DEMOCRATIC AND POPULAR ALGERIAN REPUBLIC MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
Ferhat Abass University Sétif1

Faculty of Natural and Life Sciences

Department of Biotechnology

ANALYTICAL TECHNIQUES

Course intended for 3rd-year students in Food Biotechnology



Dr.Sabrina Manel Kaddour

Academic year :2025/2026

TABLE OF CONTENTS

INTRODUCTION 1

CHAPTER I. REVIEW OF BASIC CONCEPTS

1. General Information on Good Laboratory Practice (GLP) 3

1.1. Definition 3

1.2. History 3

1.3. Scope 3

1.4. Interest 4

1.5. Principles of Good Laboratory Practice 4

1.5.1. Organisation and Personnel of the Test Facility 4

1.5.2. Quality Assurance Programme 5

1.5.3. Facilities 6

1.5.4. Apparatus, Materials and Reagents 6

1.5.5. Test Systems 6

1.5.6. Test and Reference Items 7

1.5.7. Standard Operating Procedures 7

1.5.8. Conduct of the Study 7

1.5.9. Preparation of the Study Results Report 7

1.5.10. Storage and Preservation of Archives and Materials 8

CHAPTER II. REVIEW ABOUT SOLUTIONS

2. General Information About Solutions 10

2.1. Definitions (solute, solvent, concentrations) 10

2.1.1. Dissolution Phenomenon 10

2.1.2. Ionic Aqueous Solution and Non-Ionic Aqueous Solution 10

2.1.3. Stages of Dissolution 10

2.1.4. Thermal Effects of Dissolution 10

2.2. Concentration Units 10

2.2.1. Mass Concentration of an Aqueous Solution 11

2.2.2. Molar Concentration of a Solution 11

2.3. Solubility-Saturation 13

2.3.1. Saturation of a Solution 13

2.3.2. Solubility 13

2.4. Normality and Other Concentration Units 13

3. Methods for Preparing Solutions 14

3.1. Weighing Method 14

3.2. Dilution Method 15

CHAPTER III.BASIC TREATMENTS

3. General Information on the States of Matter and Separation Methods 16

3.1. Matter 16

3.2. Mixtures 16

3.2.1. Definition 16

3.2.2. Heterogeneous Mixtures 17

3.2.3. Homogeneous Mixtures 17

3.3. Methods for Separating Mixtures 17

3.3.1. Definition 17

3.3.2. Principle of Separation 18

3.4. Separation by Extraction 18

3.4.1. Liquid-Liquid Extraction 19

3.4.2. Solid-Liquid Extraction 20

3.5. Separation of the Components of a Heterogeneous Mixture 24

3.5.1. Filtration 24

3.5.2. Decantation 27

3.5.3. Centrifugation 29

3.5.4. Crystallization 33

3.6. Heat Treatments 35

3.6.1. Sterilization 35

3.6.2. Pasteurization 40

3.6.3. Generalities on Drying 43

3.6.4. Lyophilization 47

CHAPTER IV. PARTICULAR TREATMENTS

4.1. Fermentation 49

4.1.1. Definition 49

4.1.2. History of Fermentation 49

4.1.3. Ferments 51

4.1.4. Main Food Fermentations 51

4.1.5. Microscopic Fungi and Fermented Foods 53

4.1.6. Role of Fungal Ferments 57

4.2. Wastewater 58

4.2.1. Definition 58

4.2.2. Origin of Wastewater 58

4.2.3. Composition of Wastewater 60

4.2.4. Type of Wastewater Pollution 62

4.2.5. Wastewater Treatment 63

4.2.6. Wastewater Treatment Chain 64

4.2.7. Disinfection 68

4.3. Annex Techniques 69

4.3.1. Controls 70

4.3.2. Sampling 70

4.3.3. Transport and Handling 71

4.3.4. Equipment Maintenance 71

CHAPTER V. SPECTROSCOPY

5. Atomic Absorption and Emission Spectrometry 73

5.1. Phenomena of Atomic Absorption and Emission 73

5.1.1. Atomic Absorption 73

5.1.2. Atomic Emission 73

5.1.3. Spectroscopy and Spectrum 73

5.2. Ultraviolet/Visible Spectroscopy 73

5.2.1. Principle 73
5.2.2. Spectral Range 74
5.2.3. Equipment 74
5.2.4. Application of UV/Visible Spectroscopy
5.3. Infrared Spectroscopy 76
5.3.1. Principle 76
5.3.2. Equipment 76
5.3.3. Application 77
5.4. Atomic Emission Spectrometry 77
5.4.1. Definition 77
5.4.2. Principle 78
5.4.3. Applications 78
5.5. Spectrofluorimetry 78
5.5.1. Principle 80
5.5.2. Equipment 80
5.5.3. Applications 80
5.6. Polarimetry 81
5.6.1. Polarized Light 81
5.6.2. Principle of the Method 82
5.6.3. Biot's Law 82
CHAPTER VI. CHROMATOGRAPHIC METHODS
6.1. Definition 84
6.2. Principle 84
6.3. Classification 84
6.4. Fundamental Quantities and Definitions 85
6.5. Main Chromatographic Methods 86
6.5.1. Thin-Layer Chromatography (TLC) 86
6.5.2. Two-Dimensional Thin-Layer Chromatography (2D TLC) 87
6.5.3. High-Performance Thin-Layer Chromatography (HPTLC) 88

6.5.4. Paper Chromatography 88
6.5.5. Partition Chromatography 88
6.5.6. Ion Exchange Chromatography 88
6.5.7. Exclusion Chromatography 89
6.5.8. Column Chromatography 90
6.5.9. Affinity Chromatography 91
6.5.10. High-Performance Liquid Chromatography (HPLC) 92
6.5.11. Gas Chromatography (GC) 93

CHAPTER VII. ELECTROPHORESIS

7.1. Definition 95
7.2. Principle of Migration 95
7.3. Materials 96
7.4. Main Electrophoresis Matrices 97
7.4.1. Agarose 97
7.4.2. Polyacrylamide 97
7.5. Buffers 97
7.5.1. Denaturing and Non-Denaturing Buffer System 97
7.5.2. Continuous and Discontinuous Buffer Systems
7.5.3. Loading Buffers 98
7.6. Applied Current 98
7.7. Staining and Visualization 98
7.8. Applications 99
7.9. Electrophoresis Types 99
7.9.1. Electrofocusing (Isoelectric Focusing) 99
7.9.2. Two-Dimensional Electrophoresis 99
7.9.3. Immunoelectrophoresis 100
7.9.4. Pulsed-Field Electrophoresis 102
7.9.5. Capillary Electrophoresis 102

LIST OF FIGURES

Figure 1. Preparation of solution 10

Figure 2. Principle of electrolyte dissolu 11

Figure 3. Method of preparing solutions by weighing..... 14

Figure 4. Method for preparing solutions by dilution..... 15

Figure 5. Examples of materials and pure substances 16

Figure 6. Liquid-liquid extraction protocol using a separatory funnel 19

Figure 7. Solid-liquid extraction systems. 21

Figure 8. Steam distillation and hydrodistillation systems 22

Figure 9. Preparation of a pleated filter and installation in the funnel 25

Figure 10. Simple filtration setup 26

Figure 11. Vacuum filtration setup..... 27

Figure 12.Diagram of the principle of sedimentation.....28

Figure 13. Settling of an emulsion.....29

Figure 14. Diagram of the principle of centrifugation.....30

Figure 15. Crystallization process 34

Figure 16. Drying methods 44

Figure 17. Alcoholic fermentation.....52

Figure 18. Metabolism for lactic acid bacteria (homofermentative and heterofermentative pathways).....53

Figure 19. Vinegar Fermentation.....54

Figure 20.sources of wastewater.....61

Figure 21.Schematic-of-Single-and-double-beam-spectrophotometer.....75

Figure 22. IR-Spectroscopy-Instrumentation..... 76

Figure 23. Different components of a spectrofluorometer with a xenon arc lamp.....80

Figure 24. Thin-layer chromatography and Rf values.....87

Figure 25. Two Dimensional Techniques in Paper Chromatography..... 88

Figure 26. Exclusion chromatography.....89

Figure 27. Column chromatography 90
Figure 28. Affinity chromatography 91
Figure 29. High-performance liquid chromatography 92
Figure 30. Diagram of a gas chromatography..... 94
Figure 31. Electroendosmosis current 96
Figure 32.vertical and Horizontal mounting.....97
Figure 33. Two-dimensional electrophoresis..... 100
Figure 34. Immuno-electrophoresis of Grabar and Williams. 101
Figure 35. One-dimensional electro-immunodiffusion..... 102
Figure 36. Pulsed-field electrophoresis..... 102
Figure 37. Capillary electrophoresis 103

LIST OF TABLES

Table 1. Example of mixtures 17

Table 2. Different types of homogeneous mixtures 17

Table 3. Different models of centrifuges 31

Table 4. Comparative table: Pasteurization vs Sterilization 42

Table 5.Parameter effect on crystals 35

Table 6.Deficiencies and hazards associated with crystallization..... 36

INTRODUCTION

The reliability of nutritional and health monitoring of food depends in particular on the methods used to perform analyses. Different types of analytical methods can be distinguished, depending on the analytical objective pursued (detection/quantification, characterisation, etc.) or the status of the method (standardised reference method, commercially available alternative method, internal method).

This course covers analytical techniques used in the food industry. It presents regulated instrumental analysis with an emphasis on general methods for testing and analysing food products.

In order to address regulated food analysis, understanding the term ‘food’ is a good starting point. Food refers to any substance consumed for nutritional purposes. Food contains essential nutrients such as fats, proteins, vitamins and minerals.

The choice of analytical method must take into account the nature of the results sought. It is therefore important to know the ‘internal’ performance characteristics of an analytical method during the period under consideration when using it on substances to be analysed. Any analytical method used on substances to be analysed must be applied consistently and accompanied by quality control procedures.

The course is divided into a series of chapters that enable students to acquire knowledge and skills in the analytical techniques used in the food industry for quality control.

The course will provide theoretical knowledge on the description and operating principles of analytical equipment, as well as practical experience of analytical methods through laboratory work involving the analysis of certain foods.

This course also enables students to acquire the know-how and interpersonal skills essential for food control, while complying with good laboratory practices and mastering certain basic principles such as: methods for preparing solutions and reagents and how to interpret results.

Prerequisites

To get the most out of this course, some prior knowledge is recommended, namely:

- Basic concepts in chemistry, physics and optics;

- Use of certain analytical instruments, particularly the simplest ones.

A prerequisite test will be given at the beginning of the course (in the form of oral questions or exercises) to assess students' prior knowledge, enable them to follow the course effectively and provide guidance in case of failure.

Place of the course in the programme

The Analysis Techniques course occupies an important place in the Food Biotechnology bachelor's degree programme. It is also classified under the teaching unit methodology.

Learning objectives

Methods and Techniques of Analysis aims to train students to implement current and recent analysis techniques, participate in new analytical developments, and adapt to changes in the field of analysis. In addition, the courses place a strong emphasis on general scientific and humanities training in order to prepare students for successful integration into the workplace by enriching their ‘basic knowledge’ and developing their ‘interpersonal skills’.

The course also aims to develop students understanding of the instrumental methods involved in food control. This teaching is based on three main aspects:

1- A brief theory of the method;

2- Description and operation of the equipment; 3- Interpretation of results.

As there are many instrumental methods, this course will focus on those that are widely used in the food industry.

1. General information on Good Laboratory Practice (GLP)

1.1. Definition

GLP is a set of rules written in the form of procedures, the application of which should enable work to be qualified within the framework of quality standards.

It forms a quality assurance system covering the organisation of non-clinical safety studies.

They also define staff responsibilities, equipment management and maintenance, process and method validation, and health and safety rules.

1.2. History

Good Laboratory Practice (GLP) was developed after the Food and Drug Administration (FDA) discovered a number of poor practices in toxicology laboratories in the United States. The results of the investigations showed that the studies were poorly organised, the staff were insufficiently trained and there were some cases of deliberate fraud. During its investigations, the FDA also noted that the studies were conducted without regard for protocols or procedures. The raw data generated during the study were poorly collected, non-reproducible and not recognised and approved by those responsible. The FDA decided to introduce legislation to cover the studies.

In 1978, an international group of experts, formed as part of the Special Programme on Chemical Control, established the 'OECD (Organisation for Economic Co-operation and Development) Principles of Good Laboratory Practice' by synthesising the management methods, scientific practices and experience of various national and international organisations.

The application of these GLP principles was officially recommended to member countries by the OECD Council in 1981.

In 1995 and 1996, a new Group of Experts was established to review and update these principles. The revised OECD GLP principles were reviewed by the relevant governing bodies of the organisation and adopted by the Council on 26 November 1997.

1.3. Scope

The Principles of Good Laboratory Practice shall apply to non-clinical safety testing of constituents of pharmaceuticals, pesticides, cosmetics, veterinary medicines, food and feed additives, and industrial chemicals. These substances undergoing testing are often synthetic chemicals, but may be of natural or biological origin and, in some circumstances, may be living organisms. The purpose of testing these substances is to provide data on their properties and/or safety with regard to human health and/or the environment.

1.4. Interest

Their main purpose is to help scientists obtain results that are reliable, reproducible, verifiable and internationally recognised. It enables:

• the promotion of international harmonisation for the testing of chemicals;

• assistance to countries in developing assessment methods and sharing the work;

• the evaluation of different chemicals;

• provide a framework in which countries can exchange information.

1.5. Principles of good laboratory practice

1. Organisation and personnel of the testing facility

2. Quality assurance programme

3. Facilities

4. Apparatus, materials and reagents

5. Test systems

6. Test and reference items

7. Standard operating procedures

8. Conduct of the study

9. Reporting of study results

10. Storage and retention of records and materials

1.5.1. Organisation and personnel of the test facility

- ✓ **Facility management**

Facility management must ensure that sufficient qualified personnel and appropriate facilities, equipment and materials are available to conduct the study in a timely and adequate manner.

- ✓ **Study Director**

The Study Director is solely responsible for controlling the study and assumes responsibility for the overall conduct of the study and the preparation of the final report. He or she shall approve the study plan with a dated signature.

- ✓ **Principal Investigator**

He shall ensure that the phases of the study delegated to him are conducted in accordance with the applicable Principles of Good Laboratory Practice.

- ✓ **Study Personnel**

- He shall be well informed of the parts of the Principles of Good Laboratory Practice applicable to his participation in the study.
- They shall have access to the study plan and standard operating procedures applicable to their participation in the study.
- They must record raw data promptly and accurately, in accordance with GLP, and take responsibility for the quality of this data.
- They must take the necessary hygiene precautions to minimise the risk to which they are exposed and to ensure the integrity of the study.

1.5.2. Quality assurance programme

A specific system involving the relevant personnel, which is independent of the conduct of the study and aims to provide the management of the test facility with assurance that GLP principles are being followed.

A QA programme using any useful documents that enable verification that studies are conducted in accordance with GLP principles. One or more persons designated by management must be responsible for QA. These persons must not be involved in the conduct of the study covered by the programme.

The personnel responsible for QA must: keep copies of all study plans, verify that the study plan contains the information necessary to comply with GLP principles, and conduct inspections to verify that all studies are conducted in accordance with GLP principles.

1.5.3. Facilities

The test facility must have a sufficient number of rooms or areas to ensure the separation of test systems.

It must have appropriate rooms or areas for the diagnosis, treatment and control of diseases, so that the test systems are not subjected to an unacceptable degree of deterioration.

It must have sufficient storage rooms for supplies and equipment, separate from the rooms or areas housing the test systems and adequately protected against contamination and/or deterioration.

Archive rooms must be provided for the secure storage and consultation of study plans, raw data and final reports.

1.5.4. Apparatus, materials and reagents

Apparatus used in a study should be periodically inspected, cleaned, maintained and calibrated in accordance with standard operating procedures.

Computer systems must be located in a suitable place, be of appropriate design and have sufficient capacity.

Chemicals, reagents and solutions must be labelled and their nature (including concentration, where applicable), expiry date and specific storage instructions must be indicated.

1.5.5. Test systems

A test system refers to any biological, chemical, physical or combination thereof, or animal or plant system used in a study.

Suitable conditions for the storage, handling and maintenance of biological and chemical test systems must be created and maintained to ensure the quality of the data.

Newly received animal and plant test systems should be isolated until their health status has been assessed.

Records should be kept of the origin, date of arrival and condition on arrival of test systems.

1.5.6. Test and reference items

Records must be kept detailing the characterisation of the test items, the date of receipt, the expiry date and the quantities received and used in the study.

Methods of handling, sampling and storage shall be defined to ensure that homogeneity and stability are maintained as far as possible and to avoid contamination or mixing.

1.5.7. Standard operating procedures

The test facility shall have written standard operating procedures, approved by the facility management, which shall ensure the quality of the data obtained by that facility. Revisions to the standard operating procedures shall be approved by the test facility management.

Each separate section of the test facility shall have immediate access to the standard operating procedures relevant to the work being carried out there. Documents may be used to supplement standard operating procedures.

1.5.8. Conduct of the study

For each study, a written plan shall be established before the work begins. The study plan shall include a description of the test procedure, as well as the control methods and frequency of execution. Any changes to the plan shall be recorded, stating the reason for the change, the date and the signature or initials of the person making the change.

1.5.9. Preparation of the study results report

The final report must include the raw data collected during the study. It must also include the following information:

• Start and end dates of the study

• Name of the study director

• Objectives of the study

• Description of the test system

• Details of the methods used

• Summary of the results

• Statistical analysis

• Discussion

• References

• Declaration of compliance with GLP by the study director

• Quality assurance declaration by the inspectors (Declaration)

• Signature and date

1.5.10. Storage and preservation of archives and materials

The study plan, raw data, samples of test and reference items, specimens and final report for each study; Reports on all inspections carried out in accordance with the quality assurance programme;Records of staff qualifications, training, experience and job descriptions; Records and reports relating to equipment maintenance and calibration; Documents relating to the validation of computer systems; Standard operating procedures.

2. General information about solutions

2.1. Definitions (solute, solvent, concentrations)

2.1.1. Dissolution phenomenon

When a substance is dissolved in a liquid, the result is a solution, which is a homogeneous mixture.

The dissolved substance is called the solute, and the liquid in which the substance is dissolved is called the solvent.

Note: If the solvent is water, the solution is called an aqueous solution



Figure 1. Preparation of solution

2.1.2. Ionic aqueous solution and non-ionic aqueous solution

An aqueous solution that conducts electricity contains ions (cations and anions). It is called an ionic aqueous solution. The solute is an ionic crystal. Example: The mixture of salt and water is an ionic aqueous solution containing Na+ ions and Cl- ions.

An aqueous solution that does not conduct electricity is a non-ionic aqueous solution. Example: A sucrose (sugar) solution is a non-ionic solution.

2.1.3. Stages of dissolution

The dissolution of an electrolyte in a solvent occurs in three distinct stages: dissociation, solvation and dispersion.

a. Dissociation

As the solvent is polar, its molecules behave like small electrostatic dipoles. The cations in the ionic crystal are therefore attracted to the positive sides of the solvent molecules, and the

anions to the negative sides. These attractions outside the crystal unbalance the internal interactions and weaken its cohesion: the ions on the surface of the crystal are gradually pulled away by the solvent molecules. This is referred to as dissociation of the ionic crystal. It is the phenomenon of dissociation.

Note. In the case of a non-ionic but molecular electrolyte, the same thing happens since each molecule of the electrolyte is polar and therefore has a positive side and a negative side.

b. Solvation

Once removed from the crystal, each ion is surrounded by solvent molecules. These form a shield and prevent any interaction between the ions in solution. This is the phenomenon of solvation. When the solvent is water, the solvation stage is also known as hydration.

c. Dispersion

Once solvated, the ions move freely in the solution with their entourage of solvent molecules, and the solution gradually becomes homogeneous. This stage is considerably accelerated if the solution is stirred.

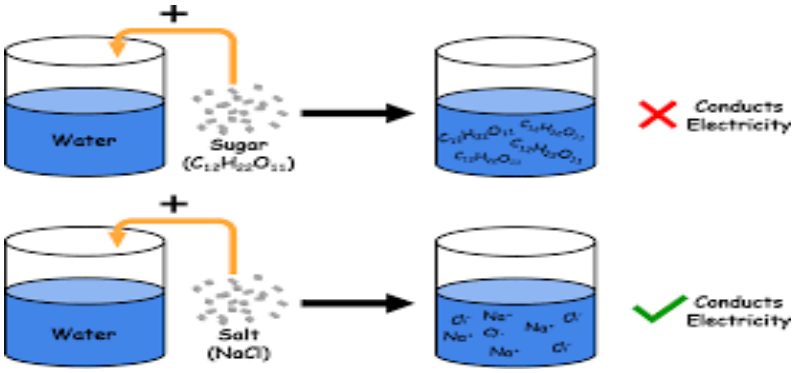


Figure 2. Principle of electrolyte dissolution.

2.1.4. Thermal effects of dissolution

The dissolution of certain solutes is accompanied by:

- An increase in temperature: the dissociation is said to be exothermic (NaOH in water).
- Or a decrease in temperature: the dissociation is said to be endothermic (NH4NO3).

2.2. Concentration units

2.2.1. Mass concentration of an aqueous solution

The mass concentration of a solution is the mass of solute contained in one litre of that solution. It is denoted by Cm and its unit is g/L.

$Cm = \frac{\mathrm{M(solute)}}{\mathrm{Vs}}$ with Cm: the mass concentration of the solution (g.L-1)

Vs: the volume the solution (L)

M: the mass of the solute (g)

2.2.2. Molar concentration of a solution.

The molar concentration of a solution is the amount of solute contained in one litre of solution. It is denoted by C and its unit is mol/L.

$C = \frac{\mathrm{n(solute)}}{\mathrm{Vs}}$ with C: the molar concentration of the solution (mole)

Vs: the volume of the solution (L)

n: the number of mole of the solute (mole)

Exercise 1:

A sodium sulphate (Na_2SO_4) solution is prepared by dissolving 3.55 g of this compound in water to obtain 250 mL of solution.

Calculate the molar and mass concentration of the solution obtained.

Example:

Preparation of 250 mL of 0.1 mol/L soda m = M C V.where

M is the molar mass of the substance to be dissolved;

C is the concentration of the solution to be prepared;

V is the volume of solution to be prepared.

Data:

molar mass of sodium hydroxide: MNaOH = 40 g.mol-1;

concentration of the desired solution: C = 0.1 mol.L-1;

volume of solution prepared: V = 250 mL = 0.25 L. hence m =……..... g.

Example:

You are asked to prepare 1 L of a 1 M hydrochloric acid solution. You have a 37% concentrated solution.

The molar mass of HCl is 36.5 g/mole. The density of the concentrated solution is 1.19.

The mass m of hydrochloric acid contained in the bottle is m = 0.37 × 1190 = 440.3 g.

The molar mass M of hydrochloric acid is: M = 36.5 mol/L.

The number of moles n contained in the bottle is:

n = m/M n = 440.3/36.5 n = 12.06 mol

The concentration c of the stock solution is therefore: c = 12.06 mol.L-1

The volume V = 1000 mL of hydrochloric acid to be prepared will contain a number of moles n such that: n = C V, n = 1 x 1 n = 1 mol

This number of moles is provided by a volume V0 of the stock solution such that:

V = n/c V = 1/12.06 V = 0.08 L

2.3. Solubility-Saturation

2.3.1. Saturation of a solution.

As dissolution progresses, the number of solvent molecules available for dissociation decreases, so dissolution becomes slower and slower. Since the number of water molecules available is limited, the amount of solute that a solution can dissolve is itself limited. When this limit is reached, the solution is said to be saturated.

