Analytical Chemistry II Laboratory

VNIVERSITAT DE VALÈNCIA

Analytical Chemistry Department

Optical methods seminar

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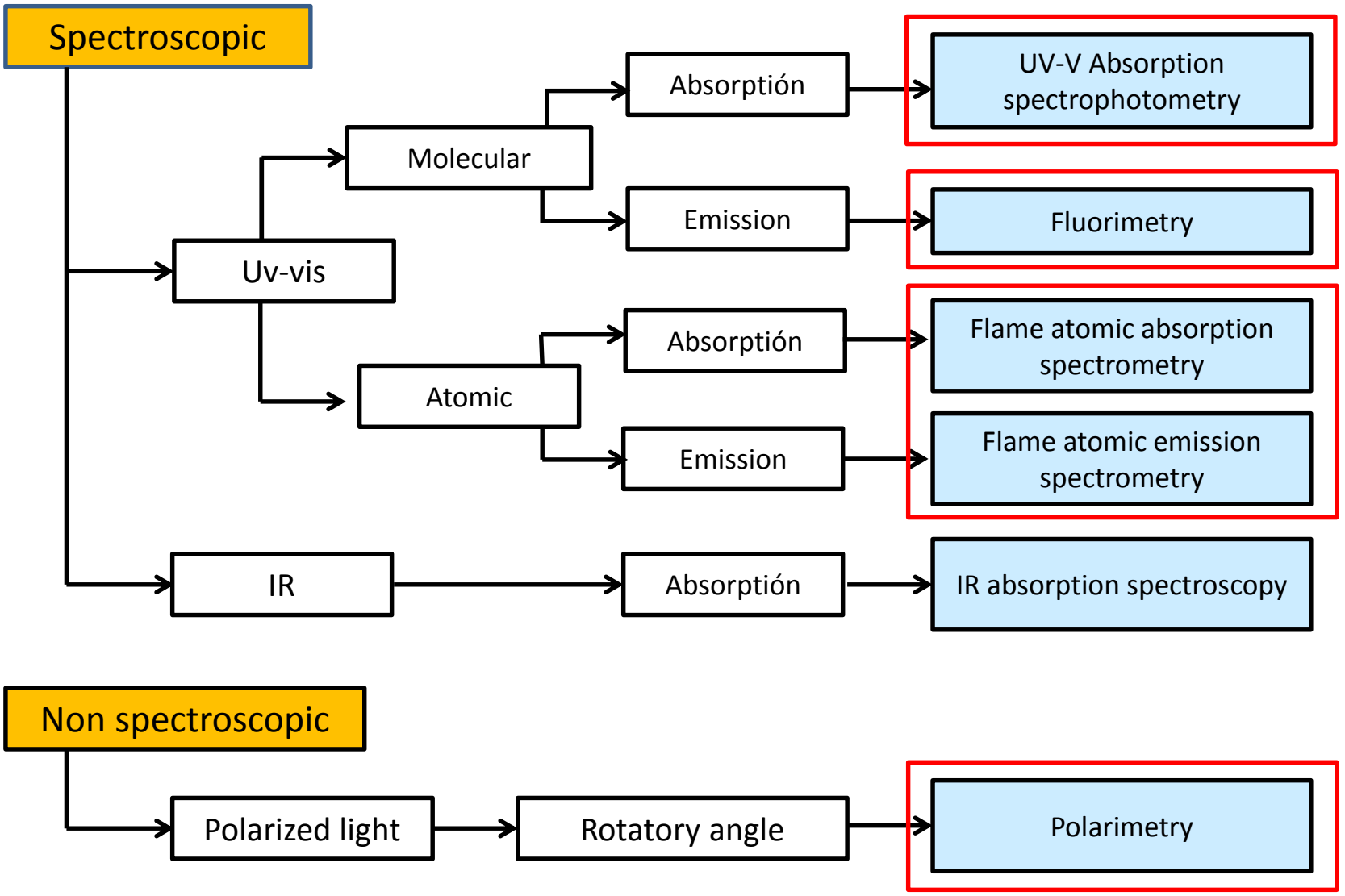
Optical methods



Practices

Practice 1

- Determination of caffeine and paracetamol in pharmaceuticals by derivative spectroscopy and multiple linear regression.

Practice 2

- Optimization of variables in molecular fluorescence: determination of quinine in tonic water.

Practice 3

- Determination of sucrose in condendensed milk by polarimetry.

Practice 4

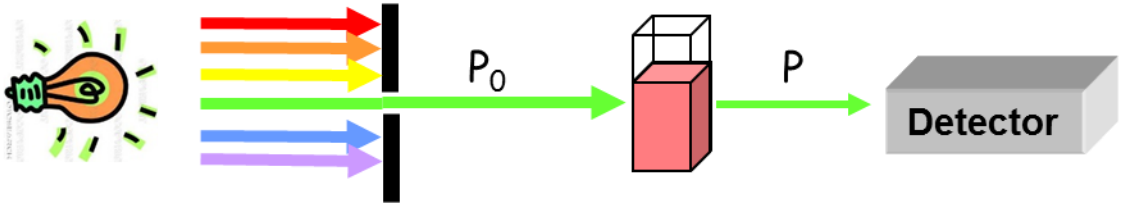
- Analysis of condensed milk. Determination of calcium by flame atomic absorption spectrometry.

Practice 5

- Determination of lithium in waters by flame atomic emission: study of the influence of variables on the analytical signal.

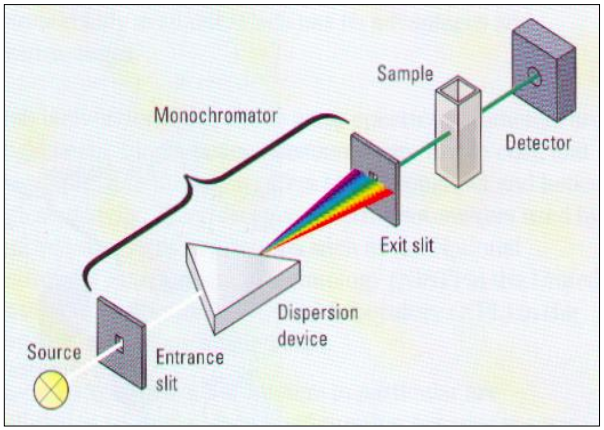
Basis: Molecular absorbance measurements in solution.

