 1927)

\[ \Delta \lambda \geq \frac{1}{\Delta \nu \cdot \Delta \mathcal{E}} \]
\[ \Delta t \cdot \Delta E \geq \frac{\hbar}{\Delta \mathcal{E}} \]
\[ \Delta t \cdot \Delta \nu \geq \frac{\hbar}{2\pi} \]

Relatively small (~10^-4 Å)

Doppler broadening

It is due to thermal agitation of atoms which position changes with respect to the observer.

\[ \Delta \lambda = \frac{v}{c} \Delta \lambda_{\text{obs}} \]

When atoms move towards the detector, the detector sees wave crests more often and detects radiation of higher frequency (shorter $\lambda$).

When atoms move away from the detector, waves are decompressed and lower frequency radiation (longer $\lambda$) is detected.

\[ \Delta \nu = \frac{u}{c} \Delta \nu_{\text{obs}} \]

\[ \Delta \nu_{\text{D}} \propto \frac{u}{m} \]

\[ \Delta \nu_{\text{D}} \approx \frac{100 \Delta \nu_{\text{obs}}}{m} \]

Lorentz accounts well for the broadening observed in the tails of lines (peaks).

Magnetic and electric fields also exert influences, Zeeman and Stark, respectively, on atomic signals.
Sample-introduction methods

The precision and accuracy of atomic methods depend critically on the atomization step and the method of introduction of the sample into the atomization region.

Method | Type of Sample
--- | ---
Pneumatic nebulization | Solution or slurry
Ultrasonic nebulization | Solution
Electrothermal vaporization | Solid, liquid, or solution
Hydride generation | Solution of certain elements
Direct insertion | Solid, powder
Laser ablation | Solid, metal
Spark or arc ablation | Conducting solid
Glow-discharge sputtering | Conducting solid

Nebulizers break-up the liquid sample into a spray of fine droplets that are conveyed into the atomizer.

a) Concentric tube: high-pressure stream of gas flowing around the tip of the tube draws the liquid sample through the capillary tube.

b) Cross-flow: the aerosol is formed by a gas flow at right angles.

c) Fritted disk: the sample solution is pumped onto a fritted surface through which a carrier gas flows. The aerosol formed is much finer than in the previous two.

d) Babington: the liquid contacts, in the surface of a hollow sphere, with an expanding jet of gas. Less prone to clogging than the others.
The sample is pumped onto the surface of a piezoelectric crystal that vibrates at a frequency of 20 kHz to several MHz. Ultrasonic nebulizers produce more dense and more homogeneous aerosols than pneumatic nebulizers do. They have, however, low efficiencies with viscous solutions and solutions containing particulates.

Ultrasonic nebulizers

A small liquid or solid sample is placed on a conductor, such as a carbon tube.

- The passing of an electric current evaporates the sample
- An inert gas (Ar) flows through the chamber and carries the vaporized sample into the atomizer

Electrothermal vaporizers (ETV)

Useful for determining As, Sb, Sn, Se, Bi, Pb

- Model reaction:
  \[ 3 \text{BH}_4^- + 3 \text{H}^+ + 4 \text{H}_2\text{AsO}_3 \rightarrow 3 \text{H}_2\text{BO}_3 + 4 \text{AsH}_3 + 3 \text{H}_2\text{O} \]
- Detection limits are enhanced by a 10 to 100 factor.

Hydride generation

Introduction of solid samples

- Direct manual insertion
- Electrothermal vaporization
- Arc, spark or laser ablation
- Slurry nebulization
- Sputtering in a glow-discharge chamber

Sample introduction

Typical basic instrumentation for flame AA
The flame is the most important and critical component. The sample aerosol when entering the flame undergoes:
- vaporization
- atomization and
- excitation of the atoms

Flame atomizer is made up of two devices: NEBULIZER and BURNER.

**NEBULIZER** transforms the aspirated sample in an aerosol or spray of very fine droplets.

**Burner** generates the flame, within which sample is vaporized, atomized and excited.

**Two distinct types of burners:**
- **Turbulent**
- **Laminar-flow or Premix**

**Total consumption**
- Premix: Laminar, silent flame
- Turbulent: Turbulent, noisy flame

- High sensitivity
- High selectivity
- Poor selectivity
- Poor sensitivity (3% aspirated)

**Sample aspiration**

**Processes occurring during atomization**
Processes occurring during atomization

Primary zone:

\[ 2 \text{C}_2\text{H}_2 + 2 \text{O}_2 \rightarrow 4 \text{CO} + 2 \text{H}_2 \]

Secondary zone:

\[ 4 \text{CO} + 2 \text{H}_2 + \text{O}_2 \rightarrow 4 \text{CO}_2 + 2 \text{H}_2\text{O} \]

Flame structure and temperature profiles

Max temperature location about 1 cm above the primary combustion zone. Optical focus to this region.

Types of flames

<table>
<thead>
<tr>
<th>Fuel</th>
<th>Oxidant</th>
<th>Temperature (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural gas</td>
<td>Air</td>
<td>2700–3000</td>
</tr>
<tr>
<td>Natural gas</td>
<td>Oxygen</td>
<td>2000–2100</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>Air</td>
<td>2000–2100</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>Oxygen</td>
<td>2000–2100</td>
</tr>
<tr>
<td>Acetylene</td>
<td>Air</td>
<td>2000–2400</td>
</tr>
<tr>
<td>Acetylene</td>
<td>Oxygen</td>
<td>2000–2400</td>
</tr>
<tr>
<td>Acetylene</td>
<td>Nitrous oxide</td>
<td>2000–2400</td>
</tr>
</tbody>
</table>

Metal atoms distribution in a fuel rich and lean flame

- Ag (not easily oxidized)
  - Continuous increase in the number of atoms from the base to the periphery of the flame

- Cr (forms very stable oxides)
  - Continuous decrease in absorbance beginning close to the burner tip, what suggests that oxide formation predominates from the start.

- Mg
  - Compromise between atomization and formation of molecular compounds

Judicious choice of flame height for the analysis in each case should be done.

Flame absorption profiles for three elements and influence of the observation height

Flame height and flow rate

Absorption profile for the Ca line in a cyanogen-oxygen flame at different sample flows.
To enforce Beer's law, the bandwidth of the source must be narrower than the bandwidth of the absorption line produced by the analyte's atomic vapour.

Hollow cathode lamp (HCL)
- Anode: W, Ni, Zr
- $\Delta V \approx 300 V$; $I = 5 - 20 mA$
- High $\Delta V$ lead to greater intensities
- Drawback: self-absorption
- Mix lamps: Ca, Mg, Al; Fe, Cu, Mn; Cu, Zn, Pb, Sn

Cross section of a HCL

Source bandwidth and absorption line bandwidth.

Electrodeless discharge lamp (EDL)
- Relatively high current: $0.5 A$
- $\Delta V \approx 30 V$
- $0.1 - 5$ torr
- $2450 MHz$
- Lifetime > 50 h
- 10 times more intense than HCL
- Inconvenient: unstability, requires cooling

Temperature gradient lamp (TGL)

Intensity: $TGL > EDL > HCL$

Useful in Atomic Fluorescence
- Commercially available for: As, Bi, Cd, Cu, Ge, Hg, K, Pb, Rh, Sb, Se, Sn, Ti, Tl, Zn
- Filament coated with oxides of Ba, Ca, Sr

EDL and TGL: are used for As and Se $\lambda < 200 nm$

$\Delta \lambda_{TGL} \equiv 0.001 nm \equiv \Delta \lambda_{EDL}$
Interferences in atomic spectroscopy

Interferences common to all three techniques
- Spectral
- Physical
- Chemical
- Temperature fluctuation
- Ionization

Interferences in AA and AF:
- Scattering of incident light

Interference proper for AF:
- Quenching

Spectral interferences: overlapping of lines.
Especially relevant in AE. Almost non-existent in AA and rare in AF

Lines < 0.1 Å cause spectral interferences

Analysis of Al: 3082.15 Å, 3092.7 Å
V has a line at 3082.11 Å. Does it interfere the quantitation of Al?
Certainly NOT

Spectral interferences: the two-line correction method
A second line from the source is used as reference. It should lie as close as possible to the analyte line but must not be absorbed by the analyte.