2.3.2. Solubility

The solubility of a substance in water is the maximum amount of that substance that can be dissolved in a given volume of water. It is a quantity that depends on temperature.

2.4. Normality

Normality is the number of gram equivalents of a solute (i) present in one litre of solution. A normal solution (or one normal) contains one gram equivalent per litre. Normality (N) and molar concentration (CM) are related by the following equation:

N = n C M

n = The gram equivalent is the amount of substance comprising one mole of the particles in question (H+, e–), the number of protons exchanged during acid-base reactions or electrons in the case of redox reactions.

Application: What is the normality of a sulphuric acid solution containing 12.25 g of acid per 500 cm^3?

Corrected:

One eq-g of H_2SO_4 = ½ mole x 98 = 49 g

Therefore: 1 eq-g ⟶ 49 g

X = ? ⟵ 12.25 g

Therefore, x= number of eq-g in the mass of 0.25g = (12.25 x 1) / 49 = 0.25 eq-g/500 cm3

Normality (by definition) is the number of eq-g/L:

500 cm3 ⟶ 0.25 eq-g

1 L = 1000 cm^3 ⟵ x= ?

Therefore, x = number of eq-g in 1 L of solution = (1000 x 0.25) / 500 = 0.5 eq-g

The normality of H_2SO_4 is therefore equal to 0.5 N or 0.5 times normal.

- **Percentage mass/volume % (m/v):** This is the number of grams of a solute in 100 ml (or 100 cm^3) of solution

Example: A 50% (m/v) solution of H_2SO_4 means that such a solution contains 50 g of H_2SO_4 in 100 ml of water.

Note: As the volume of liquids varies with temperature, the titles defined above therefore depend on temperature.

- **Molality (molal concentration):** This is the number of moles of solute per kilogram of solvent (water). It is given by the following equation:

$$\textbf{molarity} = \frac{\text{n solute}}{\text{m solvent}} = \frac{\text{n solute(mole)}}{\text{m water(kg)}}$$

Application:

We have an aqueous solution of acetic acid containing 856 g of acid per litre with a density of 1.07 kg/L. What is the molality of this solution?

Answer:

Mass of solute CH3COOH = 856 g Molar mass of solute = 60 g/mol

Number of moles of acid contained in one litre of solution:

N solute = m solute/M solute = 856/60 = 14.26 moles

1 litre of solution weighs 1070 g:

Mass of water = mass of solution – m solute = 1070 – 856 = 214 g = 0.214 kg

Molality = n solute/m water = 14.26 / 0.214 = 66.63 mol/kg.

3. Methods for preparing solutions

3.1. Weighing method

Determining the mass of solute to be weighed

Suppose we want to prepare a volume V of a solution containing species X, with molar mass M(X), at concentration [X]. In general, we need to determine the mass of species X to be weighed. Let this mass be m(X).

[X] = n (x)/v or n (x) = m (x) / M(x) Therefore [X] = m (x) / M (x) * v

And m (x) = [X] * M (x) * v



Figure 3. Method of preparing solutions by weighing

3.2. Dilution method

Diluting a solution means reducing its concentration. To do this, solvent must be added. However, the volume of solvent added must be known in order to determine the concentration of the new solution obtained. The most concentrated solution is often referred to as the 'stock solution', while the diluted solution is called the 'daughter solution'.

Principle

A volume V0 of the stock solution with concentration C0 is taken and diluted with distilled water to obtain a diluted solution with volume V1 and desired concentration C1.

Determining the volume V0 to be taken

During dilution, the amount of solute remains constant. We write: n1 = n2

Therefore: C0 . V0 = C1 . V1

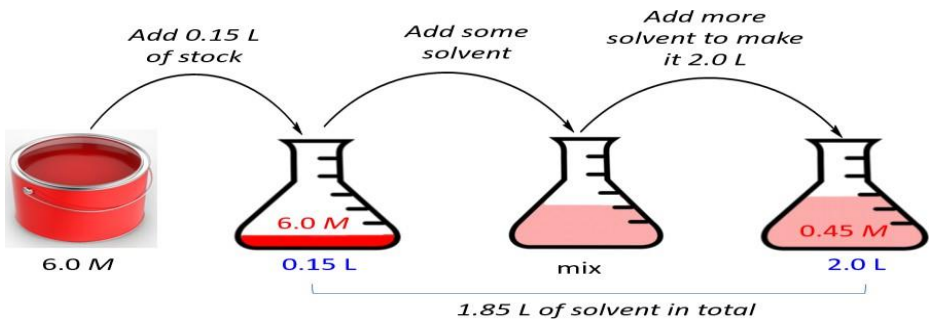


Figure 4. Method for preparing solutions by dilution

3. General information on the states of matter and separation methods

In nature, we generally find mixtures. However, chemists are interested in the properties of pure substances. They must therefore be able to separate mixtures into their constituent pure substances, i.e. sort the particles.

So, before discussing separation methods, we need to familiarise ourselves with matter, the forms it takes in nature and the different types of mixtures.

3.1. Matter

The entire universe around us is made up of matter. Matter is made up of different substances. Each substance has a mass and occupies a volume. A pure substance is a substance that consists of only one type of substance. It has characteristic, well-defined and unchanging properties.



Figure 5. Examples of materials and pure substances

3.2. Mixtures

So far, we have only considered pure substances. However, most substances found on Earth are not pure substances. For example, sea water, which covers 70% of the Earth's surface, tastes salty. It is therefore composed of at least two pure substances: water and salt. Such a combination of different pure substances is called a mixture.

3.2.1. Definition

A mixture consists of at least two pure substances. It therefore consists of at least two different types of particles. There are different types of mixtures.

3.2.2. Heterogeneous mixtures

For some mixtures, it is possible to see with the naked eye that they are mixtures. A heterogeneous mixture is a mixture in which the components can be distinguished with the naked eye. Heterogeneous mixtures have specific names that depend on the states of the constituents.

Table 1: Example of mixtures

mixture	liquid - liquid	liquid - gas	solid - gas	solid - solid
designation	emulsion	mist foam	smoke foam	/

3.2.3. Homogeneous mixtures

A homogeneous mixture is a mixture in which the components cannot be distinguished with the naked eye. Like heterogeneous mixtures, homogeneous mixtures have specific names that depend on the states of their constituents.

Table 2: Different types of homogeneous mixtures

mixture	solid - solid	solid - liquid	liquid - liquid	liquid - gas	solid – gas
designation	mixed crystals, alloys	Solution	Solution	Solution	/

3.3. Methods for separating mixtures

3.3.1. Definition

In chemistry, a separation process is a technique or technology used to transform a mixture of substances into two or more distinct components that can then be analyzed. Separation processes are based on the different physical properties of the components in the mixture, and

the choice of process depends on the mixture in question. The objectives of these operations may vary, including:

- Purification of impurities (e.g., manufacture of a drug)
- Concentration of a solution (e.g., removal of some of the water in a perfume)
- Fractionation of the mixture (e.g., distillation of fruit to obtain alcohol).

3.3.2 Principle of separation

To separate the different components of a mixture, we use the differences in physical and chemical properties between the compound of interest and the rest of the mixture. The greater the difference in properties, the easier the separation will be. Chemical compounds can be separated based on:

- Differences in melting points,
- Boiling points,
- Solubility in a carefully chosen solvent,
- Masses or even densities.

This separation is referred to as physical separation, as it is based on physical properties. Compounds can also be separated based on their different chemical affinities with a reagent (see chromatography). This type of separation is referred to as chemical separation.

3.4. Separation by extraction

Extraction is a separation process that involves extracting a chemical species, i.e., removing one or more chemical species from a solid or liquid mixture.

- **General principle**

An extraction medium is used to selectively extract one or more compounds from an initial mixture, based on chemical or physical properties. The extraction medium is not or only slightly miscible with the main components of the initial mixture, and the compound to be extracted has a greater affinity with the extraction medium than with the main components of the initial mixture.

3.4.1. Liquid-liquid extraction

This technique is used to extract a substance dissolved in a solvent (feed phase) using another solvent, called the extraction solvent phase, in which it is more soluble. The initial solvent and the extraction solvent must not be miscible. To perform liquid-liquid extraction in the laboratory, a separatory funnel or a continuous extractor such as the Kutscher-Strudel extractor can be used.

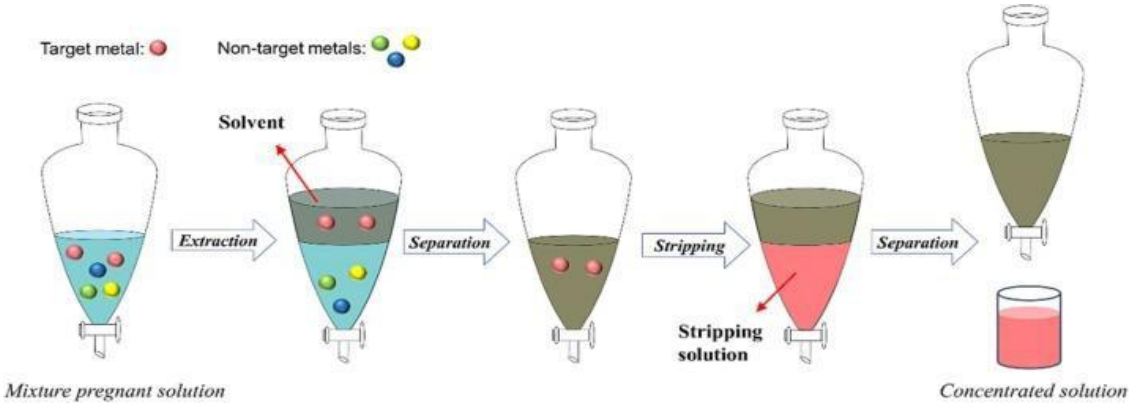


Figure 6. Liquid-liquid extraction protocol using a separatory funnel.

3.4.1.1. Properties of extraction solvents

Extraction solvents must:

- Dissolve the product to be extracted as effectively as possible.
- Be easily removed after extraction and therefore have a low boiling point. Their boiling point must be as far as possible from that of the products to be extracted. This is to avoid separation problems.
- Be chemically inert with respect to the solution to be extracted.
- Be as non-toxic as possible.
- They must not be miscible with the solvent to be extracted. They must not form a single phase.

3.4.1.2. How to improve extraction in practice

In practice, the partition coefficient of the compound to be extracted is rarely known in the solvents used. It is advisable to perform extractions with an amount of organic solvent equal

to that of the aqueous phase. To determine the number of extractions to be performed, monitoring on a thin-layer chromatography (TLC) plate is the simplest way to check that there is no more product in the aqueous phase.

The partition coefficient of the product can be modified by adding a small amount of brine (saturated aqueous NaCl solution) to the aqueous phase. This phenomenon is called “salting-out” .

3.4.1.3. Problems encountered

1. Emulsions

During separation, the interface may not be clear, or the entire mixture may form a single phase, resulting in a stable emulsion. This can happen with a lot of solvent, but it is more common when using solvents such as dichloromethane (CH2Cl2) or chloroform (CHCl3) or in the presence of surfactants. There are various tricks for breaking an emulsion.

• Wait; if there is no change after a few minutes, change the method.

• Try filtering the emulsion through glass wool or filter paper; this usually breaks the emulsion.

• The same result can be achieved with chemical agents such as the addition of an inorganic salt (usually sodium chloride (NaCl)), by modifying the relative density of the two phases.

• Centrifugation can also help to break down an emulsion that is too large or too stable.

• Ultrasound or the addition of a few drops of polar solvents (methanol, ethanol, acetone) help to restore the balance between the two phases.

2. Interface products

Interface products (filamentous, globular, etc.) can be removed by simply rotating the separating funnel. Otherwise, they are regularly removed from the organic phase and only eliminated during the final extraction in the aqueous phase.

3.4.2. Solid-liquid extraction

3.4.2.1. Principle

Solid-liquid extraction involves transferring a substance from a solid to a solvent in which it is soluble and from which it can be easily isolated. Applications for this unit operation include, for example, obtaining oil from oilseeds or washing minerals.

Note: The mass yield (R) of extraction corresponds to:

R= 100x (mass of extract/mass of solid material used)

Solid-liquid extraction is a slow process and, although batch extraction exists (maceration), continuous extraction processes are more commonly used. Pulverizing the solid increases the exchange surface area and therefore the transfer rate.

3.4.2.2. Batch extraction (maceration)

This involves maceration, which consists of soaking the solid in a solvent at room temperature, at a high temperature, or at boiling point to extract the solid components.

After filtration, the operation can be repeated on the residue with a new portion of solvent. This method is fast but not always very effective. Dissolution is always faster when the solid substance is dispersed in the solvent in divided form (crushed plants, powder, etc.) and can also be accelerated by maintaining agitation.

3.4.2.2.1. Advantages of maceration

- Maceration is a cold extraction process and therefore limits the release (and loss) of volatile chemical species into the air. It also prevents the alteration of fragile organic chemical species that can react and degrade at higher temperatures (through reaction with other species in the chemical environment, hydrolysis, oxidation on contact with air, etc.).
- Maceration does not require a heating device, so it is simpler and less expensive.

3.4.2.2.2. Disadvantages of maceration

- Maceration is often a lengthy process (several hours or even several days).
- Due to the time it takes, there is a risk of bacterial proliferation.
- Solubility is lower at low temperatures.

3.4.2.3. Continuous extraction

Continuous extraction is a much longer method than batch extraction, but it is more efficient.

• **Percolation:** this involves slowly passing a solvent through a layer of finely pulverized substance, usually contained in a thick porous paper cartridge or filter paper pouch. To ensure that the solvent remains in contact with the sample for a sufficiently long time, a Soxhlet extractor is used, for example.

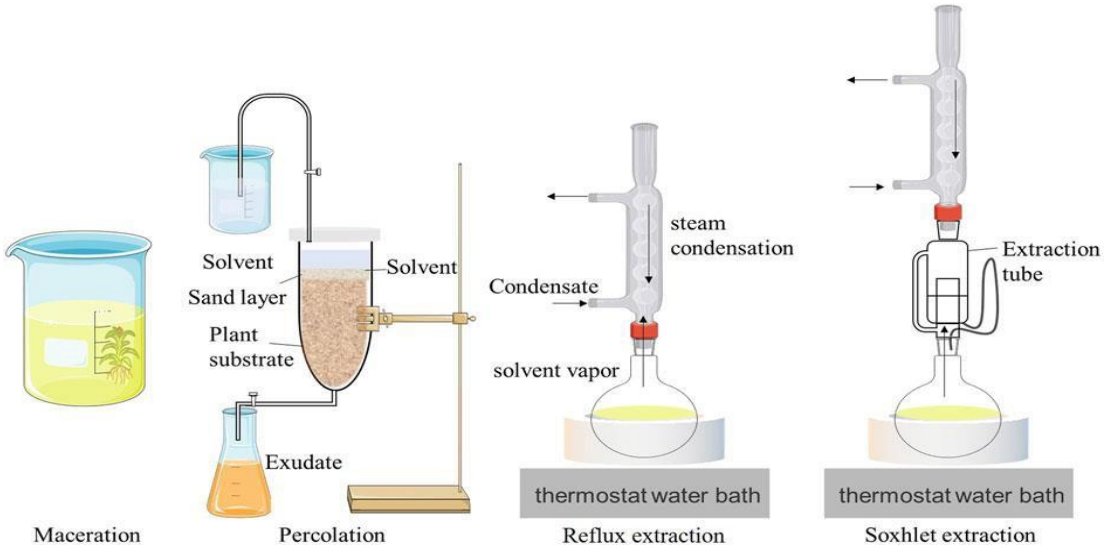


Figure 7. Solid-liquid extraction systems

• **Steam distillation and hydrodistillation:** these are two techniques based on the distillation of a heterogeneous water-organic compound mixture. They are used to isolate essential oils from plants or an organic compound located in a heterogeneous medium.

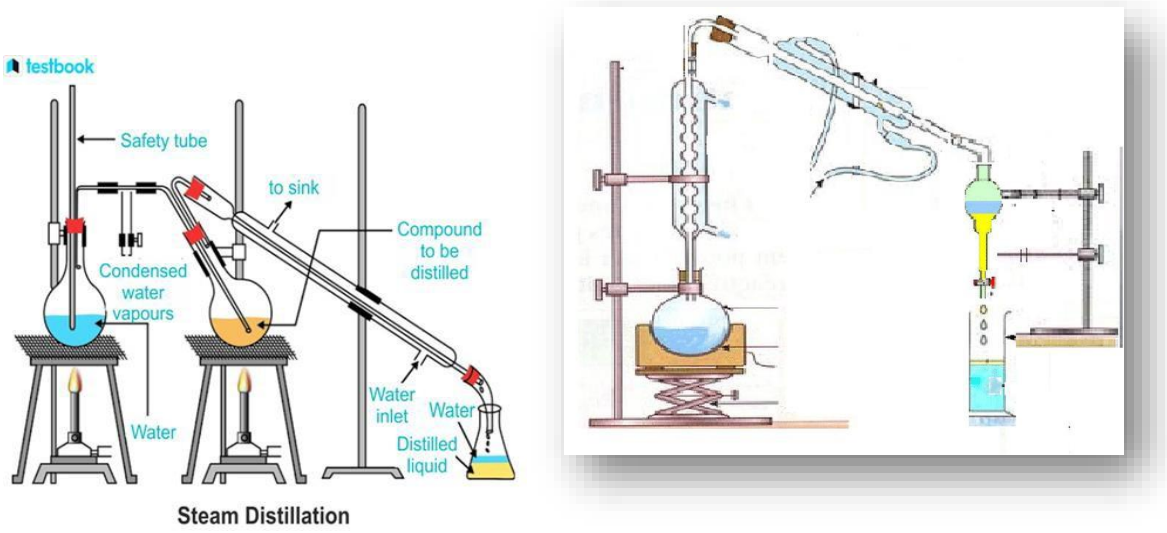


Figure 8. Steam distillation and hydrodistillation systems

3.4.2.4. Recovery of the solute

Two steps are essential for recovering the solute:

1. First, the organic phase must be washed to return it to a neutral environment.

2. Second, the solute obtained must be dried. It can then be concentrated by evaporating the extraction solvent.

3.4.2.4.1. Washing the organic phase

Washing the organic phase neutralizes the extraction solution.

• If the pH is acidic, washing with a solution of sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), or diluted soda (NaOH) is necessary.

• If the pH is basic, wash with a diluted hydrochloric or sulfuric acid solution (HCl or H2SO4).

3.4.2.4.2. Drying the organic phase

It is necessary to remove the water remaining diluted in the organic phase and therefore to dry it. This is generally done using minerals with a strong affinity for water. The action of these dehydrating agents often results from the formation of various hydrates by capturing water molecules in the solvent. The dehydrating agent must have the following qualities:

• It must not react chemically with the organic compound(s) dissolved in the solvent.

• The dehydrating action must be rapid and effective.

• Its solubility in the organic solvent must be negligible.

• It must be inexpensive.

• It must not have a catalytic effect by promoting reactions such as polymerization, condensation, or oxidation.

3.4.2.4.3. Evaporation of the extraction solvent

The evaporation operation is generally carried out using a rotary evaporator, but the solvent can also be concentrated in a conventional distillation setup after first drying the extraction

phase (organic phase) on a desiccant (unless the product is unstable at high temperatures and in the presence of oxygen).

3.5. Separation of the components of a heterogeneous mixture

Decanting, centrifugation, and filtration are techniques used to separate heterogeneous mixtures. They enable solid particles to be separated from the liquid with which they are mixed. This is achieved by allowing the mixture to settle.

3.5.1. Filtration

Filtration is the separation of particles suspended in a fluid, by pressure difference thru a porous medium or a suitable membrane (filter). The use of a filter allows for the retention of particles from the heterogeneous mixture (residue) that are larger than the holes in the filter (porosity). At the end of the operation, the solid particles remain on the filter and a homogeneous mixture is recovered (filtrate). The process is very simple to implement and does not require any phase change or chemical process.

3.5.1.1. Principle of filtration

Filter paper has a multitude of tiny pores. Liquid particles, which are able to move relative to each other, can slip through these pores. Solid particles, which are linked together in a network, are too large to pass through the pores of the filter paper.

The filter paper therefore acts like a sieve: individual particles that are free to move independently can pass through, while clusters of particles are retained. The following figure shows the correct steps for explaining how to use filter paper.

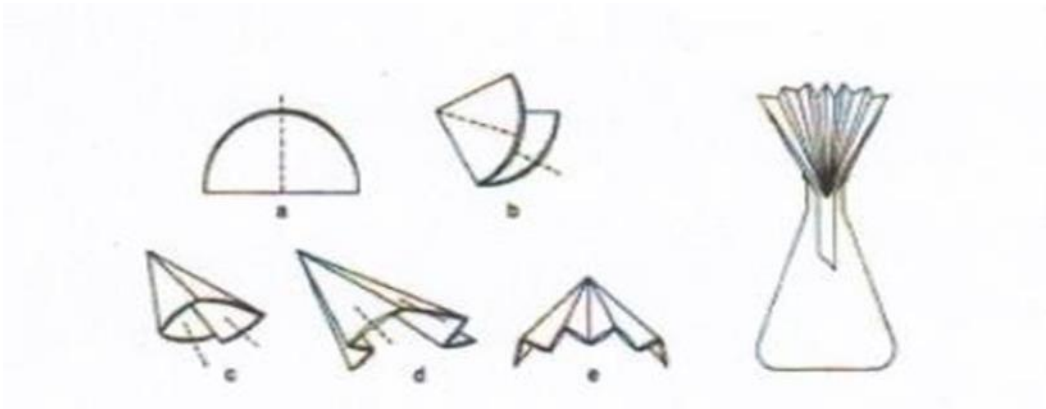


Figure 9. Preparation of a pleated filter and installation in the funnel

3.5.1.2. The objectives of filtration

The objectives of filtration are:

• Recovery of a solid, e.g., precipitation of a solid in water

• Purification of a liquid, e.g., wastewater

• Recovery and purification, e.g., recrystallization of a solid in a solvent

3.5.1.3. Filter materials

These include:

• Filter fabrics: natural textiles (cotton), metal meshes (iron, stainless steel), plastic fabrics (nylon, PVC)

• Powdered filter media: sand, charcoal

• Porous materials: porcelain, silica

• Cellulose-based products:

- Filter paper

- Cellulose pulp

- organic polymers: filter membranes: polymer discs with precisely defined porosity.

3.5.1.4. Disadvantages of filtration

• Sensitive to alkaline solutions.

• Adsorbent properties.

3.5.1.5. Filtration techniques

- Gravity filtration: the mixture is subjected only to atmospheric pressure. The liquid passes through the filter medium, which may be sand, for example, while the solid is collected on the filter medium.