Absorption

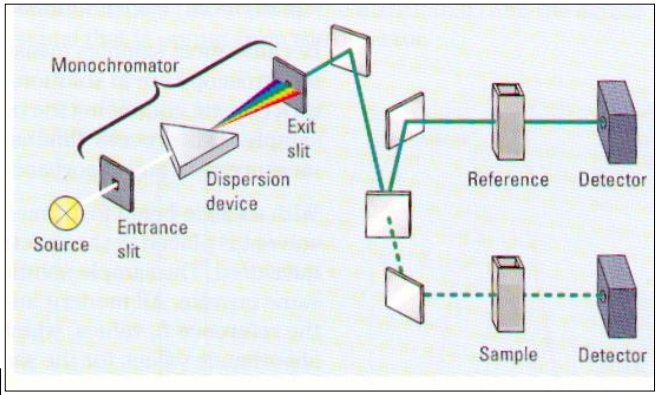


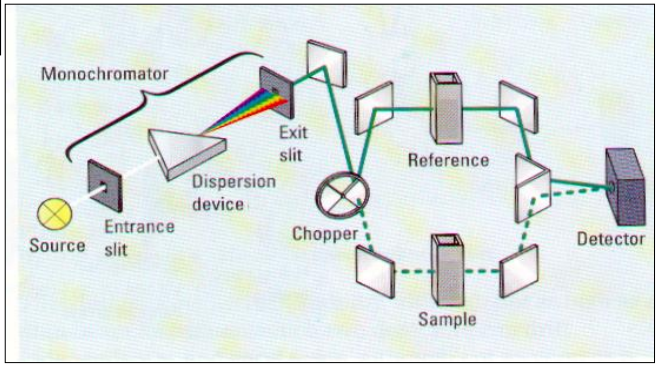
Different spectrophometer designs

simple or single beam

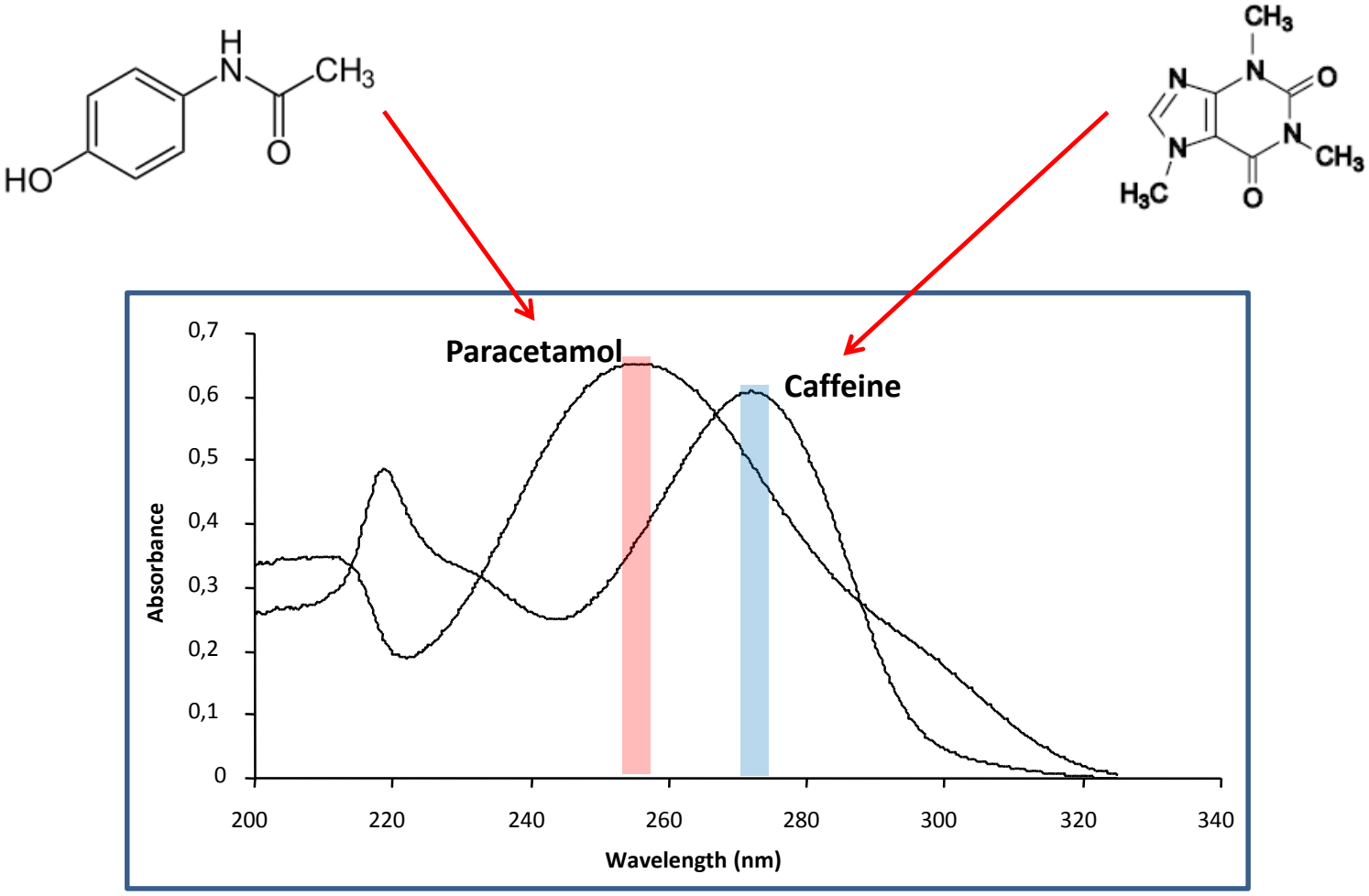


Double beam





Determination of caffeine and paracetamol in pharmaceuticals by derivative spectroscopy and multiple linear regression.



Overlapped signals: each analyte interferes in the quantitative determination of the other.

What can we do?

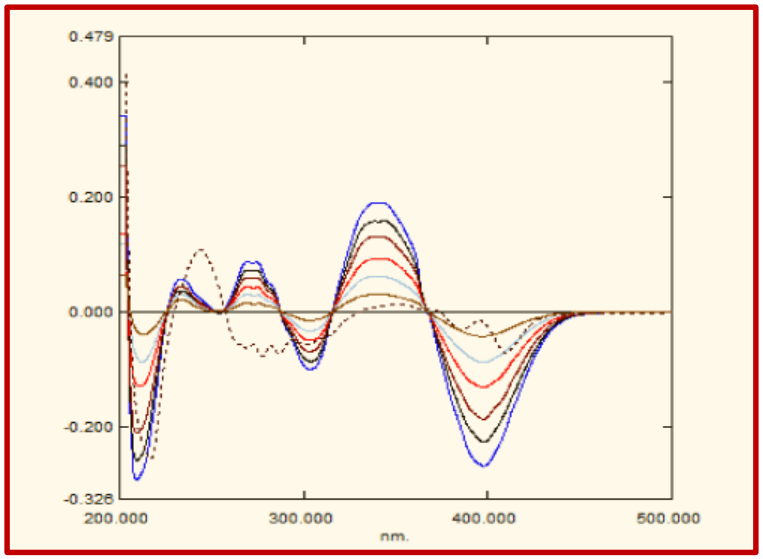
Two strategies

Multiple linear regression

$$A_{\lambda_1} = (\varepsilon_P)_{\lambda_1}\, b\, C_P + (\varepsilon_C)_{\lambda_1}\, b\, C_C$$

$$A_{\lambda_2} = (\varepsilon_P)_{\lambda_2}\, b\, C_P + (\varepsilon_C)_{\lambda_2}\, b\, C_C$$

Derivative spectroscopy



Multiple regression analysis

Beer's law can be applied to samples that contain **various absorbent** species whenever there are **no interactions** between them.

The absorbance of a multicomponent system can be determined by using the following expression, valid **for a defined wavelength**:

$$A_{total\lambda} = \Sigma A_{i\lambda} =$$

$$A_{A\lambda} + A_{B\lambda} + ... + A_{N\lambda} =$$

$$\varepsilon_{A\lambda} L c_A + \varepsilon_{B\lambda} L c_B + ... + \varepsilon_{C\lambda} L c_N$$

Where A, B, ..., N, are referred to the **different absorbing species**.

The absorbance of a system containing two absorbent species can be calculated by measuring the absorbance of the sample at two suitable wavelengths taking into account the following expression, valid at a given wavelength:

$$A_{sample\ \lambda1} = A_{A\ \lambda1} + A_{B\ \lambda1} = \varepsilon_{A\ \lambda1}\, L\, c_A + \varepsilon_{B\ \lambda1}\, L\, c_B$$

$$A_{sample\ \lambda2} = A_{A\ \lambda2} + A_{B\ \lambda2} = \varepsilon_{A\ \lambda2}\, L\, c_A + \varepsilon_{B\ \lambda2}\, L\, c_B$$

2 equations
2 variables

slopes

In our case:

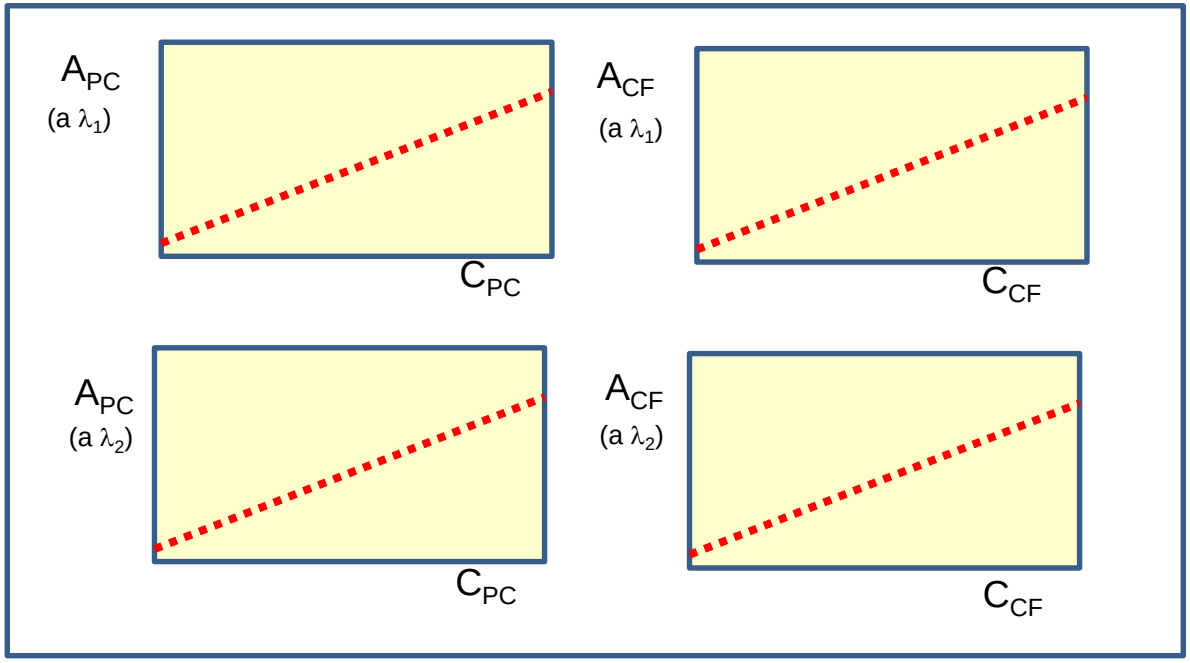
$$A_{total,sample\ \lambda1} = A_{PC\ \lambda1} + A_{CF\ \lambda1} = (slope_{PC\ \lambda1})\ c_{PC} + (slope_{CF\ \lambda1})\ c_{CF}$$

$$A_{total,sample\ \lambda2} = A_{PC\ \lambda2} + A_{CF\ \lambda2} = (slope_{PC\ \lambda2})\ c_{PC} + (slope_{PC\ \lambda2})\ c_{CF}$$

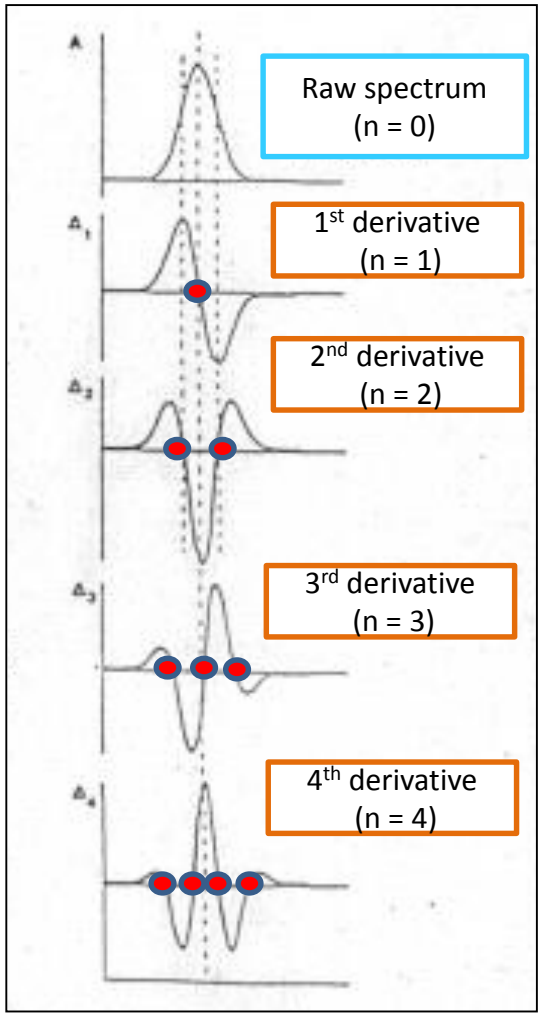
These four values are the corresponding slopes for each calibration lines. There are two for each analyte obtained from the measurements performed with the two selected wavelenghts (measurements are performed in the maximum of each analyte).

The wavelenghts that provide the maximum values for each compound are selected.

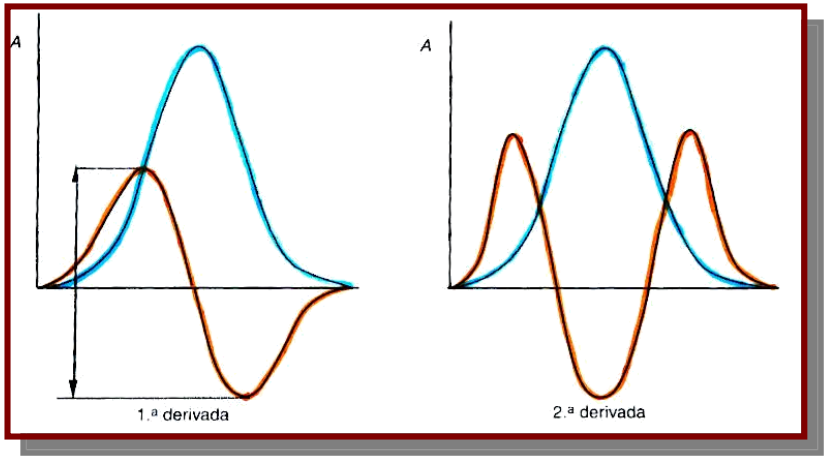
λ_1 = maximum absorption wavelength for paracetamol
λ_2 = maximum absorption wavelength for caffeine



