INSA INSTITUT NATIONAL DES SCIENCES APPLIQUÉES ROUEN NORMANDIE

TRAVAUX PRATIQUES DE SPECTROSCOPIE ET DE CHROMATOGRAPHIE

CFI3 Semestre 5

EC SPECTRO-CHRO

Contenu

In accordance with the sustainable development and social responsibility approach, we avoid printing sheets and data unnecessarily, chemicals used in laboratory manipulations are reprocessed, we choose solvents that limit exposure to chemical risks

YEAR 2024-25

ORGANISATION DES TRAVAUX PRATIQUES

Les travaux pratiques ont une durée de 4h. Ils sont organisés sous forme tournante. Les élèves doivent consulter la fiche de rotation avant le début des séances pour savoir quel TP préparer. La présence est obligatoire dès le début du TP. Les retards seront sanctionnés.

SECURITE

Avant chaque TP, les élèves doivent avoir pris connaissance des risques liés à l'utilisation des produits chimiques en consultant leur fiche FDS sur internet et en remplissant la partie produit de la fiche de sécurité donnée page 33. Il en est de même, pour les risques liés à l'utilisation des appareils. Pour ce faire, ils peuvent consulter en début de séance la fiche de risque de l'appareil qui est apposée près de celui-ci.

Cette fiche de sécurité doit être remise avec le compte rendu (elle fera partie de l'évaluation). Elle est également **vérifiée avant le début de la séance de TP**.

Les règles de sécurité inhérentes à un laboratoire de chimie doivent être respectées (Blouse, lunette, gants…).

FEUILLES DE RESULTATS

Afin d'éviter toute tricherie, les copies d'écran des feuilles de résultats sorties par les appareils **doivent être remises avec votre compte rendu** et le nom de famille d'un étudiant doit apparaitre. Nommer vos méthodes et échantillons en commençant par ce nom.

Toute absence de ces feuilles conduira **à une diminution de la note d'évaluation.**

DEROULEMENT DES SEANCES

Les étudiants doivent avoir pris connaissance du TP avant de venir et préparer les calculs de dilutions. Les notices utilisées pour programmer les appareils sont générales. Les conditions du TP priment sur les indications prises en exemple dans les notices.
Au cours de la séance, les étudiants doivent s'avancer dans le traitement des résultats et dans l'élaboration du compte rendu :

- Les droites d'étalonnage ou autre courbe doivent être tracées pendant la séance (il est conseillé apporter un portable). Cela permettra de corriger immédiatement toute erreur de calibration.
- Les calculs des teneurs finales des échantillons doivent être effectués.
- Il est conseillé de débuter la rédaction du compte rendu lors des phases d'attente.
- Nettoyer la verrerie, reconstituer le poste de travail et montrer l'ensemble des résultats à l'enseignant avant de quitter la salle.

Qualités d'eau utilisées

Le laboratoire de chimie analytique utilise 2 types d'eau : l'eau ultrapure et l'eau désionisée.
L'eau ultrapure est la plus pure mais elle coute aussi plus cher à produire.
Bien suivre les consignes des TP pour utiliser l'eau de façon optimale.

EVALUATION

La note d'évaluation de la séance de travaux pratiques est décomposée de la manière suivante :

- 5 points : comportement en séance : ponctualité, autonomie, efficacité, exploitation des résultats (tracé des courbes, calculs…), propreté de la paillasse
- 15 points : compte rendu

COMPTE RENDU

Les CR des TP 2 et 4 sont à rendre en fin de séance. Pour les autres, le compte rendu doit être remis **15 jours** après la séance sous format papier (transmission par voie électronique non acceptée).

Le compte rendu doit comporter **au minimum** les éléments suivants :

- Vos noms et prénoms, ainsi que le nom de votre encadrant
- Une présentation **succincte** du principe de la technique (5 lignes + éventuellement 1 schéma suffit).
- Il faudrait indiquer le nom de l'appareil et de ses réglages comme vous feriez en entreprise par exemple : « Chromatographe Varian 3300 équipé d'une colonne capillaire de 30 m de long (en polyethyleneglycol), de diamètre interne 0.32 mm … ». Comme ces conditions sont indiquées dans le fascicule, on vous demande uniquement de répondre à une question relative à celles-ci après avoir rappelé le fournisseur de l'appareil et le nom du logiciel utilisé.
- La préparation des solutions décrite complètement mais de façon synthétique.
- La réponse aux questions posées dans l'énoncé de TP (l'intégrer au CR).
- Le tracé des droites d'étalonnage et leur évaluation d'un point de vue statistique (passage par l'origine, linéarité).
- Le cas échéant, l'étude de la répétabilité (assortie du calcul du coefficient de variation)
- Le calcul des teneurs dans les échantillons (**pas seulement dans les solutions finales**) par exemple : mg quinine/L dans le Schweppes. Il faudra aussi calculer l'incertitude sur ces résultats. Idéalement, chaque détermination de solution inconnue doit être tripliquée (trois analyses).
- Une conclusion présentant l'intérêt du travail et ces résultats (éventuellement en comparant à des normes ou teneurs usuelles).

Un exemple type de rapport est proposé sur moodle. Merci de lire attentivement les conseils.

Pour vous aider à rédiger, votre premier CR sera relu par votre encadrant puis vous sera rendu afin que vous puissiez faire les éventuelles modifications nécessaires avant la notation finale.

- Pas de CR écrit à la main,
- Statistiques obligatoirement faites par Excel,
- Pas de sommaire

TP 1: FOURIER TRANSFORM INFRA RED SPECTROSCOPY (FT-IR)

APPLICATION TO THE DETERMINATION OF FATTY ACID METHYL ESTERS IN BIODIESEL

STUDY OF THE ROTATIONAL-VIBRATIONAL HCl GAS SPECTRUM

The instrument and its accessories are costly, please take care!!

I – Principle of Infrared spectroscopy

Infrared spectroscopy is an analytical method used for the determination of functional groups in organic chemistry. It is also used for the quantification of molecules or groups of molecules (polymer, oil, pollution, fiber…).

II. Goal of the lab session.

In this lab session, you will learn how to use a Perkin Elmer spectrum 2 FT-IR instrument (cost 27 000 €).

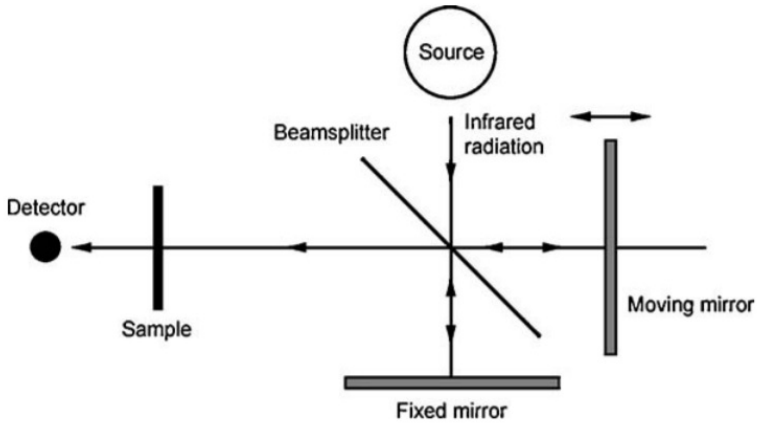
The goal is to show you the different ways to prepare the sample (gas, solid, liquid) and analyze it. We will also use this instrument for the determination of fatty acid methyl esters in biodiesel and the study of the HCl molecule in the gas phase.