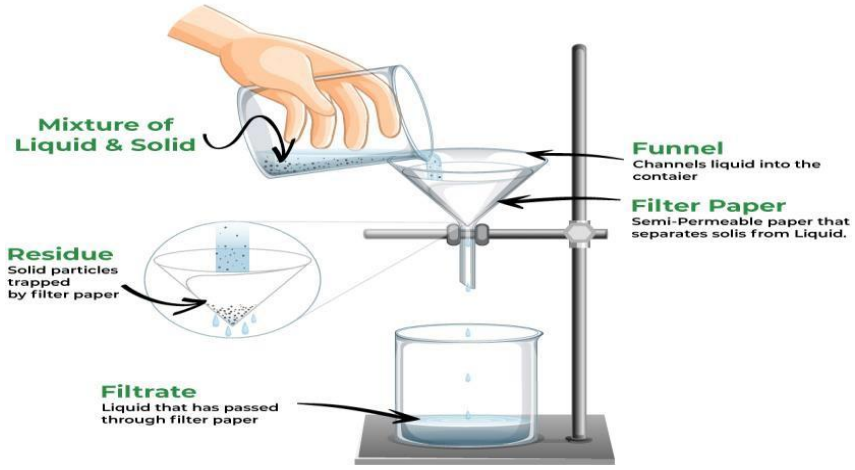


Figure 10. Simple filtration setup

- Overpressure filtration: the suspension enters the filter under pressure.
- Reduced pressure filtration: the mixture is subjected to atmospheric pressure on one side of the filter and, on the other side, where the filtrate exits, to a vacuum created by a vacuum pump .

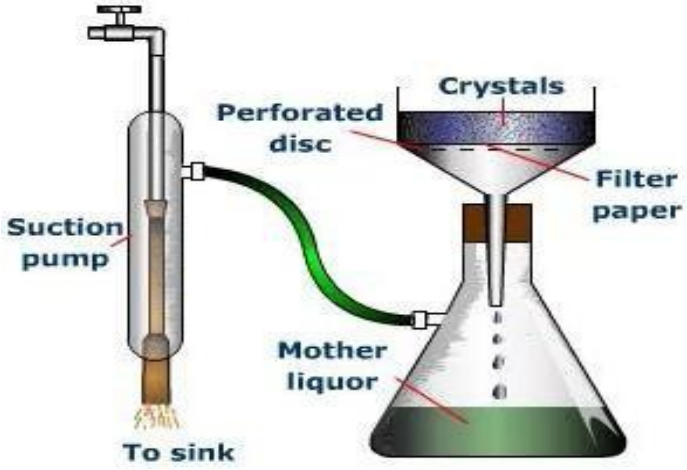


Figure 11. Vacuum filtration setup

The pressure difference between the upstream and downstream sides is very important because it regulates the filtration speed. Two types of filtration can be designed:

• Constant pressure filtration: the upstream-downstream pressure difference is regulated to a constant value. As the thickness of the cake increases over time, the filtration speed will decrease.

• Constant flow filtration: the upstream-downstream pressure difference is increased over time to maintain a constant flow rate despite the increase in pressure drop.

3.5.1.6. Applications

• Chemistry (e.g., gravimetry)

• Pharmacy (e.g., preparation of sterile solutions, analytical samples, pre-filtration of loaded solutions, or preparation of particularly pure and functional complex molecules)

• Biotechnology (preparation of culture media, purification and extraction of genetic and cellular material, etc.)

• Agri-food industry (stabilization, clarification, extraction, or concentration of products)

• Water treatment (drinking water, purified water, sterile water, wastewater)

3.5.2. Decantation

This is a mechanical process used to separate

1. Either a solid phase of suspended matter in a liquid with a lower density (solid-liquid)

2. Or two immiscible liquid phases with different densities (liquid-liquid) and different polarities

3.5.2.1. Solid-liquid separation (sedimentation)

In physics and chemistry, sedimentation (settling) is one of the processes used to separate mixtures. It involves allowing particles suspended in a liquid to settle so that they can be separated. This principle is used in some water treatment plants (settling tanks).

3.5.2.1.1. Principle of sedimentation

During sedimentation (Figure II.6) of solid particles of mass m, radius r, and density ρS in a fluid medium of density ρL and viscosity coefficient η, which is initially at rest, the particles are subjected to the action of three forces.



Figure 12. Diagram of the principle of sedimentation

The sedimentation velocity increases with the particle-fluid density difference ρS-ρL, the acceleration due to gravity g (or centrifugal acceleration rω2), and the particle diameter (its square in laminar flow). The sedimentation velocity decreases when the viscosity of the medium increases or the particle diameter decreases. Particles with a larger diameter sediment more quickly than particles with a small diameter, which gives rise to a classification method, but also leads to a cut-off threshold for a decanter.

The key factors in sedimentation are the difference in density between the solid and the liquid, the size of the particles, and the viscosity of the fluid. For particles measuring a few microns, the sedimentation rate becomes too low. Flocculating agents are then used to agglomerate the particles together and thus increase their limiting fall velocity.

3.5.2.2. Liquid-liquid decantation (two immiscible liquid phases)

Decantation is a process that separates immiscible liquids with different densities. The mixture to be separated is placed in a separating funnel. In the funnel, one liquid floats on top of the other, and the lower liquid can be collected by opening the tap.

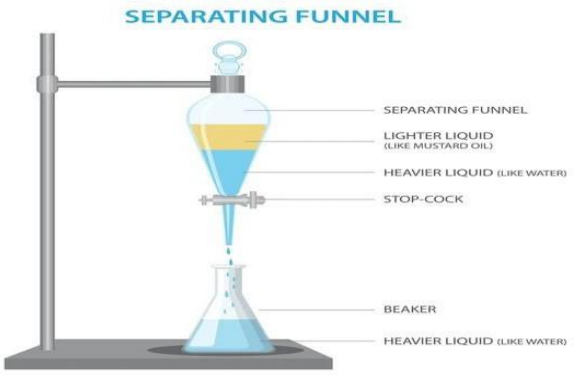


Figure 13. Settling of an emulsion

3.5.3. Centrifugation

Centrifugation is accelerated sedimentation in which g (the acceleration due to gravity) is replaced by centrifugal acceleration. Centrifugation is a mechanical separation process that uses centrifugal force to separate two or three phases in a rotating motion. It can be used to separate

• two liquid phases.

• A solid phase suspended in a liquid phase.

• Two liquid phases containing a solid phase.

• In biochemistry, it is used to separate proteins in solution that have the ability to sediment in a high centrifugal field.

3.5.3.1. Principle of centrifugation

The separation of compounds in a mixture can be achieved by decantation, under the action of gravity alone, but this sometimes takes a long time to achieve good results and is therefore often ineffective. It is therefore more efficient to use centrifugation during this separation process.

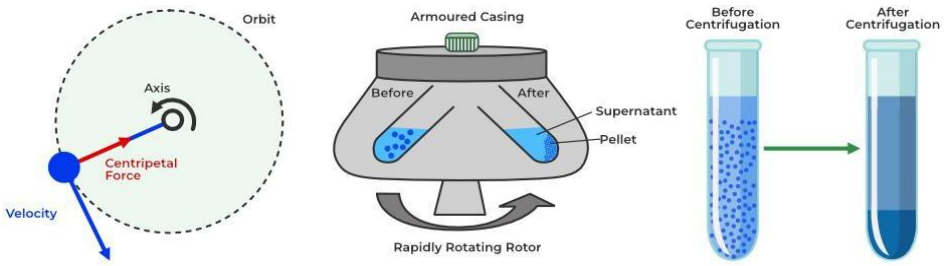
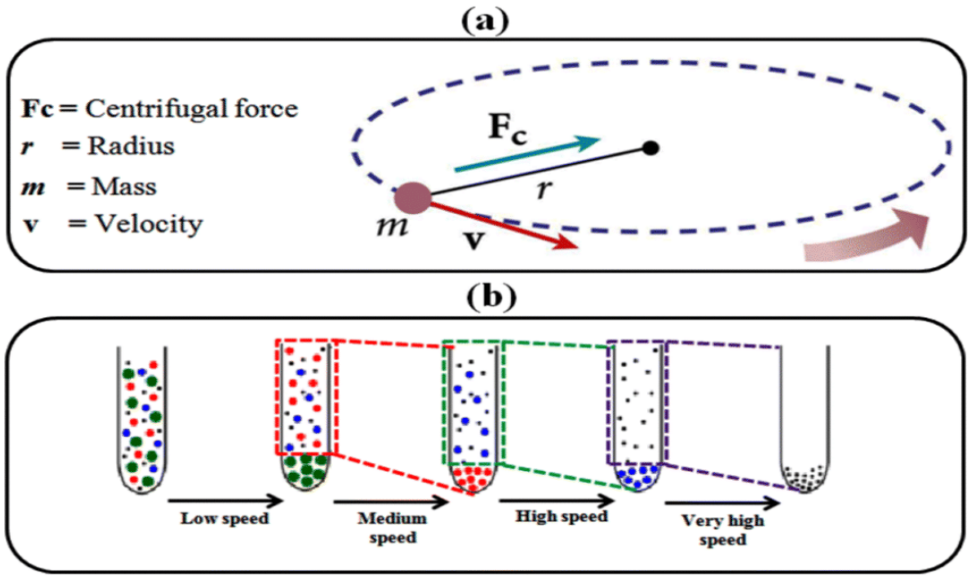


Figure 14. Diagram of the principle of centrifugation

3.5.3.2. Centrifugation equations

The compounds in the fluid located at a distance r from the axis of rotation are subjected to different forces.



Separation occurs through the action of centrifugal force Fc on the compounds. This centrifugal force, expressed in newtons, is given by the equation $Fc = m\gamma c$, where $\gamma c = r\omega^2$ in m/s^2, where:

• m is the mass of the compound to be separated

• r is the distance from the tube to the axis of rotation of the centrifuge

• The angular velocity ω expressed in radians per second or revolutions per minute.

The values used in centrifugation are approximately 400 to 10,000 g, which corresponds to rotation speeds of around 2,000 to 10,000 rpm depending on the radius of the rotors.

Centrifugation uses the centrifugal force exerted on the particles included in the solution to segregate certain components. This separation is based on the density of the particles.

3.5.3.3. Equipment

Several types of centrifuges:

• Analytical centrifuges: observation of the movement of molecules to obtain information on sedimentation constants, molecular forms and mass, size, and composition.

• Preparative centrifuges: These exploit the enrichment phenomena of the > or < zones of the tube to separate the different components of a mixture.

• Analytical and/or preparative centrifuges. All centrifuges include a rotation chamber and peripheral elements

Table 3: Different models of centrifuges

Types of centrifuges	Acceleration S	Capacity	Refrigeration system	Remarks
Tabletop centrifuges –main	1000-3000g	Small volumes 10 ml	±	-Simple model -Manual cranks
Floor centrifuges	20 000g	Large volumes 4 or 6 bottles of 250 ml	+	
Ultracentrifuges	300 000g	Dozens of 40-tube	+	Very precise analyzes
Analytical ultracentrifuges				Analyzes the size and mass of particles
Micro centrifuges	12-15 000g	Micro-volumes Micro-tubes of 1.5 ml	±	Modern Biochemistry

3.5.3.4. Types of centrifugation

3.5.3.4.1.Density gradient centrifugation

In this method, macromolecules sediment within a density gradient. However, at equilibrium, they stabilize in the gradient zone where the density of the particles is equal to the density of the solvent, which means that the gravitational force is equal to the Archimedes' buoyant force.

We will therefore use a solvent whose density will vary according to the position in the tube, allowing the different constituents to reach the zone of equivalent density to theirs: this is called a gradient. We can enhance or refine the separation methods by performing this centrifugation in a concentration gradient.

3.5.3.4.2. Differential centrifugation

The principle is to separate the different components, most often using several cycles of centrifugation with increasing acceleration. In a first centrifugation at low acceleration, the most massive elements will sediment and form a pellet at the bottom of the tube. All the other elements will remain in the supernatant. We then separately recover the supernatant and the pellet, which is equivalent to having separated the constituents that make them up. This method is, for example, commonly used to recover the formed elements (the cells) of the blood that sediment at very low accelerations (a few tens of g).

If necessary, a second centrifugation cycle can be repeated with the previous supernatant, but with a higher acceleration. Gradually, we thus separate the different constituents, ending with the smallest elements that have the least difference in density with the solvent.

3.5.3.5. Applications of centrifugation

They apply equally well to homogeneous mixtures as to heterogeneous mixtures.

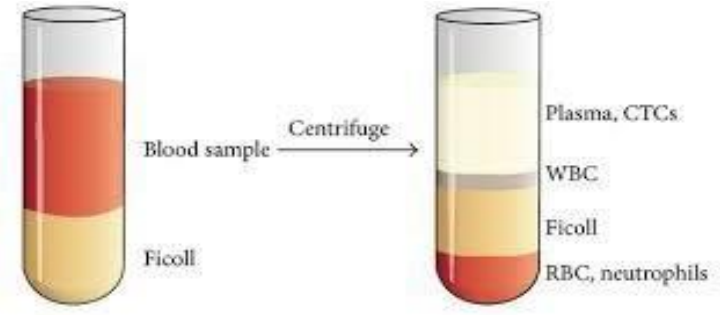
- **Preparative centrifugation:** Separations of the constituents of chemical or biological mixtures
- **Analytical centrifugation:** Study of the centrifugation of macromolecules, it is the determination of the physical constants of macromolecules:

• Measurement of the sedimentation constant Measurement of diffusion and translation coefficients

• Determination of molecular masses

• Determination of shape and dimensions

• Determination of particle density



3.5.4. Crystallization

3.5.4.1 Definition

Crystallization is a separation or transformation process that involves transitioning a solute dissolved in a solvent into an organized solid phase called a crystal.

It is involved in numerous food processing methods, notably to:

• purify a product (e.g., sugar),

• stabilize an ingredient (e.g., lactose),

• control the texture of a product (e.g., ice cream, chocolates).

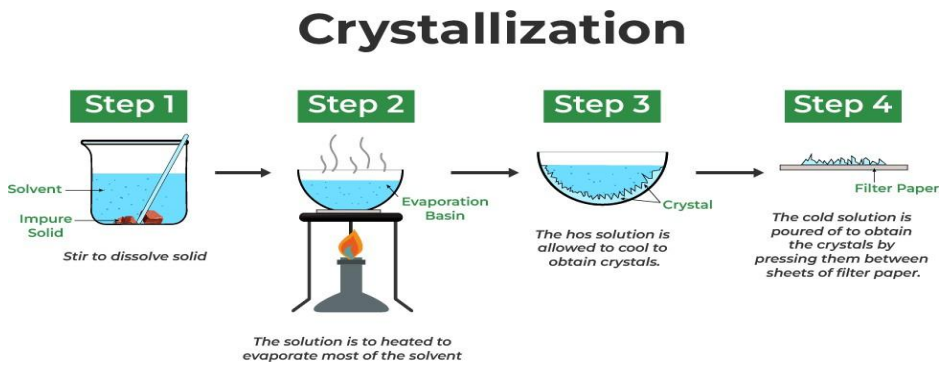


Figure15. Crystallization process

3.5.4.2. Interest in the food industry

- ✓ Allows for selective solid/liquid separation.
- ✓ Plays on texture, preservation, and microbiological quality.
- ✓ Contributes to the optimization of yield and quality in industrial production

3.5.4.3. Physicochemical principles of crystallization

3.5.4.3.1. Solubility and supersaturation

• Solubility is the maximum amount of a solute that can dissolve in a solvent at a given temperature.

• A solution becomes supersaturated when it contains more solute than the solubility allows → necessary condition for crystallization.

3.5.4.3.2 Nucleation

• Primary nucleation: spontaneous appearance of small crystals (nuclei) in the solution.

• Secondary nucleation: crystals form from contacts with other existing crystals (e.g., seeds).

3.5.4.3.3 Crystal growth

• Process by which solute molecules come to add to a nucleus.

• The shape of the crystal depends on the chemical nature, temperature, and impurities.

3.5.4.3.4 Phase diagrams

• Represent the solubility as a function of temperature.

• Example: sugar becomes less soluble when cooled → crystallization is favored

3.5.4.4. Crystallization methods used in the food industry

3.5.4.4.1 By cooling

• Very widely used (e.g., sugar, lactose).

• We cool a saturated solution → supersaturation → crystallization.

3.5.4.4.2. By evaporation

• Progressive removal of the solvent (e.g., water), typical in sugar refining.

• Allows for reaching a critical concentration.

3.5.4.4.3 With anti-solvent

• Addition of a liquid in which the solute is poorly soluble.

• Not widely used in the food industry because it is more complex and costly.

3.5.4.5. Parameters to control in industry

Parameter	Effect on crystals
Temperature	The slower the cooling, the larger the crystals
Agitation	Promotes the distribution of the solute but can break the crystals
Time	Determines the final growth.
Concentration	Influence the crystallization rate.

Impurities	Can alter the purity or shape of the crystal.

3.5.4.6. Equipment used

• Industrial crystallizers: batch or continuous, with integrated heat exchangers.

• Agitators: ensure homogeneity and prevent sedimentation.

• Temperature and concentration sensors: to continuously monitor crystallization.

• Filters / centrifuges: to separate the crystals from the rest of the solution.

3.5.4.7. Deficiencies and hazards associated with crystallization

Problem	Consequence
Too much nucleation	Small crystals → poor yield or difficult filtration
Crystals too large	Risks of blockage, poor solubility
Impurities	included Deterioration of taste, color, texture
Poor thermal control	Irregular crystals or no crystallization

3.6. Heat treatments

3.6.1. Sterilization

3.6.1.1. The history of sterilization

The history of sterilization is linked to that of surgery. Awareness of hygiene then became a priority, and the principle of sterilization only improved: an integral part of the operating room until 1960, it tended to become formalized with the drafting of best practices and regulatory and legislative texts, as well as to become structured to become a fully outsourced activity. In 1885, Dr. GASTON POUPINEL invented the first hot air sterilizer called an oven. The sterilization processes were invented by LOUIS PASTEUR.

3.6.1.2. Historical landmarks

1795 Nicolas APPERT: Sterilization of canned goods.

1851 Victor REGNAULT: Knowledge and mastery of steam.

1857 Louis PASTEUR: Demonstration of the absence of spontaneous generation. Development of modern microbiology

1879 CHAMBERLAND: Use of the first autoclave.

1885 POUPINEL: Implementation of the first hot air sterilizer in a hospital setting.

1888 TERRILLON AND TERRIER: Installation of the first autoclave in a hospital setting.

1949 PHILIPS and KAYE: Use of the sterilizing action of ethylene oxide.

1973 S. LINE: Development of sterilization by formaldehyde.

1989 JOHNSON - JOHNSON: Development of sterilization using hydrogen peroxide plasma.

1993 CENTRAL PROCUREMENT COMMISSION: Publication of the Guide to Good Sterilization Practices.

2001 GOOD STERILIZATION PRACTICES

2002 QUALITY IN STERILIZATION And 2003 Imposed on healthcare facilities

3.6.1.3. Sterilization

Sterilization is the implementation of a set of methods and means aimed at eliminating all living microorganisms carried by a perfectly cleaned object through destruction, regardless of their nature and forms. The sole performance of microbiological tests does not guarantee the sterility of a product. Ensuring sterility also involves the application of properly validated procedures as well as washing/disinfection processes. When drafting recommendations for sterilization techniques, the following aspects must be taken into consideration:

• Employment of specifically trained personnel;

• Use of appropriate premises;

• Use of appropriate equipment;

• Use of materials designed to be cleaned, disinfected, conditioned, and sterilized;

• Compliance with the necessary precautions to reduce the microbial load (bioburden) before sterilization;

• Implementation of validated methods at all critical stages of production;

• Environmental monitoring and process controls;

• Use of good storage and preservation conditions;

• Introduction of a quality assurance system.

By definition, sterilization processes are all capable of destroying all varieties of microorganisms and achieving a sterile state. Disinfection processes are effective on certain microorganisms, according to their spectrum of activity defined by standards. Some sterilization processes, and some disinfection processes are capable of inactivating prions.

3.6.1.4. Definition of sterility

"Sterility is the absence of viable microorganisms"

Sterilization is a physical or chemical process aimed at destroying all forms of microbial life, including bacterial spores, which are the most resistant forms.

sterility is actually a notion of probability. We will see that the absolute absence of microorganisms and other agents does not exist, but that practical sterilization conditions leave such a small number of infectious agents that they cannot develop and regain their pathogenic power.

3.6.1.5. Objectives of sterilization in the food industry

• Prevention of health risks (botulism, listeriosis, salmonellosis)
• Increase in shelf life without cold chain
• Maintenance of microbiological stability during storage
• Improvement of the hygienic quality of products

3.6.1.6. Sterilization methods

1. Thermal sterilization (the most common in the food industry)

a. Autoclave (steam under pressure)

• Temperature: 121°C (often used)
• Pressure: 1.1 bar above atmospheric pressure
• Advantages: reliable method, effective penetration
• Limitations: possible alteration of texture and nutrients

b. UHT Treatment (Ultra High Temperature)

• Temperature: 135 to 150°C
• Time: 2 to 5 seconds
• Products: milk, creams, fruit juices

• Aseptic packaging post-sterilization

• Advantages: long shelf life without refrigeration, minimal degradation

c. Steam or Superheated Water Treatment

• Used for canned goods or bagged products

• Applied in a rotary autoclave to homogenize the heat

2. Chemical sterilization

a. Chemical sterilizing agents

• Ethylene oxide, hydrogen peroxide, peracetic acid

• Used for heat-sensitive materials (packaging, equipment)

• Requires rinsing or residue removal

b. Advantages / disadvantages

• Little thermal impact, but potentially toxic

• Strict regulations on residues

3. Sterilization by radiation

a. Ionizing radiation

• Gamma rays (Co-60): high penetration

• X-rays, accelerated electrons: more controlled dose

• Used for spices, dry foods, medical products

b. Advantages

• No temperature increase

• Effective against all types of germs

c. Disadvantages

• High cost, limited consumer acceptability

• Potential effects on certain molecules (degradation, formation of secondary compounds)

4. Sterilization by filtration (for heat-sensitive liquids)

• Sterilizing filtration (≤ 0.22 μm)

• Used for: enzymatic solutions, vitamins, culture media

• Not applicable to particulate or viscous products



3.6.1.7. Factors influencing the effectiveness of sterilization

• Nature of the product: pH, Aw, viscosity, presence of fats

• Present contaminants: initial flora, spores

• Configuration of the treatment system: heat homogeneity, container geometry

• Thermal barrier: packaging materials, type of closure

3.6.1.8. Sterilization Control

1. Biological indicators

• Geobacillus stearothermophilus spore: very heat-resistant

• Placed in test devices (ampoules or strips) and analyzed after treatment

2. Chemical indicators

• Color changes (temperature, pressure indicators)

3. Validation and qualification

• Validation of the cycle (temperature, time)

• Thermal mapping of autoclaves

• Regular monitoring by quality

3.6.2. Pasteurization

Pasteurization is a heat treatment at temperatures between 60 and 100°C aimed at destroying all non-sporulating pathogenic microorganisms and significantly reducing the vegetative flora present in a product, without significantly altering its nutritional and organoleptic qualities. It is a limited preservation process for which the product must be hermetically packaged (with or without a modified atmosphere or under vacuum) and refrigerated (the pasteurized product

can indeed be stored at +4°C for a few days to a few weeks). It takes its name from Louis Pasteur, who demonstrated in the 19th century that heat could inactivate the microorganisms responsible for food spoilage.

Pasteurization is a technique very frequently used in the food industry. The objective is to significantly extend the shelf life of food. Pasteurization significantly reduces the biological activities of a product while avoiding changes to its organoleptic and nutritional characteristics, thus ensuring enzymatic stabilization (partial or total inactivation of enzymes). The biological activities destroyed or inactivated by pasteurization are:

•Non-pathogenic spoilage flora of food

• Pathogenic and toxigenic flora (salmonella, brucella, listeria, etc.)