Any decrease in power of the reference line from that observed during calibration arises from absorption or scattering by the matrix products of the sample (background). This decrease in power is then used to correct the analyte line

The reference line may be:
- Impurity in the HCL
- A Ne or Ar line from the gas filling the lamp
- Nonresonant emission line of the element that is being determined.

This methodology is not very frequently used due to the difficulty in finding the suitable reference line.

Spectral interferences: the continuum-source correction method
By means of a chopper, the continuum source and the HCL are passed alternatively through the atomizing area.
The slit width is sufficiently wide so that the fraction of the continuum source absorbed by the atoms of the sample is negligible.

Attenuation of the continuum source as it passes through the atomized sample reflects only the broadband absorption or scattering by the sample matrix components (background).

Interferences caused by variations of T in the atomizer
Any modification of the T on the atomizing medium implies an alteration in the number of atoms in either ground or excited state.
Its effect is more marked in AE

Atomic absorption takes place in a transition from ground state to an excited state.
Atomic emission originates in a transition from an excited state to a ground state.

Atomic emission will logically be more affected by fluctuations in T, since percentage of atoms in the excited state is strongly dependent on T.

Ex.: Calculate the quotient N*/N₀ for an atom that has 2 energy states (both monodegenerated) that differ in 3.97·10⁻¹⁹ J/atom that is exposed to a flame at 2500, 2510 and 6000 K.

2,500 K: N*/N₀ = 1.03x10⁻⁵

2,510 K: N*/N₀ = 1.05x10⁻⁵ (4% more atoms in the excited state for just a 10 K increase in T)

6,000 K: N*/N₀ = 8.27x10⁻³

Boltzmann Equation:

\[
\frac{N^*}{N_0} = \exp \left( \frac{-\Delta E}{kT} \right)
\]

Where:
- \(N^*/N_0\) is the number of atoms in the excited state relative to the ground state.
- \(\Delta E\) is the energy difference between the two states.
- \(k\) is the Boltzmann constant.
- \(T\) is the temperature in Kelvin.

Table 2-1: Effect of energy difference and temperature on number of excited states

<table>
<thead>
<tr>
<th>Wavelength difference of states (Å)</th>
<th>Energy difference of states (J/atom)</th>
<th>Excited-state fraction (N*/N₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 Å</td>
<td>3.97·10⁻¹⁹ J/atom</td>
<td>2.500 K</td>
</tr>
<tr>
<td>5 Å</td>
<td>7.94·10⁻¹⁹ J/atom</td>
<td>6.000 K</td>
</tr>
<tr>
<td>7 Å</td>
<td>3.97·10⁻¹⁹ J/atom</td>
<td>3.70·10⁻⁵</td>
</tr>
<tr>
<td>14 Å</td>
<td>2.38·10⁻¹⁹ J/atom</td>
<td>4.6·10⁻⁵</td>
</tr>
<tr>
<td>21 Å</td>
<td>2.38·10⁻¹⁹ J/atom</td>
<td>4.6·10⁻⁵</td>
</tr>
</tbody>
</table>

(Values based on the equation \(N^*/N_0 = \exp(-\Delta E/kT)\) for \(\Delta E = 3.97\cdot10^{-19}\) J/atom)
Interferences due to ionization

**Ionization interference** means that a decrease in the analytical signal is observed as a consequence of ions being produced instead of just atoms.

\[ M^+ + e^- \rightarrow M \] (especially for alkaline and earth-alkaline in hot flames)

**Alternative: Ionization suppressors**

Substances that are more easily ionizable than the analyte and generate a large population of electrons in the flame, thus preventing or at least minimizing the ionization of the analyte.

Ex.: K is an excellent suppressor in the analysis of Sr.

**Chemical interferences**

1. Formation of compounds of low volatility
   - Ca (PO$_4$$^3^-$, SO$_4$$^{2-}$): Mg(Al)
   - Alternatives: $\Delta T$ as well as using releasing and protective agents.

   Ex.: PO$_4$$^3^-$ and SO$_4$$^{2-}$ form compounds with Ca that are difficult to volatilize

   The use of a releasing or a protective agent may be helpful

   **AEDT:** is preferentially bound to Ca$^{2+}$, but it does not inhibit the atomization (protective agent)

   La$^{3+}$: reacts with the PO$_4$$^3^-$, releasing Ca$^{2+}$ (releasing agent)

2. Dissociation equilibria
   - MO $\rightarrow$ M + O
   - Alkaline metals: poorly stable oxides $\rightarrow$ predominance of atomic lines
   - Alkaline-earth: relatively stable oxides $\rightarrow$ predominance of bands

   **NaCl $\rightarrow$ Na + Cl**

   $\overset{VOx \rightleftarrows V + Ox}{V}$ shows better absorbance in the presence of Al and Ti

   **Fuel-rich flames**

   \[ \overset{AlOx \rightleftarrows Al + Ox}{Al \text{ and Ti combine preferentially with}} \]

   \[ \overset{TiOx \rightleftarrows Ti + Ox}{\text{scans Ox}} \]

**Scattering (AA and AF)**

A **deficient nebulization** may allow large drops to reach the flame, what causes scattering of radiation, resulting in:

- Decrease in the power of the radiant beam
- False, spurious, signals: unspecific absorptions

**Quenching (AA and AF)**

Deactivation through collisions with molecules of the gases being burnt in the flame. Ar, N$_2$, and noble gases in general do not quench fluorescence.

**Alternative:** purge with Ar $\rightarrow$ very low backgrounds.

---

**Table 9.2** Degree of Ionization of Metals at Flame Temperatures

<table>
<thead>
<tr>
<th>Substance</th>
<th>Induction Potential (eV)</th>
<th>$\Delta T$ (°C) at 10$^{-3}$ atm</th>
<th>$\Delta T$ (°C) at 10$^{-4}$ atm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>5.9</td>
<td>0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Sr</td>
<td>4.79</td>
<td>0.06</td>
<td>0.00</td>
</tr>
<tr>
<td>K</td>
<td>4.77</td>
<td>0.005</td>
<td>0.00</td>
</tr>
<tr>
<td>Na</td>
<td>5.19</td>
<td>0.005</td>
<td>0.00</td>
</tr>
<tr>
<td>Mg</td>
<td>5.08</td>
<td>0.003</td>
<td>0.00</td>
</tr>
<tr>
<td>Cr</td>
<td>5.00</td>
<td>0.003</td>
<td>0.00</td>
</tr>
<tr>
<td>Fe</td>
<td>4.26</td>
<td>0.002</td>
<td>0.00</td>
</tr>
<tr>
<td>Ni</td>
<td>3.98</td>
<td>0.002</td>
<td>0.00</td>
</tr>
<tr>
<td>Cu</td>
<td>3.87</td>
<td>0.002</td>
<td>0.00</td>
</tr>
<tr>
<td>Zn</td>
<td>3.72</td>
<td>0.002</td>
<td>0.00</td>
</tr>
</tbody>
</table>

---

**Calibration curve (AE)**

\[
B_v \cdot d\theta = I_v \cdot (\text{wat/m}^2) \propto C
\]

- $B_v$: flow rate of aspirated liquid sample (mL/min)
- $d\theta$: efficiency in vaporization step
- $C$: efficiency in the atomization step
- $Q$: flow rate of disappearance of vapour in the flame
- $T$: absolute temperature
- $C$: analytical solution concentration

---

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In AAS light from both the HCL and flame reach the detector

- Measurement of a small signal in the presence of a large background
- Flame background (continuum) correction is mandatory so that only the analytical signal is processed

Modulation of the source

- Double beam
  - Dark current is removed by means of a shutter
  - T is adjusted to 100% when a blank is aspirated to the flame

Instrumentation: single beam

- Reference beam corrects possible fluctuations in the lamp intensity
- Reference beam does not travel through the flame. Power losses arising from flame absorption or flame scattering remain uncorrected.