Derivative spectroscopy



Iso-differential method or zero crossing

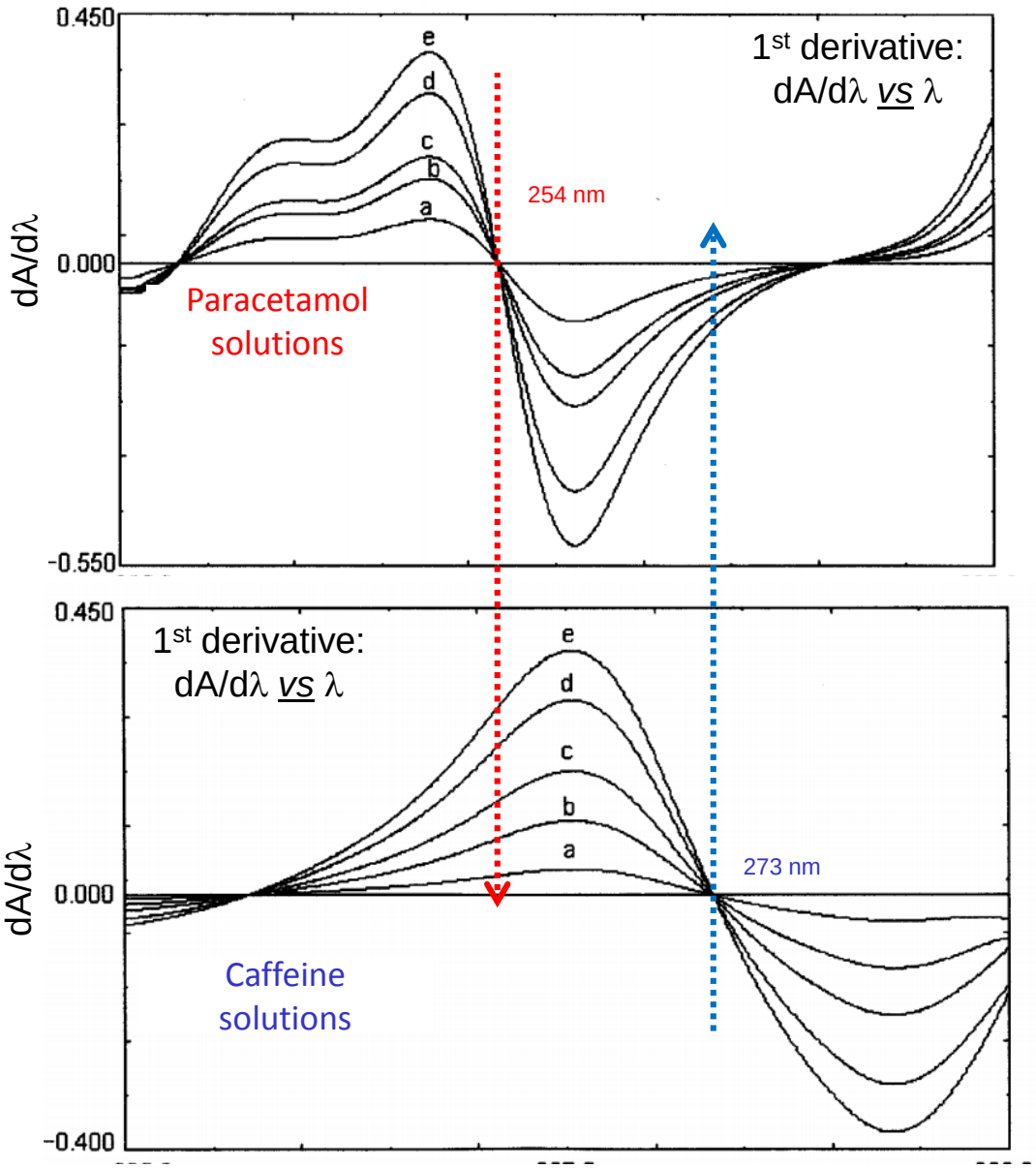


$$\frac{dA}{d\lambda} = \frac{d(\varepsilon_{PC})}{d\lambda} L\, C_{PC} + \frac{d(\varepsilon_{CF})}{d\lambda} L\, C_{CF}$$

Crossing points (signal = 0)
When we are working with an analyte standards are called:
Iso-differential points

In this sense:
Raw spectrum (**n=0**): iso-differential points = **0**, 1st derivative (**n=1**): iso-differential points = **1**, …..

Determination of caffeine and paracetamol in pharmaceuticals by derivative spectroscopy and multiple linear regression.



As an example:

Paracetamol absorbance 0 when λ_1 = 254 nm (Paracetamol iso-differential point that matchs with the maximum in the orden zero spectrum)

Caffeine absorbance 0 when λ_2 = 273 nm (Caffeine iso-differential point that matchs with the maximum in the orden zero spectrum)

Together these values we will measure:

- Paracetamol when λ_2 = 273 nm
  (caffeine absorbance = 0)
- Caffeine when λ_1 = 254 nm
  (paracetamol absorbance = 0)

Determination of caffeine and paracetamol in pharmaceuticals by derivative spectroscopy and multiple linear regression.

Working procedure

1.- Prepare caffeine calibration standard solutions spaning a concentration range between 2 and 10 ppm in presence of buffer solution (pH 10.5) from a 100 ppm caffeine standard solution.

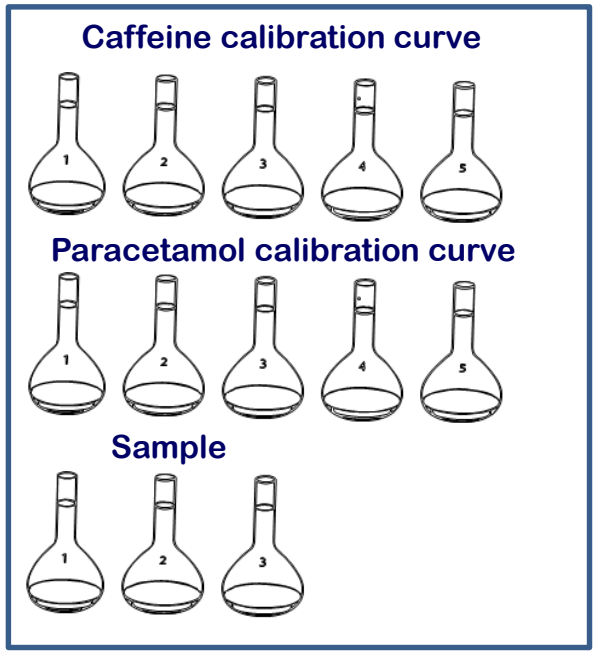
2.- Prepare paracetamol calibration standard solutions spanning a concentration range between 2 and 10 ppm in presence of buffer solution (pH 10.5) from a 100 ppm paracetamol standard solution.

3.- Prepare the sample per triplicate in a 0.05 M buffered medium and pH 10.5.

4.- Obtain the absorption spectra for standards and sample.

5.- Search the absorbance values for the two selected wavelegths in standards and samples.

6.- Obtain the order one derivative spectra and locate the wavelengths where caffeine and paracetamol values are zero.



Working procedure (*cont.*)

7.- Obtain the absorbance for sample and standards where paracetamol signal is zero in the first order derivative spectra.

8.- Obtain the absorbance for sample and standards where caffeine signal is zero in the first order derivative spectra.

Multiple linear regression

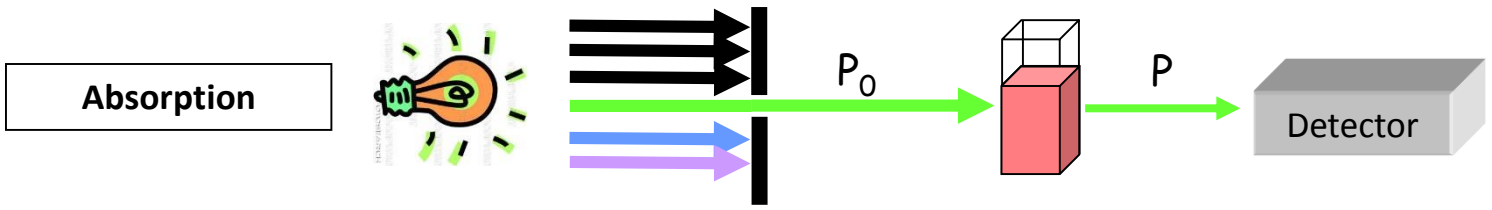
9.- Plot the calibration lines and determine the absoprtivity for caffeine and paracetamol.

10.- Solve de equation system.

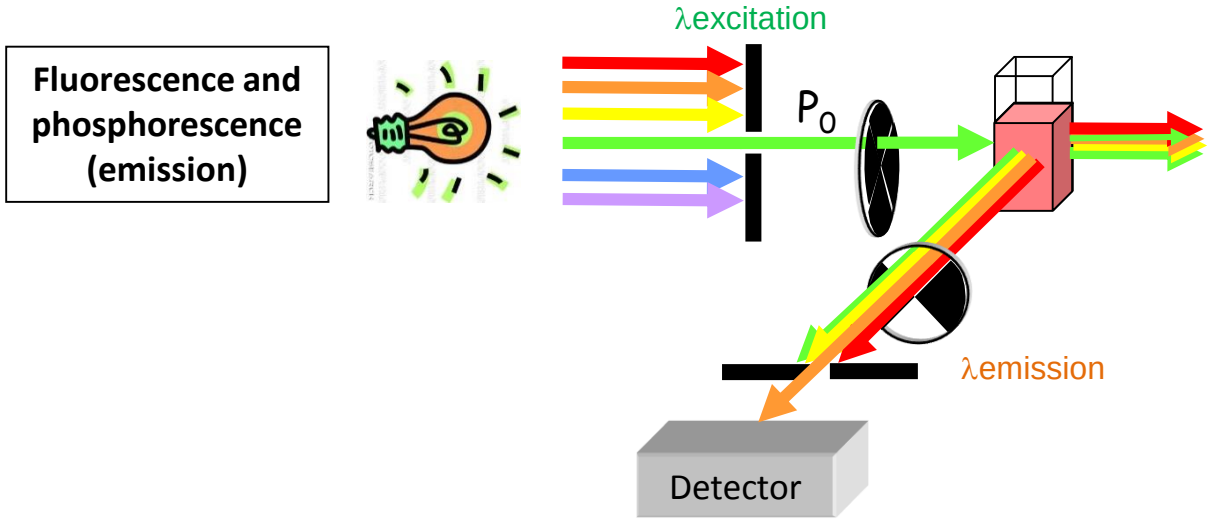
Derivative spectroscopy

11.- Plot the calibration lines and interpolate the sample signals to obtain the caffeine and paracetamol in samples.