•Endogenous enzymes such as soybean lipoxygenase (oxygenase that catalyzes the oxygenation of polyunsaturated fatty acids) or plasmin present in milk (protease with a fairly broad spectrum of action)

• Harmful intracellular enzymes. Pasteurization, like any thermal treatment, must allow:

- ✓ To preserve the nutritional aspect of the product such as the non-destruction of vitamins.
- ✓ To not alter its organoleptic qualities such as the absence of browning, discoloration, cooked tastes, emulsion breakdown, protein coagulation, etc.

3.6.2.1. The different types of pasteurization

We distinguish three types of treatment:

- Low pasteurization (62-65°C / 30 min): it can only be done in batches and is abandoned in dairies.
- High pasteurization (71-72°C /15-40 min) or HTST: it is reserved for milk of good hygienic quality. From an organoleptic and nutritional standpoint, high pasteurization has very little effect. At the biochemical level, alkaline phosphatase is destroyed; on the other hand, peroxidase remains active and the denaturation rates of serum proteins and vitamins are low. The expiration date (DLC) for milk that has undergone high

pasteurization is 7 days after packaging (glass or cardboard bottle, polyethylene or aluminum).

- Flash pasteurization (85-90°C at 1-2 s): it is performed on raw milk of average quality; phosphatase and peroxidase are destroyed.

3.6.2.2. Impacts on food

1 Advantages

• Maintenance of nutritional quality (vitamins, proteins).

• Preservation of sensory characteristics (color, texture, taste).

• Microbiological safety.

2 Disadvantages

• Partial inactivation: bacterial spores not destroyed.

• Slight loss of heat-sensitive vitamins (e.g., vitamin C).

• Need for cold chain after processing.

3.6.2.3. Industrial applications

1. Dairy products

• Pasteurized milk: widely used HTST treatment.

• Yogurts: pasteurization of milk before inoculation.

• Cream and fresh cheeses.

2. Juices and beverages

• Orange, apple, grape juice: pasteurization to eliminate yeasts/microorganisms.

3. Meat products

• Hams, pâtés: pasteurization after packaging (post-packaging heat treatment).

4. Other products

• Liquid eggs, sauces, compotes, ready-to-eat soups

Table4: Comparative table: Pasteurization vs Sterilization

Criteria	Pasteurization	Sterilization
Definition	Moderate heat treatment to destroy pathogenic and spoilage germs.	Intensive heat treatment to eliminate all microorganisms, including spores.
Temperature	60 °C to 100 °C	> 100 °C (generally 115 °C to 135 °C)
Duration	Short (a few seconds to 30 minutes)	Longer (up to several tens of minutes)
Microbiological target	Vegetative forms of bacteria, yeasts, and molds	Vegetative forms and bacterial spores
Effect on the microbial flora	Partial reduction (non-sterile	Total elimination (commercial sterility)
Need for cold chain	Yes (product sensitive to recontamination)	No (product stable at room temperature)
Shelf life	Short to medium (days to a few weeks)	Long (months to several years)
Impact on quality	Low: preservation of taste, vitamins, etc.	More importantly: possible alteration of nutritional and sensory qualities
Examples of products	Fresh pasteurized milk, fruit juices, yogurts, beers	Canned goods (prepared meals, vegetables), UHT milk, jars

Industrial applications	Fresh products, with cold chain	Long-lasting products, without refrigeration

3.6.3. Generalities on drying

The drying process is one of the oldest methods of preserving a wide range of foods. It is a thermal process that involves vaporizing a solvent to remove all or part of a liquid impregnating a body called wet. Even if it contains residual moisture, the final product is considered a dry solid. The goal is to remove water from food in order to inhibit microorganisms, stop enzymatic reactions, and preserves the biochemical and biological characteristics of these foods.

It also allows for a reduction in the weight and volume of the products to be transported, as well as for minimized storage costs. The principle of drying in the industrial field is based on two methods: entrainment drying or boiling drying. The drying process is defined as a bidirectional transfer of heat and mass.The most common drying method in the food industry is air entrainment at temperatures below that of emulation, where hot air gives the product its heat and receives the moisture that is ejected from it. The understanding of the phenomena acting on this process is mainly based on the air-product equilibrium; and the kinetics of drying and product transformation.

3.6.3.1. Drying methods

Traditional drying methods lead to complex preservation techniques. Arid or semi-arid zones are ideal places for sun drying, as the climatic conditions are very favorable: a dry season with strong sunlight, less rain, and low humidity. The drying is done in areas perfectly exposed to the sun on screens or even on flat rocky slabs, or on the roofs of houses. The results of this drying system are far from perfect because the products are soiled with sand and dust. Also vulnerable to being infected by insects and contaminated by microorganisms. The losses in terms of costs and quality will be significant. Moreover, the shelf life of these products is short and does not meet economic needs; they spoil, dry out too much or not enough, which significantly degrades their quality. It is important to know the experience and capabilities that have allowed us to adapt and circumvent environmental factors. The improvement of drying practices and equipment is always achievable in order to better meet the evolving

quality requirements of the products. There are different methods used according to the need or the aspect of the dried product:

1. **Mechanical drying**

It involves the removal of liquid by applying purely mechanical forces (pressing, centrifugation, decantation, and filtration)

2. **Chemical drying**

It is a process based on chemical treatment (osmotic solution) and the use of dehydrating agents (calcium chloride...) to extract water.

3. Thermal drying

Thermal drying involves dehumidifying the food by extracting some of its water through evaporation, which requires energy. This form of operation is a mass transfer that requires the activation of water by a particular amount of energy provided by a heat transfer. The two transfers manifest in an external phase and an internal phase:

- Internal heat transfer, from the heat source to the surface of the product.
- Internal heat transfer, from the surface to the core of the product.
- internal mass transfer, from the core to the surface of the product.
- External mass transfer from the product surface to the external environment.

3.6.3.2. Types of drying

The drying process uses many different methods. Among the most common methods are the following:

1. Hot air drying

Hot air drying is currently the most common drying method. It is simple and effective for solid industrial and food products and provides a cost-effective solution. However, this technique has the potential to completely dehydrate the surface of the product, leading to cracks or an uneven result. It can also be a slow procedure, depending on the product and the acceptable drying temperature.

2. Sun drying

Sun drying is the oldest solution adapted for drying traditional fruits and vegetables in remote areas, such as apricots and tomatoes, although it does not preserve all the properties and vitamins of the product. It is an economical technique, but this method requires a lot of space and time, and offers less control over the process.

3. Contact drying

This method involves passing the product through a heated rotating drum, and the drying process occurs through direct contact of the product with the heated conductive walls of the drum, and its rotation offers better homogeneity. It is a technique mainly intended for massive industrial drying. However, direct contact of the product with the hot walls can lead to denaturation of the dried product, and the homogeneity of the process is not guaranteed.



Figure16.Drying methods

4. Infrared drying

Infrared drying allows water or solvent to evaporate at high temperatures. It can also be combined with hot air and is used when the drying effect needs to be concentrated on the surface of the treated product to also achieve a roasting effect. This solution is far from ideal for products that should not be exposed to high temperatures.

5. Fluidized bed drying

This technique is based on mixing air with solid particles to obtain a mixture that behaves like a fluid. This method is very useful and quick for drying seeds or granular materials.

6. Dielectric drying

Microwave and radio frequency drying is based on dielectric drying technology. By simultaneously heating the entire volume of the product in a vacuum chamber with a high-frequency field that passes through it, a technique particularly recommended in applications where homogeneous and gentle drying is required. This solution is the most suitable for drying heat-sensitive products in the food, industrial, and medical fields, in order to preserve all their properties.

7. Lyophilization

Finally, the lyophilization process is based on the sublimation effect of water in a low-temperature environment (below -20°C and then brought to very low pressure, the ice will turn into vapor without passing through the liquid state). It is often recommended for drying temperature-sensitive food products because it preserves most of the organoleptic properties.



3.6.3.3. Consequences of drying on food

1. Advantages

- Long-term preservation without refrigeration
- Reduction of weight and volume

- Microbial inhibition (low water activity)
- Concentration of flavors in some cases (e.g., dried tomatoes)

2. Disadvantages

- Loss of certain heat-sensitive vitamins (vitamin C, B1)
- Hardening of the texture
- Possible non-enzymatic browning (Maillard reaction)
- Color modification (pigment oxidation)

3.6.4. Lyophilization

Lyophilization, formerly called cryodesiccation, is a cold drying process that removes water from a product. It is carried out in two stages: freezing the product and then sublimation. The latter is done by keeping the product under vacuum at a low temperature. The resulting product is called a lyophilizate. The term "lyophile" means "solvent-loving" in Greek; indeed, the lyophilizate, having a very porous structure, exhibits a great affinity for aqueous solvents. This structure most often allows it to dissolve or be penetrated by a solvent with great speed. To obtain the initial product again, a third rehydration step is necessary.

3.6.4.1. Lyophilization Technique

This operation is carried out in three phases:

1. Freezing the product between – 30°C and – 50°C;

2. Primary drying or sublimation: During this so-called sublimation stage, carried out under vacuum, the water contained in the product transitions from the solid state (ice) to the gaseous state (vapor). This operation takes place at a temperature above – 20°C.

3. Secondary desiccation or final drying: This step of the process is always carried out under vacuum at a temperature not exceeding +50°C. The final residual moisture content of the product is between 4 and 6%. The average duration of a freeze-drying cycle is between 60 and 72 hours.

3.6.4.2. Advantages

• Preservation of the quality of heat-sensitive foods

• Rapid rehydration due to the porous structure of the powders

• Very low residual moisture

3.6.4.3. Applications

• Biological products (proteins, enzymes, microorganisms...)

• Pharmaceutical and cosmetic active ingredients

• Food products

4.1. Fermentation

4.1.1. Definition

It is a natural phenomenon that occurs during the decomposition of organic matter. It is a biochemical reaction that involves releasing energy from an organic substrate under the action of microbial enzymes and discarding products. This reaction does not involve oxygen (O2), so it takes place in the absence of air (anaerobiosis).

Fermentation transforms the substrate by favorably altering its properties. The nutritional value can be improved by destroying toxic or indigestible substances, by the appearance of growth factors (vitamins, amino acids), or more generally by a favorable modification of the chemical composition. The organoleptic qualities can be modified by transformation or the appearance of tastes and odors in a favorable direction.Finally, the shelf life can be improved thru product stabilization by eliminating substances prone to the development of undesirable contaminants (due to the mass effect of the technological flora on the establishment of contaminants), or by producing substances with stabilizing or antimicrobial effects (acids, alcohols).

4.1.2. History of fermentation

the use of fermentation by humans began empirically. It was initially used to preserve food, prepare bread, alcoholic beverages...Studies show that the Sumerians (Lower Mesopotamia) were already using it 8000 years before Christ. However, it would take until the 18th century to discover the microorganisms involved .

In 1789, A. Lavoisier wrote the first article on fermentation. He describes "vinous fermentation" as a division of sugar into two portions (alcohol and carbon dioxide) following the reaction of a "ferment." Many scientists will then embark on research on fermentation and propose different hypotheses regarding its causes and triggers. In 1836, three scientists discovered that yeast is a living organism that reproduces by budding.

It was Pasteur, in 1857, who established that alcoholic fermentation is due to the metabolic activity of Saccharomyces cerevisiae (brewer's yeast). He would then study acetic, butyric, and lactic fermentations, and demonstrate that fermentation is a chemical and biological reaction by cultivating the bacteria and yeasts involved. It is also him who will give the first general theory of fermentations: "any fermentation of a sugar solution or organic matter

results from the metabolic activity of a specific microorganism, and is accompanied by the formation of characteristic products (alcohols, acids, ketones, and carbon dioxide)" .

Nowadays, fermentation is used in numerous industrial processes and is present in the diet of people all over the world. According to the AFNOR NFX 42-000 standard of December 1986: "biofermentations that use microorganisms," fermentation is defined as "the degradation of carbohydrate substrates without the use of oxygen."

More recently, and following the discoveries of the 21st century, fermentation is characterized as "the oxidation of organic compounds without the use of oxygen, thanks to characteristic enzymatic systems, with various organic compounds as electron acceptors, often without the ejection of electrons outside the cell."

4.1.3. Ferments

Microorganisms are often considered as pathogens. In food, they are often responsible for product spoilage (e.g., mold on fruits). However, these microorganisms (bacteria, yeasts, and molds) are useful and necessary for the production of certain foods and are classified into two types of flora:

- **The beneficial flora**

it is the positive flora that we will seek to promote. It will allow for the different fermentation processes. It can be found naturally in the food, or added during the manufacturing processes.

- The undesirable flora: it is the negative flora, which we seek to inhibit. It is also divided into two categories:

  - **The spoilage flora**

These microorganisms can grow and cause physical and chemical changes in the food. This leads to unpleasant odors and flavors, mucus formation, gas accumulation, and liquid release. There is then a degradation of the gustatory and/or esthetic quality.

  - **Pathogenic flora**

These microorganisms can be dangerous for human consumption, even at low concentrations. In case of ingestion, the food can be a source of food poisoning.

The challenge in the food industry is therefore to select the beneficial flora and eliminate the undesirable flora. Moreover, microorganisms can already be present in food, or they can be added. In this case, the microorganisms that can be used in the food industry must have the following common characteristics:

- ✓ not posing a risk to human and animal health
- ✓ not posing a risk to the environment
- ✓ having rapid development
- ✓ being easily usable and controllable.

4.1.4. Main food fermentations

4.1.4.1. Alcoholic fermentation

The catabolism of simple sugars in anaerobiosis with the final production of ethanol and carbon dioxide constitutes alcoholic fermentation. Alcoholic fermentation is very common among yeasts, Saccharomyces, Kluveromyces, Brettanomyces. Few bacteria (Zymomonas mobilis) carry out this fermentation. The yeast strains ensuring alcoholic fermentation tolerate

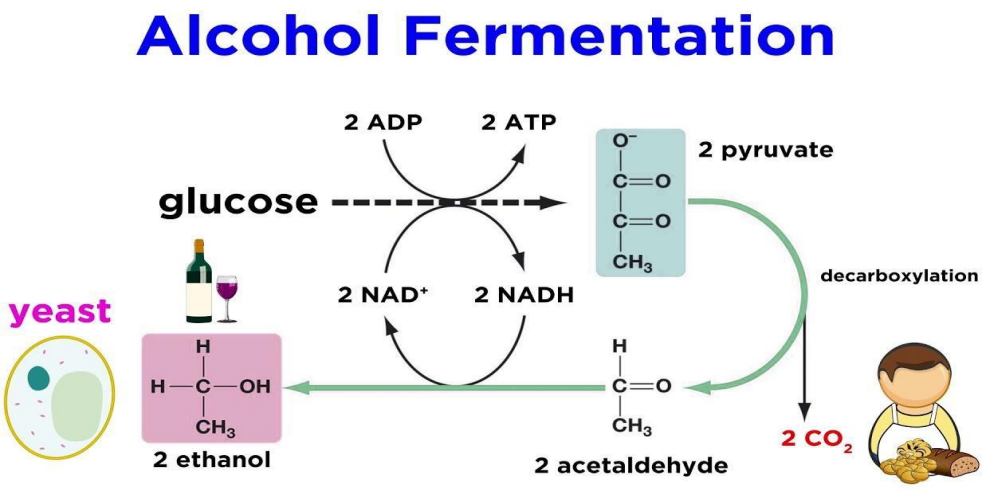


Figure17. Alcoholic fermentation

4.1.4.2. Lactic fermentation

It is caused by numerous bacteria (rarely by molds). Lactic fermentation is an essential step in the production of cheeses and yogurts, but also of many fermented vegetable products (silage, sauerkraut, olives, pickles…) and charcuterie (sausage, ham). Lactic fermentations, in

addition to their organoleptic role (acidification, aromatic by-products), play a significant role in stabilization (through pH reduction and antibiosis phenomena) and, above all, a major role in food quality, as lactic ferments are a source of growth factors.

Lactic acid is industrially prepared to be used as a food additive. Depending on the case, homo (Streptococcus, Lactococcus, some Lactobacillus, etc.) or heterolactic (Leuconostoc, other Lactobacillus) bacteria or a mixture are used, much more rarely molds. In cheese making, the development of lactic ferments causes acidification, which contributes (along with rennet when used) to coagulation. Thru the production of CO2, hetero fermentative lactic acid bacteria cause the formation of cavities (openings) that are very important for certain types of cheese. In blue cheeses, it is in the cavities that *Penicillium roquefortii* will develop. Other microorganisms play a significant role in cheese making: yeast, micrococci, proteolytic bacteria, and especially molds. They carry out transformations after lactic fermentation during ripening. Among the transformations, let's mention the maturation of the curd thru proteolysis and lipolysis, the opening of Emmental-type cheeses by CO2 production during propionic fermentation by *Propionibacterium shermanii.*

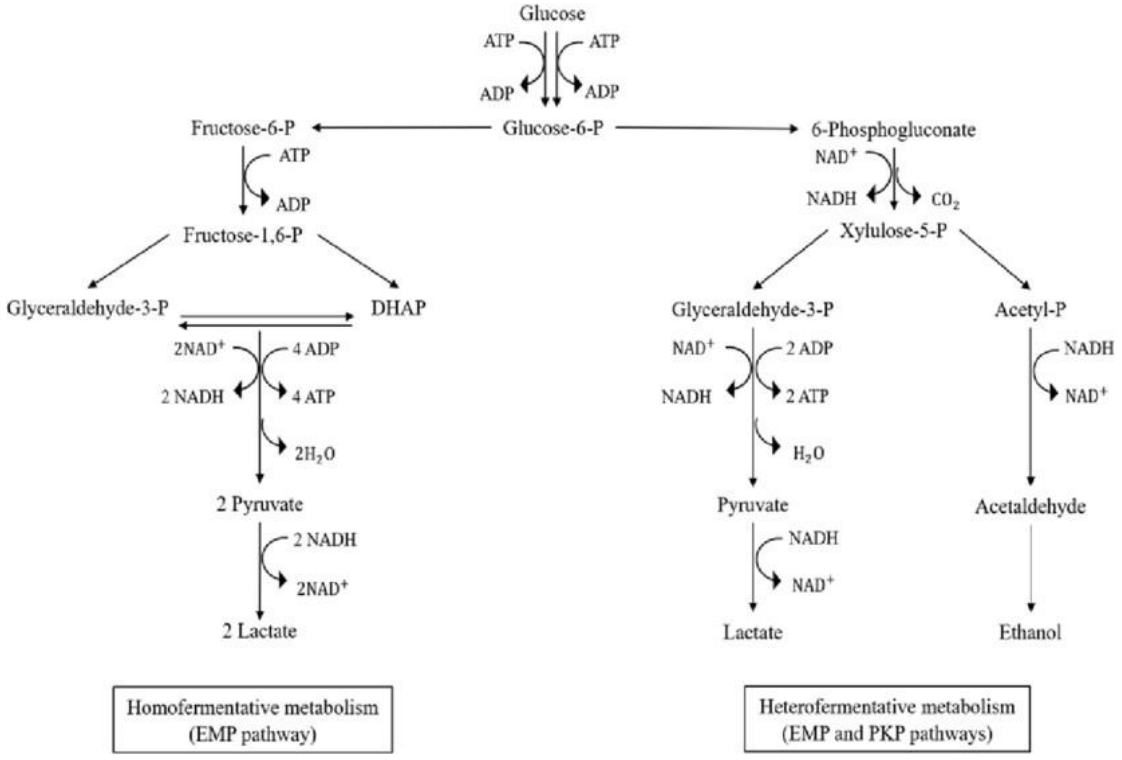


Figure 18. Metabolism for lactic acid bacteria (homofermentative and heterofermentative pathways)

4.1.4.3. Acetic fermentation

It occurs in the production of vinegar and is due to Acetobacter. This fermentation requires very strong aeration. The alcohol is breathed into acetic acid. Acetic bacteria only come into play if the ethanol content is low. Their action can be favored by the intervention of yeasts that oxidize ethanol and thus lower its concentration. Vinegar is a product stabilized by its pH; it is used as a preservative. The simplified acetic fermentation reaction is:

$CH_3\text{-}CH_2\text{-}OH + O_2 \rightarrow CH_3\text{-}COOH + H_2O + Energy$

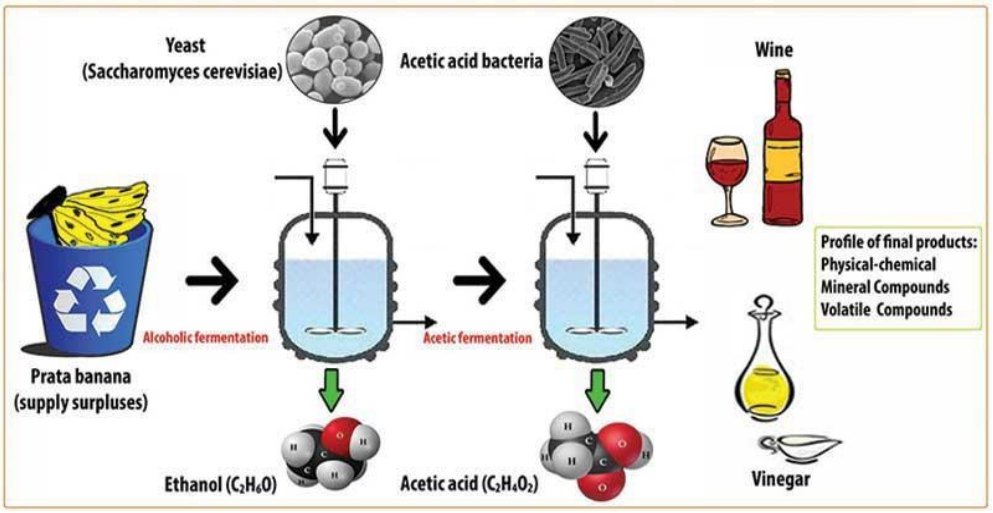


Figure19. Vinegar Fermentation

4.1.4.4. Other fermentations

Various other types of fermentations are encountered in many products, particularly of exotic type: cocoa, soy sauce, miso, tempeh koji. In the case of cocoa, the fermentation of the beans is due to yeasts, then to acetic bacteria. The first ones destroy the mucilage and produce the ethanol that will serve the second ones. The acetic acid formed is an essential element in the transformation of the cocoa bean content. Eastern fermentations often involve an initial step due to the amylolytic or proteolytic action of molds.

4.1.5. Microscopic fungi and fermented foods

4.1.5.1. Yeasts

Yeasts have been used for over 4000 years in the production of beer, wine, but also bread thanks to sourdough. Sourdough is a natural yeast discovered 4000 years ago in Egypt, it is a symbiotic culture of yeast and bacteria in a mixture of whole wheat flour and water.

Today, yeasts are the most widely used microorganisms by humans in numerous fields such as the food industry, health, agronomy...

- ***Saccharomyces cerevisiae***: is the most common species. Alcoholic fermentation is also used in baking: bread making is based on alcoholic fermentation, which allows the dough to rise due to the formation of carbon dioxide bubbles. Yeasts participate in cheese ripening by deacidifying the paste by reducing lactic acid, which allows for the establishment of a flora of bacteria and others that contribute to the formation of the taste. They hydrolyze certain peptides with a bitter taste, thereby enhancing the flavor of the cheese. Yeasts can also catabolize lipids and proteins, thereby releasing aromatic compounds that contribute to the flavor of the product. The yeasts most commonly found on the surface of cheeses belong to the genera Saccharomyces, Candida, and Debaryomyces.