III –Instrument

The original infrared instruments were of the dispersive type: it was a double-beamed instrument (reference and sample) with a constant energy on the reference beam and the light was dispersed by a grating. On these old instruments, the time of acquisition was around 10 minutes for a full spectrum (4400 - 450 cm^{-1}).

Justify the choice of wave number range for the molecules analyzed in this lab session.

Today, instruments are of the Fourier transform type: the dispersion in space is replaced by a modulation in time using a mobile mirror. The use of a Michelson interferometer and Fourier transform allow a very fast acquisition (4s).



The IR Spectrum is always corrected from the background spectrum (air or other) by making the ratio between the transmitted energy of the sample and the blank. The background must be done

in the same conditions as the sample (redo it anytime you change the acquisition parameter or the accessory).

On the spectrum, the y-axis is the transmission or the absorbance. Generally, spectra are presented as %T (transmission)=f(s), but for quantitative determination, only absorbance is proportional to the concentration (Beer-Lambert Law).

Follow the instructions in the manual to operate the instrument.

IV – IR SPECTRUM OF A POLYSTYRENE FILM.

The instrument being a single beam one, we have to correct the signal from the blank first. So, the first thing to do is to acquire the background (blank).

Check that the set-up conditions for the scan are the following:

- resolution: 4 cm^{-1}
- Spectral range: 4500 - 450 cm^{-1}
- Number of scans: 4
- apodisation: strong

Print the spectrum as a pdf file.
Note the CO_2 and H_2O absorption bands that are present in the air. Why don't we see the absorption of O_2 and N_2?
Delete after printing the spectrum
Place the polystyrene film and acquire with the same conditions as the background
Label the peaks and print the transmission spectrum as a PDF file. Assign main peaks on the spectrum.
Transform the transmission spectrum into an absorbance spectrum and print it as a pdf file.

V- PREPARATION OF SAMPLES FOR IR-FT SPECTRUM ACQUISITION.

V- 1) Methods used for a liquid sample:

We take the example of the IR spectrum of methyl oleate.

a) Liquid sample: film between 2 KBr or NaCl pellets

For qualitative study (determination of functional groups in organic synthesis), the acquisition can be performed on a liquid film between two KBr or NaCl pellets. Why do we use this material?
Conditions: range 4500-450 cm^{-1}.
Capture the background on air (4 scans) with the two KBr or NaCl pellets. Why do we have to perform a background again?
Measure the IR beam's **energy** before and after placing the accessory.
Place a drop of the liquid on one pellet of KBr or NaCl, place the second pellet on top, and make a turn to obtain a film.
Capture the film spectrum of methyl oleate, label the peaks, print it, and assign the main peaks on the spectrum.
CAUTION: These materials are very moisture-sensitive. These crystalline pellets should be stored in a desiccator. They should be washed with dichloromethane after use (never water!). Call the instructor to help you.

b) Liquid sample: HATR.

The HATR method (Horizontal Attenuated Total Reflectance Infrared) is used here. This method can be used either for liquid, powder, or paste. The IR beam goes through a ZnSe (high refractive index) crystal where it undergoes several reflections. There is contact of the beam with the sample on several points where the radiation penetrates slightly in the liquid.

However, as these several reflections create interferences in the IR spectrum, a mathematic correction must be applied to the spectrum to get the absorption spectrum.
Call the instructor to place the accessory in the FT-IR instrument.
Measure the IR beam's **energy** after you place the accessory. Compare the energy with the 2 accessories.
Capture the background spectrum with the instrument in place.

- Resolution: 4 cm^{-1}
- 4 SCANS between 4500 and 600* cm^{-1}

*600 cm^{-1} because ZnSe crystal absorbs below.
Place the liquid sample on the crystal. CAUTION! DO NOT SCRATCH THE CRYSTAL!
Capture the spectrum using the same conditions as the blank, label the peaks, print the spectrum as a PDF file, and assign the main peaks on the spectrum.
Wash the crystal with dichloromethane. Call the instructor to help you.

V - 2) Methods for a solid sample:

Call the instructor to help you.

a) solid sample: pellet of the solid compound diluted in KBr or NaCl

❶ Preparation of the pellet to obtain the background spectrum:
Transfer quickly in a mortar 10 mg of KBr and grind. The sample must be finely divided. Transfer the mixture between two stainless disks and use the manual press to apply pressure and make a thin transparent solid

Change the SETUP: range 4500 to 450 cm^{-1}, (4 scans);
Measure the **energy** of the IR beam after you place your pellet.
Capture the background spectrum on air

❷ Preparation of the pellet containing methyl hydroxybenzoate: $C_8H_8O_3$:
Add around 1% of the sample to the mortar and then grind again. Transfer the mixture between two stainless disks and use the manual press to apply pressure and make a thin transparent solid film.

Capture the sample spectrum.
Correct the baseline
Label peaks, print the spectrum as a PDF file, and assign main peaks on the spectrum.
Don't forget to wash the mortar (with water).

b) solid sample: ATR

The principle is close to HATR with a single reflection. It can be applied to liquids (provided their viscosity is sufficient to obtain a thick film) or solids.

We won't use this method in this lab session to avoid too many manipulations of the accessory (cost: 10000 €) but you will use it in your organic chemistry lab sessions. Ask the instructor to show you what it looks like and how it works.

VI. Rotational – Vibrational spectrum of HCl.

H : 1,0 g.mol^{-1} et Cl : 35,5 g.mol^{-1}

As seen in the spectroscopy course, diatomic molecules in the gas phase give a rotational-vibrational spectrum. These peaks can be used to determine the concentration of some gas (CO_2, CO, …) in the air.

Diatomic molecules have rotational (J) and vibrational (v) energy levels. The expression of their energy is:

$$E(v,J) = h\nu_0(v+\frac{1}{2}) + hcBJ(J+1) \quad (1)$$

where $\nu_0 = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}$ $\quad B = \frac{h}{8\pi^2 Ic}$ in these expressions:

-μ is the reduced mass $\mu=(m_1 x m_2)/(m_1+m_2)$ where m_1 and m_2 are the masses of the atoms in kg.

- I is the moment of inertia ($I=\mu r^2$ where r is the intermolecular distance).

- k is the force constant (the system is considered a harmonic oscillator)

In expression (1), we use a harmonic potential for the description of the vibration without any interaction between rotation and vibration.

The use of a more complex model results in the following expression:

$$\frac{E}{hc} = \frac{\nu_0}{c}(v+\frac{1}{2}) - C_0(v+\frac{1}{2})^2 + BJ(J+1) - D_0 J^2(J+1)^2 - \alpha_0(v+\frac{1}{2})J(J+1) \quad (2)$$

C_0 is due to the use of an anharmonic potential (Morse potential). D_0 is the centrifugal distortion constant (the intermolecular distance increases with the rotating speed). Eventually, α_0 comes from the interaction between the vibrational and rotational motion.