Instrumentation: double beam

- Dynamic system of high T
- Tremendously stable, reproducible, reliable
- Few memory effects
- A smart combination of fuel and oxidizing agents provide ample T ranges and high atomization rates are achieved.
- Cheap and lasting atomizer
- High performance in terms of sensitivity and selectivity for more than 70 elements measured between 200-800 nm

Flame as atomizer system: advantages

- Sample is dissolved and expanded in a large flow (up to 10 L/min) of gasses in the flame: dilution and loss of sensitivity
- Elements with a tendency to form refractory oxides show a low atomization yield, what adds to the scarce time of residence of the analyte in the flame.
- Although there is a certain margin to modify the ratio of oxidant, there’ll always be the need to employ oxidants in the flame.
- Solid samples cannot be introduced in the flame.

Flameless atomizer: advantages

1. Increase in sensitivity (>10^3)
   - Atoms do not undergo expansion
   - Absence of O_2 (purge with Ar)
2. Microsamples may be introduced (5, 10, 20 µL)
3. Untreated solid samples may be analyzed
4. Purge with noble gases make unspecific absorptions to vanish (supposedly)
5. Discrete, transient, signals

Flame as atomizer system: practical disadvantages

- The minimum volume required is relatively large (1.5 – 2 mL)
- Premix chamber has to remain filled in order to guarantee a stationary regime
- Nebulization yield in aqueous solutions tends to be low due to lower surface tension as compared with organic solvents.
- Large drops reaching the flame: unspecific absorptions
- Need to use bottled gases (relative risk)

Flame as atomizer system: intrinsic disadvantages

- Sample is dissolved and expanded in a large flow (up to 10 L/min) of gasses in the flame: dilution and loss of sensitivity
- Elements with a tendency to form refractory oxides show a low atomization yield, what adds to the scarce time of residence of the analyte in the flame.
- Although there is a certain margin to modify the ratio of oxidant, there’ll always be the need to employ oxidants in the flame.
- Solid samples cannot be introduced in the flame.
**Tantalum boat (1968): abandoned for its lack of reproducibility**

**Delves cup (Ni): first attempt to confinement of atomic vapour in the pathlength. Determination of Pb in blood**

**Atom trap: quartz cylinder that traps atoms when cooled and releases those adsorbed atoms when heated.**

---

**Alternatives to the flame: hydride generation**

**Appropriate for groups IV, V, VI B:**

\[3 \text{BiH}_3 + 3 \text{H}^+ + 4 \text{H}_3\text{AsO}_3 \rightarrow 3 \text{H}_3\text{BO}_3 + 4 \text{H}_2\text{O} + \text{Sb} + \frac{3}{2} \text{H}_2\]

- **Great sensitivity:** no portion of analyte is lost. It is an absolute method
- **These elements tend to form molecular compound rather than atoms in the flame**
- **The resonant lines (\(\approx 190 \text{ nm}\)) coincide with the strong background absorption by the flame (\(<200 \text{ nm}\))**
- **Great bioinorganic relevance**

**Alternatives to the flame: cold vapour AF for Hg**

**Hg: just the one known metal that - thanks to its high vapour tension - is able to form monoatomic vapour at room temperature as noble gases do (not even \(\text{N}_2\) nor \(\text{O}_2\) do the same)**

- No flame is necessary: \(\text{Hg}^0\) is carried in atomic state
- **Improved sensitivity in AF determinations**

---

**Electrothermal atomizers**

- **Based on early works by L’vov**
- **Massmann: first model commercialized**

**Introduction of sample (solid, liquid, gas, slurry)**

- **Purge with Ar avoids C oxidation.**
- **Need of cooling.**
Electrothermal atomizers: graphite furnace

**Typical composition of the furnace:** Graphite

**GFAAS:** Graphite Furnace Atomic Absorption Spectroscopy

- Length: 18 - 28 mm
- Volume of sample: 5 - 100 μL
- Furnace lifetime: 200 - 1000 cycles
- Maximum temperature: 3000°C to prevent decomposition of graphite
- C may act as a reducer of metallic ions
- Flowing Ar: avoids oxidation of graphitic C

Other materials: Ta, W, Pt

- High fusion point required
- No radiance emitted when heated at high T (disadvantage of W and Ta)

Heating programme

- 0-20 s: sample (dilute or diluted); or mg of solid (direct analysis of solids)
- It is common to use matrix modifiers that either stabilize the analyte or induce the volatility of the matrix
- Samples are generally acidified in order to avoid adsorptions on containers

**Heating ramps:** up to 1000 ºC/s

**Limited lifetime:** 200-300 firings

**Possible memory effects**

**Amenable to automatization:** autosamplers

**Good reproducibility** demands the use of batches of furnaces

**Sensitivity:** LOD >10³ AA

Electrothermal atomizers vs. flame

+ Sensitivity: improved LODs (long residence time of the vapour in the atomizer)
+ Smaller sample volume
+ Wide range of sample states
+ Less dependence of the signal on the physical characteristics of the sample (viscosity, surface tension and density)
+ Purge with Ar diminishes the possibility of oxides formation and enhances a larger atomic vapour population
  - Serious matrix effects, possibility of inter-elemental compounds to be formed, and memory effects
  - Worsened reproducibility (5-10%)
  - Longer analysis time
  - Higher cost; it demands a more specialized maintenance
**Chemical**

Ascribable to sample
- Volatilization of analyte in the form of volatile salts
- Decrease in the efficiency in atomization

Ascribable to the apparatus or the method
- Formation of carbides
- Condensation
- Formation of nitrides
- Memory effects

**Spectral (Physical)**

Light emission by incandescent graphite

Unspecific absorptions:
- Light scattering
- Formation of molecular species
- Formation of stable halides

**Matrix effects detected by standard addition method**

![Graph showing matrix effects](image)

**1. Addition of matrix modifiers**

Substances that help the atomization step to happen in the right time and in the most homogeneous possible way.

“Recipes” specific for given elements and matrices:

- \( \text{NH}_4\text{NO}_3 \) volatile, facilitates the elimination of the matrix during the charring step and helps to homogenize the atomic vapour.
- \( \text{NH}_4\text{H}_2\text{PO}_4 \) acts as a retainer of substances that otherwise could get eliminated in the charring stage.
- \( \text{O}_2 \) when adsorbed in the inner walls of the tube, enhances a smooth atomization (besides helping to burn the remaining organic matter)

**2. Graphite furnace surface**

Often, the use of pyrolytic graphite (less porous) makes atomizations more reproducible.
Graphite furnace with L’vov platform (1982)

Vaporization of MX deposited on the platform is delayed until wall and filling gas are hot enough as to completely atomize MX.

Graphite furnace with L’vov platform (1982)

Background correction based on the Zeeman effect

1. Unpolarized radiation from a HCL (A) is passed through a rotating polarizer (B)
2. The beam is separated into two components that are plane-polarized and 90º to one another (C)
3. These beams pass into a tube surrounded by a permanent 11-kG magnet, originating 3 absorption peaks (D)
4. Analyte absorbs only radiation that is plane polarized with the field, whereas broadband molecular absorption and scattering by the matrix products occur during both half cycles (E)
5. This results in a cyclical absorbance pattern (F)
6. The subtraction of the absorbance during the perpendicular half cycle from that for the parallel half cycle provides for background correction.
Background correction based on the Zeeman effect

- DC in atomizer
- AC in atomizer
- DC in source

The magnetic field gives rise to different splitting models depending on the multiplicity of the state.

\[ \sigma^- \rightarrow \pi^- \rightarrow \pi^+ \rightarrow \sigma^+ \]

\[ B = 0 \neq 0 \]

They only absorb plane polarized radiation parallel to the field.

