Instrumental analysis of fish, seafood products, feed and fish habitat

1. General principles of instrumental analysis

In classical qualitative and quantitative analysis the methodology is based on the chemical reactions of the analyte, and the reactions are followed by gravimetric or titration. If, on the other hand, the analysis is based on the ability of the analyte either to absorb or emit electromagnetic radiation, its electrochemical properties or its affinity to certain solid matrixes we talk of instrumental analysis, because these phenomena can be measured and recorded by specific instruments.

Generally, when the absorption or emission of electromagnetic radiation is measured, we talk about spectrometry. The electrochemical properties are measured by potentiometric and conductometric methods. When we isolate and analyze compounds on the basis of their differential adhesion or binding to specific matrices, then the term chromatography is used.

2. Spectrometry

2.1. Electromagnetic radiation

Electromagnetic radiation consists of oscillating waves of radiation with different wavelengths. The radiation also carries energy, and the smaller the wavelength, the higher the associated energy according to the equation:

$E = hc/\lambda$ or $E = h\upsilon$

(E = energy, c = the speed of light, 300 000 000 m/s, λ = wavelength, and h = Planck's constant, 6.626×10^{-34} Js).

The spectrum of electromagnetic radiation is presented in Figure 1.

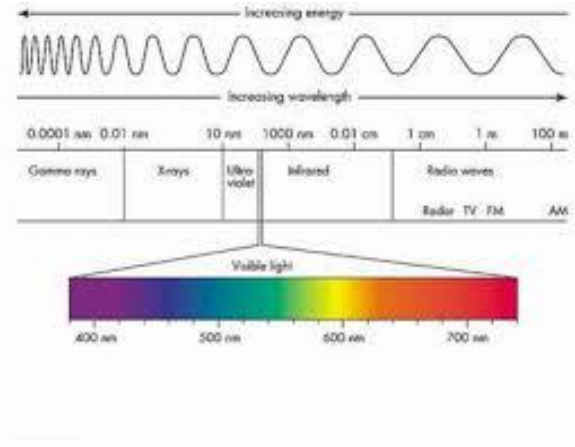

Figure 1: The spectrum of electromagnetic radiation. The range of visible light (wavelengths 400 – 800 nm, from blue to red) is indicated

2.2.The interaction of radiation and matter

Because of the atomic structure, and of the electrons having each their characteristic, discrete energy levels, an electron can absorb energy from electromagnetic radiation and shift from one energy level to another. If, on the other hand this kind of exited electron returns to its basic energy level, it emits energy (as light or heat). Both absorption and emission can be instrumentally followed and recorded by spectrofotometers. By measuring the absorption of a compound at various wavelength, a characteristic absorption spectrum is obtained (Figure 2).

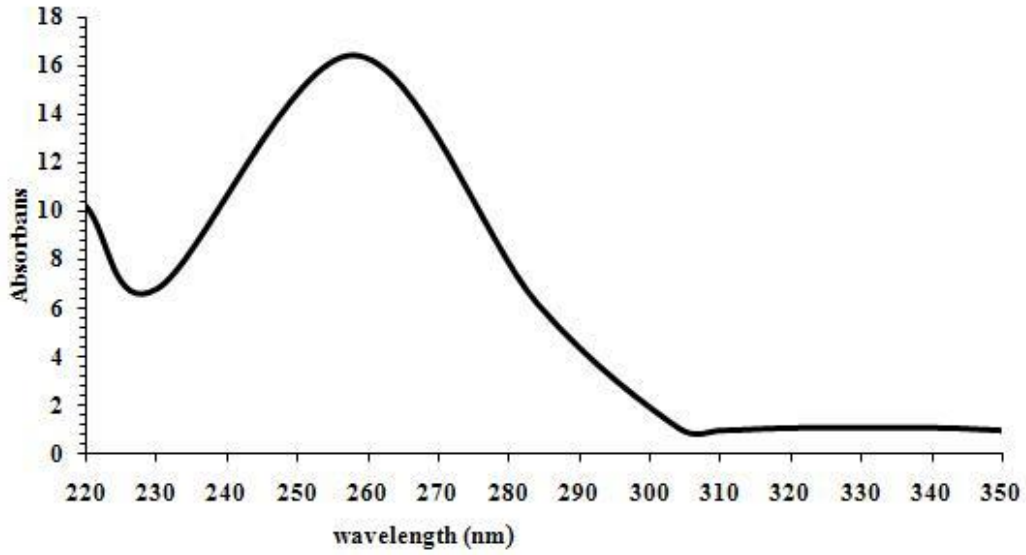

Figure 2: The absorption spectrum of deoxyribonucleic acid (DNA), with the characteristic absorption maximum at the 257 nm wavelength in the ultraviolet-region of the electromagentic radiation.

2.2.1. The operating principle of a spectrophotometer

In a spectrophotometer the sample is irradiated with light with a known wavelength (monochromatic light). Usually the wavelengths used range from ultraviolet to visible light (UV-VIS-spectrophotometry). The molecules in the sample absorb the light, and consequently the intensity of the light is decreased. The ratio of the entering and leaving radiation is called transmission. Its reciprocal is called absorbance. The absorbance is dependent of the concentration of the absorbing molecules according to the Beer's law:

$A = \epsilon bc$

(A = absorbance, b = the length of the light path through the sample, c = concentration as M/l, ϵ = the molar absorptivity specific to each molecule, dimensions M^{-1} cm^{-1}). The length of the light path in standard spectrophotometers is usually 1 cm.