For low values of v and J, C_0 and D_0 terms are negligible. We will thereby not consider them in this work.

The selection rules in IR spectroscopy state that: Δv=+1 and ΔJ=+ or -1. Moreover, the transition can only occur if there is a change in the dipole moment during the vibration.

In our case, this leads to a spectrum with two branches:

Transition (v=0, J) to (v=1, J-1) branch P:

$\Delta E(J)=E(v=1, J-1)-E(v=0, J)=h\nu_0+(2\alpha_0-2B)hcJ-\alpha_0 hcJ^2$ with J= 1, 2, 3… (3)

Transition (v=0, J) to (v=1, J+1) branch R:

$\Delta E(J)=E(v=1, J+1)-E(v=0, J)=h\nu_0+2B-3\alpha_0+(2B-4\alpha_0)J-\alpha_0 J^2$ with J= 0, 1, 2, 3… (4)

We will define m= J+1 for the R-branch and m= -J for the P-branch. New expressions are:

$\Delta E(m)= h\nu_0+2(B-\alpha_0)\, m-\alpha_0 m^2$ with m=...-3,-2,-1,1,2,3… (5)

Hence $\sigma(m) = \frac{\Delta E}{hc} = \frac{\nu_0}{c} + 2(B-\alpha_0)m - \alpha_0 m^2$ (6)

1. Filling the gas cell.

Caution: safety glasses and gloves must be worn for this manipulation. Ask your instructor to help you.

The goal, here, is to fill the gas cell to capture the IR spectrum of HCl(g).
The cell will first be purged with $N_{2(g)}$ (Why?) to make a blank sample. Capture the background spectrum: resolution 4 cm^{-1} range 2000 to 4000 cm^{-1}, 4 scans.
Then fill the cell with HCl(g). HCl(g) is generated by adding sulfuric acid to NaCl(s).
Capture the HCl(g) spectrum in the absorbance mode, select a spectrum region, and double-click on it. Label the peaks and print as a pdf file.

2. Calculations and report

a) Assign m values and P and R branches on the spectrum.
b) Trace $\Delta\sigma=\sigma(m+1)-\sigma(m)$ and find the numeric value of $(2B-3\alpha_0)$ and $2\alpha_0$
c) Deduce B and α_0
d) Determine the numeric value of ν_0
e) Using these constants, calculate the intermolecular distance of HCl(g) and the force constant k.

VII. Determination of fatty acid methyl ester in Diesel by FT-IR.

Biofuels have a vital role to play in the global transition to sustainable, renewable energy. There is a lot of research nowadays on advanced liquid biofuels (second-generation biofuel) that answer to sustainability risks associated with changing land use and competition over food production. Indeed, researchers use agricultural residues, forest residues, or seaweeds as raw Material.

As regards industrial biofuels, there are two main biofuel production chains nowadays: the "gasoline" biofuel chain using mainly bioethanol and the "diesel" biofuel chain using FAMEs.

FAMEs are produced in France by the transesterification of rapeseed oil that yields to fatty acid methyl esters. This mixture of compounds is called biodiesel. It can mostly be used as such in vehicles (B100 blend), but most often it is mixed with diesel in different proportions, for example, 30% (B30 blend). According to the European standard EN 590, the products sold as diesel (with no particular label) can contain up to 5% of FAMEs. This is what we propose here to check using a method close to the standard

method EN 14078 which provides a determination of FAME in Diesel by infrared spectroscopy using a transmission cell.

1. Preparation of the calibration curve.

Prepare five standard solutions (10 mL each) with the following concentrations in methyl oleate: 0.25%, 0.5%, 1.0%, 1.5% et 2.0% (volume percentage). The dilution solvent will be cyclohexane. You will have to prepare a more concentrated intermediate solution in a 20 mL flask.

2. Preparation of the Diesel solution.

Take 2 mL of Diesel and add to a 10 mL volumetric flask, complete with cyclohexane. Triplicate this sample.

3. Analysis.

Ask the instructor to put the transmission cell and the peristaltic pump in place.
Perform the background on pure cyclohexane.
Acquire the IR spectrum of each solution
resolution: 4 cm-1, range 2000 to 1000 cm^{-1}, 4 scans (Stop the pump during acquisition).

Why do we choose this range of wavelength?

The quantification will be done using de C=O band. Follow the manual to calculate the area of the selected peak.
After the manipulation, rinse the system with cyclohexane solution then dry it with air. You will not forget to drain the trash flask.

- Explain the use of the C=O band for calibration
- Draw the calibration curve and perform statistical analysis.
- Calculate the fatty acid methyl esters content in the diesel and calculate the confidence interval on this result.

WARNING: WASH GLASSWARE WITH ACETONE!!! NO WATER.

AND PUT BACK THE GLASSWARE TO THE BOX AFTER WASHING.

Main points to discuss:

- For each sample present your interpretation of peaks in a table with wavenumbers, functions, bonds, and vibration mode
- Interpret the values of energy transmitted with and without each accessory. Interpret the values obtained
- You have changed the number of scans and range of wavenumbers. Explain why in your conclusion

TP 2: UV-VISIBLE SPECTROMETRY ON A SHIMADZU UV-1900i
Determination of Co^{2+} in the presence of Cr^{3+}

UV-visible spectroscopy is widely used for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. There are many environmental applications such as the quantification of chromium or cobalt in industrial effluents.

Focus on chromium: Cr(III) is essential to human beings while Cr(VI) is carcinogenic. Unfortunately, Cr(VI) (or hexavalent chromium) has widespread applications in various industrial processes such as electroplating, printing, dyeing, tanning, and metallurgy. Cr(VI) submitted to authorization by the REACH regulation. It is the reason why there are nowadays many industrial efforts to replace (CrVI) but it often reveals to be very difficult. Cr(VI) is thus still widely used and it is thus crucial to quantify separately Cr(III) and (CrVI). Our UV-visible method is not specific to Cr(III) or Cr(VI). ICP-MS coupled with ionic chromatography is the current method for the **speciation** analysis of Cr(III) and Cr(VI).

GOALS

- Plot a UV-VISIBLE Spectrum.
- Build and use a calibration curve.
- Correct interference during the spectrometric determination of a compound quantity.

II. ACQUISITION OF A SPECTRUM

Refer to the manual for the software.

CAUTION !!!!! : All solutions will be prepared in 0.25 M H_2SO_4, this solvent is very corrosive for the user and the instrumentation !!

Two stock solutions of Co^{2+} ($Co(NO_3)_2,6H_2O$) and Cr^{3+} ($Cr(NO_3)_3,9H_2O$) are provided with concentrations of 0.2 and 0.1 mol L^{-1} respectively.

Prepare and capture the spectrum of the following solutions:

- a) Cr^{3+} (Cr) 0.025 mol.L^{-1}

- b) Co^{2+} (Co) 0.075 mol.L^{-1}

You will use 0.25 M H_2SO_4 for all dilutions.