Background correction based on the Zeeman effect

- Good for the UV-VIS zone without the need to recourse to any other type of lamp.
- It corrects up to 3 units of absorbance
- Economic and simple to handle
  - Lower sensitivity (30 - 40% less)

Solid sample analysis

- Pretreatment of sample is minimized or avoided
- Analyte is not retained in insoluble residues
- Contamination sources of sample are reduced
- Reagents are not necessary
  - It is likely that some amount of sample be either lost or contaminated in the transfer to the atomizer
  - Every replicate has to be weighed separately
  - The calibration curve is more elaborated
  - Matrix modifiers are not as effective due to a worsened interaction between the modifier and the analyte included within the solid.
  - It is practically impossible to dilute a solid sample

Self-inversion with self-absorption

Any absorption taking place during the high current pulse is assumed to correspond to the background and it is subtracted from signal measured under low current, thus obtaining the analyte signal.
Atomic Emission Spectrometry (AES)

• Early 19th century: alcohol flame (Brewster, Herschel, Talbot, Foucault)
• Mid 19th century: Discovery of Cs, Tl, In, Ga by AES (Bunsen, Kirchoff)
• 1877: Gouy designs the pneumatic nebulizer
• 1920s: Use of arcs and sparks for AES
• 1930s: First commercial spectrometer (Siemens-Zeiss)
• 1960s: Plasma sources (commercialized in the 70s)

Quasi-instantaneous relaxation of excited species is accompanied by emission of UV-VIS radiation at discrete wavelengths (lines) that prove useful for qualitative and quantitative analysis.

Excitation sources can be:
- Plasma
- Flame
- Electric discharge (arc and spark)
- Glow discharge
- Laser ablation

In AES light emitted from radiational deactivation of excited atoms is measured, which intensity is proportional to the concentration of excited atoms and, accordingly, to the concentration of atoms in the ground state.

Atomic Emission Spectrometry (AES)

Numerous spectral interferences

- Lines:
  - <CO bands between 205 to 245 nm
  - CN, CO, OH, NH bands between 380 to 780 nm
  - H + OH → H₂O + hv
  - CO + O → CO₂ + hv

- Bands:
  - H₂O → OH + hv

Recombination reactions:

Typical LODs in FAES

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>Power</th>
<th>LOD (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>228.8</td>
<td>N/A</td>
<td>0.02</td>
</tr>
<tr>
<td>Au</td>
<td>329.3</td>
<td>N/A</td>
<td>0.05</td>
</tr>
<tr>
<td>Cu</td>
<td>324.8</td>
<td>N/A</td>
<td>0.005</td>
</tr>
<tr>
<td>Fe</td>
<td>325.4</td>
<td>N/A</td>
<td>0.00005</td>
</tr>
<tr>
<td>Mg</td>
<td>285.2</td>
<td>N/A</td>
<td>0.001</td>
</tr>
<tr>
<td>Ni</td>
<td>334.2</td>
<td>N/A</td>
<td>0.00005</td>
</tr>
<tr>
<td>Zn</td>
<td>327.4</td>
<td>N/A</td>
<td>0.0005</td>
</tr>
<tr>
<td>Co</td>
<td>320.3</td>
<td>N/A</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ni</td>
<td>332.3</td>
<td>N/A</td>
<td>0.00005</td>
</tr>
<tr>
<td>Cu</td>
<td>324.8</td>
<td>N/A</td>
<td>0.0005</td>
</tr>
<tr>
<td>Al</td>
<td>396.1</td>
<td>N/A</td>
<td>0.0005</td>
</tr>
<tr>
<td>Zn</td>
<td>327.4</td>
<td>N/A</td>
<td>0.00005</td>
</tr>
<tr>
<td>Mg</td>
<td>285.2</td>
<td>N/A</td>
<td>0.001</td>
</tr>
<tr>
<td>Fe</td>
<td>325.4</td>
<td>N/A</td>
<td>0.00005</td>
</tr>
<tr>
<td>Si</td>
<td>251.6</td>
<td>N/A</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Typical characteristics of arcs and sparks

- Cheap
- Limited in features
- Very useful for alkali and alkaline earth
- Low temperatures to achieve atomization of many other elements
- Clearly displaced by atomic absorption (AAS)

Arc: Electrical discharge between two conducting electrodes (1-30 A)
Spark: High voltage intermittent discharge (few µs)
A) **Continuum:** arising from the thermal radiation

B) **Bands:** groups of lines very close among themselfs that accumulate in a maximum intensity zone (head of band), caused by radiational emission of electrically excited molecules (e.g.: OH, cyanogen CN, SiO, CaF₂)

C) **Lines:** Emission by electrically excited atoms.

"arc lines": all those lines belonging to the atomic spectrum (neutral atom)

"spark lines": those corresponding to the monovalent ionic spectrum (M⁺)

<table>
<thead>
<tr>
<th>Atomizer</th>
<th>Type of spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flame</td>
<td>Atomic (albeit and ablation)</td>
</tr>
<tr>
<td>Arc</td>
<td>Atomic</td>
</tr>
<tr>
<td>Spark</td>
<td>Ionic</td>
</tr>
<tr>
<td>Plasma</td>
<td>Ionic</td>
</tr>
</tbody>
</table>

Practical hints

Spectra fisonomy will depend on:

A) Nature of excited species

B) Type of electrical excitation source

↓P⁰ionization ↓P⁰arce resonant ↑ richness of lines

**Bunsen:** Li, Na: 2 lines; K: 3 lines; Rb: 4 lines; Cs: 6 lines

**Arc and spark** (10 eV): capability to excite those atoms with excitation potential less than 10 eV (~70 elements)

**Arc emission**

Powdered sample located in the graphite cup

- Low potential (~ 40 V)
- High intensity (~ 10 A)

Arc is kept up to the total consumption of the sample

Since low energy is used as excitation source, atomic spectra are generally obtained.

**Low precision** due to erratic movement of arc

Different metals are volatilized in diverse ways and, thus, multiple analysis and quantitation is difficult.

**Spark emission**

High voltage (~ kV)

Low intensity (~ mA)

Intermitent signal (µs - ms)

Temperature up to 40,000 °C

Due to the high potential used, the spectrum is generally ionic in nature.

- the movement of the spark over the surface provides a representative sampling with good precision (0.1-1% RSD), but poor sensibility (LD ~0.01%)
- Electrically noisy
- Signal has to be averaged over time

Arc and spark: instrumentation

Internal standard calibration curve

---

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Sensitivity

<table>
<thead>
<tr>
<th></th>
<th>300 nm</th>
<th>400 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>signal AA</td>
<td>10^3</td>
<td></td>
</tr>
<tr>
<td>signal AF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>noise AA</td>
<td>10^4</td>
<td></td>
</tr>
<tr>
<td>noise AF</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Selectivity

AA > AF > AE

Main noise sources:
- sputtering in the lamp
- oscillations in the flame
- shot noise in the detector

In AF:
- lamp noise is not registered.
- limiting noise is the shot noise.
- lamp power can be increased as much as desired: improved sensitivity.

Analytical features compared

AA:
- Precision < 1% at trace level (ppm)
AE:
- Precision 2 - 5 %
AF:
- Precision 2 - 3 %

Applicability

Plasma: electrically conducting gaseous mixture containing a significant concentration of cations and electrons (with a zero net charge) which characteristics are strongly dependent on the degree of ionization.

Three primary types:
- Inductively coupled plasma (ICP)
- Direct current plasma (DCP)
- Microwave induced plasma (MIP)

Inductively Coupled Plasma (ICP):
- Ionization of the flowing Ar gas and production of electrons is initiated by a spark from a Tesla coil.
- The resulting ions, and their associated electrons, interact with the fluctuating magnetic field generated by the induction coil and they get accelerated describing closed annular paths.
- The temperature of the plasma is kept very high (6000-10000 K) by means of the energy produced by the induction coil and absorbed by ions and electrons.
- Tangential flow of Ar isolates the inner walls of quartz (cooling effect) and radially centers the plasma.