The structure of a spectrophotometer is shown in Figure 3:

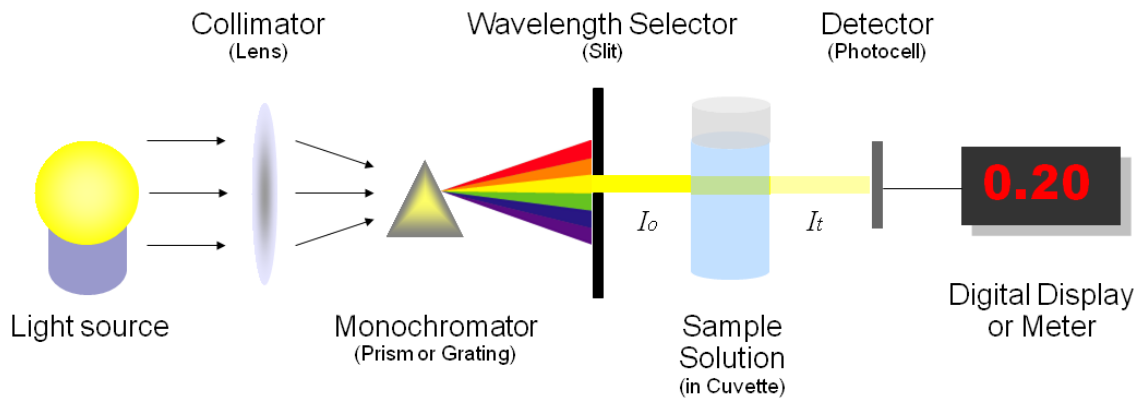

Figure 3. The general principle of a spectrophotometer. From a light source a specific wavelength is selected using a monochromator, and the sample solution, which is placed in a cuvette, is illuminated. The intensities of the incoming irradiation (I_o) and the transmitted light (I_t) are measured and the absorbance recorded.

2.2.2. Examples of spectrophotometric analysis: Nitrite and Iron

Nitrite (NO_2^{-1}) is formed in the environment and water ecosystems,when bacteria oxidize ammonia (NH_4^{+1}). Nitrite reacts with a compound called sulfanilamide, forming a compound called N-(1-Naphtyl)-ethylenediammonium. This compound absorbs light at the wavelength of 543 nm.

In practice, a standard curve is first made by allowing known amounts of nitrite to react with sulfanilamide and the absorbance at 543 are plotted against the nitrite concentration. The curve should be linear (Figure 4).

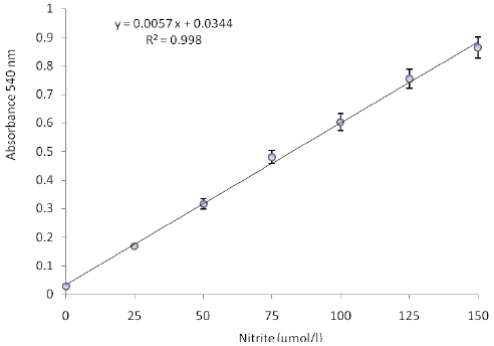

Figure 4. The standard curve of nitrite measured as the absorbance of the reaction product of nitrite and sulfanilamide having the at 540 nm.

When the absorbance of the unknown sample is measured after the reaction with sulfanilamide, the corresponding nitrite concentration can be estimated from the curve.

The iron content of natural waters can vary greatly. Iron can be spectrophotometrically determined as ferro ions (Fe^{+2}). This means, that the ferric ions (Fe^{-3}) in the sample must first be reduced using a suitable oxidizing reagent (typically hydroxylamine hydrochloride) in the presence of sulphuric acid.

Subsequently Fe^{+2} ions are allowed to to react with 1.10- phenantronline hydrochloride to form a red compound that can be analyzed spectrophotometrically at 510 nm. The procedure is similar to the one with nitrite. A standard curve is first determined, and the unknown ferro-ion content can be estimated on the basis of the absorbance measured.

2.3. Other types of spectrometry

2.3.1. Infrared (IR) spectrometry

The infrared (IR) region of the electromagnetic radiation spectrum (wavelengths 700 nm – 1 mm) is also called heat radiation. The energy of this radiation interacts with the molecular bonds between the atoms of a molecule. The advantage of this type of spectrometry is that, in addition to gaseous and liquid samples, also solid samples (usually as finely ground powder in mineral oil) can be analyzed. IR spectrometry can, therefore, be used to analyze, for example the nutrients in a feed sample.

IR spectrometry is particularly useful for the determination of the molecular structure of the compounds present in the sample, because the different chemical bonds and functional groups have their characteristic adsorption peaks. Thus, for example, the bond between oxygen and hydrogen (O-H) absorbs IR with wavelengths 2.7 - 4 µm, and a triple bond between two carbons (C=C) has its absorption peak at 6 – 6.7 µm.[1]

The procedure of IR spectrometry can be analogous to UV-VIS spectrometry with monochromatic radiation, or alternatively, the whole IR can be used.. In the latter case the primary data must be processed with the so called Fourier transformation (FTIR-spectrometry).