The parameters for the acquisition are the following:

- Absorbance (y-axis): 0 to 1.0 uA

- Spectral range to scan: 800 to 310 nm.

Why do we use quartz cells in this study?

III. DETERMINATION OF Co^{2+} CONCENTRATION USING A CALIBRATION CURVE

Using the spectrum, choose the best wavelength for the determination of Co^{2+} concentration.

Then prepare the following solutions:

1	(Co)	=	0.020 $mol.L^{-1}$
2	(Co)	=	0.040 $mol.L^{-1}$
3	(Co)	=	0.050 $mol.L^{-1}$
4	(Co)	=	0.060 $mol.L^{-1}$
5	(Co)	=	0.070 $mol.L^{-1}$
6	(Co)	=	0.080 $mol.L^{-1}$
7	(Co)	=	0.090 $mol.L^{-1}$

Determine the absorbance of each solution (refer to the manual).

Use solutions 1, 3, 4, 5, and 7, to build the calibration curve. (3 measures per solution, the cell will be filled three times).
Make a linear regression and check for linearity and crossing at the origin.

Check for accuracy: Determine, using the calibration curve, the concentration of solutions 2 and 6. Calculate the confidence interval. Is this correct? Is there a systematic error?
You are now given a real unknown solution A that only contains Co^{2+} in 0.25 M H_2SO_4. Find a method to calculate its concentration and calculate the confidence interval.

IV. DETERMINATION OF QUANTIFICATION AND DETECTION LIMIT OF Co^{2+}

LOD (Limit of detection) is the lowest quantity of a substance that can be detected with a given confidence level.
LOQ (Limit of quantification) is the lowest quantity of a substance that can be quantified with a given confidence level.
If a result inferior to the LOD (LOQ) is obtained then the numerical value must be replaced by <LOD (<LOQ).
We will see 2 different ways to estimate the LOD and LOQ.

1. First method

Measure ten times a blank sample. Calculate the standard deviation of absorbance measures $\sigma_{abs.}$

The limit of detection (LOD) is defined by:

$$LOD = 3 \times \frac{\sigma_{abs}}{a}$$

The limit of quantification (LOQ) is defined by:

$$LOQ = 10 \times \frac{\sigma_{abs}}{a}$$

Where "a" is the slope of the calibration curve.

2. Second Method

Another method can be used to estimate the limit of detection and quantification. It may be defined by:

$$LOD = 3 \times \frac{s_{a0}}{a} \qquad LOQ = 10 \times \frac{s_{a0}}{a}$$

Where "a" is the slope of the calibration curve and s_{a0} is the standard deviation of y-intercept (may be calculated using the function droitereg in Excel).

Calculate LOD and LOQ using both methods and conclude.

V. QUANTIFICATION IN THE CASE OF INTERFERENCES

Explain why Cr^{3+} is an interferent in the quantification of Co^{2+}.

V-1) Method

If the absorbances of the analyte (index 1) and the interferent (index 2) are recorded at several wavelengths λ_i, show that the following relation is followed:

$$\frac{A_{m,\lambda i}}{A^0_{1,\lambda i}} = \frac{c_1}{c_1^0} + \frac{A^0_{2,\lambda i}}{A^0_{1,\lambda i}} \frac{c_2}{c_2^0}$$

c_1^0 and $A^0_{1,\lambda i}$: concentration and absorbance at the wavelength λ_i of the solution of analyte without interferent

c_2^0 and $A^0_{2,\lambda i}$: concentration and absorbance at the wavelength λ_i of the solution of interferent without analyte

c_1 and c_2 : concentration of analyte and interferent in the mixture

$A_{m,\lambda i}$: Absorbance of the mixture at the wavelength λ_i

How can c_1 and c_2 be obtained using a linear regression?

it is advisable to work on exercise 3 of chapter 5 of the spectroscopy course before the practical session.

V-2) Experiment

Capture, in the same conditions as in paragraph II, the spectrum of 2 mixtures of unknown concentrations in Co^{2+} and Cr^{3+}.

Using the database of these spectra and the ones obtained in II, choose 6 wavelengths that fit the 2 conditions :

- molar extinction coefficients between the analyte and the interferent are quite different, that is to say choose different ratios $\frac{A^0_{2,\lambda i}}{A^0_{1,\lambda i}}$.

- The absorbance of the analyte and the interferent are significantly different from 0

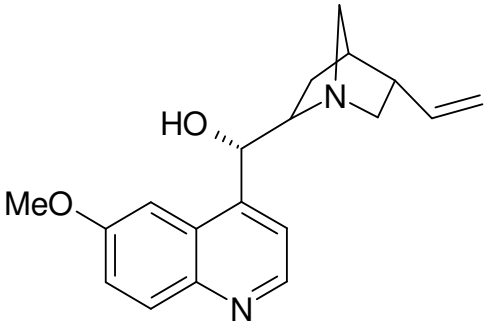
For each selected wavelength, read on the spectra the absorbance of the solution of the analyte (Co^{2+}) at the concentration of 0,075 mol/L, the absorbance of the solution of interferent (Cr^{3+}) at the concentration of 0,025 mol/L, and the absorbance of each unknown mixture B and C.

Apply the method presented in paragraph V-1 to obtain the concentration of Co^{2+} and Cr^{3+} of the 2 unknown solutions.

Think of the limit(s) of the method used.

TP 3: MOLECULAR EMISSION SPECTROPHOTOMETRY ON A SHIMADZU RF-6000

STUDY AND DETERMINATION OF QUININE IN SODA



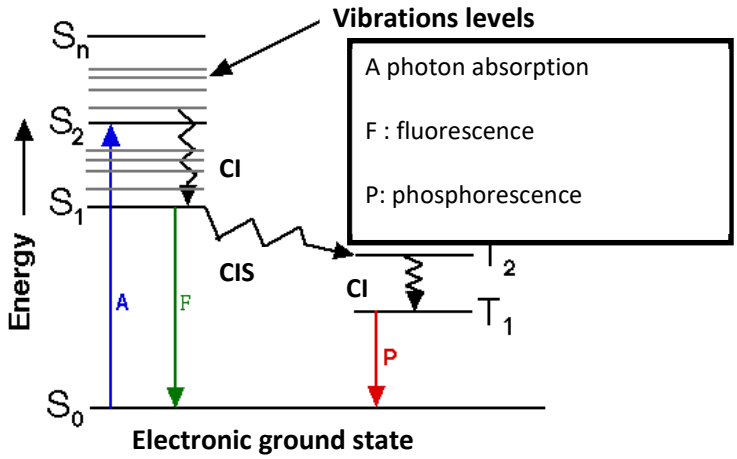
Quinine

The work aims to determine the quinine content in a soda using a UV fluorescence spectrophotometer.

NB: the quinine determination in urine is used to supervise the heroin concentration in bodies (quinine can be detected until 2 weeks after injection). Quinine is used to dilute heroin.