Inductively Coupled Plasma (ICP): advantages
- Long residence time (~2-3 ms): efficient desolvation and volatilization
- High temperatures: complete atomization and more effective excitation
- Inert Ar atmosphere: prevents formation of oxides, lengthening the analyte lifetime
- Small emission zone: higher intensity and absence of self-absorption
- Ionization relatively large: amenable to coupling with MS

Inductively Coupled Plasma (ICP): Argon consumption

Analytical zone (blue)
Initial radiation zone (red)
Induction zone
Quartz torch
Auxiliar gas flow (0.5 L min⁻¹)
Ar forming and sustaining the plasma (30-20 L min⁻¹)
Axial flow of aerosol containing the sample (1 L min⁻¹)
Introduction of sample in the ICP

1 mL min⁻¹

Analytical features of ICP

- All elements (but, obviously, Ar) can be identified and determined.
- True simultaneous multi-elemental analysis feasible in a short time (30 s) in uniform atomization-excitation conditions for all elements.
- Elements such as B, P, W, U, Zr and Nb, that are able to form refractory oxides (highly resistant to thermal decomposition) can be analyzed.
- Non-metallic elements such as Cl, Br, I and S, can also be determined (although <180 nm)
- Wide linear calibration curves (up to 6 orders of magnitude) thanks to the relatively constant T in the plasma section that prevents self-absorption to take place.
- High T guarantee minimum chemical interferences
- Spectral interferences are avoidable by using alternative lines
- Several possibilities to introduce the sample
- More sensitive than AF and sensitivity similar to GFAAS
- Diverse degrees of automation possible
- It spans over wider spectral range (190 – 900 nm)

Fluctuations in either viscosity or surface tension may lead to very imprecise results, especially in:
- samples containing dissolved solids
- highly acidified samples
- Formation of salts in the tip of the nebulizer alters nebulization step and affects the flow of aerosol

Remedies
- Sample dilution
- Use of a peristaltic pump
- Recourse to an internal standard
- Use a better nebulizer (ultrasonic)

ICP: physical interferences

ICP: disadvantages

- very complex spectra (hundreds to thousands of lines)
- large number of experimental variables to be optimized (simplex methodology)
- high resolution and high quality optical parts
- expensive instruments and highly trained and specialized personnel

ICP: sequential instrumentation

2400 grooves/mm: 160-380 nm
1200 grooves/mm: 380-850 nm
Entrance slit, exit slits and the grating surface are located along the circumference of a Rowland circle, the curvature of which corresponds to the focal curve of the concave grating.

Radiation from each of the fixed slits impinges on the PMT.

The slits are factory configured to transmit lines for selected elements.

![Rowland's circle image]

**Figure 10.7** Schematic of an echelle polychromatic system.

**Table 21-2** Comparison of detection limits for Ni\textsuperscript{2+} ion at 231 nm

<table>
<thead>
<tr>
<th>Technique\textsuperscript{a}</th>
<th>Detection limits for different instruments (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICP atomic emission (pneumatic nebulizer)</td>
<td>3–50</td>
</tr>
<tr>
<td>ICP atomic emission (autosonic nebulizer)</td>
<td>0.3–4</td>
</tr>
<tr>
<td>Graphite furnace/atomic absorption</td>
<td>0.02–0.06</td>
</tr>
<tr>
<td>ICP mass spectrometry</td>
<td>0.001–0.02</td>
</tr>
</tbody>
</table>

\textsuperscript{a} ICP = inductively coupled plasma.


**Table 21-4** Comparison of atomic analysis methods

<table>
<thead>
<tr>
<th>Flame absorption</th>
<th>Furnace absorption</th>
<th>Plasma emission</th>
<th>Plasma-mass spectrometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection limit (ng)</td>
<td>Linear range</td>
<td>Precision (1–10 min)</td>
<td>Precision (1–10 min)</td>
</tr>
<tr>
<td>ICP</td>
<td>0.05–1</td>
<td>0.1–10</td>
<td>0.5–5%</td>
</tr>
<tr>
<td>ICP</td>
<td>0.1–10</td>
<td>1–10%</td>
<td>1–10%</td>
</tr>
</tbody>
</table>

Sample conscious:
- Very few
- Very many
- Many
- Few
- Some
- Many
- Small
- Medium
- Large

Sample volume:
- Very small
- Medium
- Large

Health and safety:
- Inert gases
- Medium
- Large

*Source: Adapted from ICP Solutions, PerkinElmer, MA.*

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Comparison of analytical performances: LOD’s

<table>
<thead>
<tr>
<th>Element</th>
<th>AA</th>
<th>ICP-AES</th>
<th>LOD</th>
<th>AES</th>
<th>AES</th>
<th>AES</th>
<th>AES</th>
<th>AES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>70</td>
<td>0.02</td>
<td>0.0005</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>70</td>
<td>0.02</td>
<td>0.0005</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>100</td>
<td>0.02</td>
<td>0.0005</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>100</td>
<td>0.02</td>
<td>0.0005</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>100</td>
<td>0.02</td>
<td>0.0005</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

AA and ICP are complementary techniques rather than competitive techniques.

Comparison of detection limits for several atomic spectral methods

| Number of Elements Detected at Concentrations of |
| Method | <1 ppb | 1-10 ppb | 10-100 ppb | 100-500 ppb | >500 ppb |
| ICP       | 12 | 11 | 10 | 9 | 8 |
| GF-AAS    | 15 | 14 | 13 | 12 | 11 |
| ICP-AES   | 18 | 17 | 16 | 15 | 14 |

GF-AAS vs. ICP-AES

-更好的精度
-更低的检测限

The joint use of both techniques allows ca. 70 elements to be determined below the ppb level

Direct Current Plasma (DCP)

- T as high as 10000 K are reached
- Ar is also used to form the plasma
- Lower consumption of Ar and DC source es simpler and less expensive
- Graphite rods are to be removed every few working hours; comparatively, ICP requires less maintenance
1. Classification of Electroanalytical Techniques.
2. General Laws.
3. Electrode Phenomenon.
4. Limit of Electroactivity.
5. Potentiostatic Assembly.

Summary of common electroanalytical methods

- **Differential Pulse Voltammetry (DPV)**
- **Electroimmunoassay (EIA)**
- **Ionic Chromatography-Electrochemical Detection (IC-ED)**
- **Amperometric Biosensors**
- **Flow Injection Analysis-Electrochemical Detection (FIA-ED)**
- **Stripping Voltammetry**
- **High Performance Liquid Chromatography-Electrochemical Detection (HPLC-ED)**

Main analytical modes for electrolytic cells:

- **Potentiometry**
  - An indicator electrode responds to the activity of the analyte
  - The $E_{cell} = E_{ind} - E_{ref}$ is measured
- **Amperometry**
  - Set $E_{applied}$ so that a desired reaction occurs
  - Stir the solution
  - Measure current
- **Voltammetry**
  - Quiet or stirred solution
  - Vary ("scan") $E_{applied}$
  - Measure current
  - Indicates reaction rate
  - Reaction at electrode surface produces concentration gradient with bulk solution
  - Mass transport brings unreacted species to electrode surface

Properties of membranes

- **Non-crystalline**
  - Glass
  - Liquid immobilized liquid
- **Crystalline**
  - Single crystal
  - Polycrystalline or mixed crystal

The inherent sensitivity and selectivity of the membranes are due to:

- Minimal solubility
- Some electrical conductivity (small migration of singly charged ions within the membrane)
- Selective reactive layer thickness of the analyte
  - Ion exchange
  - Crystallization
  - Complexation
Divalent cation electrodes

Membrane electrode vs. Glass electrode

Membrane response

Response of a liquid-membrane electrode to variations in the concentration and activity of calcium ion

Characteristics of Liquid-Membrane Electrodes

- Respond preferentially to one species in a solution.
Gas-permeable electrodes for a variety of analytes (gas sensors)

Disadvantage of gas electrodes:
Time of response relatively long:
1-7 min to reach equilibration