MOLECULAR FLUORESCENCE SPECTROSCOPY

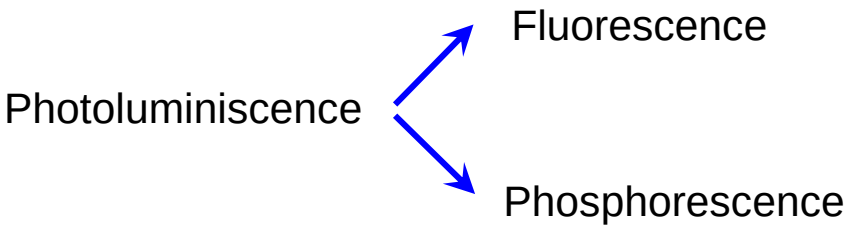


$$A = \varepsilon L c$$



$$I_f = P_0 \; K \; A = P_0 \; K \; (\varepsilon \; L \; c)$$

MOLECULAR FLUORESCENCE SPECTROSCOPY

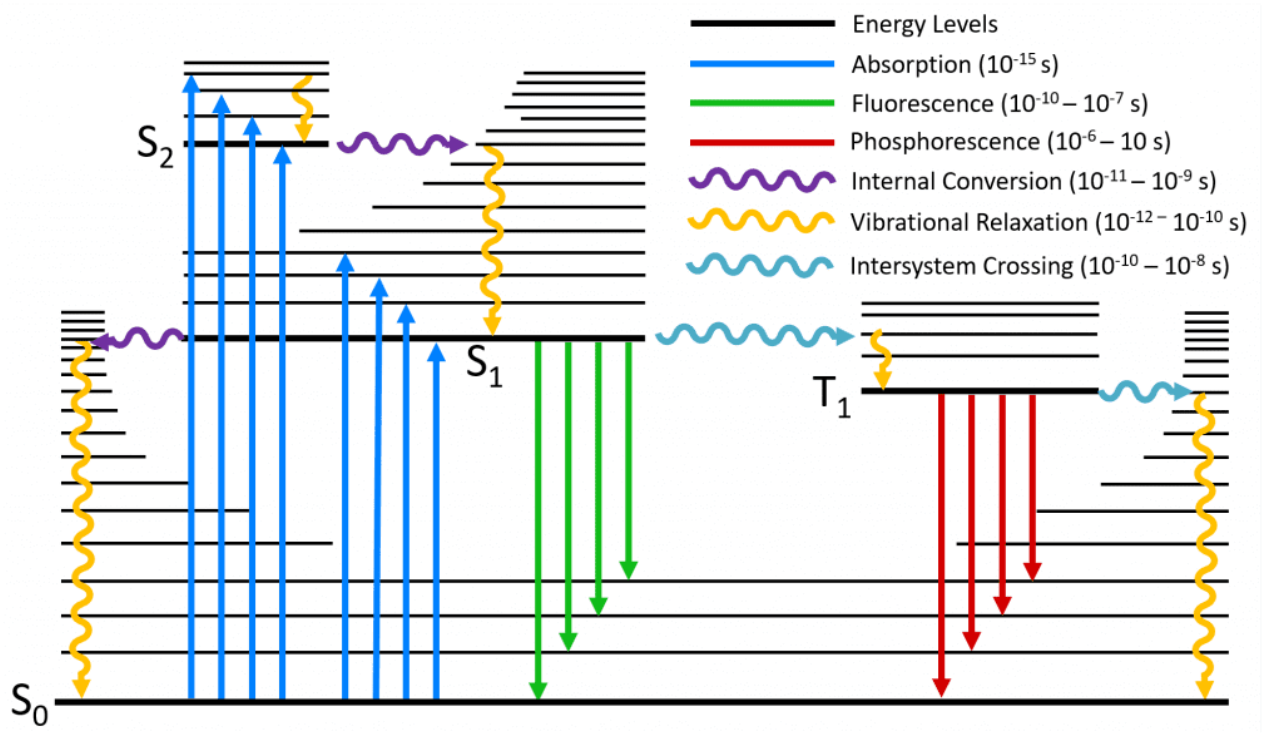


Excitation produced by electromagnetic radiation absorption

Return to the fundamental state by **emission** of excess energy as **photons**

Fluorescence → • Short life-time

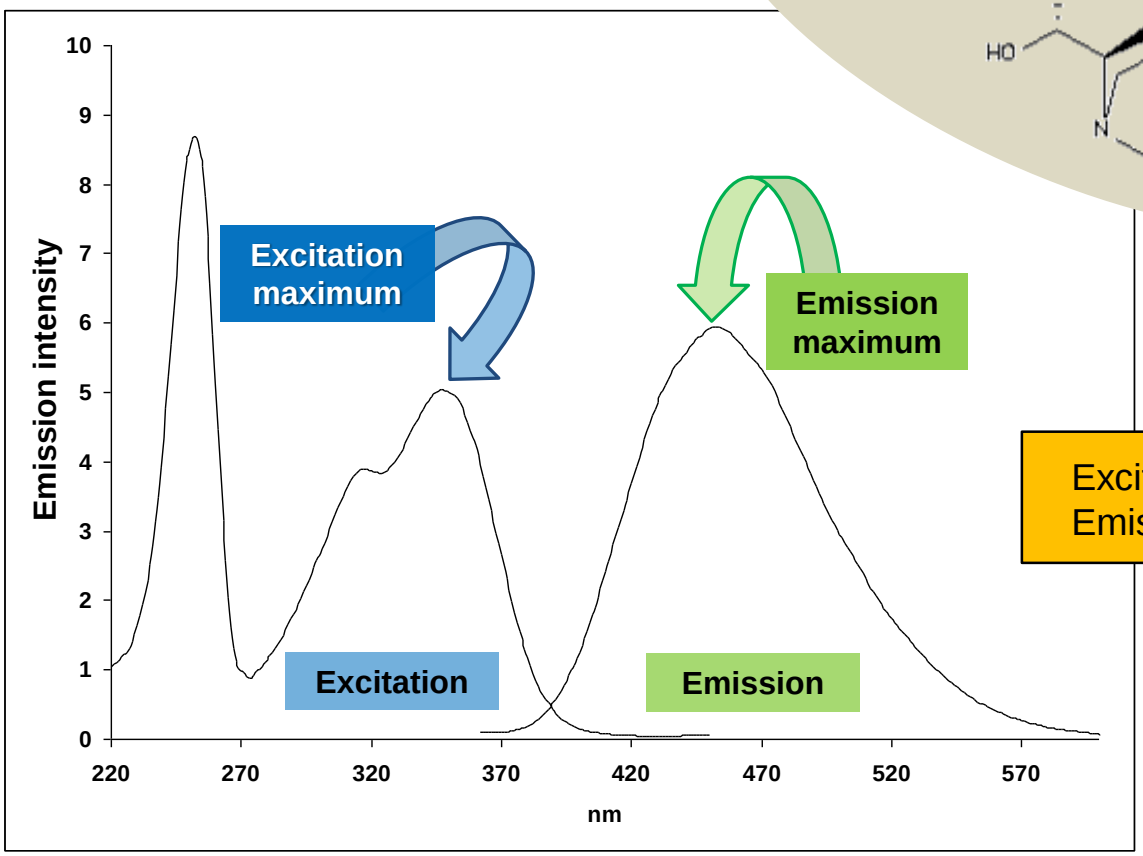
Phosphorescence →
- Longer life-time than fluorescence
- Lower intensities, less aplications in Chemical Analysis

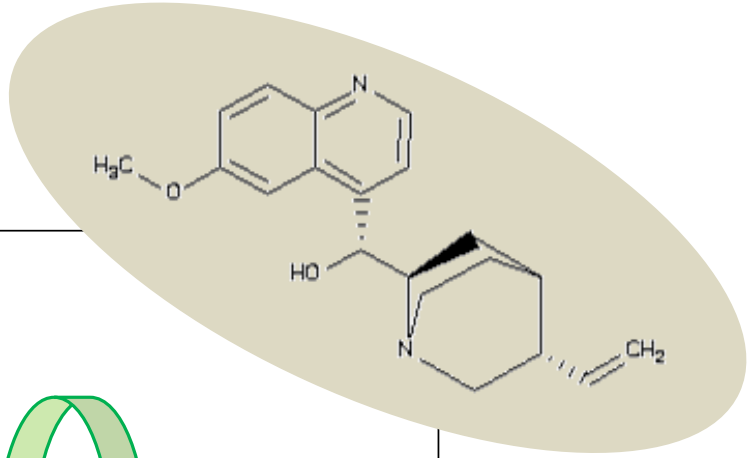


Mechanisms of relaxation to the fundamental state

- Vibrational relaxation
- Fluorescent relaxation

Optimization of variables in molecular fluorescence: determination of quinine in tonic water.