- **Alcoholic beverages:** in virtually all cultures, we have seen the development of alcoholic beverages; in this case, the desired product is obviously not carbon dioxide, but ethanol.In the West, these are beer, wine, and fermented fruit juices. In Asia, the most important alcoholic beverage is sake (wine obtained by rice fermentation), in Central Asia, kumys made from fermented milk, in the Middle East, kvass made from grains, and pulque made from agave juice in Central and South America.

- **Wine:** comes from the fermentation of grapes or grape juice by yeasts, which constitute the most important flora: *Saccharomyces cerevisiae*, *Saccharomyces oviformis, Hanseniospora uvarum*, **Torulopsis stellata**, Schizosaccharomyces sp, Candida sp.

- **Cider:** is a beverage obtained by fermenting a sweet must made from apples. The fermentation by yeasts transforms the sugars in the juice into alcohol. We find the yeasts: *Hanseniospora* and *Saccharomyces cerevisiae*. There are secondary fermentations that generate aromas produced by hetero fermentative lactobacilli.

- **Beer:** is obtained thru the alcoholic fermentation of a wort made by macerating barley malt. The fermentation of the wort can be achieved spontaneously (Belgian Gueuze-type beer) or more often by massive inoculation with yeast. The yeasts used for beer production are not all identical.

4.1.5.2. Molds on dairy and meat products

Molds can be found in all environments of the food industry, where they are considered undesirable pollutants and therefore eliminated as best as possible. But, there are useful species that are seeded in the dairy industry, in salting, and in fermented soy products.

1.Dairy products

Milk, due to its composition, is a food of choice: it contains fats, lactose, proteins, minerals, vitamins, and 87% water. Its pH is 6.7.It is a very favorable substrate for the development of microorganisms. It can be transformed, thru enzymatic or microbial actions, into products that have acquired new nutritional and organoleptic qualities and exhibit increased preservation. We call fermented milk, pasteurized milk transformed solely by the action of microorganisms, and not subjected to draining: yogurt, kefir, acidified milk. We call cheese a product obtained by draining, after coagulation of milk by rennet (an enzyme of animal origin, extracted from the abomasum (stomach part) of the unweaned calf) or a related protease: a cheese can be fermented or not.

A. Cheese making

Cheese making is more or less complex. They undergo various operations depending on the type of cheese desired:

1) Acidification and coagulation of the milk due to lactic bacteria and rennet. Molds do not play a role in this phase. After curdling, draining can be spontaneous or enhanced by pressing, cutting, stirring, filtration, centrifugation...

2) Neutralization by consumption of lactic acid ensured by yeasts, Geotrichum candidum, and Penicillium.

3) Refining: involves numerous biochemical transformations and corresponds to a partial digestion of the curd's constituents by enzymes of microbial origin, there is a more or less advanced degradation of casein but also of fats:

- ✓ A proteolysis of the curd, which leads to the final texture of the product and plays an important role in flavoring. Fungal ferments are of great importance here.
- ✓ A lipolysis of fat that provides free fatty acids, important taste compounds, and precursors of various aromatic products. Here too, fungal ferments play a crucial role.

- ✓ Finally, the synthesis of aromas from molecules resulting from these degradations, such as fatty acids that are transformed into methyl ketones, characteristic aromas of cheeses, and this is thanks to the enzymatic potential of fungal ferments.

B. Types of cheese

1) **Fresh cheese:** these are drained cheeses obtained by centrifugation or filtration. They primarily undergo lactic fermentation and are not aged.

2) **Soft cheeses:** these are cheeses obtained by the action of rennet that undergo ripening after lactic fermentation, but whose paste is neither cooked nor pressed: draining is slow and achieved by simple cutting (stirring). We distinguish:

-Soft-ripened cheeses with a bloomy rind (Camembert, Brie, Carré de l'Est…)

-Soft-ripened cheeses with washed rind (Munster, Livarot, Pont-l'Évêque…)
-Soft blue-veined cheeses (with internal molds (Roquefort and other "blues"...).

3) **Pressed cheese:** these are cheeses obtained by the action of rennet, which undergo ripening after lactic fermentation, and are obtained by draining with curd cutting, stirring, and pressing. We distinguish:

-Uncooked hard cheeses (pressed and ground paste) (Cantal…)

-Uncooked pressed cheese with washed rind (St Paulin, Reblochon)

-Uncooked pressed cheese with moldy rind (St Nectaire, Tomme de Savoie…)

-Uncooked pressed cheese with an artificial rind (Edam…)

- Cooked pressed cheeses with openings (Emmental, Comté…)

- Cooked pressed cheeses without holes (Beaufort…)

- Very hard pressed cheese (very stirred) (Cheddar...)

2. Meat products

Dry sausage is a raw and fermented product. It is composed of an animal casing into which a mixture of minced meat, salt, sugar (glucose, lactose...), spices, and ferments is introduced. The formed product then undergoes a 72-hour incubation phase at a temperature between 20

and 25°C, followed by a drying phase at 13 to 14°C of variable duration, between 15 and 75 days. These two phases having the same objective of drying the product and developing the flora. The two ferments added to the preparation are:

- Lactobacilli, anaerobic Gram-positive bacteria. They break down lactose and glucose into lactic acid thru fermentation, which leads to an acidification of the environment.

- Micrococcaceae, of which Staphylococcus is the genus most adapted to the sausage environment. They reduce nitrates to nitrites and thus play a role in the coloration of the product. They also perform lipolysis, allowing for the synthesis of aromatic molecules (ketones).These two ferments, in addition to developing the taste, texture, and color of the sausage, also inhibit the growth of microorganisms that could alter the taste of the sausage or be pathogenic. This inhibition is made possible by the acidification of the environment and the development of positive flora, which is in addition to the lowering of water activity caused by salting.

- Other ferments, yeasts, and molds are also deposited on the surface of the sausages. Their development helps contribute to the drying of the sausages, their maturation, the development of aromas, and the overall appearance of the product. By its physical presence on the surface of the sausages, this flora also acts as a deterrent to the growth of undesirable microorganisms.

4.1.6. Role of fungal ferments

1. Interest of fungal ferments on the appearance of the product

The appearance of a cheese obtained by seeding a strain depends on many other parameters: seeding rate, the medium, and especially the protein/carbohydrate ratio, product humidity (aw), and temperature.

2-Interest of fungal ferments in ripening (taste and texture)

Cheeses are fermented foods that undergo numerous biochemical transformation processes thanks to the enzymes produced by microorganisms. The molds used as fungal ferments play a crucial role in these processes. The three species considered: *Penicillium camembertii*, *Penicillium roquefortii*, and *Geotrichum candidum* assimilate lactic acid very well, most often in the form of lactate. Deacidification is initially due to yeasts and *Geotrichum candidum* when it is present, then to Penicillium, which have a slower growth. The mechanisms of

proteolysis, lipolysis, and aroma production (amino acids and fatty acids) are largely ensured by the fungal species used as ferments.

3-Interest of fungal ferments in the fight against pollutants

In cheese making, one can find numerous species of microorganisms responsible for pollution and manufacturing accidents. The massive inoculation with fungal ferments helps combat this pollution by occupying the environment as quickly and extensively as possible. The risk of mycotoxin production is not negligible. These contaminations can have various causes, but most often they come from the ambient air; these species produce dry spores or xerospores that are easily carried by the air. In addition to disinfection measures, a massive inoculation with the selected strain of fungal ferment helps to eliminate them. In general, the development of pollutants is hindered by: acidic pH levels, a combined action between proper acidification and the use of fungal ferments is often sufficient to eliminate them.

4.2. Wastewater

4.2.1. Definition

Urban wastewater (ERU), or sewage, is water loaded with pollutants, soluble or not, primarily originating from human activity. Wastewater is generally a mixture of pollutants falling into these categories, dispersed or dissolved in water that has been used for domestic or industrial needs. So, under the terminology of residual water, we group waters of very diverse origins that have lost their purity; that is to say, their natural properties due to the effect of pollutants after being used in human activities (domestic, industrial, or agricultural).

4.2.2. Origin of wastewater

We can classify as wastewater, urban-origin waters consisting of domestic water (personal and laundry washing, premises cleaning, kitchen water) and sewage water loaded with feces and urine; this entire mass of effluents is more or less diluted by street washing water and rainwater. Depending on the case, industrial and agricultural wastewater may also be added.

The water, thus collected in a sewer network, appears as a cloudy liquid, generally grayish, containing suspended matter of mineral and organic origin in extremely variable concentrations. In addition to rainwater, urban wastewater is primarily of domestic origin but may contain industrial wastewater of extreme diversity. So, urban wastewater (ERU) consists of:

- ✓ Residual water or wastewater of domestic, industrial, and/or agricultural origin,
- ✓ Rainwater or urban runoff.

4.2.2. 1.Industrial origin

Industrial waste and effluents largely define the quality and pollution rate of these wastewaters. Industrial establishments use a significant amount of water which, while remaining necessary for their smooth operation, is only actually consumed in a very small part; the rest is discharged. Nevertheless, we can classify the main industrial discharges according to the nature of the inconveniences they cause:

- ✓ Pollution due to mineral suspended solids (Coal washing, quarrying, sand and gravel screening, phosphate fertilizer producing industries…)
- ✓ Pollution due to mineral dissolved solids (Pickling plant, galvanizing…)
- ✓ Pollution due to organic matter and fats (Agri-food industries, rendering plants, paper pulp…)
- ✓ Pollution due to hydrocarbon and various chemical discharges (Oil refineries, pig farms, pharmaceuticals…)
- ✓ Pollution due to toxic discharges (Untreated radioactive waste, radioactive effluents from nuclear industries…).

Industrial wastewater generally has a more specific composition and is directly related to the type of industry considered. Regardless of the load of organic or mineral pollution, whether they are putrescible or not, they may exhibit specific toxicity characteristics related to the transported chemicals.

4.2.2.2. Domestic origin

Domestic effluents are a mixture of waters containing human waste: urine, feces (sewage), and water from washing and cleaning floors and food (household water).These waters are generally composed of degradable organic matter and mineral matter, these substances are in dissolved or suspended form. They mainly consist of toilet drain valve water. And domestic wastewater from the drainage of kitchens, bathrooms.They mainly come from:

- ✓ Kitchen water containing suspended mineral matter from washing vegetables, food substances based on organic matter (carbohydrates, lipids, proteins), and detergents used for dishwashing, which have the effect of solubilizing fats;
- ✓ Laundry water mainly containing detergents;
- ✓ Bathroom water loaded with products used for personal hygiene, generally hydrocarbon-based fats;
- ✓ Wastewater from toilets (W.C), heavily loaded with hydrocarbon-based organic matter, nitrogenous compounds, phosphates, and microorganisms.

4.2.2.3 Agricultural origin

These are waters that have been polluted by substances used in the agricultural field. In the context of high-performing and intensive agriculture, the farmer is led to use various industrial or agricultural products, some of which present or may present risks to the environment, particularly to water quality. These are mainly:

- ✓ Fertilizers (commercial mineral fertilizers or animal waste produced or not on the farm);
- ✓ Phytosanitary products (herbicides, fungicides, insecticides,…)

4.2.2.4. Rainwater and runoff

Rainwater and runoff are taken into account in cases where the wastewater collection system is unitary, particularly during treatment at the wastewater treatment plant. Rainwater is likely to carry atmospheric pollutants and contaminate surface and groundwater thru infiltration and runoff. The main pollutants involved are SO2, NO and its derivatives, and dust.

4.2.3. Composition of wastewater

The composition of wastewater is extremely variable depending on its origin (industrial, domestic, etc…). The composition of raw urban wastewater essentially depends on: Human activity (household water and sewage); The composition of drinking water supply and, incidentally, the constitution of water pipes, for chemical compounds; The nature and quantity of industrial effluents possibly discharged into the urban network. Urban wastewater contains

solid materials, numerous substances in solid or dissolved form, and microorganisms. These substances can be classified into four groups.

a) Microbiological elements

The microorganisms found in wastewater are divided into 4 groups: viruses, bacteria, protozoa, and helminths. They come from the environment and fecal matter. They have various effects on health: they are the cause of mild infections (such as gastroenteritis) as well as deadly diseases. They have various effects on health: they can cause mild infections (such as gastroenteritis) as well as deadly diseases.

b) Suspended solids (TSS)

The suspended solids associated with dissolved substances are the cause of the turbidity of wastewater. The determination of S.S. is essential to evaluate the distribution between dissolved pollution and sedimentable pollution, whose fate is taken into account in a treatment system. The reference method for determining TSS is the AFNOR method no. 90-105, by filtration on a glass disk. They are expressed in mg/l of insoluble dry matter.

c) Nutrients

Their presence in wastewater can have a negative impact on human health and the quality of surface water, and to a lesser extent, a beneficial impact on crops in the case of agricultural reuse. Nitrogen, phosphorus, potassium, and trace elements essential for plant life are found in appreciable quantities, but in highly variable proportions relative to the vegetation's needs, in treated or untreated wastewater.

d) Organic and non-organic micropollutants

Micropollutants are elements present in infinitesimal quantities in wastewater.Some micropollutants, such as heavy metals or pesticides, can accumulate in the tissues of living beings, particularly in cultivated plants.

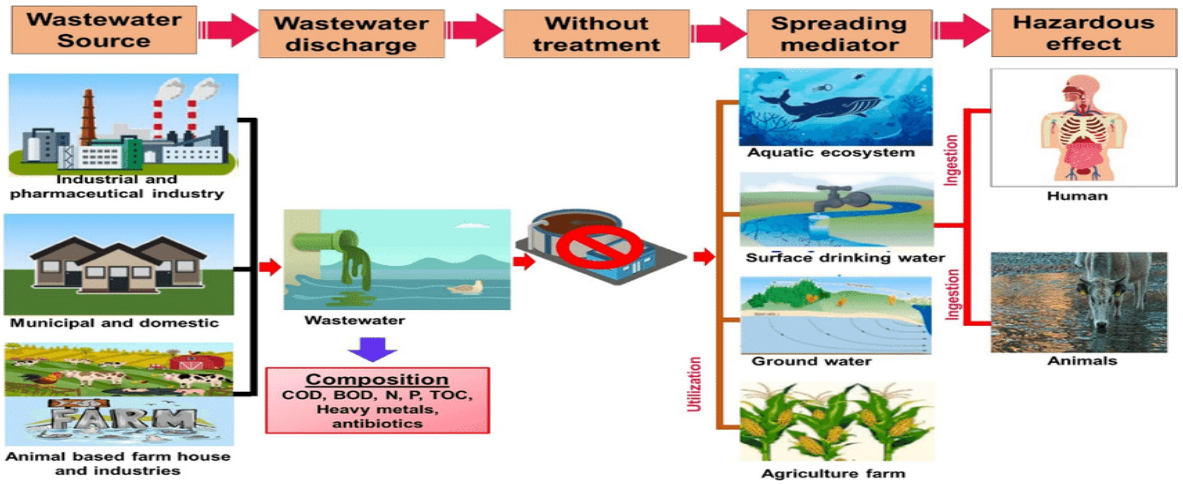


Figure20. sources of wastewater

4.2.4. Type of wastewater pollution

- **Organic pollution**

Organic pollution often constitutes the largest fraction, especially since, in its broadest sense, this form of pollution can be considered as resulting from various activities (urban, industrial, artisanal, and rural), each activity releasing specific compounds that may or may not be biodegradable.

- **Mineral pollution**

Mineral pollution of water can disrupt plant growth or cause physiological disorders in animals. The mineral pollutant is mainly heavy metals and nutrient mineral elements.

- **Microbiological pollution**

Sewage water contains a multitude of living organisms brought by excrement of human or animal origin. The water may contain pathogenic microorganisms (viruses, bacteria, parasites). They are dangerous to human health, and therefore limit the uses we can make of the water.

- **Toxic pollution**

The toxicity present in wastewater can be organic or mineral, toxic organic substances include pesticides, hydrocarbons, and synthetic organic industrial products (aldehydes, phenols, nitrogenous products, etc.). Toxic mineral substances are: salts at high concentrations, metal ions discharged by industrial effluents.

❖ **Thermal pollution**

The water discharged by factories using a cooling circuit for certain installations (thermal power plants, nuclear power plants, refineries, steel mills...) has a temperature of around (70 to 80) °C. It decreases to (40 to 45) °C when it comes into contact with the waters of aquatic environments, leading to a warming of the water, which affects the solubility of oxygen.

The main objective of treatment is to produce treated effluents at an appropriate and acceptable level from the perspective of risk to human health and the environment. In this regard, the most appropriate wastewater treatment is the one that reliably provides effluents of the required chemical and microbiological quality for a specific use, at a low cost and with minimal operational and maintenance needs.The treatment of wastewater has therefore become necessary to preserve the quality of natural environments, particularly surface and groundwater. The treated water is currently mostly discharged.

The widespread reuse of this water can therefore help to partially fill the deficit of first-hand water. The recycling of water in industries and the use of water for irrigating recreational spaces can therefore help reduce the pressure exerted on high-quality natural resources. The use of unconventional water in agriculture can contribute to the creation of new irrigated areas or serve as a supplementary source for existing areas. It is practiced in both developed and developing countries.

4.2.5. Wastewater treatment

The wastewater treatment sector recommends various techniques at different technological levels, often very elaborate. These are illustrated as classical treatment methods; as well as new techniques aimed at environmental protection and the preservation of the natural environment have emerged, demonstrated under the terms of lagooning or phytoremediation.

4.2.5.1. Classical treatment methods

The complete wastewater treatment line can be schematically divided into two streams:

- The water stream in which the water is cleared of all pollutants before its discharge into the natural environment;
- The sludge stream in which the residues generated by the water stream are treated and dehydrated before their disposal.

The water treatment process generally includes:

- A pretreatment for the removal of objects ranging in size from 0.1 to 50 mm (screening, sieving), fats, and sand,
- A primary treatment for the removal of easily settleable suspended solids,
- A secondary treatment consisting of a biological reactor for the removal of biodegradable organic pollution BOD5 or mineral pollution (NH3, NO3-, P).
- Some stations are also equipped with tertiary treatment for the removal of microorganisms or residual phosphorus.

The sludge from the primary clarifier (primary sludge) and biological treatment (biological sludge) will then be treated and conditioned in the sludge stream.

4.2.6. Wastewater treatment chain

4.2.6.1. Physical pretreatments

a. Screening

This involves passing wastewater thru a screen with bars that are more or less spaced apart, retaining the coarsest elements. After cleaning the screens by mechanical, manual, or automatic means, the waste is disposed of with household garbage.

The screens can be vertical, but are most often inclined at 60 to 80° relative to the horizontal.

b. Desanding

The desanding process removes sand from wastewater to prevent its subsequent sedimentation.The flow of water at a reduced speed in a basin called a "desander" causes them to settle at the bottom of the structure.

These particles are then sucked up by a pump. The recovered sands are then drained, then washed before being either sent to a landfill or reused.The desanding process concerns mineral particles with a diameter greater than approximately 0.2 mm.

c. Degreasing-deoiling

Degreasing-deoiling aims to eliminate fats and oils in wastewater, which can hinder the effectiveness of subsequent biological treatments. The operation is carried out by flotation. The injection of air at the bottom of the structure allows the fatty substances to rise to the surface. The fats and oils are scraped off the surface, then stored before being disposed of (landfill or incineration).

4.2.6.2. Primary Treatments

a. Natural Sedimentation

The "primary" treatment relies on natural physical processes, possibly combined with physicochemical processes, such as coagulation-flocculation.The suspended solids settle at the bottom of the basin and are mechanically thickened in a pit located under the lamellae. The materials resulting from primary sedimentation constitute primary sludge.They are extracted by pumping to the sludge treatment line. The clarified water is collected on the surface by gutters and directed toward biological treatment.

b. Physico-chemical sedimentation

The turbidity and color of water are mainly caused by very small particles, called colloidal particles. These particles, which can remain suspended in water for very long periods, can even pass thru a very fine filter. To eliminate them, we use coagulation and flocculation processes.

- **Coagulation**

The main purpose of coagulation is to destabilize suspended particles, that is, to facilitate their agglomeration. In practice, this process is characterized by the injection and rapid dispersion of chemical products: cationic mineral salts (iron or aluminum salts).

- **Flocculation**

The objective of flocculation is to promote, with the help of slow mixing, the contact between destabilized particles. These particles come together to form a floc that can be easily removed by the sedimentation process.

4.2.6.3. Secondary Treatments (Biological)

In the vast majority of cases, the removal of carbonaceous and nitrogenous pollution relies on biological processes, based on the growth of microorganisms at the expense of "biodegradable" organic matter, which serves as food for them.

a. Anaerobic treatments

Anaerobic treatments involve bacteria that do not use oxygen, particularly methanogenic bacteria, which, as their name suggests, lead to the formation of methane from organic matter, and to a lesser extent, CO2.It is a delicate operation that requires significant monitoring. Indeed, the temperature must be maintained at a very stable and sufficiently high level. It is also necessary to avoid sudden pH changes and substances that inhibit bacterial growth, such as cyanides, heavy metal salts, and phenols.

b. Aerobic treatments

The microorganisms used require a constant supply of oxygen. We distinguish five essential methods:

1. Activated sludge (Free cultures)

In practice, the process involves feeding a stirred and aerated basin (aeration basin) with the water to be treated (pre-treated effluent, see previously settled) within a continuous flow of wastewater, the aerobic bacteria are subjected to prolonged action of strong oxygenation obtained by introducing air regularly distributed in the effluent, these bacteria absorb and degrade the organic matter and form large flocs (biofloc) that settle, which in turn, constitute sludge or flocculent masses called activated sludge.The mixture of wastewater + biofloc forms a mixed liquor.

2. Bacterial beds (Fixed cultures)

A bacterial bed appears as a circular column that can reach 4 to 5 meters in height, in which there is a material (Pumice or Plastic).The effluent trickles over the surface of the biological film that proliferates on the support (interstices), which contains a high concentration of bacteria and fungi. These organisms absorb and metabolize the organic matter from the effluent, gradually depleting it during its passage.The oxygen supply is naturally provided thru aeration vents, thus ensuring the oxygen needs of the biomass.

3. Biological Disks (Fixed Cultures)

The wastewater, previously settled, feeds a structure in which disks fixed on an axis are rotated at a slow speed. On these plastic biological disks, a bacterial film then develops. During their emergence, these bacteria take the oxygen necessary for their respiration, and during immersion, they absorb the dissolved pollution they feed on. As soon as the biological film exceeds a thickness of a few millimeters, it detaches and is carried toward the final clarifier where it is separated from the purified water. The trapped sludge is periodically pumped back to the headworks for storage and digestion.

4. Lagooning

- **Natural lagooning (Fixed cultures)**

Lagooning is a natural purification process that uses aquatic vegetation as a purifying agent for polluted water.Aquatic plants are used here as a substrate for bacterial colonies, ensuring the effective purification of water that slowly passes thru the established plant colonies.Polluting elements such as nitrates are thus absorbed by the plants to return good quality water at the outlet of the lagooning.

- **Aerated lagoon**

In a first basin, bacteria intervene to eliminate waste (organic matter) and transform it into mineral salts and gas. Subsequently, in a second basin, these products are recovered by the plants to allow their development.These will then produce oxygen (through photosynthesis). The micro-algae (phytoplankton) will be consumed in the last ponds by the zooplankton (microscopic animals). At the end of this stage (approximately 80 days after entering the first pond), the waters are suitable for discharge into the natural environment.