[1] In the IR spectrometry the units are usually expressed as reciprocals of the wavelength with units as cm^{-1}. Thus the wavelengths used in the examples correspond values of 3750 – 2500 cm^{-1} and 1675 - 1500 cm^{-1}, respectively.

2.3.2. Atomic Absorption Spectrometry (AAS)

In AAS spectrometry the sample is heated at temperatures high enough to break it down to atoms. These are then illuminated with a lamp that emits light that is able to selectively interact with atoms of certain elements, which results in the absorption of the radiation. This can be recorded. An outline of the atomic absorption spectrometer is presented in Figure 5.

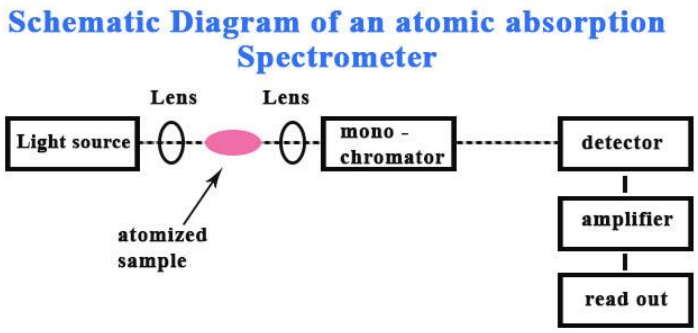

Figure 5. The principle of AAS. The radiation from the light source excites the atoms of the sample, and the resulting absorption of the radiation is recorded

In traditional AAS instruments the atomization of the sample was done using acetylene flame. Nowadays, often a graphite furnace is used for the same purpose.

Metals as well as arsenic (As) and selenium (Se) can be analyzed by AAS. Consequently AAs is much used in food analysis to detect and quantify heavy metals (Hg, Pb, Cd) and As.

2.3.3. Mass spectrometry (MS)

MS is based on completely different principles from the other spectrometric methods, because the sample does not interact with radiation. Instead, it gets ionized by a bombardment with electrons resulting in the ionization and fragmentation of the molecule. The ionized molecules/fragments travel then through a variable electric field, which is adjusted so that molecules/fragments of certain sizes will reach the detector and get recorded. The result is the mass spectrum of the analyzed molecule.

The main importance of MS in food analysis is that it is often applied in detectors of chromatographic instruments (see Section).

3. Electrochemical analysis

3.1. The electrochemical cells and electrolysis

The electrochemical properties of metals have been already discussed in the chapter on qualitative and quantitative analysis. In that chapter also the simple electrochemical cells (Daniell's cell) were described. Also the concept of the hydrogen electrode being as the standard electrode (or "zero-electrode"),having the normal potential to which the electric potential of other types of electrodes are compared.

A Daniell's cell can be constructed also by replacing the salt bridge between the two electrode wessels by a porous barrier allowing the movement of ions between the electrodes. This type of a cell (with, in this case, zink and copper electrodes) is described in Figure 6. In this system Cu^{2+} ions that have a higher tendency to catch electrons than Zn^{2+} ions, are reduced to metallic copper precipitating on the surface of the copper electrode, while the metallic zink from the zink electrode gets oxidized to Zn^{2+} ions. In this case the copper electrode (where reduction occurs) is called a cathode and the zink electrode as anode.

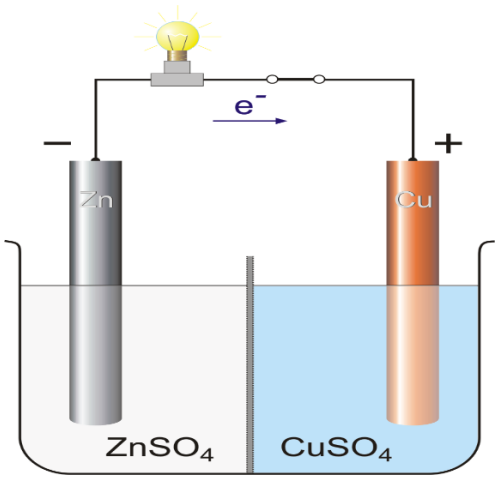

Figure 6. An electrochemical cell formed by copper and zink electrodes immersed in $CuSO_4$ and $ZnSO_4$ solutions, respectively, and separated by a permeable barrier allowing for the movement of ions. The Cu^{2+} ions get reduced to metallic copper, while metallic Zink is oxidized to Zn^{2+}.

In electrochemical cells the electric current is spontaneously formed between electrode pair having different electrode potential. In electrolysis an external power source is used to drive the oxidation-reduction reactions. An electrolysis cells is shown in Figure 7. In this case molten NaCl solution is electrochemically converted to gaseous chlorine and metallic sodium.