Luminescence phenomena are represented in the following scheme:

Jablonski diagram



The energy gap corresponding to the fluorescent emission of a compound is always lower than that corresponding to the absorption, because of vibrational relaxation phenomena in both ground and excited states.

It can be demonstrated that the fluorescence of low-absorbing solutions is described by the following quantitative approximate expression:

$$F = K . I_0 . C$$

With F the solution fluorescence [arbitrary unit], K a constant, I_0 the light source intensity, and C the concentration of the fluorescent compound. There is a linear relation between fluorescence and concentration, as long as the light source has a constant light intensity. There are however 2 limitations to checking this relation.

- This expression stands for low-absorbing solutions. Indeed, if this is not the case, a decrease in fluorescence due to the strong absorption by the solution itself should be observed. It is the auto-absorption process.

- Besides, in the presence of other compounds in substantial concentration, the fluorescent molecule may lose its energy in a non-radiative way, by collisions with these compounds. Thus, fluorescence intensity decreases. It is the quenching process.

The relation giving fluorescence intensity with and without interfering ions is the following:

$$F_0 = F. (1 + K_Q . [Q])$$

With F_0 the fluorescence without interfering ions, F the fluorescence with interfering ions, K_Q the quenching constant, and [Q] the concentration of interfering ions.

In this practical lab session, the quinine fluorescence will be monitored in 3 dimensions to find the excitation and emission analytical wavelengths for this compound. These optimum wavelengths will be used to determine quantitatively the quinine content of a soda with 2 methods. The quenching of quinine due to chloride ions could have been studied, and the fluorescence quenching constant deduced, but it won't be done for a question of time.

EQUIPMENT AND SOLUTIONS

-RF6000 (Shimadzu) spectrofluorimeter coupled with a computer. See the notice on the instrument.

- Measurement cell in **quartz** with **4 transparent faces.** Why are 4 transparent faces required?

Each measurement must be **triplicated.**

- At your disposal:

  - HNO_3 solution 10^{-2} mol.L^{-1}

  - Quinine solution 1.10^{-3} mol.L^{-1} in HNO_3 10^{-2} mol.L^{-1} (prepared from **quinine sulfate**).

I. DETERMINATION OF ANALYTICAL PARAMETERS

Prepare a solution (50 ml) containing quinine 1.10^{-7} mol.L^{-1} in HNO_3 10^{-2} mol.L^{-1}.

(Keep this solution for other studies).

Capture the emission spectra of this solution between 350 and 600 nm, for excitation wavelengths between 270 and 390 nm (see the user notice of the instrument). Once the spectrum acquisition is over, observe (see notice) and take a screenshot for your report. Select excitation and emission wavelengths so that fluorescence is the highest possible.

II. STUDY OF THE LINEAR-RANGE

Prepare 5 solutions (50 mL volumetric flasks) of quinine concentration: 10^{-4}, 10^{-5}, 10^{-6}, $10^{-7,}$ and 10^{-8} mol.L^{-1} in HNO_3 10^{-2} mol.L^{-1}. Do the blank. Then measure the fluorescence of each solution (mother solution included) and plot the curve F=f([quinine]). Deduce the useful concentration range.

Note: the instrument beeps and puts a message on the software when the saturation of the detector is reached.

III. DETERMINATION OF QUININE IN A SODA

1. External calibration method

It is possible to obtain the quinine concentration in a soda using the external calibration method.
Prepare 6 standard solutions (50 ml) containing increasing concentrations of quinine (from 10^{-7} to 3 10^{-6} mol.L^{-1}) in HNO_3 10^{-2} mol.L^{-1}.
Do the blank. Then measure the fluorescence of quinine sulphate standard solutions. Plot the calibration curve: F = f ([quinine]).
Prepare and dilute, if necessary, the soda in HNO_3 10^{-2} mol.L^{-1}, so that the quinine concentration is optimum for the determination.
Measure fluorescence three times (mean value, standard deviation of fluorescence, 95% confidence interval on the quinine concentration).

2. Standard addition method

A video https://youtu.be/1T4D1Opgowk explains how this method works.

Prepare a solution S_a with quinine concentration of 10^{-5}mol.L^{-1} in HNO_3 10^{-2}mol.L^{-1}.
Prepare a solution S_i (50 mL) containing 5 mL of soda, completed with HNO_3 10^{-2}mol.L^{-1}.
Prepare 6 volumetric flasks (50 mL) according to the following table.

Flask	**1**	**2**	**3**	**4**	**5**	**6**
S_a (mL)	0	2	4	6	8	10
S_i (mL)	5	5	5	5	5	5
Qsp HNO_3 10^{-2}mol.L^{-1}	50 mL	50 mL	50 mL	50 mL	50 mL	50 mL

Main points to discuss:

- Explain the principle of fluorescence analysis and the device.
- Why is a 4-sided transparent cell used?
- Analyse the spectrum (explain all peaks)
- Check the linearity range
- Check the calibration curve (linearity, origin) and repeatability on a calibration solution.
- Principle and interest of the standard addition method?
- Calculate quinine content in soda and confidence interval using external calibration and standard addition method*.
- The quenching phenomenon is of huge importance in UV fluorescence. Is it significant in the case of quinine in Schweppes?

* Statistic formula to apply in the standard addition method :

$$-\frac{a_0}{a_1} \pm \frac{t\left(1-\frac{\alpha}{2}, n-2\right)s}{|a_1|}\sqrt{\frac{1}{n}+\frac{\bar{y}^2}{a_1^2\left(\sum(x_i-\bar{x})^2\right)}} \qquad s^2 = \frac{\sum_i (y_i-\bar{y})^2 - a_1^2\sum_i (x_i-\bar{x})^2}{n-2}$$

TP 4: FLAME ATOMIC ABSORPTION SPECTROMETRY: Study of the chemical interference of Al on the determination of Mg

I. Principle

Flame atomic absorption spectrometry is an analytical technic used for the determination of metals in solution. This very efficient method will be described in the course SPECTRO.

The principle is the following: the solution containing metallic ions is pumped up with a constant flow by a pneumatic nebulizer and transformed into an aerosol which is introduced in a spray chamber. It is then mixed with fuel (acetylene) so that the finest droplets enter into a flame.

If the flame temperature is sufficient, after several steps the ion will be atomized (see the course on Moodle "CFI3/MRIE3 Spectroscopies'). In some cases, these steps can be difficult and can result in interference (decrease of the sensibility).

A light beam of a specific wavelength (corresponding to the element we determine) is passed through the flame. The absorbance is measured and converted to concentration thanks to the Beer-Lambert law (using a calibration curve).

II. Goals

The goal of the lab session is to study the effect of chemical interference on the determination of a metallic ion. Then we will show how this interference can be corrected.

III. Instruments

Spectrometer: **Thermo AA Series Spectrometer** and Acquisition software: **Solaar 32**

IV. Flame ignition

Open the Solaar 32 software (on the desk) and follow the manual.

The air pressure should be 2.1 bar. The air provides the aspiration using the pneumatic nebulizer. Check that the inlet flow is around 5-6 mL/min.

What would be the effect if the flow was lower?