5. Tertiary treatments

Also called complementary treatments that aim at the elimination of nitrogen and phosphate pollution as well as biological pollution from domestic wastewater.Tertiary treatments refine or improve secondary treatment, which aims to enhance the overall water quality for subsequent reuse in industry or irrigation.These treatments are carried out when the receiving environment requires it. We generally distinguish the following operations:

- Nitrification-denitrification and biological or mixed (biological and physico-chemical) dephosphorization
- Bacteriological and virological disinfection.

4.2.7. Disinfection

Most pathogenic microorganisms are removed from the water during the previous treatment phases. However, water disinfection is still necessary to prevent drinking water from being harmful to our health. Disinfection is a treatment that eliminates microorganisms likely to transmit diseases; this treatment does not necessarily include sterilization, which is the destruction of all living organisms in a given environment.

Disinfection can be carried out by adding a certain amount of a chemical with germicidal properties to the water. The most commonly used chemicals are: chlorine, chlorine dioxide, ozone, bromine, iodine, and potassium permanganate. We can also disinfect water using physical means: boiling, ultrasound, ultraviolet light, or gamma rays.Infectious diseases caused by pathogenic bacteria, viruses, and protozoan parasites are among the most common and extend the health risks of drinking water. People are contaminated by these microorganisms thru contaminated drinking water, aerosols, and washing or bathing.

Disinfection is the ultimate step in the treatment of drinking water before distribution. It allows for the elimination of pathogenic microorganisms from the water. However, some common germs may still remain in the water, as disinfection is not sterilization (sterilization = destruction of all germs present in an environment).Disinfection is a treatment aimed at eliminating pathogenic microorganisms, bacteria, viruses, and parasites, as well as the majority of less resistant common germs.It is the means to provide bacteriologically potable water, while maintaining a sufficiently high disinfecting power to prevent bacterial resurgence.

4.2.7.1. Different modes of disinfection

Not all disinfection processes and products are equivalent, so the most appropriate process must be chosen, taking into account certain particular conditions (characteristics and uses of the water, types of microorganisms to be eliminated, quality of the distribution network, etc.) and knowing that a disinfectant or disinfection process must:

a) Not be toxic to humans or animals

b) Be toxic, at low concentrations, to microorganisms

c) Be soluble in water

d) Form a homogeneous solution with water

e) Be effective at normal drinking water temperatures (0 to 25°C).

f) Be stable, in order to promote the maintenance of a certain residual concentration over long periods of time

g) Not react with organic matter other than that of microorganisms

h) Not deteriorate metals or damage clothing during washing

i) Eliminate odors

j) Exist in large quantities and be sold at an affordable price

k) Be easy to handle and not pose any danger to operators

l) Allow for easy measurement of its concentration.

Chlorine disinfection accounts for 80% of disinfection worldwide, as this disinfectant has several disadvantages listed above. However, the addition of this product can lead to undesirable side effects that, in some cases, require the use of other disinfectants. Thus, chlorine reacts with the organic matter in the water, which can sometimes lead to the formation of carcinogenic substances (trihalomethanes) or unpleasant odors (chlorophenols). Moreover, chlorine is not powerful enough to completely eliminate certain highly resistant microorganisms such as viruses and protozoa. In order to address these shortcomings, chlorine dioxide or ozone is used. These disinfectants, much more powerful than chlorine, nevertheless have the disadvantage of being unstable.

4.3. Annex Techniques

In industrial, pharmaceutical, agri-food, or chemical sectors, certain operations complementary to the main activities play a fundamental role in the smooth running of processes. These operations are called ancillary techniques.They include several actions such as quality control, sampling, handling and transportation of materials (solids, liquids, or gases), as well as equipment maintenance (cleaning, disinfection, protection).Although often

considered secondary, these techniques are essential to ensure safety, product quality, compliance with standards, and the proper functioning of facilities. Mastering these techniques directly contributes to the performance and reliability of operations.

4.3.1. Controls

1. Definition

Controls are systematic checks carried out throughout the production or handling process of a product. They ensure quality, traceability, and compliance with regulatory and health standards.

2. Types of controls

- ✓ **Visual inspection:** appearance, color, cleanliness.
- ✓ **Physical inspection:** temperature, pressure, mass, volume.
- ✓ **Chemical control:** pH, chlorine level, residue analysis.
- ✓ **Microbiological control:** search for pathogenic or contaminating germs.

3. Objectives

- ➢ Ensure the safety of users and consumers.
- ➢ Maintain a consistent quality of products.
- ➢ Comply with the current standards (ISO, HACCP, etc.).

4.3.2. Sampling

1. Definition

Sampling consists of taking a representative quantity from a batch of product (solid, liquid, or gas) to perform analyzes.

2. Methods

- ✓ **Random sampling:** at random, without a precise method.
- ✓ **Systematic sampling:** at regular intervals (e.g., every 30 minutes or every 10 liters).

- ✓ **Stratified sampling:** by dividing the lot into several homogeneous parts.

3. Precautions

- ➢ Use of clean and suitable containers
- ➢ Respect for hygiene conditions to avoid contamination
- ➢ Clear labeling: date, time, origin, name of the collector.

4.3.3. Transport and Handling

Move products safely and without compromising quality. Reduce the risks of contamination, breakage, or loss

1. Manipulation of Product Shapes

a) Solids

- ✓ Use of bins, bags, containers, pallets.
- ✓ Beware of dust, moisture, and shocks.

b) Liquids

- ✓ Pipes, pumps, drums, tanks.
- ✓ Verification of tightness, temperature, chemical compatibility.

c) Gas

- ✓ Pressurized bottles, distribution networks
- ✓ Specific risks: explosion, leak, asphyxiation → mandatory use of PPE.

3. Technical means

- ➢ Pallet jacks, forklifts, conveyors.
- ➢ Must be maintained and used according to safety guidelines.

4.3.4. Equipment Maintenance

1. Cleaning

- **Frequency:** regular, after each use or at the end of the shift.
- **Methods:** manual washing, machine, steam, high pressure.
- Use of detergents suitablefor the type of dirt.

2. Disinfection

- ✓ Must follow cleaning (because dirty equipment cannot be effectively disinfected).
- ✓ Use of approved products (disinfectants, virucides, bactericides).
- ✓ Respect the contact time, rinse if necessary.

3. Protection

- ✓ Equipment stored and protected from dust, moisture, and impacts.
- ✓ Wearing personal protective equipment: gloves, glasses, masks depending on the products used.

4. Traceability

- ✓ Cleaning and maintenance tracking sheets.
- ✓ Verifications by quality or safety managers.

The mastery of ancillary techniques is essential for:

- ✓ Ensure the sanitary safety of the products.
- ✓ Preserve the quality of production.
- ✓ Comply with the current regulations.
- ✓ Ensure the safety of operators and the longevity of equipment.

5. Atomic absorption and emission spectrometry

Spectral methods include both molecular and atomic methods. Molecular methods are represented by band spectra and include UV-Visible spectroscopy and Infrared spectroscopy. As for atomic methods, they are represented by line spectra and include atomic emission spectroscopy and atomic absorption spectroscopy.

5.1. Phenomena of atomic absorption and emission

Atomic absorption spectrometry (AAS) and atomic emission spectrometry (AES) are two widely used techniques for the analysis of more than 70 elements, sometimes in trace amounts.

5.1.1. Atomic absorption

Atomic absorption is the phenomenon observed when an atom in its ground state absorbs electromagnetic radiation at a specific wavelength and transitions to an excited state. This results in a spectrum of dark lines on a light background (Absorption Spectrum).

5.1.2. Atomic emission

Atomic emission is the phenomenon observed when electromagnetic radiation is emitted by excited atoms or ions that return to the ground state. This results in a spectrum of bright lines on a black background (Emission Spectrum).

5.1.3. Spectroscopy and spectrum

Spectroscopy is the study of the electromagnetic spectrum of a phenomenon, visually. The spectrum is the distribution of energy, power, intensity, absorbance, transmission, etc. as a function of wavelength or frequency. We distinguish three types of spectra:

- Continuous spectra, for which there is a "signal" for every wavelength or frequency
- Discontinuous spectra, or line spectra, or discrete spectra, which only have a signal for certain specific frequencies or wavelengths, characteristic of the irradiated matter.
- The combined spectra, which consist of a superposition of a continuous spectrum and a discrete spectrum.

5.2. Ultraviolet/Visible Spectroscopy

UV-Visible spectroscopy is the oldest and most widely used analytical method in laboratories. It notably allows for quantitative applications thru the application of Beer-Lambert's law. However, it provides very little structural information (Qualitative Analysis) compared to other spectroscopic methods (IR, NMR, and MS).

5.2.1. Principle

In a molecule, UV-visible electronic transitions involve the most significant energies in chemistry (160 to 665 kJ·mol-1). The order of magnitude of the energies involved is that of the bonding energies of the molecules, and these radiations can sometimes cause bond breakages. More generally, they cause electronic transitions between the different energy levels of the molecules.

The absorption of photons results in transitions of electrons involved in molecular orbitals (the electrons of σ and π bonds as well as non-bonding n electrons) from the ground state to the unoccupied levels of excited states. Each transition is characterized both by its wavelength λmax and by its molar absorption coefficient: Єmax at that wavelength.

5.2.2. Spectral range

In a molecule, electronic transitions occur in the ultraviolet region (approximately 400-10 nm) and the visible region (800-400 nm).

• **visible:** 800 nm - 400 nm.

• **near-UV:** 400 nm - 200 nm

• **Far UV:** 200 nm - 10 nm.

Usable UV range in analysis: 190 –400 nm. So the far UV is not accessible for measurements under these conditions. Visible range usable in analysis: 400 – 800 nm

- ❖ The choice of solvent is important in this technique. It must be: - Inert with respect to the solute. - Transparent at the wavelength used.Some examples of solvents:

- Cyclohexane: Transparent beyond 210 nm.
- Hexane: Transparent beyond 210 nm.
- Distilled water: Transparent beyond 200 nm.
- Chloroform: Transparent beyond 230 nm.
- The solvent must be rid of all its impurities before being used.

5.2.3. Equipment

The study of absorptions requires the use of a device called a spectrometer. The following figure represents the principle diagram of a single-beam UV-visible absorption spectrometer.

We dissolve the substance to be analyzed in a solvent and the resulting solution is poured into a cuvette designed to be placed in the spectrometer. In order not to distort the measurements, the cuvette and the chosen solvent must not absorb the radiation emitted by the spectroscope.

- **Source:** The role of the source is to provide light radiation. In the UV region, the source is a deuterium discharge lamp. Or hydrogen lamp. A tungsten filament lamp is used for the region ranging from the visible.
- **Monochromator:** its role is to disperse the polychromatic radiation coming from the source and obtain monochromatic radiation.
- **Analysis cell:** UV: quartz cell, Visible: quartz or glass cell.
- **Detector:** is a photomultiplier tube that converts the received light into electrical current.

- **Recorder:** Recording of measurements. It allows for the plotting of an absorption spectrum of the analyzed sample.

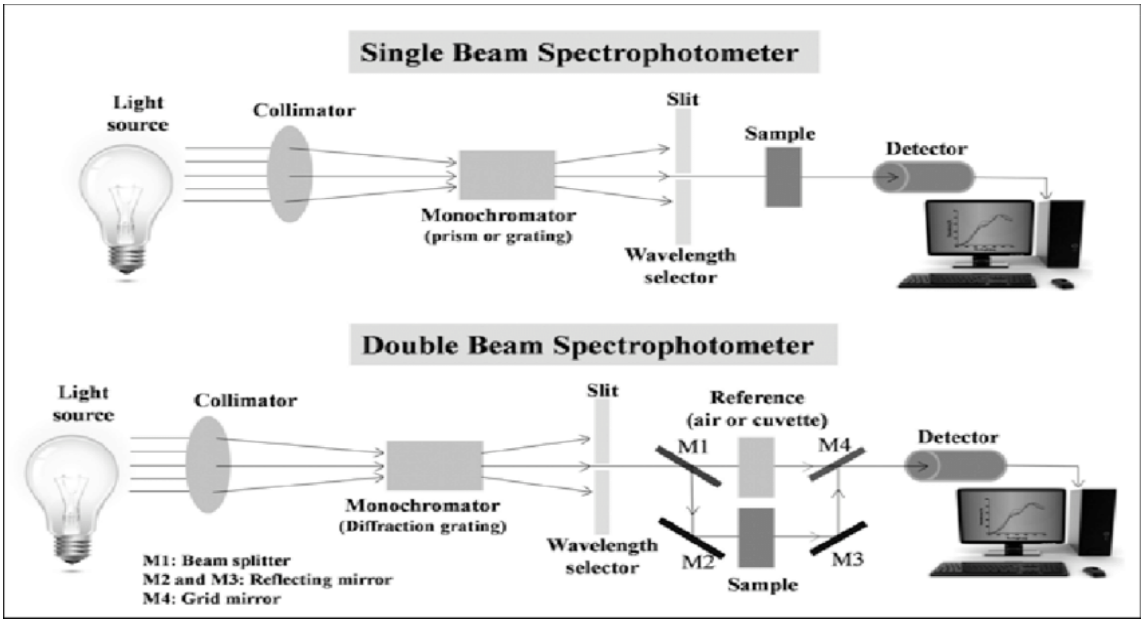


Figure 21. Schematic-of-Single-and-double-beam-spectrophotometer

5.2.4. Application of UV/Visible spectroscopy

Large field of applications (Analytical chemistry, mineral, organic, biochemistry), 90% of medical analyzes, quantitative analyzes (Beer-Lambert law) - high sensitivity, simplicity, speed.

a) **Quantitative analysis** (Identification of the concentration of an unknown substance)

must first choose its specific absorption band.

b) **Qualitative analysis** (identification of an unknown substance)

UV-VIS spectrophotometry can be used to identify various classes of compounds both in their pure state and in biological preparations. This is done by plotting the absorption spectrum curves.

c) **Enzymatic assays**

Enzymatic activity can be easily and quickly calculated when the substrate or product is colored or absorbs light in the UV range. In these cases, the rate of appearance or disappearance of a light-absorbing product or substrate can be measured using a spectrophotometer.

d) **Structural Application - Purification Control**

This is one of the most important applications of UV-VIS spectrophotometry. Impurities can be detected very easily by testing if the compound does not show its characteristic absorption spectrum. - Determination of molecular weight – Turbidimetry.

5.3. Infrared Spectroscopy

Infrared absorption spectroscopy studies the vibrations and rotations of molecules when they are irradiated by an electromagnetic wave with a frequency within the infrared range. This identification technique is mainly used for the qualitative analysis of a molecule by highlighting the presence of bonds between atoms (functions and groups).

Infrared radiation (IR) was discovered in 1800 by Frederick William Herschel. These radiations are located between the visible spectrum region and microwaves. The infrared range extends from 12500 cm-1 to 10 cm-1. It is divided into 3 categories:

- The near infrared: 12500-4000 cm-1.
- The mid-infrared: 4000-400 cm-1.
- The far infrared: 400-10cm-1.

5.3.1. Principle

IR spectroscopy is based on the interaction of IR light with the electron cloud of chemical bonds. Generally, in the majority of optical spectroscopies such as UV/visible spectroscopy, the absorption of energy allows an electron from a chemical bond to transition from a ground state to an excited state. In the case of IR absorption spectroscopy, the radiation emitted by the polychromatic source is generally not energetic enough to cause electronic transitions, but it induces transitions between vibrational and rotational energy levels. This will therefore lead to vibrational and rotational movements within the molecule. So, for each group of atoms undergoing these movements, there will correspond an absorption band characterized by a well-defined wavenumber v.

Also, the exciting light will cause, for each vibrational transition, a multitude of rotational transitions, which will give the vibrational transition peak the appearance of an absorption band. The majority of applications are located between 4000 cm-1 and 400 cm-1 (mid-IR). This range corresponds to molecular transitions of vibration and rotation type, which will lead to absorptions. The transitions between rotational levels appear in the far IR.

5.3.2. Equipment

The elements of an IR spectrometer are an infrared radiation source, a radiation separation system (monochromator), and a signal detector.

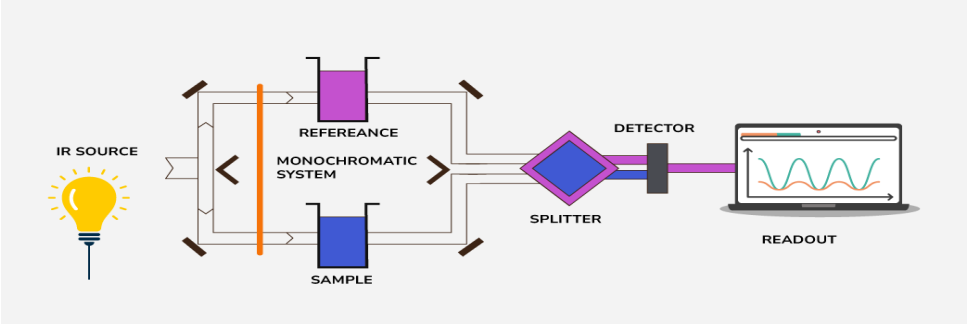


Figure 22. IR-Spectroscopy-Instrumentation

1. The source:

Most infrared spectroscopy devices are equipped with thermal sources.

In the near-infrared, halogen lamps with a tungsten filament are used.

In the mid-infrared, we generally use a Globar source based on silicon carbide (SiC).

In the far infrared, we use quartz lamps with mercury vapor under high pressure.

2. Sample holder

3. The Monochromator

4. The detector: the signal detection occurs thru the conversion of the incident radiation into an electrical signal. The detector used is of the thermal type. It detects temperature variations and transforms them into intensity variations.

5. Recorder: Recording of spectra.

5.3.3. Application

The use of this technique has widely spread in a large number of industries, leading to very diverse analytical applications: food industry, pharmaceutical industry, materials fields (polymers...), petroleum industry, textile industry….etc.The study of an I.R spectrum allows:

a) To identify an unknown compound or its functional groups

b) Verify the purity of a known product by the absence of bands indicating the presence of foreign compounds.

c) To follow a reaction process by studying the appearance or disappearance of characteristic bands of certain functional groups.

5.4. Atomic emission spectrometry

5.4.1. Definition

It is a method of measuring certain metallic elements. This method uses the ability of certain excited atoms to de-excite by emitting photons of determined energy (thus of determined wavelength).

Atomic emission photometry measures the emission of UV or visible electromagnetic radiation due to the de-excitation of atoms that have been excited by the energy provided by the transfer to a very high temperature (introduction of the sample into a flame or plasma). The quantitative measurement of the emission allows for dosages. The flames used in flame measurement devices reach 2000 to 3000 °C and allow for the emission by atoms of the alkali series (Na, K, Li), some alkaline earth metals (Ba), and a few other metals. Plasma devices that reach over 7000 °C allow for the expansion of the range of measurable atoms.

5.4.2. Principle

Thru the flame or plasma, whose temperature is very high, the objective is to break down the molecular structures and to convert the element to be measured (at least partially) into atomic vapor form. Under the effect of high temperatures, some of the atoms will be excited and their electrons will move to higher energy levels. The excited levels are unstable, and the return to the fundamental level of minimal energy will lead to a release of energy in the form of electromagnetic radiation with a wavelength characteristic of the atom that is de-exciting. It is the measurement of this emission at a wavelength characteristic of the atom to be measured that forms the basis of atomic emission photometry.The sample is introduced at the atomizer, which plays a dual role:

- Production of atomic vapors.

- Excitation of atoms.

After excitation, the return to the fundamental state is accompanied by the emission of specific radiations from the element to be measured.The intensity of the emitted radiation is proportional to the concentration of the analyte being considered. Whereas, in AAS measurements are taken at a wavelength, selected by the bandwidth (the spectrum presents a single absorption band).

5.4.3. APPLICATIONS

This essential analysis method has also found various applications in several fields (industrial, environmental, metallurgical, forensic, pharmaceutical...etc.). Alkali metals, which produce colored flames, are easily quantified by emission. We can therefore use flame emission in mineral analysis and biology to measure lithium, sodium, and potassium (ionogram) and also certain alkaline earth metals (Ba). We can perform these analyzes in the visible or ultraviolet range. In bromatology, it can be used for control (e.g., measuring sodium and calcium in milk).

5.5. Spectrofluorimetry

Some organic or mineral compounds, liquid or solid, whether pure or in solution, emit light when excited by photons in the UV-Visible range. Among the applications in the analysis of this phenomenon called photoluminescence is fluorimetry.

It is an analytical technique using the phenomenon of fluorescence. The latter is defined as the emission of electromagnetic radiation by molecules (in all directions) without heat release. This phenomenon is associated with a whole terminology characterizing the emission (fluorescence, phosphorescence) or the excitation (chemiluminescence, bioluminescence, radioluminescence, cathodoluminescence, photoluminescence, thermoluminescence).

Fluorescence covers a very wide range of wavelengths, the most well-known being molecular fluorescence, whose emission spectrum lies in the visible or ultraviolet range. For the study of heavy metals, X-ray fluorescence has been developed, whose emission spectrum is formed of X-rays.

Fluorochromes or fluorophores are chemical substances capable of emitting fluorescence light after excitation.

5.5.1. Principle

When the molecule is in an excited state following the absorption of a photon, it returns to the lowest energy level of the excited state thru relaxation (energy transfer with the solvent, notably due to collisions). The lifetime of these non-radiative vibrational relaxations is on the order of 10-12 seconds.

The molecule then returns to the ground state either thru internal conversion (direct return to the ground state) or external conversion (collision with other molecules) or by light emission. This emission, when it occurs, is called fluorescence. Indeed, the emitted energy is generally lower than the excitation energy. This is because the center returns to the ground state from the lowest vibration level of the excited state. This difference is called the Stokes shift. The emission spectrum (fluorescence emission intensity as a function of the wavelength of the emitted photon) depends on the nature of the fluorescent molecule and the interactions between this molecule and its surroundings.

For fluorescence to occur, several characteristics are necessary:

- ✓ Absorption in the UV and visible.
- ✓ High maximum absorption probability (εmax).
- ✓ Low electronic transition energy.
- ✓ Absence of atoms or groups that promote non-radiative phenomena in the molecule.
- ✓ Absence of atoms or groups that promote non-radiative phenomena in the molecule.

Fluorescence is often the privilege of cyclic, rigid molecules with π bonds. It is increased by the presence of electron-donating groups and decreased by electron-withdrawing groups.

5.5.2. Equipment

The fluorescent compound behaves like a source that radiates in all directions.

The excitation source is often made up of a xenon arc lamp. The measurement is done by a photomultiplier.