Table 25: Selected ionic species for gas permeable electrodes

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Equilibrium reaction</th>
<th>Sensing electrode</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₃</td>
<td>NH₃ + H₂O ⇌ NH₄⁺ + OH⁻</td>
<td>Glass, pH</td>
</tr>
<tr>
<td>CO₂</td>
<td>CO₂ + H₂O ⇌ HCO₃⁻ + H⁺</td>
<td>Glass, pH</td>
</tr>
<tr>
<td>HF</td>
<td>HF ⇌ H⁺ + F⁻</td>
<td>Ag, pH</td>
</tr>
<tr>
<td>H₂S</td>
<td>H₂S ⇌ HS⁻ + H⁺</td>
<td>Ag, pH</td>
</tr>
<tr>
<td>NO₂</td>
<td>2NO₂⁺ + H₂O ⇌ NO₃⁻ + H⁺</td>
<td>Ion exchanger, pH</td>
</tr>
</tbody>
</table>

Equilibrium in internal solution:

\[
K = \frac{[\text{H}^{+}]_{\text{int}}[\text{HCO}_3^-]_{\text{int}}}{[\text{CO}_2]_{\text{int}}} = L = 0.059 \log K'_{1}[\text{CO}_2(aq)]_{\text{test}}
\]

Global:

\[
\text{CO}_2(aq) + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+
\]

Gas probe response:

\[
\text{E}_{\text{glass}} = L + 0.059 \log K'_{1}[\text{CO}_2(aq)]_{\text{test}}
\]

Gas sensors: kinetics of equilibria

"A biosensor is a tool or analytical system comprising an immobilized biological material (such as an enzyme, antibody, whole cell, organelle, or combinations thereof), in intimate contact with a suitable transducer system which converts the biochemical signal into a quantifiable electrical signal."

"Gaswa"
**Potentiometric biosensor**

\[
\text{(NH}_2\text{)}_2\text{CO} + 2\text{H}_2\text{O} + \text{H}^+ \rightarrow 2\text{NH}_3^+ + \text{HCO}_3^{-}
\]

\[
\text{NH}_3^+ \rightleftharpoons \text{NH}_3 + \text{H}^+
\]

**Enzymatic electrodes: urea sensor**

- **Potentiometric vs. Amperometric transduction**
  - Zero-current cell potential
  - Fast response time
  - Non-perturbing measurement device (the analyte is not consumed)
  - Measuring ion activities rather than total concentrations

**Biological specificity of enzyme catalyzed reactions**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Reaction</th>
<th>ISF detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urease</td>
<td>( \text{Urea} \rightarrow 2\text{NH}_3 + \text{CO}_2 )</td>
<td>NH(_3^+) glass or polymer, pH gas sensor, H(_3) glass or polymer, pH gas sensor</td>
</tr>
<tr>
<td>Creatinase</td>
<td>( \text{Creatine} \rightarrow \text{Creatinose} + \text{NH}_3 )</td>
<td>NH(_3^+) glass or polymer, pH gas sensor, H(_3) glass or polymer, pH gas sensor</td>
</tr>
<tr>
<td>l-Glutamate</td>
<td>( \text{L-glutamate} \rightarrow \text{CABA} + \text{CO}_2 )</td>
<td>CO(_2) gas sensor, pH gas sensor</td>
</tr>
<tr>
<td>Angiotensin</td>
<td>( \text{Angiotensin} \rightarrow \text{ANGIII} + \text{CO}_2 )</td>
<td>CO(_2) gas sensor, pH gas sensor</td>
</tr>
<tr>
<td>Glucose</td>
<td>( \text{Glucose} \rightarrow \text{Glucose} ) (unreacted)</td>
<td>N(_2) gas or polymer, pH gas sensor, H(_3) gas or polymer</td>
</tr>
<tr>
<td>Penicillin</td>
<td>( \text{Penicillin} \rightarrow \text{Penicillin} ) (unreacted)</td>
<td>N(_2) gas or polymer, pH gas sensor, H(_3) gas or polymer</td>
</tr>
</tbody>
</table>
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:

$$\text{LaF}_3 \rightarrow \text{LaF}_2^+ + F^-$$

Solid (membrane surface) Solution

The side of the membrane with the lower $a_F^-$ becomes positive relative to the other surface:

$$E_{\text{mem}} = L + 0.0592 \log \frac{1}{a_F^-}$$

$$E_{\text{mem}} = L + 0.0592 \log a_{F^-}$$

$$E_{\text{mem}} = L + 0.0592 \log a_F$$

$E_{\text{mem}} = L + 0.0592 \log F^-$

Fluoride Electrode
- Usually ionic compound
- Single crystal
- Crushed powder, melted and formed
- Operation similar to glass membrane

Total Ionic Strength Adjustment Buffer ($TISAB$)
$5<pH<8$

Electrodes based on silver salts
Membranes prepared from single crystals or pressed disks of various silver halides are selective toward silver and halide ions

AgCl; AgBr: ion Ag$^+$ mobile
Alternative: mixing in a 1:1 ratio with Ag$_2$S

Ag$_2$S + AgX ($Cl^-$, $Br^-$, $I^-$)

Ps AgX > Ps Ag$_2$S ($\approx 10^{-52}$)

Crystalline membranes are also available that consist of a homogenous mixture of Ag$_2$S with sulfides of Cu, Pb or Cd

Crystalline membranes
- Electroanalytical methods based on electrolytic oxidation or reduction of an analyte for sufficient period to assure quantitative conversion to new oxidation state:
  1. Constant-Current Coulometry
  2. Electrogravimetry

In the first, quantity of electricity needed to complete the electrolysis serves as measure of amount of analyte present. Total charge, $Q$, in coulombs passed during electrolysis is related, according to Faraday’s law, to the absolute amount of analyte:

$$Q = nF/N$$

For electrogravimetry, product of electrolysis is weighed as a deposit on one of the electrodes.
Typical apparatus for electrogravimetry

**Constant current coulometry**

- The current is kept constant until an indicator signals completion of the analytical reaction.
- The quantity of electricity required to attain the end point is calculated from the magnitude of the current and the time of its passage.
- Controlled-current coulometry, also known as amperostatic coulometry or coulometric titrimetry.
- When called coulometric titration, electrons serve as the titrant.

Controlled-current coulometry has two advantages over controlled-potential coulometry:

**First**, using a constant current leads to more rapid analysis since the current does not decrease over time. Thus, a typical analysis time for controlled current coulometry is less than 10 min, as opposed to approximately 30-60 min for controlled-potential coulometry.

**Second**, with a constant current the total charge is simply the product of current and time. A method for integrating the current-time curve, therefore, is not necessary.

Other necessary instrumental components for controlled-current coulometry is an accurate clock (a digital clock provides accurate measurement of time, with errors of ±1 ms) for measuring the electrolysis time, t, and a switch for starting and stopping the electrolysis.

### Karl-Fischer reaction: determination of H₂O

Three-components mixture dissolved in CH₃OH comprising:

$$I₂ \quad C₄H₄N \quad SO₂$$

1 : 10 : 3

It is carried out in a methanolic medium:

$$N SO₃ + CH₃OH \rightarrow NH₂CH₂SO₃$$

So as to avoid the non-stoichiometric reaction:

$$N SO₃ + H₂O \rightarrow NH₂SO₃$$

### Coulometric in-situ generation of reagent

- **Anode**: Iodine production by oxidation
  $$2 I^- \rightarrow I₂ + 2 e^-$$

- **Cathode**: Hydrogen production by reduction
  $$2 H^+ + 2 e^- \rightarrow H₂$$

Side reaction:
Reduction of sulfur components.
After 1-2 weeks, smells like mercaptans.

Change catholyte every week!
Resolution: 0.1 µg water
Detection limit: 5 µg water for 5 g sample → 1 ppm
Measuring range: 10 µg - 100 mg water/sample
1 ppm - 5 % water

The cell with the diaphragm uses two solutions, one in the cathode chamber and the other in the anode chamber.

The cell without the diaphragm uses one solution that has all the reagents needed for K-F titration (e.g. HYDRANAL-Coulomat AD).