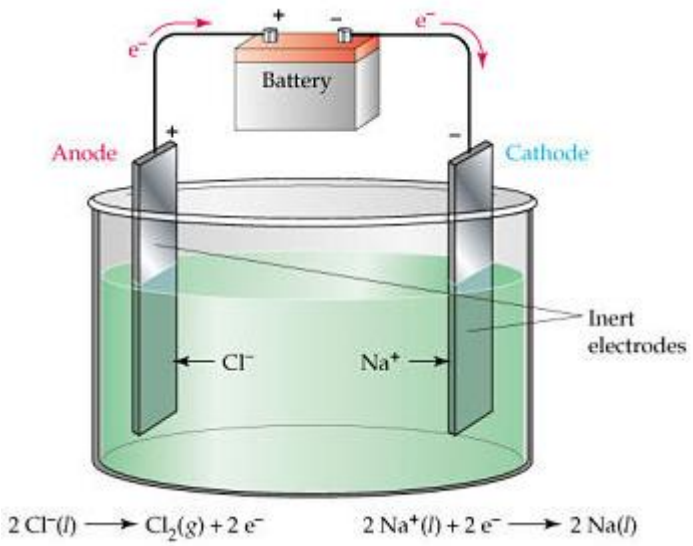

Figure 7. An electrolysis cell in which NaCl is converted to metallic sodium and gaseous chlorine in a reaction driven by an external power source. Na^+ ions receive electrons from the external power source and Cl^- ions loose them in order to maintain the current in the closed circuit.

In electrolysis the amount of electricity (Coulombs, C) is the product of the currency I (in Amperes, A) and time t (seconds, s) according to the equation Q = I x t. One mole of electrons transferred convey the electric amount of one electron multiplied by Avogadros's number, or $6.022137 \times 10^{23} \times 1.602177 \times 10^{-19}$ C = 96485 C. This amount is also called the Faraday's constant (F)

Thus a following equation can be written for an electrolysis reaction:

I x t = n x z F , where I is the current, t is the time, n is the molar amount of oxidized or reduced substance, and z is the number of electrons involved in the oxidation-reduction reaction expressed in moles.

For example, if $AlCl_3$ is electrolytically converted to metallic aluminium by applying an electric current of 10 A for 2 hours (or 7200 s), the following reaction occurs:

1) At chatode Al^{+3} + 3 electrons → Al (metallic)
2) At anode 2 Cl^{-1} → Cl_2 (gas) + 2 electrons

Therefore for each mole of metallic aluminium three moles of electrons are needed, thus z is three.

By rearranging the terms of the electrolysis equation one obtains:

n = (I x t)/(z x F) = (10A x 7200s)/(3 x 96485C) = 72000/289455 = 0.25 M metallic aluminium.

Electrolytic reactions can be utilized in quantitative of metals by measuring gravimetrically the amount of metal formed as the result of the reduction of metallic ions. However, this type of analysis has only limited use in food research.

3.2. pH measurements and potentiometric analysis

The accurate measurement of pH is of great importance to chemical and microbiological laboratories. This is generally done using potentiometric instruments instead of time consuming titrations. A typical pH meter consists of an ion selective electrode and a reference electrode. In this case the ion selective electrode is designed for H^+ ions. A typical electrode of this type is presented in Figure 8. The overall reaction at the electrode is the following:

$AgCl\ (solid) \leftrightarrow Ag\ + Cl^-$

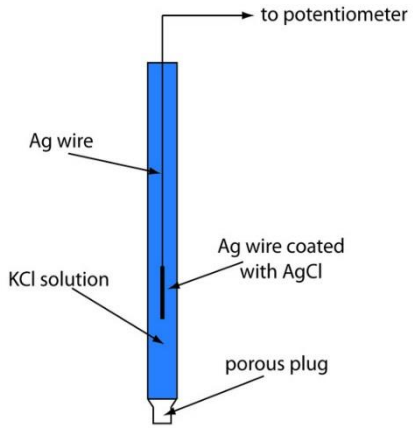

Figure 8. A silver electrode that is usually used as the ion selective electrode in pH measurements. The extent of the reactions at the electrode depends of the amount of protons available. Protons can pass through the porous glass plug at the tip of the electrode.

In addition to the proton selective silver electrode a reference electrode having a constant potential is needed. It is usually also based on AG/AgCl but it is not permeable to protons. The potential difference between the reference electrode and the proton selective electrode is proportional to the pH, which thus can be measured. In modern pH meters the ion selective electrode and the reference electrode are within the same glass shell as a combination electrode, as shown in Figure 9.

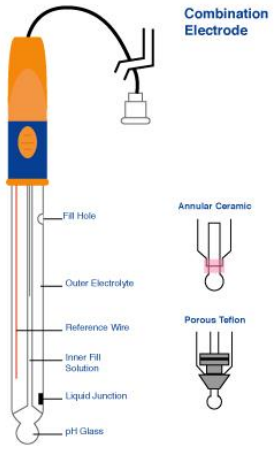

Figure 9. A combination pH electrode containing both the reference electrode and the proton selective electrode within the same shell.

The reactions are temperature dependent, and the pH meters are generally designed to give the most accurate readings at 25 °C. In modern pH meters there is an automatic temperature

compensation. The pH meters should regularly be calibrated using buffer solutions with known pH.

Although pH measurements are the most common examples of potentiometric analysis, ion selective electrodes can be used for other purposes, too. There are, for example selective electrodes for fluoride and sodium ions. In these cases the determination of the ionic concentrations is based on the standard curves obtained by plotting the electrode potential readings with solutions containing known amount of the ions to be measured.

3.3. Conductometric analysis

In conductometric studies the conductance or the ability of the electric current to pass through a solution is measured. This is done by applying two electrodes in the solution and measuring the conductance after turning on the electric current. It is important to use alternating current to avoid electrolysis happening in the solution. The voltage applied is usually 5 – 10 V, and its ffrequency 50 Hz. The conductometric device (figure 10) should be calibrated with a suitable solution, generally 0.01 M KCl.