The air is also used for the combustion of acetylene. Modification of the flow of acetylene and air will change the flame stoichiometry and thereby change its temperature and redox properties.

- Low acetylene flow: oxidizing flame.
- High acetylene flow: reducing flame

V. Study of the interference occurring in magnesium determination

Standard solutions **$Mg(NO_3)_2,6H_2O$; $Sr(NO_3)_2$; $Al(NO_3)_3,9H_2O$**:

Mg^{2+}: 0,01g/L* Al^{3+}: 1g/L* Sr^{2+}: 1g/L*

* See real concentrations on the bottles.

Small equipment: 50 mL volumetric flasks, a graduated buret for each ion

V.1. Study of magnesium determination alone

Prepare a series of 6 solutions containing 0.1 to 0.6 mg/L of Mg^{2+}.

Plot the calibration curve using the software as explained in the manual. The blank solution will be HQ water. Check for linearity and origin.

V.2. Interference with aluminum

In the flame, Aluminum reacts with magnesium and forms a mixed oxide which is very stable. This compound atomizes only at very high temperatures, so the number of Magnesium atoms in the flame is significantly reduced.

a) Study the influence of a concentration of 20 mg/L Al^{3+} on Mg^{2+} solutions (0mg/L, then 0.1 to 0.6 mg/L). Measure the absorbance of these solutions.
b) Same study on solutions of 0.5 mg/L Mg^{2+} but the concentration of Al^{3+} varies from 0 to 500 mg/L (0, 20, 50, 100, 150, 200, 300, 500 mg/L).
c) Complete the Excel sheet and discuss your results.

V.3. Interference correction

The addition of an element that can substitute the magnesium in the mixed oxide releases magnesium and can correct the chemical interference. In the present study, we use Strontium.

a) Study the influence of Sr^{2+} by measuring the absorbance of solutions containing 0,5 mg/L Mg^{2+}, 20 mg/L Al^{3+}, and concentration of Sr^{2+} from 0 to 150 mg/L (0, 5, 10, 20, 50, 100, 150 mg/L).
b) Plot the calibration curve (0mg/L, then 0.1 to 0.6 mg/L in Mg^{2+}) the presence of 20 mg/L of Al^{3+} and 100 mg/L of Sr^{2+}.
c) Complete the Excel sheet and discuss the results obtained.

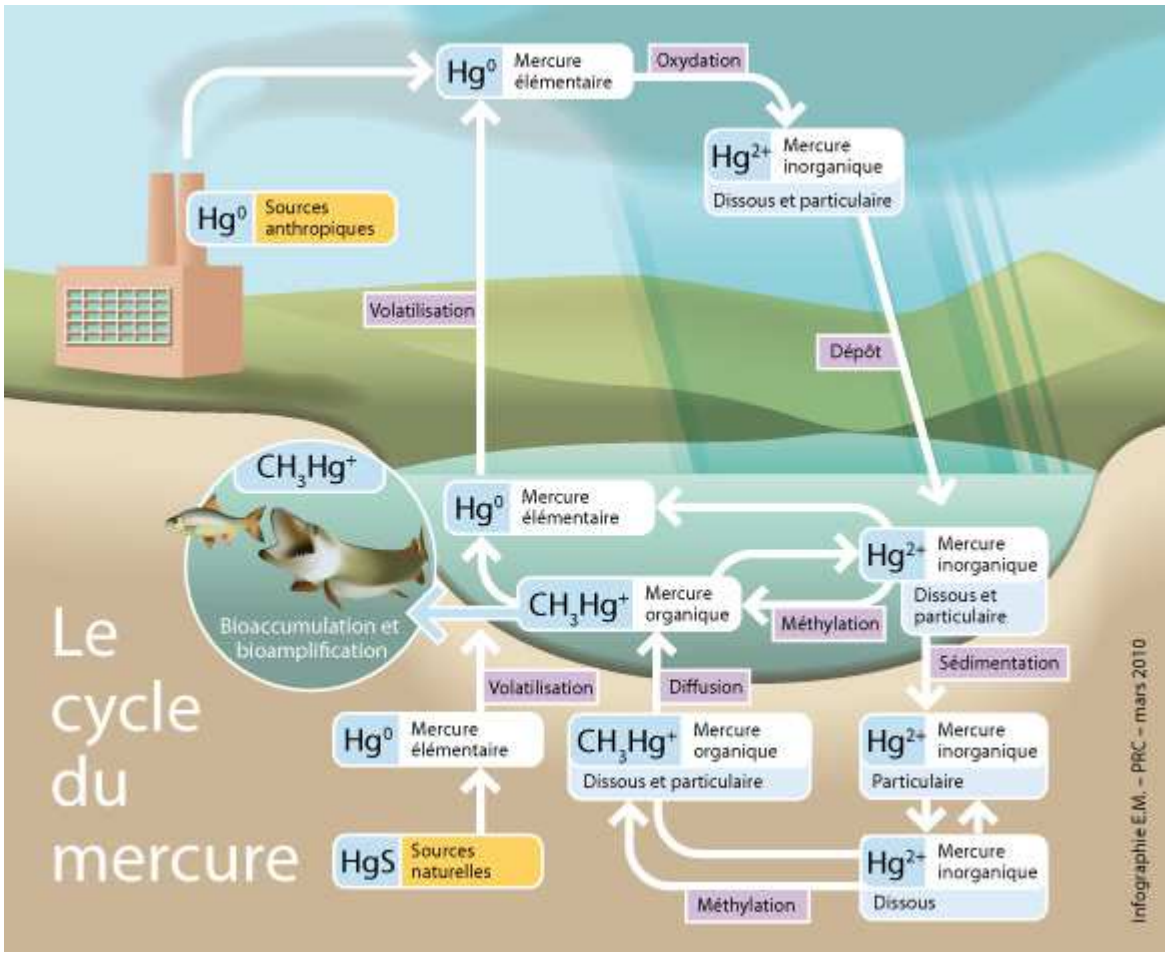
VI. Determination of Magnesium in mineral water.

Check on the label that there is no aluminum inside. Determine the magnesium concentration of the mineral water in mg/L. Triplicate the preparation of the sample. Give the concentration and the confidence interval. Would you use the same instrument if you had to quantify several elements?

TP 5: DETERMINATION OF THE CONTENT OF ZINC, MAGNESIUM, AND CALCIUM IN SHRIMPS

Fish has always been appreciated for its culinary and nutritional qualities and is among the healthiest foods. We propose, in this regard, to quantify its magnesium and calcium content. However, in recent years, some species of sea fish have been the subject of special attention due to their high content of heavy metals. In the aquatic environment, fish ranging at the end of the food chain, such as tuna, swordfish, sharks and other large fish accumulate these elements.

Below, we can see the example of the bioaccumulation of mercury but we will quantify zinc during our lab session.



Objectives of the work:

This work aims to measure calcium, magnesium, and zinc in shrimps sold in cans. Thus, wet digestion of samples will be conducted first so that they can be analyzed afterward by ICP-OES (Inductively Coupled Plasma/Optical Emission Spectroscopy).