Bi-pontentiometric end-point detection

On the double platinum electrode:
- Constant current of e.g. 20 µA or 50 µA
- EP 200 mV

Electrodes for voltammetry

Counting electrode
- Reference electrode
- Working electrode
Faraday's first law

Faraday's second law

**Faraday's first law**

\[\text{Ox} + n\ e^- \rightarrow \text{Red}\]

\[N_{\text{Ox}} \text{(moles)} = \frac{Q}{nF}\]

\[m_{\text{Ox}} \text{(grams)} = \frac{QM}{nF}\]

When \(i\) const., \(Q = \int i dt\)

**Kinetics:**

\[i = \frac{dQ}{dt} = nF \frac{dN_{\text{Ox}}}{dt} = nFA \frac{dN_{\text{Ox}}}{A \cdot dt}
\]

\[\Psi = \text{mol cm}^{-2} \text{ s}^{-1} = \text{V}u\]

**Faraday's second law**

\[\int_{t_0}^{\infty} idt = nF\]

**Kinetics:**

\[\text{d}N_{\text{Ox}} = \text{d}N_{\text{Red}} = nFA \Psi = nFAu\]

**General laws of electrolysis**

When \(i\) const.,

\[\text{Ox} + ne^- \rightarrow \text{Red}\]

- Mass transfer from the bulk of the solution to the interface
- Previous and subsequent coupled chemical reactions
- Electroodic charge transfer on the surface of the electrode

**Electrode reaction**

\[\text{Electroregion} \rightarrow \text{Bulk of the solution}\]

**Mechanisms and transport regimes in electrochemical cells**

<table>
<thead>
<tr>
<th>Transport mechanisms</th>
<th>Transport conditions</th>
<th>Time regime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular diffusion</td>
<td>Still electrode in still solution</td>
<td>Transient</td>
</tr>
<tr>
<td>Convective diffusion</td>
<td>Forced convection</td>
<td>Transient</td>
</tr>
<tr>
<td>Ionic migration</td>
<td>Dynamic electrode techniques: suppressed by background electrolyte</td>
<td>Ionic migration, physical basis of the measure</td>
</tr>
</tbody>
</table>

**Mass transport towards the electrode**

\[\Psi = D \frac{dc}{dx} \bigg|_{x=0} - u\frac{d\phi}{dx} \bigg|_{x=0} + Cv\]

- **Nernst-Planck**

- **Diffusional flow by concentration gradient**

- **Ionic migration**

- **Convective transport**

- **\(u = \frac{zFD}{RT}\):** mobility of ionic species

- **\(\text{grad} \ \phi = vz\):** intensity of the electric field

- **\(v\):** fluid hydrodynamic velocity
The interface IPE-solution behaves as a capacitor.

\[ q = \sigma \epsilon \]

Electroactivity windows

**Classic assembly**

Three-electrode potentiostat assembly

**Electrolyte solutions**

Potential ranges for three types of electrode materials in various supporting electrolytes:

- Pt
  - 1 M H₂SO₄ (P)
  - pH 7 Buffer (P)
  - 1 M NaCl (P)
  - 1 M H₂SO₄ (H)
  - 1 M NaCl (H)
  - 0.1 M HCl (H)

- Ag
  - 1 M HNO₃ (C)
  - 0.1 M HCl (C)

\[ E, V \text{ vs. SCE} \]
1. Stages in the electrochemical reaction: diffusional control
2. Diffusion layer
3. Nernst' hypothesis
4. Diffusional and convective flow
5. Fick's laws.
6. i-E curves.
7. Equilibrium, mix and limit potentials.

Lesson 7.

Two types of processes can conduct current across an electrode / solution interface. One kind involves a direct transfer of electrons via oxidation/reduction reaction at the electrode / solution interface. Processes of this type are called faradaic processes because they are governed by faradaic laws which state that an amount of chemical reaction at one electrode is proportional to the current i.e. faradaic current.

In a non-faradaic process, the charge do not cross the interface, but rather stays there. Anyway, external currents may flow as a consequence of changes occurring in potential, electrode area or solution concentration. These currents are always present as main contribution to background current.

\[ \text{Faradaic and non-faradaic processes.} \]

\[ N_{eq} \text{(moles)} = \frac{Q}{nF}; \quad m_{eq} \text{(grams)} = \frac{Q}{nF} \]

Diffusional and convective processes.

Mass transport towards the electrode:

\[ \Psi = D \cdot \frac{\partial C}{\partial x} - u \cdot C \cdot \frac{\partial \phi}{\partial x} + Cv \]

Nernst-Planck

Diffusional flow by concentration gradient

 Ionic migration

 Convective transport

\[ \text{Diffusional flow by concentration gradient} \]

\[ \text{Ionic migration} \]

\[ \text{Convective transport} \]

\[ \text{mobility of ionic species} \]

\[ \text{intensity of the electric field} \]

\[ \text{fluid hydrodynamic velocity} \]

\[ \text{Distance from electrode, cm} \]

\[ \text{Distance from electrode, cm} \]
Effect of time on the concentration profile of an electroactive species at a given distance from the electrode ($X_1$) and at an $E_{app}$ that guarantees that a diffusion-controlled current is reached at a time $t_1$.

**Diffusion-limited current**

The electrochemical reaction rate is a function of the flow of substance that reaches the electrode per unit time and unit surface:

$$
\Psi = \frac{dC}{dx}, \quad \text{plane diffusion}; \quad \frac{dC}{dx} = 0
$$

**First Fick's Law** expresses the change in concentration over time for each distance from the electrode:

$$
\frac{\delta C}{\delta t} = -D \frac{\delta^2 C}{\delta x^2}
$$

The profile of the concentration gradient at the interface is obtained by integrating this expression, and it results to be:

$$
C(x,t) = C_0 \exp \left( \frac{-t}{\delta} \right)
$$

**Cottrell**

$$
i = nFAD \frac{C^*}{\sqrt{4Dt}}
$$

Quiet solution; $E$ constant; plane geometry

**Diffusion-limited current**

That which is obtained for a maximum flow of substance.

$$
\Psi = -D \frac{dC}{dx} \frac{C^*}{\delta} \frac{C_0 - C(x,t)}{\delta}
$$

when $C(x,0) = 0$:

$$
\Psi_{max} = C^* \frac{C_0}{\delta} \Rightarrow i_{max} = nF \Psi_{max} = nFAD \frac{C^*}{\delta}
$$

For intermediate values of $i$:

$$
i = \pm nFAD \frac{C^*}{\delta} \left(C_0 - C(x,t)\right) = \pm nd(C^* - C(x,t))
$$

$$
d = \frac{FAD}{\delta} = \frac{D}{\delta} \cdot K_d \text{[cm s}^{-1}] \quad \text{Rate constant of mass transfer.}
$$

**Quiet solution; $E$ constant; plane geometry**

The maximum oxidation and reduction currents will be written:

$$
i_{max} = nFAD \frac{C^*}{\sqrt{4Dt}}
$$

Substituting $C_{Red}(x=0)$ and $C_{Ox}(x=0)$, and re-arranging:

$$
i_{max} = nFAD \frac{C^*}{\sqrt{4Dt}}
$$

Catho-anodic $i-E$ curve

$$
i = \frac{i_{max}}{1 + \exp \left( \frac{nF\left(E - E^0\right)}{RT} \right)}
$$

Anodic $i-E$ curve

$$
i = \frac{i_{max}}{1 + \exp \left( \frac{nF\left(E^0 - E\right)}{RT} \right)}
$$

Cathodic $i-E$ curve

$$
i = \frac{i_{max}}{1 + \exp \left( \frac{nF\left(E - E^0\right)}{RT} \right)}
$$

When $E \gg E^0$, $i \rightarrow i_{max}$
When \( E \ll E^0 \), \( i \to i_{Ox} \)

\[
i = \frac{i_{Ox}}{1 + \exp\left(\frac{nF}{RT}(E - E^0)\right)}
\]

when \( E \ll E^0 \), \( i \to i_{Ox} \)

\[
i = i_{Ox} \exp\left(\frac{nF}{RT}(E - E^0)\right)
\]