Excitation spectrum (λ_{em} = **450 nm**)
Emission spectrum (λ_{ex} = **350 nm**)

Emisión fluorescente

- Transición del nivel de vibración más bajo del estado electrónico excitado E_1 al estado fundamental E_0
- Transición del nivel de vibración más bajo del estado electrónico excitado E_1 a cualquier nivel de vibración de E_0
- Menor energía que la que acompaña a la excitación (desplazamiento de Stokes)
- Espectro de excitación, imagen especular de su espectro de emisión

Optimization of variables in molecular fluorescence: determination of quinine in tonic water.

Working procedure

1.- Prepare a 200 ppm (500 mL) quinine standard solution from quinine sulphate solid reagent. (Remember that quinine mole = 2 x quinine sulphate mole, $MW_{quinine}$ = 324.42 g/mol and $MW_{quinine\ sulphate}$ = 782.96 g/mol).

2.- Prepare a 5 ppm quinine standard solution in 0.05 M sulphuric medium (25 mL) and obtain the absorption spectrum between 220 and 450 nm. Locate the maximum absorption wavelength.

3.- Employing this maximum absorption wavelength as excitation wavelength (λ_{em}), obtain the emission spectrum. Locate the maximum excitation wavelength (λ_{ex}). Then, obtain the excitation spectrum and locate the maximum excitaton wavelength (λ_{ex}).

4.- Study the influence of emission wavelength on excitation espectrum and the excitation wavelength on emission espectrum. For this prourpose, select three λ_{em} and obtain their corresponding excitation spectra. In the same way, select λ_{ex} and obtain the emission spectra. Select two excitation-emission wavelengths as measurement conditions.

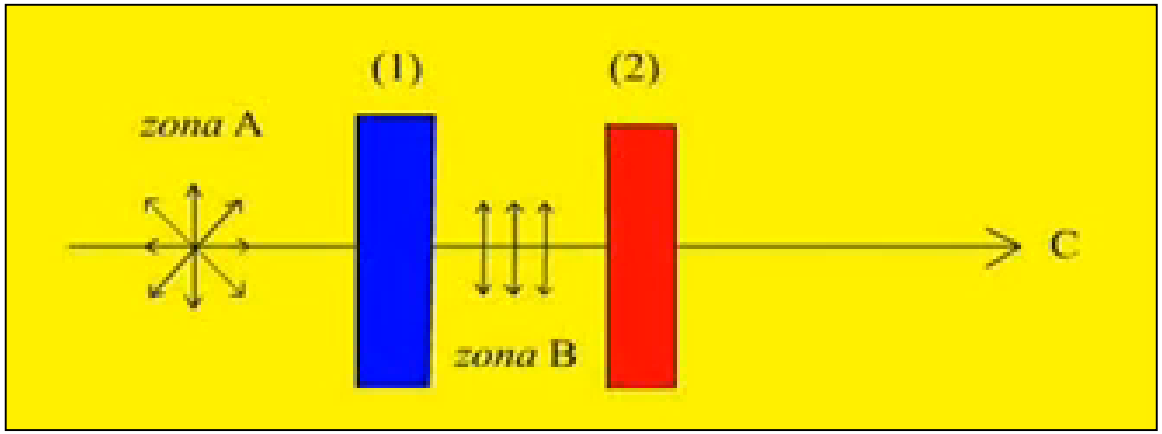
Working procedure (*cont.)*

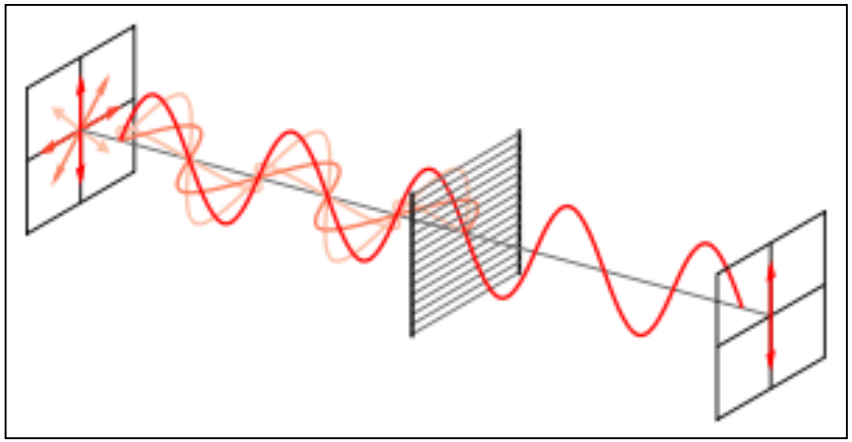
5.- Prepare calibration standard solutions in 0.05 M sulphuric medium. Prepare, per triplicate, removing first the gas by sonication and with the appropiate dilution for the calibration curve range.

6.- Measure the emission signals fpor standards and samples. In addition, measure ten times the blank in order to determine the limit of detection.

Polarized light measurements

"**Polarized light**" is the result of pass the light through a polarizing filter (**polarizer**) that forces electromagnetic waves to vibrate in a single plane.

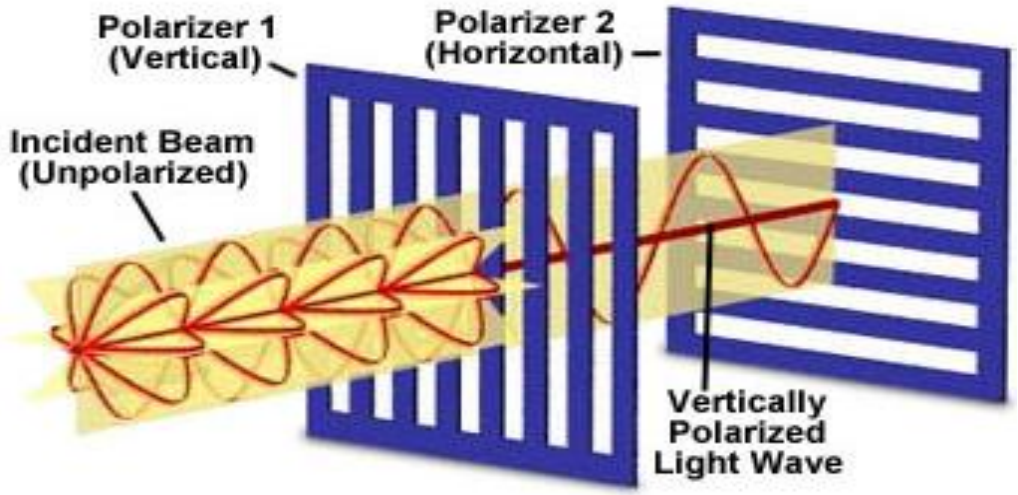




POLARIMETRY

When the polarized light in a plane passes through an **"optically active"** substance, the **polarization plane rotates** with an **angle** that is **characteristic of this substance**.

Polarimeters detect the position of the polarization resulting plane and compares it with the original position. "**Polarimetry**" is based on the measure of the ROTATION of light (α).