The unit of conductance is Siemens (S), which is defined as the reciprocal of resistance. The unit of resistance (R) is Ω or 1 V/A. Thus, one Siemens is 1 A/V. For example, the conductance of the0.01 M KCl calibrating solution at 25 °C is 1.143 mS/cm (mS = milli Siemens)

The resistance between the electrodes depends of the conductivity (κ) of the solution, according to the formula:

R = k/κ, where k is cell constant or L/A [L = the distance between the electrodes (m), A = area of the electrode]. The units of κ are S/m. The conductometers are selected on the basis of their cell constants. The smaller the expected conductance, the smaller the cell constant.

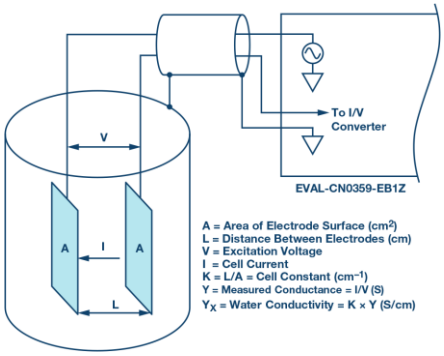

Figure 10. A conductometric cell and its key parameteres

Conductometry is widely used in water analysis. Generally, the less impurities there are in the water, the smaller conductivity. The conductivity of ultrapure water is appr. 0.01mS/m at 25 °C. The conductance is a very important parameter in the characterization of natural waters in fish habitaits.

3.3.1. Conductometric titration

The conventional acid-base titration can in some cases be replaced by conductometric titrations, for example, when the solution to be titrated is so deeply colored that the use of acid base-indicators is not practical.

The titration is performed by measuring the conductivity of the solution after each addition of acid or alkaline. The end point of titration is the marked by a sudden change in the conductivity. This is due to the relative amounts of protons in the solution. When, for example, an acidic solution is titrated with NaOH, the Amount of free protons at first decreases due to the neutralization reaction, and the conductivity decreases (Na^+ ions move slower than the protons, and therefore do not cause an equal conductivity. When the equilibrium point is reached, NaOH starts to accumulate and the free OH^{-1} ions cause the conductivity to increase again. The titration curve therefore has the characteristic V-shape (Figure 11.).

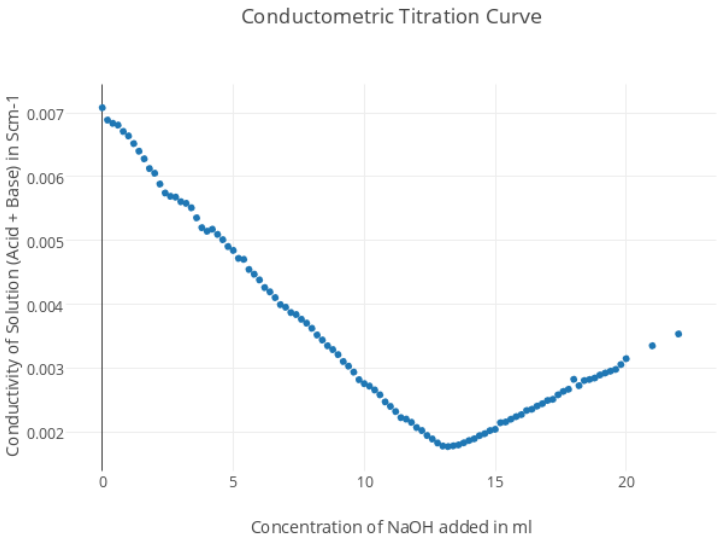

Figure 11. Conductometric titration curve of dilute HCl by NaOH. The equivalence point is the NaOH volume, after which there is a sudden increase of the conductivity

4. Chromatographic methods

An important aspect of analytical chemistry is the separation and purification of individual components of complex mixtures. This is usually done by chromatography. In the chromatographic process the sample or its solution forms the so called mobile phase, which moves through the stationary phase (a substance that is able to retain the components of the sample). In the classical column chromatography (Figure 12) the stationary phase (which can be some resin, silica particles, cellulose, or some other relatively inert material) is packed within a column. The sample is the adsorbed on the top of the column and then eluted with a suitable solvent. The eluent travels through the column and leaves as eluate. During this process the individual chemicals present in the sample are eluted at different time points depending on their affinity to the immobile phase and to the eluent.