When \( E \ll E^0 \), \( i \to i_{Ox} \)

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Reversible i-E curves under connection

\[
E_{eq} = E^0 + \frac{RT}{nF} \ln \frac{C_{Ox}^{\text{eq}}}{C_{Red}^{\text{eq}}}; \quad i = 0
\]

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\]

Evolution of \( C_{Ox} \) and \( \phi \) as a function of \( E_{\text{applied}} \) in stationary diffusion regime

Curves i-E obtained for the anodic oxidation of species Red under stationary diffusion regime

Half-wave potential

That potential for which the intensity is half the sum of the oxidation and reduction limiting intensities.

\[
E = E^0 + \frac{RT}{nF} \ln \frac{D_{Red}}{D_{Ox}} + \frac{RT}{nF} \ln \left(\frac{i_{Ox} + \frac{i_{Ox}}{2}}{i_{Red} - \frac{i_{Ox}}{2}}\right)
\]

\[
i = \frac{i_{Red} + \frac{i_{Ox}}{2}}{2}
\]

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E_{eq} = E^0 + \frac{RT}{nF} \ln \frac{D_{Red}}{D_{Ox}} + \frac{RT}{nF} \ln \left(\frac{i_{Ox} + \frac{i_{Ox}}{2}}{i_{Red} - \frac{i_{Ox}}{2}}\right)
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\]
Anodic oxidation of Ag and reduction of Ag⁺ ion in HClO₄ medium (reversible curves): a) in the absence of its ions, b) in the presence of its ions

\[ \text{Anodic oxidation of a metal} \]

\[ M^{n+} + ne^- \rightarrow M; E_{f}^{0} \]

\[ E = E_{f}^{0} + \frac{RT}{nF} \ln \left( \frac{C_{M^{n+}}}{C_{M^{0}}} \right) \]

\[ i = nF \frac{RT}{nF} \exp \left( \frac{nF}{RT} (E - E_{f}^{0}) \right) \]

Anodic oxidation of Ag and reduction of Ag⁺ ion in HClO₄ medium (reversible curves): a) in the absence of its ions, b) in the presence of its ions

Mixed Potential

Potential types: equilibrium, mixed, limiting

Limiting potential
1. Amperometric and potentiometric titrations.
2. Electrochemical systems as indicators of equilibrium reactions.
3. Coulometry: determination of H₂O by Karl-Fischer

### Curves i-E obtained along a titrimetry

#### Fe²⁺ + Ce⁴⁺ ⇌ Fe³⁺ + Ce³⁺

Fe²⁺ + 1e⁻ → Fe³⁺    E° = -0.3V

#### Ce⁴⁺

Pre-equivalence

#### Fe²⁺ + Ce³⁺ ⇌ Fe³⁺ + Ce⁴⁺

Fe³⁺ + 1e⁻ → Fe²⁺    E° = 0.3V

#### Pre-equivalence

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Amperometría y potenciometría de indicación:

Amperometría (E = 0.6 V)

Potenciometría (i = 0)

Potenciometría (i ≠ 0) anóodo
Pre-equivalence

Equivalence

Post-equivalence

Amperometric and potentiometric indications

Amperometry (E = 0.6 V)

Equivalent point

Amperometry (E = 0.1 V)

Equivalent point

Potentiometry (i = 0)

Potentiometric indication at i ≠ 0

Potentiometry (i ≠ 0; anode)

Negative error

Potentiometry (i ≠ 0; cathode)

There is no indication

Start
Amperometric and potentiometric indications

Equivalence

Post-equivalence

Pre-equivalence

Amperometry (E = 0.6 V)

Amperometry (E = 0.1 V)

Potentiometry (i = 0)

Equivalent point

Equivalent point
Potentiometry (i ≠ 0; anode)

Potentiometry (i ≠ 0; cathode)

No indication whatsoever

Equivalence

Post-equivalence

Amperometric indication

Amperometry (E = 0.6 V)

No indication provided

Amperometry (E = 0.1 V)
Potentiometry with two indicator electrodes of the same kind. Titration of M with X in the presence of N. Potentiometric indication at \( i \neq 0 \). Evolution of the potentials of the indicator electrodes.

Curves i-E corresponding to the titration of M with X in the absence of N. Evolution of the potentials of the indicator electrodes.

Constant current potentiometry with two indicator electrodes of the same kind. Curves i-E corresponding to the titration of M with X in the absence of N. Potentiometric indication at \( i = 0 \).

Potentiometry with two indicator electrodes of the same kind.

Curves i-E corresponding to the titration of M with X in the presence of N. Evolution of the potentials of the indicator electrodes.
Constant current potentiometry with two indicator electrodes of the same kind
Curves $i-E$ for the $\text{Fe(CN)}_{6}^{3-}/\text{Fe(CN)}_{6}^{4-}$ system on a platinized platinum electrode.

Constant current potentiometry with two indicator electrodes of the same kind
Curves $i-E$ for the $\text{As(V)}/\text{As(III)}$ and $\text{Br}_2/2\text{Br}^-$ systems on a bright Pt electrode in an acidic medium

Curves $i-E$ for the $\text{As(V)}/\text{As(III)}$ and $\text{Br}_2/2\text{Br}^-$ systems on a bright Pt electrode in an acidic medium

Once all $\text{AsO}_3^{3-}$ has reacted, the excess of $\text{BrO}_3^-$ reacts with the $\text{Br}^-$:

$$\text{BrO}_3^- + 5\text{Br}^- + 6\text{H}^+ \rightarrow 3\text{Br}_2 + 3\text{H}_2\text{O}$$

Karl-Fischer reaction: determination of $\text{H}_2\text{O}$

Three-components mixture dissolved in $\text{CH}_3\text{OH}$ comprising:

$$\text{I}_2 : \text{C}_4\text{H}_4\text{N} : \text{SO}_2 = 1 : 10 : 3$$

It is carried out in a methanolic medium:

So as to avoid the non-stoichiometric reaction:
Side reaction: Reduction of sulfur components. After 1 - 2 weeks, smells like mercaptans.

\[
\text{Anode: Iodine production by oxidation} \\
2 \text{I}^{-} \rightarrow \text{I}_2 + 2 \text{e}^{-}
\]

\[
\text{Cathode: Hydrogen production by reduction} \\
2 \text{H}^+ + 2 \text{e}^{-} \rightarrow \text{H}_2
\]

Resolution: 0.1 µg water
Detection limit: 5 µg water for 5 g sample
Measuring range: 10 µg - 100 mg water/sample
1 ppm - 5 % water

\[
\text{Coulometric in-situ generation of reagent} \\
\text{Coulometric cell with diaphragm} \\
\text{Coulometric cell without diaphragm}
\]

Excess I₂ determination in an indirect titrimetry of a reducing agent.
Amperometry with a constant potential difference applied across two indicator electrodes

Titration curve for the complexometry of M with X (in the absence of N) by using an amperometric indication with a constant potential difference applied across two identical indicator electrodes.

\[
\Delta E_1, \Delta E_2, \Delta E_3
\]

Zoom of the potential axis where the two indicator electrodes (thanks to the magnitude of the potential difference forced across them) show the residual current (I ≈ 0) when x = 1.

\[
\text{Current flowing through each of the indicator electrodes at different stages of the titration:}
\begin{align*}
\text{a)} x = 0; & \quad \text{b)} x < 1; & \quad \text{c)} x = 1; \\
\text{d)} x > 1
\end{align*}
\]
Theoretical $i$-$E$ curves along the titration of $\text{Fe}^{2+}$ with $\text{Ce}^{4+}$.

Warning: sign criteria are reversed; doesn't matter since absolute values of flowing currents are measured.

Curves $i$-$E$ for the systems $5\text{O}_4^{2-}/2\text{S}_2\text{O}_3^{2-}$ and $\text{I}_2/\text{I}^-$ registered on a Pt electrode in diluted acidic medium.

Amperometry with a constant potential difference applied across two indicator electrodes.