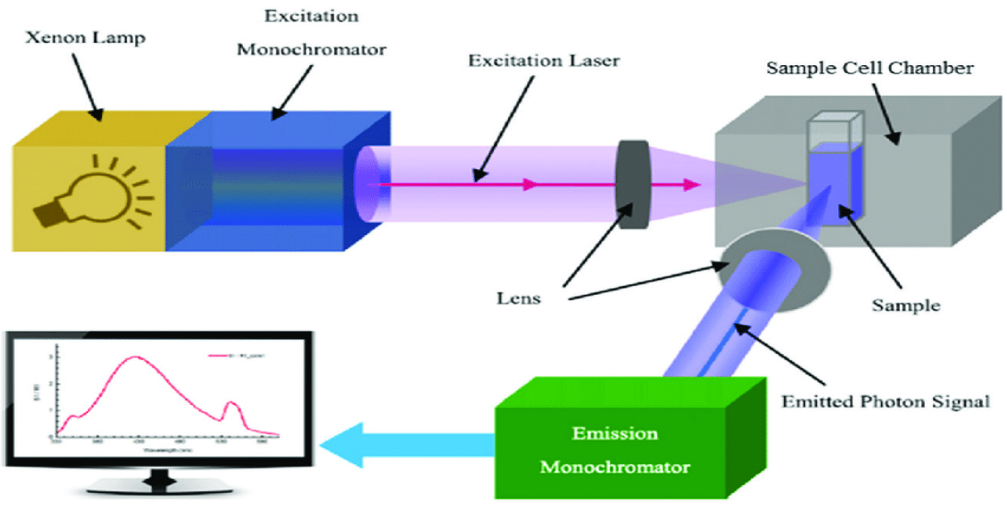


Figure 23. Different components of a spectrofluorometer with a xenon arc lamp.

a) A light source

- ✓ Xenon arc lamps: continuous emission between 220 and 700 nm. Their energy varies depending on the wavelength. They are the most efficient.
- ✓ Mercury vapor lamps: emit only a discontinuous spectrum (lines between 254 and 366 nm).
- ✓ Laser sources: recent appearance, still not widely used.

b) An excitation monochromator (prism or grating).

c) A quartz cuvette.

d) An emission monochromator: allows for the selection of a narrow band of emission wavelengths (because after excitation, the sample emits in all directions).

e) A photomultiplier/photodiode: transforms the emitted light into an electric current. f. A signal reading system.

5.5.3. Applications

- ➢ Sensitive and specific method (trace identification) such as certain trace gasses in the Earth's atmosphere that are quantified based on their fluorescence.
- ➢ Determination of the absorption spectrum of a substance under conditions where UV-VIS spectrometry is unsuitable.
- ➢ Allows for the plotting of the excitation spectrum of a molecule at very low concentrations or in the presence of interfering absorbing substances.
- ➢ Analysis of drugs or natural substances at often low concentrations in biological media.
- ➢ Analysis after excitation at a determined wavelength.
- ➢ Direct analysis of naturally fluorescent substances (e.g., quinine and quinidine).
- ➢ Weakly fluorescent molecules are amplified by heterocyclization.

Non-fluorescent molecules can be made fluorescent thru fluorescence derivatization. Among these derivatizing compounds, fluorescamine, bromomethoxy-coumarin, and 7-hydroxycoumarins.

In biochemistry, fluorescence finds numerous applications for quantifying proteins or nucleic acids using reagents that specifically bind to these compounds.

- ➢ Fluorimetry is also usable within the framework of immunological methods in homogeneous or heterogeneous phase, the tracer being the molecule to be measured marked by a fluorochrome.

5.6. Polarimetry

Polarimetry is an experimental technique based on measuring the deviation of the polarization plane of polarized light passing thru a solution composed of one or more chiral molecules.

This method is only applicable to optically active (chiral) molecules. It was discovered by Biot in 1812 on crystals and then in 1815 on organic molecules.

Brief reminders

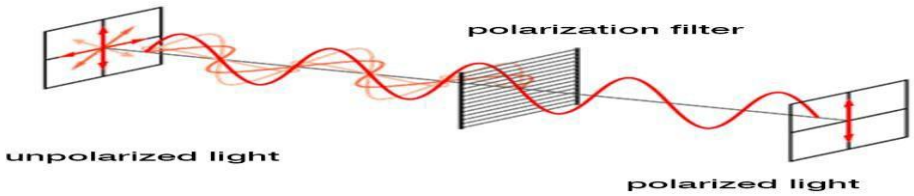
We recall the basic vocabulary:

- Chiral molecule: molecule possessing at least one asymmetric carbon (or stereogenic carbon).
- Stereoisomers: these are molecules of a given (developed) formula whose spatial structure is different.
- Enantiomer: an enantiomer is one of the stereoisomers of a chiral molecule.
- Optically active solution: solution containing chiral molecules.
- Racemic mixture: it is a solution containing an equimolar mixture of two enantiomers. Its rotatory power is zero.

5.6.1. Polarized light

Polarized light was discovered by Malus in 1809. "Normal" light is composed of an electric field and an electromagnetic field: we also talk about an electromagnetic wave. Let's limit ourselves to the electric field, the one to which the eye is sensitive. This field can take any direction. In polarized light, the electric field is limited to a single direction.

This polarized light is obtained using a polarizer or Polaroid, composed of a polymer that filters the components of the electric field according to the direction perpendicular to the "polymer lines."



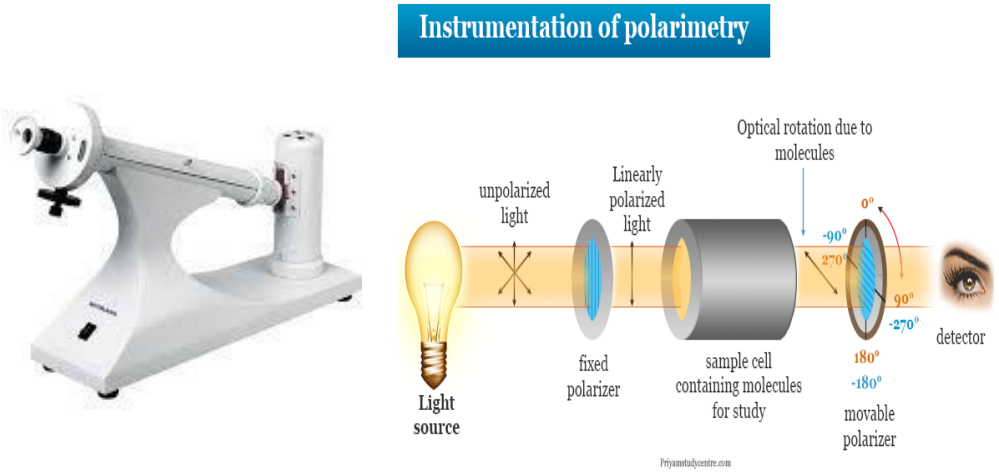
Two Polaroid sheets placed in front of a light source will let the light pass thru if they are parallel, and will block it when they are perpendicular.

5.6.2. Principle of the method

A chiral molecule has the property of deviating the plane of polarization of incident polarized light. It is therefore sufficient to place a solution containing the chiral substance between the two polaroids to check if it deflects the plane of polarization of the light.

If that's the case, the angle at which extinction is achieved corresponds to the angle of rotation specific to the chiral substance. The measurement of this angle is therefore a characteristic of the substance. This is the principle of the polarimeter.

There are several types of polarimeters. There is the famous Laurent polarimeter, and others built on the same principle (Lippich, Zeiss polarimeters). In polarimeters, the light source is a sodium vapor lamp placed in front of the polarimeter.



5.6.3. Biot's Law

Biot's law states that the angle of rotation is proportional to the length of the cuvette and the concentration. The constant of proportionality is called the specific rotation of the substance. It depends on the temperature and the wavelength at which the experiment was conducted. We can therefore write Biot's law in the form:

$\alpha = [\alpha]T \cdot l \cdot c$

Where:

α: angle of rotation observed in degrees.

l: length of the tank in dm.

c: concentration of the solution in g/mL.

[α] T: specific rotation defined at a temperature T and measured for a given wavelength, expressed in g-1.mL.dm-1.

The angle of rotation being a function of the concentration (Biot's law), we can therefore use polarimetry to monitor reaction kinetics.

Note. The specific rotation [α]T is a characteristic quantity of the chiral molecule. If a chiral molecule has a stereogenic carbon (that is, it has two enantiomers), its two enantiomers will have specific rotational powers of the same value but with opposite signs. This is no longer true in the case of molecules with multiple stereogenic centers or in the case of epimers.

Chromatographic methods

6.1. Definition

Chromatography is a technique used to separate the components of a mixture and to purify substances. Although this technique was originally developed to separate colored substances (hence the name, Chroma which means color in Greek). Chromatography is nowadays used to separate all types of substances, including those that are colorless. Therefore, an effective detection technique must be employed to observe the compounds (UV, refractive index, complementary reagent, mass after evaporation of the eluent...).

6.2. Principle

The basic principle of chromatography lies in the difference in affinity of a solute toward a stationary phase (the adsorbent) and a mobile phase (the eluent). Obviously, these phases must not react chemically with each other. Upon contact with these two phases, a solute will be separated from other solutes according to its greater or lesser affinity for these phases.

Chromatography relies on the transport of a dissolved sample by a mobile phase thru a stationary phase. This retains the substances contained in the diluted sample more or less strongly depending on the intensity of the low-energy interaction forces (such as Van der Waals forces, hydrogen bonds, etc.) between the different molecular species and the stationary phase.

The different components of the sample generally have a characteristic speed that allows them to be separated, or even identified. This separation speed is highly dependent on the nature of the mobile phase and the stationary phase.

Often, the sample is analyzed by comparison with substances already known in the sample or by comparison with the results of the analysis of a standard solution (commercial solution containing known substances at well-known concentrations). These substances serve as references and allow for the identification or quantification of each species by comparing their separation rates. This is analytical chromatography. In other cases, we are content with separating the fractions, collecting them to identify them by other techniques: this is preparative chromatography.

6.3. Classification

Chromatographic methods encompass a wide variety of techniques that can be classified according to three different modalities.

6.3.1. Classification according to the nature of the phases

In this classification, the mobile phase is a fluid that can be either liquid or gaseous, and the stationary phase can be either a finely pulverized solid or a liquid immobilized on a fixed phase.

6.3.2. Classification according to the nature of the phenomena involved

This classification is based on the nature of the stationary phase and its interaction with the molecules to be separated (adsorption ,partition ,ion exchange ,exclusion and affinity chromatography)

6.3.3. Classification according to the technique used

According to the chromatography support, we distinguish:

- ✓ Column chromatography, where the stationary phase is contained in a metal or glass cylinder.
- ✓ Planar chromatography (paper chromatography or thin-layer chromatography).

6.4. Fundamental quantities and definitions

6.4.1. Stationary (fixed) phase

The stationary phase can be solid or liquid. The solids, treated silica or alumina, allow the separation of the components of mixtures due to their adsorptive properties. They can be used as column packing (gravity chromatography and high-performance chromatography or HPLC) or spread in a thin layer on a glass, aluminum, or plastic sheet (thin-layer chromatography or TLC). The stationary phase can also be made up of a liquid impregnating a solid support or even a carbon chain attached to a support (grafted phase). Thus, in paper chromatography, the stationary phase is formed by the water that the cellulose molecules of the paper adsorb, whereas in gas chromatography, it consists of a low-volatility and thermally stable liquid impregnating a porous granule.

6.4.2. Adsorption

It is the binding of dissolved molecules by the stationary phase. This fixation is due to the establishment of secondary surface bonds between the adsorbent and the adsorbed molecule: dipole-ion bond, or dipole-dipole bond, or Van der Waals bond…

6.4.3. Adsorbents

These are highly divided solids (adsorption being a surface phenomenon, the adsorbent must present the largest possible useful surface area). The quality of an adsorbent depends on its purity, its surface area, its homogeneity, and its water content.

6.4.4. Solvents or eluents

For a given chromatographic system, the eluting power depends on the polarity of the solvent. The more polar an eluent is, the more easily it will carry a polar substance. On the other hand, a non-polar solvent will have poor eluting power toward polar substances but will easily carry a non-polar component. We use either a pure solvent or a mixture of several solvents to "adjust" its eluting power to the chromatographic system being studied.

6.4.5. Notion of time

6.4.5.1. Retention time tr

A constituent is characterized by its retention time tr, which represents the time elapsed between the moment of injection and the moment that corresponds on the chromatogram to the maximum of the peak associated with it. The retention time is independent of the injected quantity.

6.4.5.2. Dead time tm

The dead time tm is the time it takes for a solute not retained by the stationary phase to pass thru the column (time spent in the mobile phase).

6.4.6. Elution volume or retention volume Vr

The elution (retention) volume Vr of each solute represents the volume of mobile phase necessary to make it migrate from one end of the column to the other. It corresponds on the chromatogram to the volume of the mobile phase that has flowed between the moment of injection and the one corresponding to the peak maximum. If the flow rate D is stationary, Vr = tr.D

6.5. Main chromatographic methods

6.5.1. Thin-layer chromatography (TLC)

Recent developments in thin-layer chromatography, TLC or (TLC, for thin-layer chromatography), have allowed it to transition from a semi-quantitative method to a quantitative method, capable of producing very low results. It is now an instrumental technique just like other forms of chromatography.

Thin-layer chromatography (TLC) is primarily based on adsorption phenomena: the mobile phase is a solvent or a mixture of solvents, which progresses along a stationary phase fixed on a glass plate or on a semi-rigid sheet of plastic or aluminum. After the sample has been deposited on the stationary phase, the substances migrate at a speed, essentially by capillarity (Fig. 31). Furthermore, each component of the sample moves at its own speed behind the solvent front. This speed depends on the one hand on the electrostatic forces retaining the component on the stationary plate and on the other hand on its solubility in the mobile phase. The compounds thus move alternately from the stationary phase to the mobile phase, the retention action of the stationary phase being mainly controlled by adsorption phenomena. Generally, in thin-layer chromatography, substances of low polarity migrate more quickly than polar components.

The identification of isolated substances is done using different methods: Directly if the substances are colored, or with the help of developers if they are colorless in order to transform them into colored spots; the products are often detected by their classical functional reactions: amino acids by ninhydrin which gives a blue-violet color with most, organic acids by colored indicators, sugars by Molisch's reagent which uses the reducing power of sugars. Some reagents like iodine or permanganate give non-specific colorations with most organic compounds. The Rf is characteristic of a product in a given eluent and for a given stationary phase

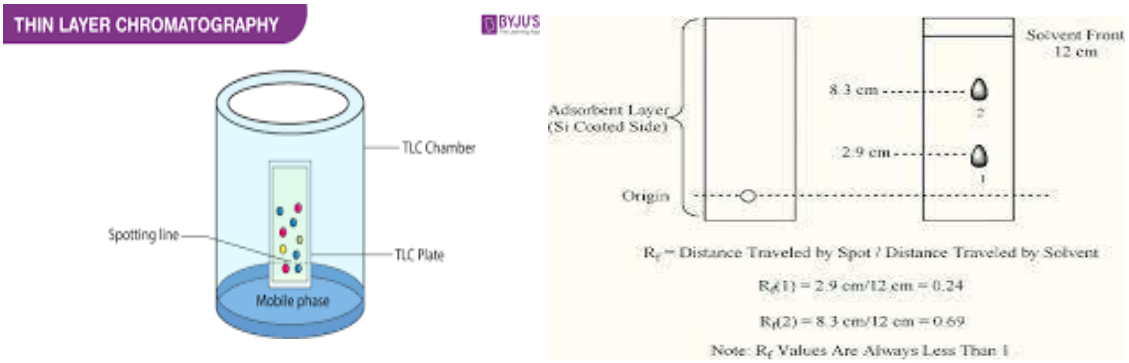


Figure 24. Thin-layer chromatography and Rf values

6.5.1.1. Applications

- TLC allows for easy and rapid monitoring of the purity of an organic compound.
- Continue the progression of a reaction (indicates the number of components in a mixture).
- TLC is also the technique usually used to search for the best solvent before undertaking a separation by column chromatography.

6.5.2. Two-dimensional thin-layer chromatography (2D TLC)

2D TLC is a technique used when the separation of the components of a mixture is not sufficient with classical chromatography. It involves using a square plate on which the sample is applied near a corner. A first development is carried out with a solvent, then, after drying, the plate is rotated by 90° and a second development is performed with another solvent. This method allows for better separation, even for very similar compounds, on a small surface (10 cm x 10 cm).

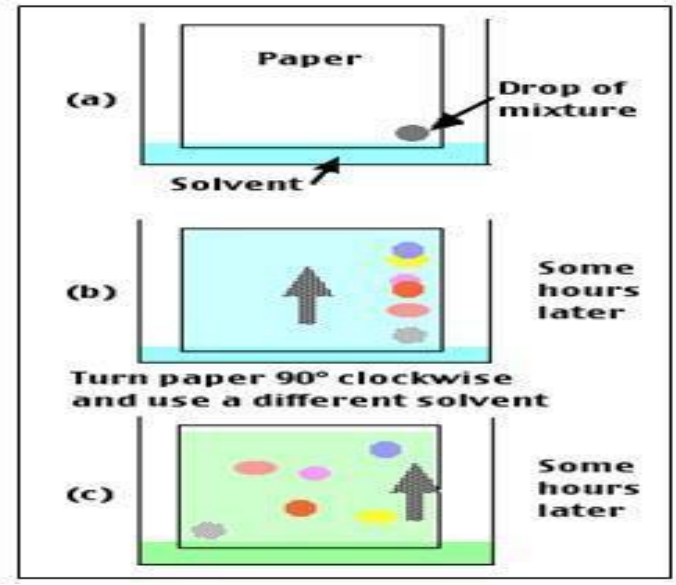


Figure25. Two Dimensional Techniques in Paper Chromatography

6.5.3. High-performance thin-layer chromatography (HPTLC)

HPLC is an improved version of thin-layer chromatography, offering higher resolution thanks to better quality adsorbents (purified or modified silica gel with 3-5 µm particles). This technique allows for faster separations, uses small amounts of samples (100-200 nL) forming very compact spots, and allows for more samples to be deposited on the same plate. It offers up to 5000 theoretical plates, surpassing classical TLC. The precise introduction of the sample, often via an iridium-platinum capillary, is an essential step to ensure the quality of the results.

In quantitative analysis by TLC-HPLC, direct photometric measurements are often performed using scanning densitometers. These devices analyze the spots on the plate by reflection or absorption of a

light beam. The difference in light intensity between the adsorbent and the spots generates a series of peaks on the recording, the area of which is proportional to the amount of substance present.

6.5.4. paper chromatography

Paper chromatography is a liquid phase chromatography method based on a partitioning phenomenon between two phases: a stationary phase (water adsorbed on the cellulose of the paper) and a mobile phase (often a mixture of water and organic solvent). The sample is placed on the paper, and the components migrate at different speeds depending on their solubility and affinity with water. Highly polar compounds, such as amino acids, sugars, and polyfunctional compounds, are particularly well analyzed by this technique.

6.5.5. partition chromatography

Partition chromatography is a liquid-liquid chromatography technique based on the difference in solubility of substances in two immiscible liquids. One liquid is retained on an inert support (stationary phase) and the other moves (mobile phase). The separation is based on the partition coefficient of the solutes between the two phases: the compounds more soluble in the mobile phase migrate more quickly.

We distinguish two types according to the polarity of the phases:

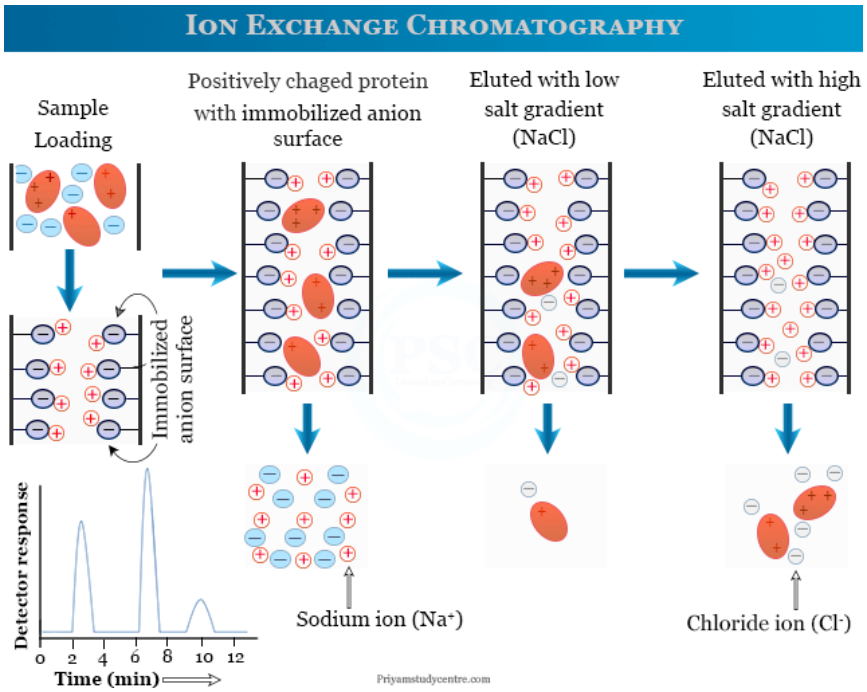
- **Classical partition chromatography:** polar stationary phase and non-polar mobile phase.
- **Reversed-phase chromatography:** nonpolar stationary phase and polar mobile phase (e.g., water/methanol).

6.5.6. ion exchange chromatography

Ion exchange chromatography is a separation technique based on the selective binding of anions or cations by ion exchange resins, which are insoluble macromolecules bearing ionized groups (positive or negative). It is generally performed in a column, but can also be done on a thin layer or in batch.

The stationary phase is a solid, stable, insoluble resin, often in the form of spherical particles, containing ionized functional groups capable of exchanging ions with those in the solution.

The principle is based on the fixation of the sample's ions on the resin, followed by a buffer wash, then a gradual elution according to pH, allowing the fixed ions to be detached. The detection is generally done by conductometry.



❖ **Applications**

Ion exchange has been used in the laboratory since the preparation of demineralized water to the analysis of ionic species in a sample. This chromatographic technique is particularly applicable to the analysis and separation of mineral salts, amino acids (peptide hydrolysates), peptides (particularly hormonal ones), proteins, nucleotides, nucleic acids, ionized lipids, ionized carbohydrates, etc.

It is also used for decalcification, decarbonation of drinking water, elimination of bitter compounds from fruit juices, deacidification of citrus juices, acidification of milk in cheesemaking, demineralization of whey, and decolorization of syrup in refineries.

6.5.7. Exclusion chromatography

Exclusion chromatography, or gel filtration or gel permeation or molecular sieving, is based on the "retention" of solute molecules according to their size due to their penetration into the pores, filled with solvent, of an appropriate stationary phase. If we assume that the solute molecules have no affinity for the walls of the particulate stationary phase, the large molecules will not be able to penetrate the pores; they will migrate more quickly than the small molecules, which can, in turn, penetrate a greater number of pores.

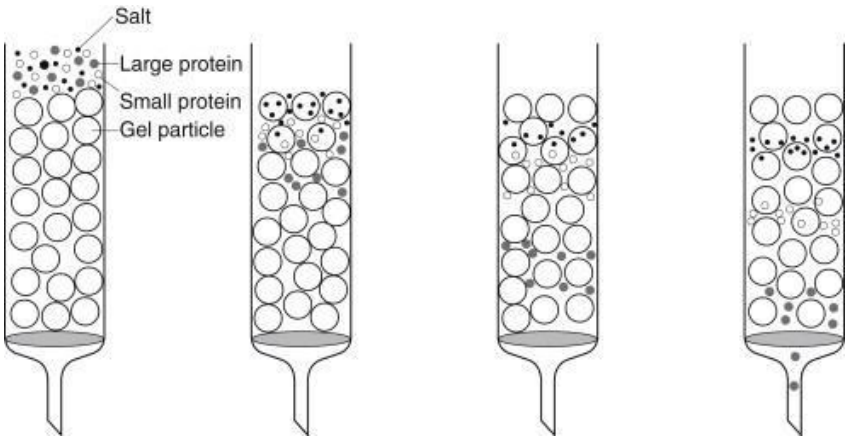


Figure 26. Exclusion chromatography

❖ **Applications**

The applications of molecular sieving are analytical or preparative, for example:

- The demineralization of protein solutions.

- The separation of peptides, proteins, triglycerides, osides, dispersion of organic products.

- Separation of viruses.