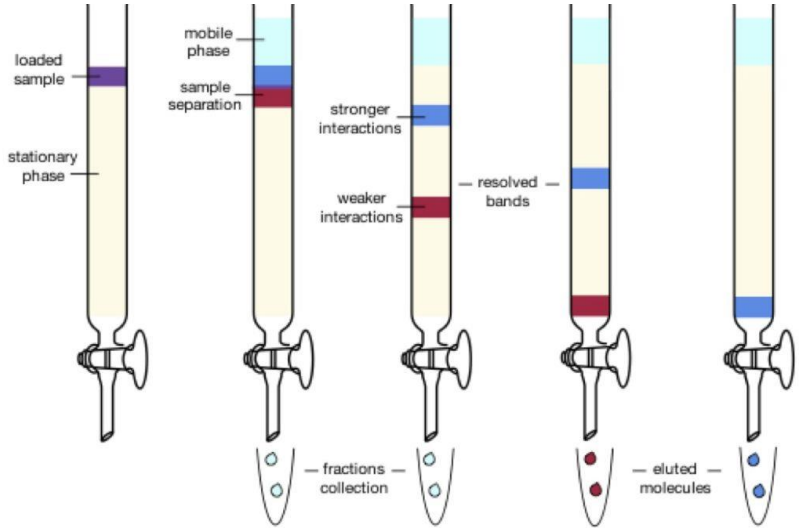

Figure 12. The principle of column chromatography. The sample is loaded on the top of the stationary phase in the column. Eluent is allowed to pass through the column, and the sample components start to separate due to their different affinities and partition between the mobile and stationary phases

In addition to analytical purposes, chromatography can also be used to obtain large amounts of some chemical for research or commercial purposes. Then the process is called preparative or semipreparative chromatography.

4.1. Different mechanisms of chromatographic separation

The separation of the different sample fractions can occur according to several different mechanisms. In adsorption chromatography the sample components have specific adhesion to the particles of the stationary phase. In partition chromatography the stationary phase consists of a

thin liquid layer on supporting particles that fill the column, and the sample components get dissolved into this liquid layer, while they pass through. In ion exchange chromatography the stationary phase has charged groups that interact with the charged groups of the sample components. In gel filtration, the stationary phase is porous material into which small particles migrate while the large ones are excluded and are eluted out.

4.2. Different types of chromatographic techniques

Column chromatography is the oldest form of chromatography, and it can be used to purify relatively large amounts of samples. Indeed, column chromatography can be scaled up to industrial dimensions. The stationary phases are often silica gel or aluminum oxide, which have affinity to electrically charged molecules. Therefore, the elution is often done with solvents like hexane, chloroform or dichloromethane, in which electrically charged molecules have a poor solubility. Gel filtration or exclusion chromatography is another common type of column chromatography, commonly used in biochemistry, for example in protein fractionation.

In the thin layer (TLC) chromatography (Figure 13) the stationary phase forms a thin layer on a solid surface (usually glass or plastic plate). The stationary phase can be silica, cellulose or some specially designed material. The mobile phase enters the stationary phase by capillary forces. The electrophoretic run is done in a special chamber ("tank") with the eluent on the bottom and the chromatographic plate in vertical position and partially immersed in the eluent. Several parallel samples can be spotted on the plate. The method is especially suitable for small samples.

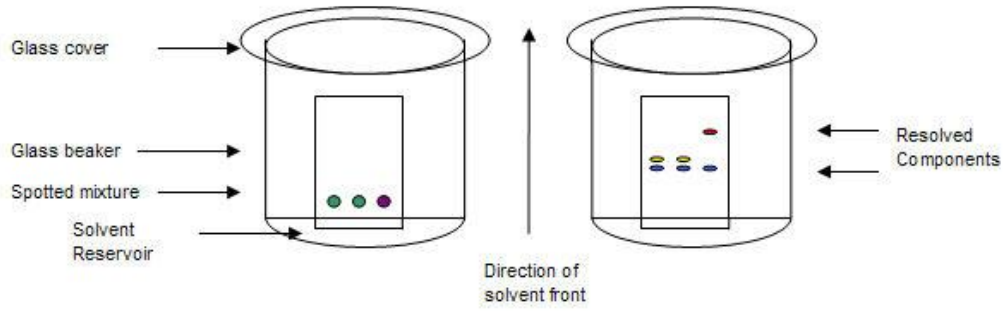

Figure 13. The principle of thin layer chromatography (TLC). The solvent migrates from the bottom of the tank towards the top of the chromatographic plate, and the different components of the samples get separated during the process.

Liquid chromatography (LC) is based on the forced movement of the mobile phase, which is pumped through a column packed with an adsorbent. The absorbent may be coated with a liquid phase (partition chromatography). High Performance Liquid Chromatography (HPLC, Figure 14) is nowadays a very common method, in which high pressure is used to force the mobile phase through the stationary phase consisting of a powder with a very fine particle size. The columns have a diameter of only 1 – 5 mm and the length of 5 – 30 cm. However, also HPLC can be scaled up to industrial scale.

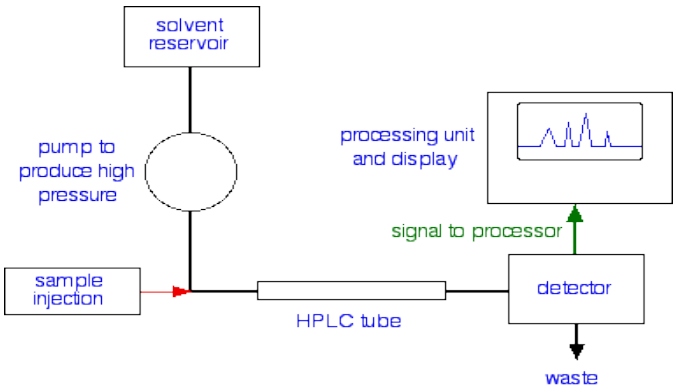

Figure 14. The principle of High Performance Liquid Chromatography (HPLC).