POLARIMETRY

Rotation (α) when a polarized light passes throught a length d cell, containing a solution of known concentration (C), responds to Biot's law:

$$\alpha_{i\lambda} = [\alpha]_{i\lambda} \; d \; C_i$$

Where, C = analyte contentration expressed as g/mL and d = cell length expressed in en dm.

Usually α is measured at λ = 589 nm (Sodium light).

To calculate the analyte concentration, the theoretical specific rotatory value $[\alpha]_{i\lambda}$ at a fixed measurement wavelength (λ) is employed, being this value expressed as g mL^{-1} dm^{-1}

$$C_i = \alpha_{i\lambda} / ([\alpha]_{i\lambda} L) \text{ g/mL} = 100 \, \alpha_{i\lambda} / ([\alpha]_{i\lambda} L) \text{ \% (m/v)}$$

Working procedure

Weight approximately 50 g of condensed milk in a 250 mL beaker and add approximately 60 mL of deonized water (at 80-90 ºC).

Shake by rotation to avoid the foam formation, cool to ambient temperature and transfer quantitatively to a 250 mL volumetric flask.

DON´T TAKE THE VOLUME TO THE MARK: do not add more than 150 mL in the volumetric flask.

Add:

+ 5 ml NH_3 1:10, shake by rotation and let resting 15 minutes.
+ 3-4 of phenol red solution and shake by rotation (the content will turn in a pink colour).
+ 10% acetic acid solution till the neutralization, shaking by rotation (the content will turn in a yellow colour).
+ 15 mL $Zn(CH_3COO)_2$ solution, shaking by rotation.
+ 15 mL $K_4Fe(CN)_6$ solution, shaking by rotation.

Working procedure (*cont.*)

TAKE TO THE MARK with deionized water.

If there is foam, add few drops of ethanol BEFORE TAKE TO THE MARK.

Let resting 15 minutes.

Filter through a dry pleated filter paper. Take the filtrate in a beaker discarding the 25 mL first elution volume. Close the beaker with a watch glass and label as "MILK FILTRATE"

USE ONLY A 50 mL of "MILK FILTRATE"
TO PERFORM THE POLARIMETRIC DETERMINATION

WARNING:
Reserve the other part of the filtered to perform the
Calcium determination

Determination of sucrose in condendensed milk by polarimetry.

Working procedure (*cont.*)

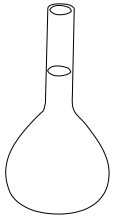
40 mL milk filtered

3 ml HCl (c) (12 M)

Hydrolisys: performed in water bath at 60ºC, 10 min, little shaking

Let cool, Transfer and take to the mark.

50 mL



Label as: "**HYDROLIZED MILK FILTERED**"

Let resting for 1 hour

When the hydrolysis is performed the sacarose transforms in **invert sugar** (glucose and fructose mix)

$C_{12}H_{22}O_{11} + H_2O$ (m. HCl) ⇆ $C_6H_{11}O_6$ + $C_6H_{12}O_6$

sacarose **glucose** **fructose**

1.000 g sacarose → 1.053 g invert sugar

Working procedure (*cont.*)

Direct analysis

Sacarose, Lactosa, Glucose +Fructose (invert sugar)

$$P_D = 0{,}665\,d\,m\,x/v + 0{,}525\,d\,my/v - 0{,}202\,d\,mz/v$$

(x, y, z sacarose, lactose and invert sugar percentages in milk)

Crystalized lactose [α-lactose].

Rotational power +89 mL dm^{-1} g^{-1}

Condensed milk dilution

Mutarotation: α-lactose ⇆ β-lactose

Rotational power: α-lactose +89° mL dm^{-1} g^{-1}

β-lactose +35° mL dm^{-1} g^{-1}

Equilibrium time ([β-lactose]/[α-lactose]=1.63) 24 h

Rotational power in equilibrium +52,5 ° mL dm^{-1} g^{-1}

Ammonia accelerates mutarotation process:
the analysis time decreases

Working procedure (*cont.*)

Hydrolysis

$$C_{12}H_{22}O_{11} + 2\ H_3O^+ \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6 + H_2O$$

Glucose + Fructose (invert sugar)

$$P_I = 1{,}053\,(-0.202)\,d\,m\,x/v' + 0.525\,d\,m\,y/v' - 0.202\,d\,m\,z/v'$$

v': final volume
v: initial volume $\longrightarrow$ f = v'/v

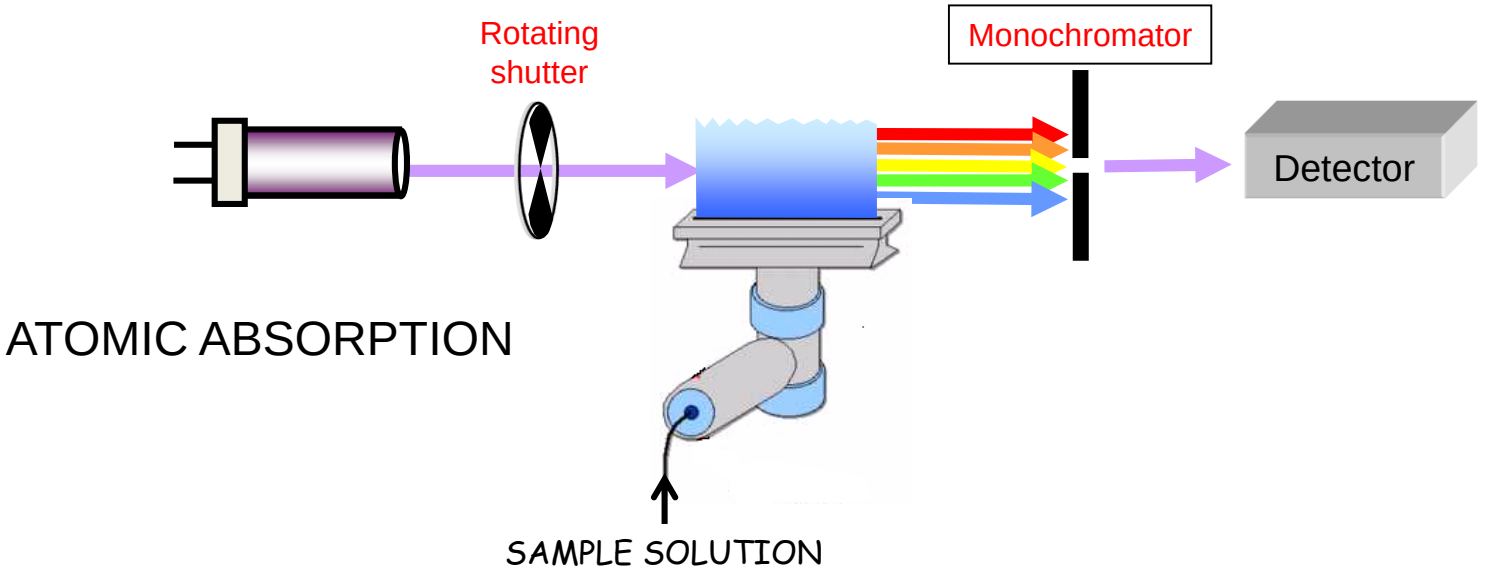
$$P_I f = 1{,}053\,(-0.202)\,d\,m\,x/v + 0.525\,d\,m\,y/v - 0.202\,d\,m\,z/v$$

$P_D - P_I f \longrightarrow$ Sacarose (%) $= \dfrac{(V - \Delta V)(P_D - fP_I)}{0{,}878dm}$

$$\Delta V = \frac{m}{100}(1{,}08G + 1{,}55P)$$

FLAME ATOMIC ABSORPTION SPECTROSCOPY

BASIS: Absorbance or emission intensity provided by the atoms formed in the flame measurements



The monochromator selects the element under study absorption wavelength.

Due to atomic absorption and emission wavelengths are coincident, a rotating shutter is required (the light sent will be discontinuous) to discriminate between transmitted light (after absorbing part of the light sent by the lamp) and emitted light (it doesn't come from the lamp, it will be continuous).

Calcium determination in milk by FAAS: Troubleshooting

INTERFERENCES :

Proteins:
Physical interference.
Solution: precipitate and filter → Done!!!!