Educational objectives:

- Become familiar with sample digestion.

- Use an inductively coupled plasma optical emission spectroscopy ICP-OES ICAP 6300 in the simultaneous mode for Zn, Ca, and Mg determination.

The organization of working time:

- Launch the wet digestion simultaneously of 3 shrimps samples and one blank.
- During 1 h of the wet digestion, ask the technician to open the gas on the ICP, check with the teacher or technician the preparation of your dilutions, and then prepare solutions for external calibration.
- Once the digestion is performed, prepare your samples, and analyze them using ICP.

I- Digestion under sonication

The protocol was adapted from Niwat Manutsewee, Wanlapa Aeungmaitrepirom, Pakorn Varanusupakul, Apichat Imyim Determination of Cd, Cu, and Zn in fish and mussel by AAS after ultrasound-assisted acid leaching extraction Food Chemistry 101 (2007) 817–824

Wearing nitrile gloves (blue gloves) in addition to a lab coat and security glasses is essential in the preparation of the sample.

Sample preparation (done by the technical personnel): Weigh then dry shrimps at 100°C in an oven for 20h. Weigh dry shrimps then ground them into small pieces (<300µm) and keep them in a closed vessel in a desiccator with the indication of the water content.

Sample digestion: Weigh precisely around 0,2 g of dried shrimps in 50 mL plastic tubes. Add in each plastic tube and also in an empty plastic tube (method blank*) 2 mL of concentrated nitric acid (4M), 2 mL of hydrochloric acid (4M), and 0,25 mL of 35% hydrogen peroxide. Put an unclosed cap. Place all 4 tubes in a beaker containing water then place it in the sonicated bath for 30 min. Then close the cap and centrifuge the tube at 4000 rpm for 10 min. Then carefully transfer the liquid phase in a 50 ml volumetric flask by filtration on filter paper. Then wash the solid residue with 5 mL of water, put it in the sonicated bath for 10 min, and centrifuge again at 4000 rpm for 10 min. Add the liquid phase to the previous flask by filtration on filter paper and complete to the mark with ultrapure water. These flasks are called $A_{shrimps}$. Then dilute at 1/10 each previous solution to give respectively $B_{shrimps}$.

* A method blank (MB) is an analyte-free matrix that is processed exactly in the same manner as the samples. Its main function is to check there is no contamination resulting from the analytical process. It is different from instrument zero (reference).

Measure the solutions $A_{shrimps}$, $B_{shrimps,}$ and the method blank.

II-Setting up of the ICP - AES ICAP 6300

Calcium, magnesium, and zinc will be analyzed simultaneously on an ICP - OES (Inductively coupled – plasma-optical emission spectroscopy). The analysis will be performed in the axial mode.

Element	Wavelength: λ (nm)	Mode
Ca	184,0	Axial
Mg	202,5	Axial
Zn	202,5 /206,2 / 334,5	Axial

Refer to the manual, available near the instrument, for the creation of the method. The technical staff or teacher is with you and helps with the plasma ignition.

What are the gases used during analysis and what is their function(s)?

III- Preparation of calibration solutions

3 stock solutions (magnesium, calcium, zinc) 1000 mg/L are available.

From these solutions, prepare the following range of solutions in 50 mL volumetric flasks:

Flask	1	2	3	4	5
Ca mg/L	0,5	1	2	5	10
Mg mg/L	0,5	1	2	5	10
Zn mg/L	0,05	0,1	0,2	0,5	1

Note: Intermediate solutions will be necessary. Don't use glass pipettes with a volume inferior to 0.5 mL (low reliability).

IV-Analysis and Results

Ask the Professor or the technician to come with you for ICP analysis.

Prepare your method on the software by following the manual indications. Switch the plasma on and let it equilibrate.
Using the manual, pass your solutions on the spectrometer (ask the teacher or the technical personnel to monitor operations).
Use the external calibration method with the Blank sample, $A_{shrimps,}$ and $B_{shrimps}$. Check for spectral interferences (peak overlapping) on these samples with the help of your instructor.
Results will be quickly processed with Excel (you need to prepare your calculation sheet).
Make your linear regression for each element and wavelength. **Calculate** the detection limit and quantification limit using method with the formula

$$LOD = 3 \times \frac{s_{a0}}{a_1} \qquad LOQ = 10 \times \frac{s_{a0}}{a_1}$$

The content of magnesium and calcium in shrimps will be expressed in mg/kg.

Is it possible to quantify zinc? If yes, express its content. Give the name of an instrument that has far lower detection limits and would be a better choice for this application.

Main Points to discuss

- In the introduction, give the main sources of zinc, explain the main effect(s) on health, and indicate the maximum content with the name of the associated European regulation.
- Explain the principle of ICP-OES. Present spectral interferences. Are there spectral interferences at the wavelengths chosen to quantify Zn, Mg, and Ca in shrimps, justify with relevant screenshots.
- Explain the digestion step
- Check the linearity and estimate the limit of detection and quantification in the case of Zn.
- Choose for each element, the most relevant dilution, then determine independently the content in Ca and Mg of the 3 shrimp samples analyzed. Express it in mg/kg of dry and ***wet*** products (same question for zinc but in µg/kg)
- Determine the confidence intervals and interpret them.

TP 6: GAS PHASE CHROMATOGRAPHY: ETHANOL ASSAY IN WINE, BEER, AND EAU DE TOILETTE

We want to determine ethanol in aqueous solution. This assay will be applied to obtain the alcohol content of a wine, beer, and eau de toilette.

The internal standard method with isopropyl alcohol as a chemical standard will be used. Comment precisely on this choice.

I) OPERATING CONDITIONS

Scion GC8300 Chromatograph equipped with a flame ionization detector (FID).
See the equipment and software notice.
The choice of this type of detector is due to the fact that as aqueous solutions are studied, there will be no solvent peak. (Why?)

- Stationary phase: ZB5 5% diphenyl 95% polydimethylsiloxane, diameter 0.32 mm, length 30 m, Stationary phase thickness 0,25µm. Tmax=340°C.

- **Temperatures**: injector: 250°C - detector: 250°C

- **Carrier gas**: Helium: flow: 1 mL/min

- **Injector Split**: split factor 30:1

Why is the injector temperature so high and why do we use a split factor?
Prepare an aqueous solution containing 5% volume of ethanol and 2% of isopropyl alcohol. Inject 0,2 µL. Optimize the oven temperature to obtain a good separation in a suitable time.
We will check the repeatability of retention times, areas, and area ratios for this solution after optimization. (Inject 4 times the same solution). Conclude.

II) ETHANOL ASSAY

We will use the internal standard method to perform the calibration (Why?). (See annex)

N.B.: with the notations in the annex, A is the molecule studied (here ethanol), and E is the standard (here isopropyl alcohol).

II-1) Calibration

Prepare a series of six calibration solutions in 25 mL flasks for which the ratio r changes from 0,2 to 1,2

r = ethanol volume/isopropyl alcohol volume

As the ionization flame detector has a high sensitivity, solutions need to be enough diluted to obtain good results.