In gas chromatography (GC, Figure 15) the mobile phase is a gas (hydrogen, helium or nitrogen), which carries the volatile compounds through the long column (typically with a coil shape) which can be coated either with liquid, solid particles or solid particles covered with liquid. The carrier gas can be hydrogen, nitrogen helium or argon. The length of the column is typically around 30 m, and this length ensures the efficient separations of compounds during the long travel. Glass capillary columns are usually the most efficient for separation. The temperatures inside the colon can reach up to 400 °C, and very often the temperature is gradually raised during the chromatographic run to ensure the optimal separation of the components.

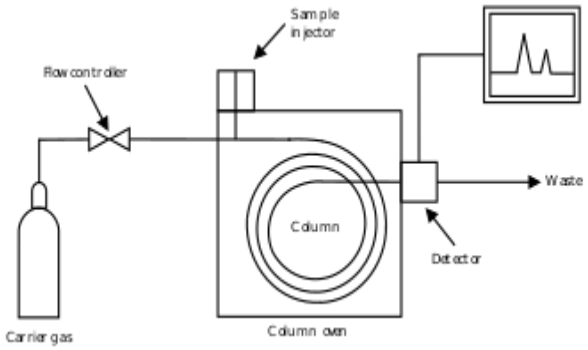

Figure 15. The principle of gas chromatography (GC). Note the long, spiral shaped coil containing the stationary phase

4.3. Eluents used as the mobile phase

The eluents that are used in chromatography must be selected according to the type of the sample. In standard column chromatography the eluent can be a buffer solution with some specific pH and ionic strength, although organic solvents are also often used.

In liquid chromatography the eluents are usually organic solvents with different polarities, such as pentane, toluene, diethyl ether, chloroform, dichloromethane, acetone, ethyl acetate, isopropanol, ethanol or methanol. When a single eluent is used throughout the chromatography, the elution is isocratic. However, in many analytical runs an elution gradient is applied by gradually changing the composition of the eluent (for example starting with a highly nonpolar organic eluent and gradually changing it to a more polar solvent). By this gradient the elution of compounds having different polarities is maximized.

The gradient from polar to less polar solvent is typical for reverse phase chromatography, in which the stationary phase is a non-polar or weakly polar solvent.

4.4. Analyte derivatization

Sometimes the samples have to be pretreated in order to improve either the separation or especially the detection of the different compounds. Derivatization of sample molecules with some easily detectable chemical moieties is a common technique, especially in GC andLC.

For, example, silylation or replacing certain reactive hydrogen atoms in the functional groups of the analyte with trimethylsilyl group to increase the volatility of the analytes and to decrease there surface absorption is typical for GC. Different fluorescent groups are often introduced to compounds to be separated by HPLC, mainly to improve their detection (see 4.4).

4.4. Detection of the eluted compounds

The detection of compounds separated during the chromatographic run is, of course, an essential part of the analysis. Sometimes, when the compounds are colored, they can be seen as discrete colored band migrating at different speeds in the chromatographic columns. More commonly, however, the elute is continuously collected as small fractions of constant volumes (for example, if a chromatographic column is eluted with 100 ml of mobile phase, the eluate is collected as 100 one ml fractions). From each of the fractions the substances are analyzed by appropriate techniques, often spectrometrically.

In thin layer chromatography, the plates often have to be developed after the run by spraying with chemicals reacting with the individual components and forming a colored product that can be seen as a spot. For example, ninhydrin reagent is commonly used to detect amino acids (Figure 16). In some cases the spots can be visualized by their fluorescence under ultraviolet light UV-illumination.

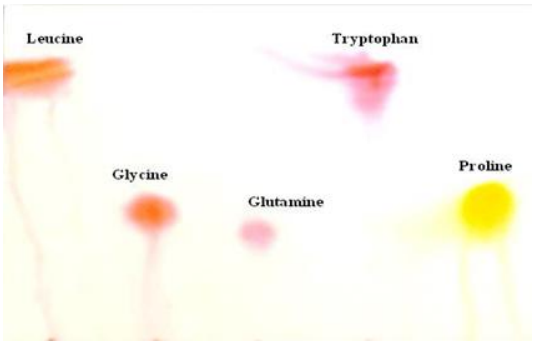

Figure 16. A thin layer chromatogram of amino acids Leucine, Glycine, Tryptophan, Glutamine and Proline after staining with ninhydrine reagent. Each amino acid has migrated at different speed and formed a colored spot after the ninhydrin treatment.

In GC and LC the detection of separated compounds is based on different types of detectors must be used. The detectors (see figures 14 and 15) are located at the end of the column and follow on line the elution process giving a signal each time a different compound is eluted from the column.

There are many different types of detectors. Some common ones are listed in Table 1.