Phosphates:
Chemical interference.
Solution: La (III) masking

Ionization:
Sensitivity loss
Solution: La (III) ionization buffer addition
La^0 is easily ionized than Ca^0 :

$$Ca^{2+} \xrightarrow{\text{atomization}} Ca^0 \xrightarrow{\text{ionization}} Ca^{+n} + ne^-$$

$$La^{3+} \xrightarrow{\text{atomization}} La^0 \xrightarrow{\text{ionization}} La^{+n} + ne^-$$

When we don't have any additional chemical interference, we can use a external traditional calibration.

But if we suspect that another type of chemical interference can be present in the determination, a standard addition calibration is needed.

Working procedure

Calcium concentred standard solution = 1000 μg/mL prepared in 50 mL volumetric flask

- Weight $CaCO_3$ (analytical balance) in a beaker.
- Add 10 mL HCl 1 M and dissolve (employ heat if it is necessary).

  Note: HCl 1M (each 2 pairs)
  approximately 25 mL HCl 1M (from HCl 12M)
- Let cool, transfer to 50 mL volumetric flask and take to the mark.

Label as "Calcium – x μg/mL", where x is the exact concentration (according your weight) expressed with 4 decimal places.

Calcium intermediate standard solution = 50 μg/mL calcium prepared in a 100 mL volumetric flask

- Shake the concentrated standard solution. Transfer 20-25 mL in a dry beaker.
- Take the appropriate volume from the beaker and take to the mark.

Label as ""Calcium – x μg/mL", where x is the exact concentration expressed with 4 decimal places.

Working procedure (*cont.*)

External traditional calibration

- Employing 25 mL volumetric flasks, prepare a calibration line in a concentration range between 0 to 8 μg mL^{-1} with:
  - 2.5 mL La (III) (0.1%) solution
  - Corresponding volumes of intermediate calcium solution
- Label as P0, P1, P2, P3, P4

Standard addition calibration

- Employing 25 mL volumetric flasks, prepare a calibration line in a concentration range between 0 to 10 μg mL^{-1} (take into account that condensed milk contains approximately 2 mg of calcium per gram) with:
  - 2.5 mL La (III) (0.1%) solution
  - Corresponding volumes of intermediate calcium solution
  - 1 mL of prepared sample

Label as: M + P0, M + P1, M + P2, M + P3, M + P4

Working procedure (*cont.*)

Standard addition sample preparation:

- Weight 2.0000 g of milk in a 50 mL beaker.
- Add 20 mL of 80C heated deionized water and mix by rotation.
- Cool and transfer to a 50 mL volumetric flask. Take to the mark.

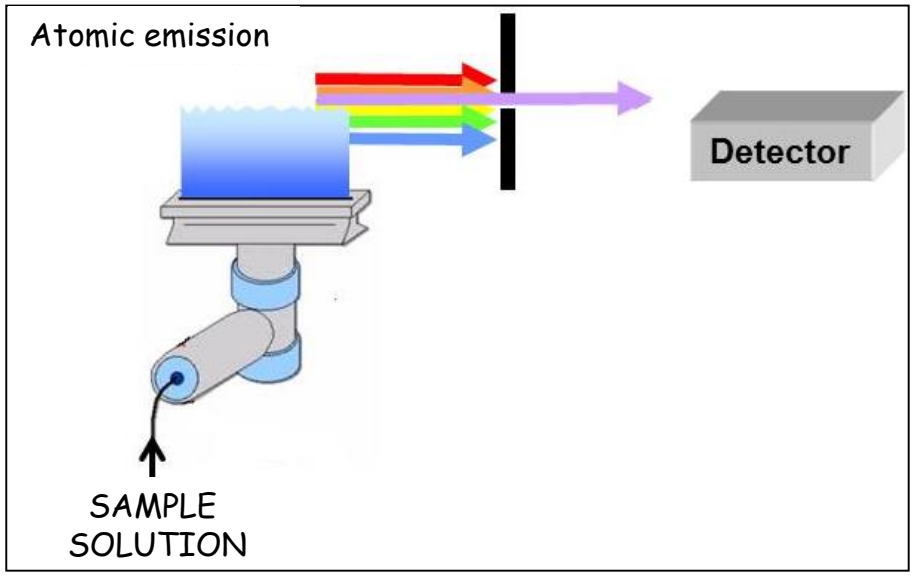
External calibration sample:

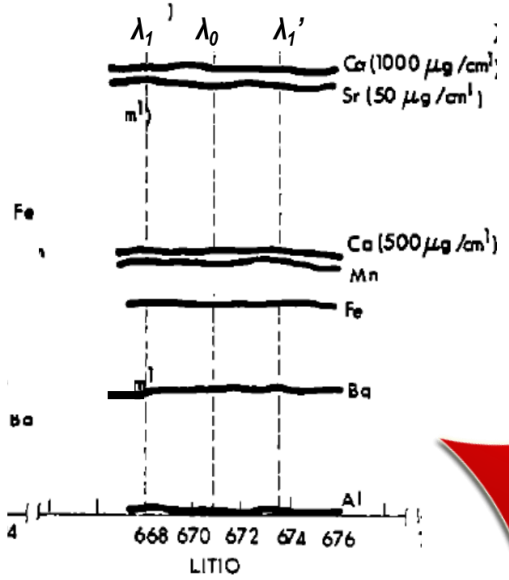
- Take 10 mL of “MILK FILTRATE” and dilute till 50 mL in a volumetric flask with deionized water.
- Take 1 mL of the previous solution in a 25 mL volumetric flask and add 2.5 mL of La (III) 0.1%.
- Take to the mark with deionized water.

Determination of lithium in waters by flame atomic emission: study of the influence of variables on the analytical signal.

BASIS: Emission intensity provided by the

atoms formed in the flame measurements





$Li^{+}_{(aq)} \rightarrow Li^{o}_{(gas)} \rightarrow Li^{*}_{(gas)} \rightarrow Li^{o}_{(gas)} + h\upsilon$

Possible interferences in Li determination:

- Spectral interferences (Ca, Ba, Sr): Remove by precipitation
- Ionization interference: ionization buffer

Working procedure

Ca, Ba and Sr influence

- From the 100 μg/ml lithium standard solution, prepare a 2.5 μg/ml Li intermediate standard solution in a 50 mL volumetric flask.
- Calculate the required volumes to prepare the solutions indicated in Table 1 employing 25 mL volumetric flasks and the Li intermediate standard solution. K (1%), Ca (1%), Ba (1%) and Sr (1%) standard solutions are previously prepared by the laboratory technicians.

Table 1: Interference studies

Solución	Li	K	Interferent
Tunning	1.0 μg /ml	0.2 %	-
1	0.2 μg /ml	0.2 %	-
2	0.2 μg /ml	0.2 %	Ca^{2+} 100 μg /ml
3	0.2 μg /ml	0.2 %	Ca^{2+} 3000 μg /ml
4	0.2 μg /ml	0.2 %	Ba^{2+} 100 μg /ml
5	0.2 μg /ml	0.2 %	Ba^{2+} 3000 μg /ml
6	0.2 μg /ml	0.2 %	Sr^{2+} 100 μg /ml
7	0.2 μg /ml	0.2 %	Sr^{2+} 3000 μg /ml

Working procedure (*cont.*)

Sample solutions

Per triplicate:

- Use a beaker:
  - 2.5 mL sample
  - 25 mL deionized water
  - 2.5 mL precipitant solution

- Boil the mixture till the volume decrease to the third part of the initial one. Allow to stand for 30 minutes and filter with paper in a 25 mL volumetric flask. Clean the paper with water and take to the mark with deionized water.

Working procedure (*cont.*)

Calibration standard solutions

Employing 25 mL. volumetric flasks, prepare solution with 0, 0.05, 0.10, 0.15 y 0.20 μg/ml of Li using:

- the required volumes of 2.5 μg/ml Li standard solution
- 2.5 mL de precipitant solution.

Adjust the photomultiplier with the most concentrated calibration standard solution.

Measure the emission intensity of the standard solutions and samples.