Solutions will be prepared as follows:

- 1mL isopropyl alcohol (internal standard)

- x mL ethanol

- deionized water until 25 mL

II-2) Assay in wine, beer, and an eau de toilette.

NB: the beer was degassed.

We will make sure first that isopropyl alcohol is **absent** from the studied solutions.
After reading the etiquette, sample a known volume, add isopropyl alcohol, and complete your volumetric flask with water. The value of r must be inside the calibration curve defined above.
Prepare 3 samples for each assay.
Determine the ethanol concentration in wine, beer, and eau de toilette in %vol and give the confidence interval* (confidence level 95%) for each assay.
* Be careful that the confidence interval calculated is related to $V_{EtOH}/V_{internal\ standard}$

Main points to discuss:

- Explain the principle of gas chromatography
- Using the commercial name of the column, find on the internet the nature of the stationary phase and for which type of chemicals it is suitable
- Why is water not detected?
- Explain the choice of isopropyl alcohol as the internal standard
- Explain the relative retention time of compounds
- Check the repeatability (CV) of retention times, areas, and areas ratio. Conclude about the use of an internal standard
- Draw the calibration curve and make a statistical check
- Calculate results for samples and give CVs and confidence intervals.

ANNEXE TP6: THE INTERNAL STANDARD METHOD

IN QUANTITATIVE CHROMATOGRAPHY

In chromatography, the area under the peak is in general proportional to the quantity injected.

For any molecule A, the area S_A under the peak A can be written:

$S_A = k_A^m \times m_A$ if a mass m_A of A has been injected

Or $S_A = k_A^v \times v_A$ if a volume v_A of A has been injected

We use m_A or v_A depending on the way solutions are prepared:

- Mass titer: M_A being the mass of A in the sample of total volume V.

if v is the injected volume: it contains a mass m_A of A, with:

$$S_A = k_A^m M_A \frac{v}{V}$$

- Volume titer: V_A being the volume of A in the sample of total volume V.

if v is the injected volume: it contains a volume v_A of A, with:

$$S_A = k_A^v V_A \frac{v}{V}$$

We could think of performing a classical calibration by preparing solutions of known mass or volume titer and injecting always the same volume v.

But, given the small value of v: tenth of µL, the reproducibility of the injected volume is too weak to obtain precise results.

The internal standard method gives results independent from the injected volume, as long as the answer of the detector is in its linearity area.

Response factor:

The internal standard must be soluble in the sample, give an isolated peak not far from the analyzed peak, and not react with the sample. We write it with the E index.

The injection of a fraction v of V gives: $S_A = k_A^m M_A \frac{v}{V}$ as long as $S_E = k_E^m M_E \frac{v}{V}$, where MA, ME, (or VA and VE), v and V are known and SA and SE measured.

Thus $\frac{S_A}{S_E} = \frac{k_A^m}{k_E^m}\frac{M_A}{M_E}$ or $\frac{S_A}{S_E} = \frac{k_A^v}{k_E^v}\frac{V_A}{V_E}$

So $\frac{S_A}{S_E}$ is independent of v.

We call the response factor the value of k_A^m or k_A^v divided by the standard one, the standard response factor taken equal to 100.

So: $F_A^m = \frac{k_A^m}{k_E^m} \times 100 = \frac{S_A}{S_E}\frac{M_E}{M_A} \times 100$ or $F_A^v = \frac{k_A^v}{k_E^v} \times 100 = \frac{S_A}{S_E}\frac{V_E}{V_A} \times 100$

Calibration

The calibration curve can be obtained by preparing known solutions of A and E, containing variable ratios $r_m = \frac{M_A}{M_E}$ or $r_v = \frac{V_A}{V_E}$. A straight calibration curve is obtained by plotting:

$\frac{S_A}{S_E} = f(r)$, ie $\frac{S_A}{S_E} = \frac{F_A^m}{100} \times r_m$ or $\frac{S_A}{S_E} = \frac{F_A^v}{100} \times r_v$

To analyze an unknown solution I, one adds a volume V_{ci} (known volume of the unknown solution) or a mass M_{ci} (known mass of the unknown solution) to a volume V_{cEi} (known volume of the standard in the unknown solution) or a mass M_{cEi} (known mass of the standard in the unknown solution).

The ratio $\frac{S_{Ai}}{S_{Ei}} = \frac{S_A}{S_E}$ gives the corresponding value of r.

Thus: $r_v = \frac{V_{Ai}}{V_{cEi}}$ or $r_m = \frac{M_{Ai}}{M_{cEi}}$

The A titer can be calculated as:

- In volume %: $= \frac{V_{Ai}}{V_{ci}} \times 100$

- In mass % $= \frac{M_{Ai}}{M_{ci}} \times 100$

- In g/l: $= \frac{M_{Ai}}{V_{ci}}$

- In molar concentration: $= \frac{M_{Ai}}{(Molecular weight\ of\ A \times V_{ci})}$

Direct Calculation

In the case, we are sure that the calibration curve is a straight curve passing through the origin, a direct calculation can be performed after the calculation of response factors

2 steps are required:

-1) known solution of A and E to calculate F_A

-2) To a given volume V_{ci} (or mass M_{ci}) of the unknown solution I, one adds a volume V_{cEi} (or a mass M_{cEi}) of the standard.

We obtain:

$V_{Ai} = \frac{S_{Ai}}{S_{Ei}} \times \frac{100}{F_A^v} \times V_{cEi}$; the titer is calculated as before

Or $M_{Ai} = \frac{S_{Ai}}{S_{Ei}} \times \frac{100}{F_A^m} \times M_{cEi}$; the titer is calculated as before

Notes:

-a) The standard can be used to assay several molecules simultaneously, as long as the separation is correct.

-b) The standard concentration must be in the same magnitude order as the one of the assayed molecules: adapt its titer.

-c) As long as possible, the standard molecule and the analyte must have close structures.

FEUILLE DE SECURITE (à photocopier une par TP, à remplir avant et à rendre avec le compte rendu).

RISQUES et SÉCURITÉS LIÉS AUX PRODUITS


***LA BLOUSE ET LES LUNETTES DE SECURITE SONT OBLIGATOIRES EN LABORATOIRE. Ne pas les indiquer dans la rubrique sécurité**

Réactifs, Solvants et solution	Risques (préciser sa catégorie et sa signification)	Sécurité : proposer la meilleure solution pour se protéger pour chaque risque (prévention ou protection) en plus de *	Matières incompatibles (donner 1 à 2 exemples)
Exemple : *Acide acétique concentré*	*1-Liquide et vapeur inflammable (catégorie 3)* *2- Brûlures de la peau et graves lésions des yeux (catégorie 1A)*	*1--Tenir à l'écart de toute source de chaleur (flamme,…)* *2-Travailler sous sorbonne pour se protéger des vapeurs corrosives* *2-Porter des gants adaptés*	*Les matières oxydantes (ex : permanganate de potassium)*
		Select the most important information for your use	

RISQUES LIÉS AUX MACHINES (compléter en séance)

Appareils	Risques (Il faut faire attention à quoi ?)	Sécurités (Comment s'en prémunir ?)