Detector	Principle of detection	Type of chromatography
Thermal conductance detector (TCD)	Measures the characteristic thermal conductance of each substance	GC
Flame-ionization detector (FID)	The eluate is burned in a mixture of hydrogen and air. This leads to the formation of positively charged ionic organic products that are detected by a measuring device	GC
Electron capture detector (ECD)	Sensitive to substances containing halogens (chlorine, fluorine, bromine and iodine). The carrier gas is ionized by high-energy electrons, which creates a constant current. Molecules eluted may catch some of the ionizing electrons and thus reduce the current. This change is recorded	GC
UV/VIS detector	Measures the absorption of UV or visible radiation of the elute	LC

Refractive index detector	Measures the changes in refractive index. Has generally rather low detection limit, and is not suitable for gradient elutions	LC
Mass spectrometry (MS)	Measures the mass spectra of the eluted compounds (see Section 2.3.3). Very sensitive and not only detects but also identifies the eluted compounds. Nowadays one of the most common detectors in analytical procedures requiring high sensitivity.	LC (LC-MS)

4.5. Chromatograms

When the intensities of the signals obtained from the detectors (or the concentrations of the compounds present in the fractions collected from column chromatography) are plotted against the length of the run, a chromatogram is obtained. A typical chromatogram consists of peaks of variable intensities, each peak corresponding to a separate eluted compound (Figure 17).

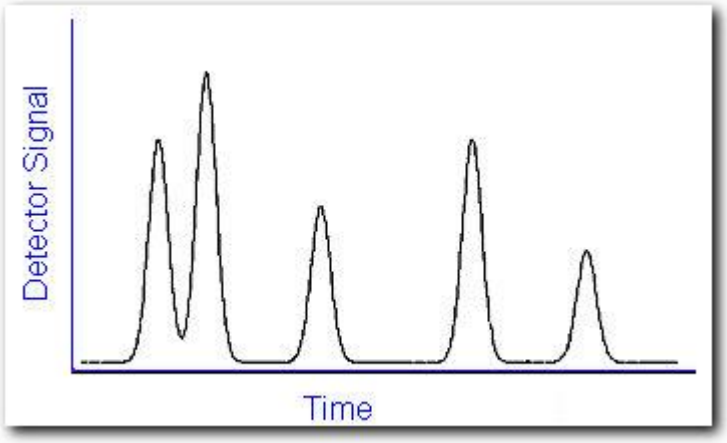

Figure 17. A chromatogram of five eluted compounds ("peaks"), each having a characteristic retention time (the time point coinciding with the maximum value of the peak)

Retention time is one of the central parameters of a chromatogram. It means the time point at which each compound emerges from the column. With compound having nearly similar retention times some degree of overlap may occur between the corresponding peaks. These problems can often be eliminating by changing the composition of the mobile phase or the other parameters of the chromatographic run.

4.6. Chromatographic methods in fish and seafood analysis

Chromarographic methods can be used in the nutritional assessment of foodstuffs (for example in qualitative and quantitiative fat analysis). They provide a particularly powerful approach in the analysis of different contaminants in foods, such as pesticide and drug residues or environmental pollutants. Some examples of chromatography applied specifically to fish and fishery products are given below.

4.6.1. Methyl mercury analysis

Mercury is an important environmental pollutant accumulating in fish mainly in the form of methylmercury. Mercury levels in fish are regulated both in national and international legislation. For example, in the EU the allowed mercury levels in different types of fish are 0.5 – 1.0 µg/kg. Mercury analysis can be done using AAS, but also gas chromatographic methods are widely used.

Mercury can first be extracted from the muscle tissue by a suitable solvent such as hexane, and this extract is subjected to the GC-MS chromatography. Alternatively, a fish tissue sample can first be digested using a suitable caustic chemical like methanolic KOH solution, followed by derivatization with tetraethylborate before solvent extraction and GC analysis (in this case as ethylmercury).

4.6.2. Analysis of antibiotic residues

Antibiotics of the tetracycline family (oxytetracycline, doxycycline, tetracycline and chlortetracycline) can be analyzed from fish and seafood extracts by HPLC using a gradient mobile phase consisting of organic solvent [such asmethanol:acetonitrile (1:1, v/v)] and a special buffer as an aqueous mobile phase and using a specific fluorescence detector.

Reversed-phase HPLC method with mass-spectrometric detection can be used for the simultaneous analysis of quinolone and fluoroquinolone antibiotics (oxolinic acid, flumequine, piromidic acid, enrofloxacin, ciprofloxacin, danofloxacin, sarafloxacin and orbifloxacin) in extracts of fish and crustacean tissues.

4.6.3. Analysis of histamine

Histamine is a biogenic amine formed by bacterial metabolism from the natural aminoacid histidine. Histamine has strong physiological activities, and can cause histamine-food poisonings, protein rich foods, such as fish, cheese and meat products being frequently implicated.

Histamine can be analyzed by an isocratic reversed phase HPLC from fish and seafood extracts made by homogenizing in the trichloroacetic acid and enriched by ion-exhange chromatography. Alternatively, a simple TLC method using cellulose as the stationary phase and an ammonia-ethanol mixture as a mobile phase can be used for a quick screen for the presence of histamine. Histamine spots can be visualized with a special reagent (Pauly's reagent).

5. Concluding remarks

Instrumental analysis provides accurate and time saving possibilities both to quantitative and qualitative analysis. Some of the instrumental methods, such as pH measuring using a potentiometric pH meter, are so commonplace that they hardly are considered as analytical methods, any more.

Spectroscopic and chromatographic methods vary greatly in their sophistication. While spectrophotometric measurements and the ability to perform basic HPLC and GC runs belong to the repertoire of modern food laboratory technicians, the accurate interpretation of the results and the planning of the chromatographic parameters require the expertise of an experienced analyst.

Future reading

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