-The determination of the molecular masses of macromolecules, especially proteins

6.5.8. Column chromatography

Column chromatography is a preparative method. It is a technique based on adsorption phenomena. The solid phase, most often alumina or silica, fills a column of variable length and cross-section; the sample, in concentrated solution, is placed at the top of the column, and the separation of the components results from the continuous flow of an eluent, passing thru the column by gravity or under the effect of low pressure. One can use a single solvent as the eluent or gradually increase the polarity of the eluent to accelerate the movement of the compounds.

The molecules are pulled downwards at varying speeds depending on their affinity for the adsorbent and their solubility in the eluent. The chromatogram develops by forming a succession of cylindrical zones that separate as they migrate downwards. As each zone flows out of the column, it is collected.

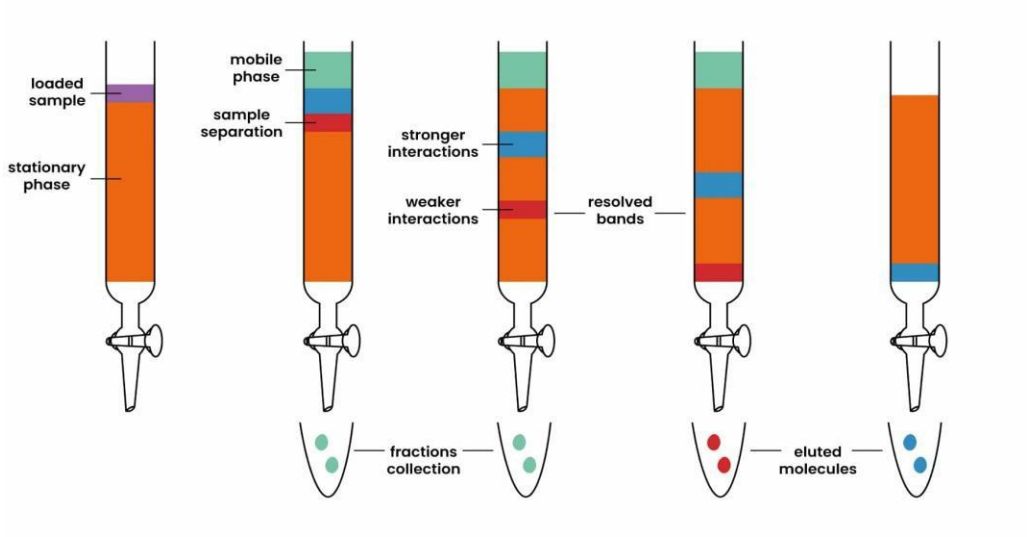


Figure 27. Column chromatography

6.5.9. Affinity chromatography

In this type of chromatography, the stationary phase is a chemically inert macromolecular support onto which an effector is grafted that exhibits a biological affinity for a solute in the sample to be analyzed. Three types of affinities are used: Enzyme-substrate affinity, ligand-receptor affinity, and antigen-antibody affinity. Very often, the molecule being fixed will be the substrate, the ligand, or the antibody. This will allow for the purification of the enzyme, the receptor, or the antigen, respectively.

- **Applications**

The technique is suitable for either the analysis or the preparation of biological substances. It is practiced, depending on the case, in a column or in a batch. It is used:

- In enzymology, for the extraction of enzymes and the purification of enzymatic extracts.
- In immunology, for the purification of antibodies.
- In protein chemistry, for the study of membrane proteins.
- In nucleic acid chemistry, for the fractionation of various nucleic acids (mRNA, rRNA….etc).

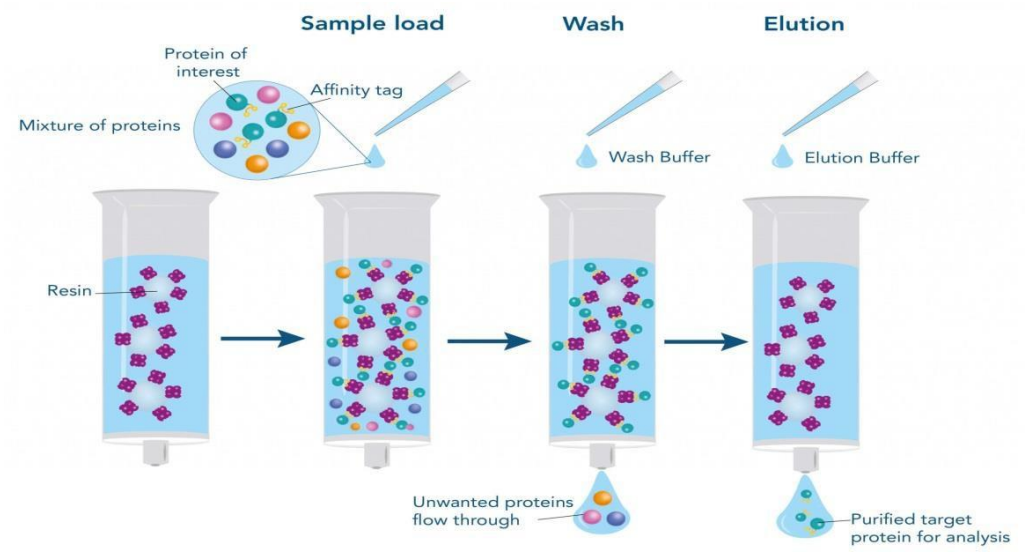


Figure 28. Affinity chromatography

6.5.10. High-performance liquid chromatography (HPLC)

High-performance liquid chromatography, often referred to by its abbreviation HPLC – CLHP in French, is a very general analytical technique. It derives from the oldest form of column liquid chromatography, whose performance, in terms of selectivity and resolution, has been greatly improved by miniaturization and the use of highly sophisticated stationary phases. These phases, composed of spherical micro-particles with a diameter ranging from 2 to 5 micrometers, lead to a significant pressure drop in the column. Which led to applying high pressure on the mobile phase to achieve a suitable flow rate. To mark this particularity of the technique, the letter P in the acronym HPLC has for a long time corresponded to the word pressure. Today, the P has been assigned to Performance to mark the innovation of this separation technique.

The sample is injected into a column filled with a stationary phase of fine granulometry. The flow rate of the mobile phase is high (increase in pressure). This high flow rate reduces the time needed to separate the components. The fine particle size of the stationary phase allows for better separation of the components. The peaks obtained are narrower, so the resolution is improved. The combination of high speed and high resolution - leads to the designation: high performance.

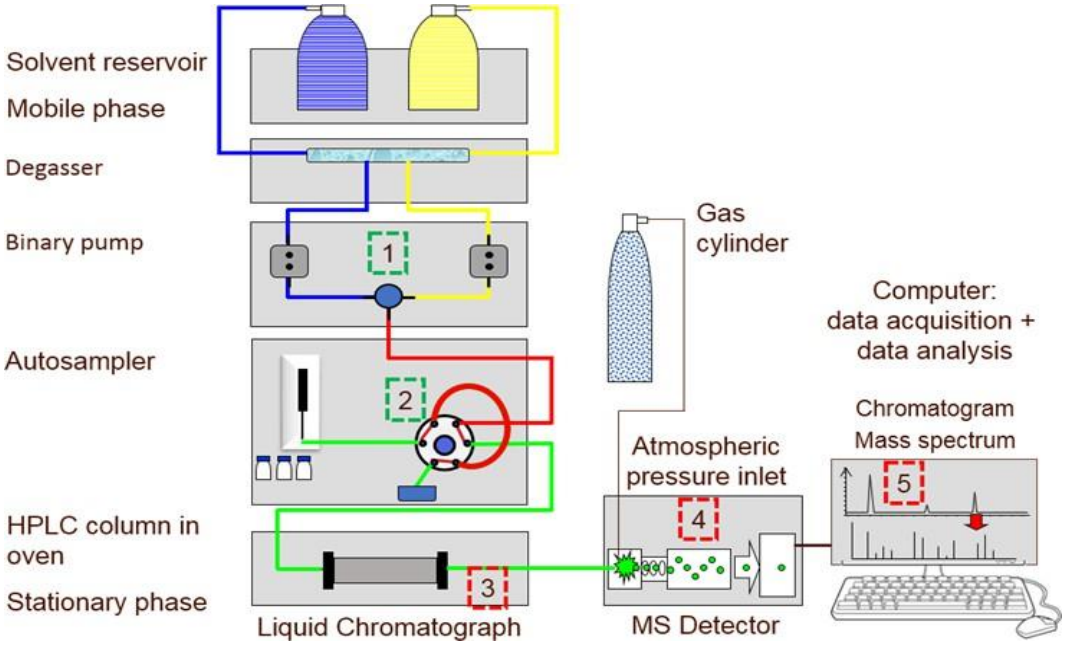


Figure 29. High-performance liquid chromatography

6.5.11. Gas chromatography (GC)

Gas chromatography (GC) is a technique that allows for the separation of different components of a very complex mixture of gasses or liquids by passing a mobile gas phase over a solid or liquid adsorbent stationary phase. It mainly applies to gaseous compounds or those likely to become volatile by heating but thermostable. There are two types of gas chromatography based on the stationary phase:

- ✓ Gas-solid chromatography (GSC): It is an adsorption chromatography; the stationary phase is a porous solid, reserved for the analysis of mixtures of gasses or liquids with low boiling points.
- ✓ Gas-liquid chromatography (GLC): It is a partition chromatography; the stationary phase is a liquid immobilized on a solid support by impregnation or grafting.

The principle of separation is based on a difference in the distribution of the compounds of a mixture between two phases, a mobile phase (the carrier gas) and a stationary phase. The mixture to be analyzed is injected into the column in the form of a fluid and is vaporized in the injector. The carrier gas carries it into the thermostated separation column. The compounds of the mixture distribute differently in the two phases, thus moving at different speeds. This phenomenon of interaction thus causes the separation of the components of the mixture. An adequate detection system at the column's outlet allows for the creation of a signal that is recorded. The intensity of the signal is proportional to the amount of compounds detected. At their exit, they are detected and peaks appear on the recorder.

- ❖ **Instrumentation**

A GC device consists of three parts: injector, column, and detector thru which a carrier gas carries the substances of a mixture to be separated. The most commonly used gas is helium, the others are

hydrogen, nitrogen, or argon. It must be very pure and especially contain neither oxygen nor water. The gas flow is adjusted by a regulator.

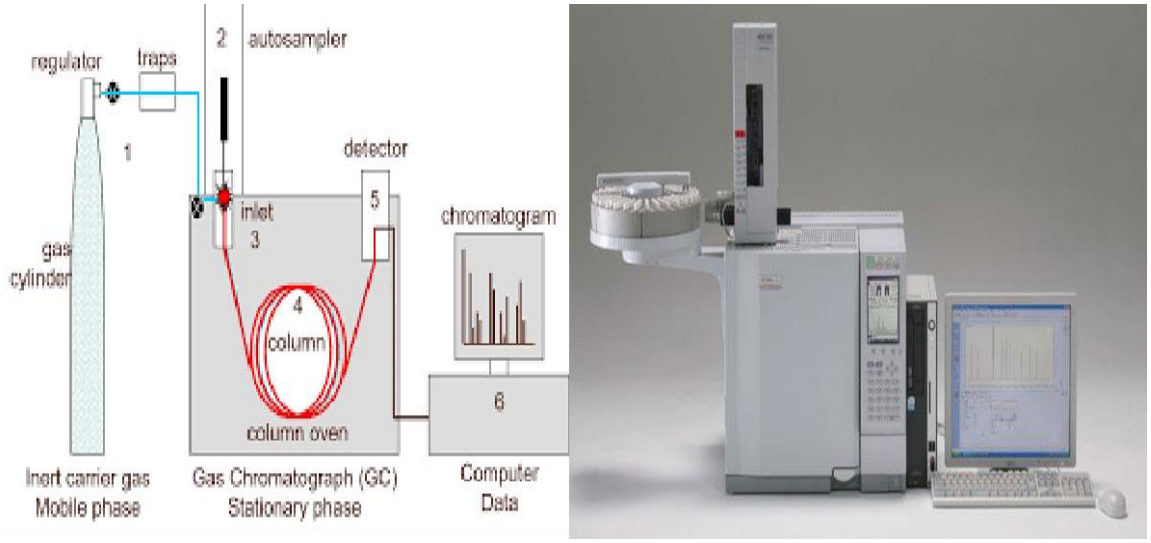


Figure 30. Diagram of a gas chromatography.

- **Application**

Gas chromatography (GC) is widely used across various industries for the separation, identification, and quantification of volatile and semi-volatile compounds. In environmental science, it helps monitor air and water pollution by detecting harmful organic compounds. The pharmaceutical industry uses GC for drug purity testing, residue analysis, and pharmacokinetic studies. In petrochemicals, it analyzes the composition of fuels, natural gas, and crude oil. GC is also essential in the food and beverage industry for flavor analysis, detecting pesticide residues, and verifying additives. Forensic scientists use it to detect drugs, alcohol, and explosives, while clinical and biomedical applications include blood alcohol testing and disease diagnosis. Additionally, it plays a key role in quality control and process monitoring in chemical manufacturing, and in regulatory testing for tobacco and cannabis products.

Electrophoresis

7.1. Definition

Electrophoresis is a qualitative and quantitative analytical method, as well as a preparative method (identification and quantification) and fractionation method based on the differential migration of electrically charged particles under the influence of an electric field. According to the nature of the charged particles, we distinguish:

- ✓ **Ionophoresis:** The charged particles are mineral or organic ions: mineral cations, amino acids, boric esters of sugars, sulfuric and phosphoric esters of sugars….etc.
- ✓ **Electrophoresis:** The charged particles are macromolecules bearing ionizable groups.
- ✓ **Cataphoresis:** The migrating particles are neutral and the charge is acquired by adsorption of foreign ions.

7.2. Principle of migration

Migration depends on several factors:

- **Electrophoretic mobility μ**

The electrical mobility is the speed at which particles move in the suspension, and it depends on the charge and geometry of the particle.

- **The charge Q**

The charge depends on the isoelectric pH of the particle and the pH of the buffer.

The difference pH - pHi determines the sign of the charge Q of a particle:

- if pH > pHi net negative charge (anion) migration toward the anode
- if pH < pHi net positive charge (cation) migration toward the cathode
- if pH = pHi i net zero charge no migration

The difference between pH and pHi determines the intensity of the charge Q of a particle.

- **The size**

The size of the molecules has a certain influence, particularly in the case where the molecules pass thru a porous matrix. The smaller the size of the molecules, relative to that of the pores, the more easily the molecules will move, which obviously implies that they will migrate quickly.

- **The affinity of the molecules for the matrix.**
- **Electric field E**

➢ **Liquid currents:** They include the Electrolysis Current that occurs in the tanks and leads to changes in the pH of the compartments, and the Evaporation Current that is accompanied by

a heating of the support. To limit this phenomenon, the tank is closed with a lid; refrigerated tanks are also used.

- **Electro-endosmosis current:** Under experimental conditions, the support becomes negatively charged; a mobile layer of positive charges forms in the solvent, in contact with the support, and overall drives the liquid phase toward the cathode. This current accelerates or slows down the migration of molecules.

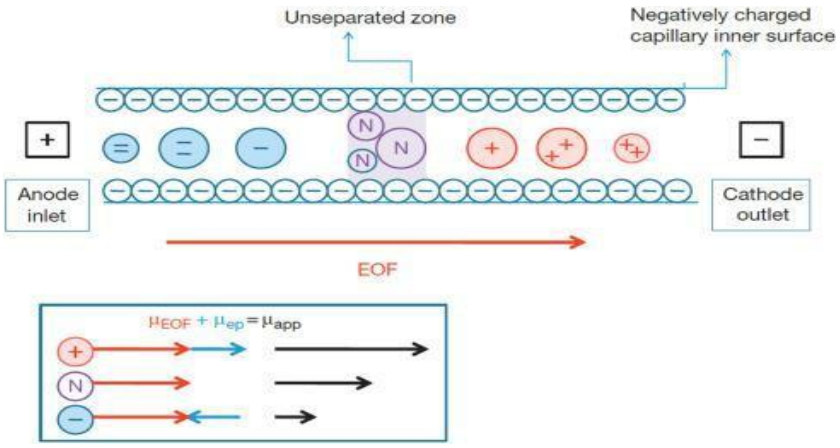


Figure 31. Electroendosmosis current

7.3. Materials

7.3.1. Mounting There are two types

1. Horizontal mounting

This type of setup is mainly used for matrices such as cellulose acetate or paper where the samples move on its surface (the matrix is in the form of a narrow strip). Horizontal setups are also used for agarose matrices during nucleic acid electrophoresis or immunoelectrophoresis.

2. Vertical mounting

This type of setup is mainly used for matrices such as polyacrylamide gels or, more rarely, agarose gels. The samples generally move within the matrix.



Figure 32. vertical and Horizontal mounting

7.4. Main electrophoresis matrices

The matrix of an electrophoresis is the physical support on which or in which the sample will be deposited and its molecules will migrate. There are several types of matrices: Paper, Cellulose acetate, Starch, Agarose, and Polyacrylamide.

7.4.1. Agarose

Agarose, a natural colloidal polysaccharide extracted from algae, agarose gels have large "pores" and are primarily used to separate large molecules with a molecular mass greater than 200 kDa. It is the matrix very often used for the electrophoresis of nucleic acids. linear. Immunoelectrophoresis is also performed on agarose gel. More and more, this matrix is used for the separation of serum proteins.

7.4.2. Polyacrylamide

Polyacrylamide gels are obtained by polymerizing the acrylamide monomer in the presence of another bifunctional and thus cross-linking monomer, N,N'-methylene bisacrylamide (or an equivalent). The polymerization is generally initiated by ammonium persulfate in the presence of the accelerator N,N,N',N'-tetramethylethylenediamine (TEMED). TEMED catalyzes the formation of free radicals from persulfate. Free radicals initiate polymerization.

Generally, the acrylamide/bisacrylamide/solution mixture is poured between two glass plates and thin spacers. The polymerization is completed in a few minutes.The porosity of the obtained gels depends on the total monomer concentration and the proportion of cross-linking monomer agent (bisacrylamide).

7.5. Buffers

The effective separation by agarose or polyacrylamide gel electrophoresis depends on the effective maintenance of the pH in the matrix. The different categories of available buffer systems for electrophoresis are:

7.5.1. Denaturing and Non-Denaturing Buffer System

The separation based on molecular weight requires the inclusion of denaturing agents, which unwind the strands of nucleic acids and eliminate the influence of shape on their mobility. Denaturing requires the disruption of these hydrogen bonds. The most commonly used denaturing buffer systems include urea and formamide as DNA denaturants. Denaturing buffer systems for proteins include the use of sodium dodecyl sulfate (SDS). SDS is an anionic detergent that denatures secondary and tertiary structures (but not disulfide bonds), and applies a negative charge to each protein in proportion to its mass.

Heating the samples to at least 60 °C denatures the molecules by helping the SDS to bind. As a result, when these samples are electrophoresed, the proteins separate according to mass only, with very little effect from compositional differences. DNA molecules are negatively charged; therefore, the addition of SDS to the gel is solely for the purpose of increasing the resolution power of the bands.

7.5.2. Continuous and discontinuous buffer systems

In continuous buffer systems, the identity and concentration of the buffer components are the same in the gel and the reservoir. Although continuous buffer systems are easy to prepare and provide adequate resolution for some applications, the bands tend to be wider and consequently result in poorer resolution in these gels. These systems are used for most forms of agarose gel electrophoresis of DNA.

Discontinuous systems use different buffers for the reservoir and the gel, and often two different buffers in the gel. Discontinuous systems concentrate the samples in a very narrow zone before separation, leading to improved resolution and band sharpness. The gel is divided into a concentration gel (pH 6.8) at the top with a low percentage of acrylamide and a separation gel (pH 8.8) at the bottom with a higher percentage of acrylamide.

The stacking and separating gels contain only chloride as the mobile anion, while the reservoir buffer contains glycine as its anion, at a pH of 8.8. The main advantage of the discontinuous system is that this gel system can tolerate larger sample volumes.

7.5.3. Loading buffers

The loading buffer contains glycerol or sucrose, which makes the sample denser and thus allows it to stay at the bottom of the loading well. It also contains dyes that allow for tracking the migration of samples thru the gel, such as bromophenol blue.

7.6. Applied current

The voltage of the current depends on the length of the fragments and the concentration of the gel. The higher it is, the faster the samples migrate. However, high voltage causes a huge increase in the temperature of the buffer and the current in a very short time. Therefore, it is recommended not to exceed 5-8 V/cm and 75 mA for standard-sized gels or 100 mA for minigels.

7.7. Staining and visualization

The simplest way to visualize proteins is to stain them. Before proceeding with this staining, the proteins must be fixed by placing the gel in a diluted acid such as acetic acid or 1 to 10% trichloroacetic acid.

The most commonly used dye is Coomassie blue. There is also Ponceau red, Amido-schwartz (black), and Lissamine green.

The decolorization is done by transferring the gel into an acidic methanolic solution. We obtain a transparent gel with colored protein bands.

The most commonly used intercalating agent to make DNA bands visible under ultraviolet light is ethidium bromide. The dye can be included in both the buffer and the gel.

7.8. Applications

Electrophoresis allows for the separation of charged molecules: proteins, peptides, amino acids, nucleic acids, and nucleotides. Under certain conditions (use of micelles of ionic detergents), it allows for the separation of non-ionic molecules, such as steroid hormones for example.

- Separation of serum proteins on cellulose acetate
- Polyacrylamide gel electrophoresis SDS-PAGE

It is a widely used technique in biochemistry, forensics, genetics, and molecular biology to separate proteins based on their electrophoretic mobility (depending on the length of the polypeptide chain or the molecular weight). SDS-PAGE of samples with identical charge due to SDS binding leading to migration by size fractionation.

7.9. Electrophoresis types

7.9.1.Electrofocusing

Electrofocusing or isoelectric focusing advantageously uses the charge that each protein possesses at a given pH. It consists of a migration, induced by an electric current, of proteins in a pH gradient until they reach a pH equivalent to their pHi, at which point they stop migrating, since their net charge is zero. We use a gel of high porosity (polyacrylamide or agarose) so that size does not influence the migration. The pH gradient is generated by ampholytes, synthetic amphoteric molecules introduced into the gel at the time of its preparation. These molecules migrate quickly in the gel until they reach an area where their charge becomes neutral. They generate a substantially linear pH gradient along the gel.

This technique, which separates polypeptides based on their charge rather than their mass, is very sensitive: it can distinguish proteins with a pHi difference as small as 0.01 pH units.µ

Two-dimensional electrofocusing is a method that allows for the determination of a titration curve for a given protein (charge = function of pH). We prepare a gel in which we establish the pH gradient, then we deposit the protein solution in a central trough, after which we turn the plate by 90° and run an electric current again

7.9.2. Two-dimensional electrophoresis

Two-dimensional electrophoresis is one of the most powerful and common methods for separating hundreds to thousands of proteins extracted from tissues, cells, etc. The principle of protein separation in two-dimensional electrophoresis is carried out in two main steps: 1st dimension and 2nd dimension. In the 1st dimension, the proteins are resolved based on their isoelectric point and separated into a narrow band in a pH gradient using isoelectric focusing (Fig.47). In the 2nd dimension, the proteins are separated according to their molecular weight by SDS-PAGE. Both separations are performed in polyacrylamide gel. Proteins are more effectively separated by two-dimensional electrophoresis rather than SDS-PAGE.

A remarkable feature of two-dimensional electrophoresis is that the resolution obtained during the first separation is not lost when the gel is joined to the SDS-PAGE gel in the second electrophoresis.

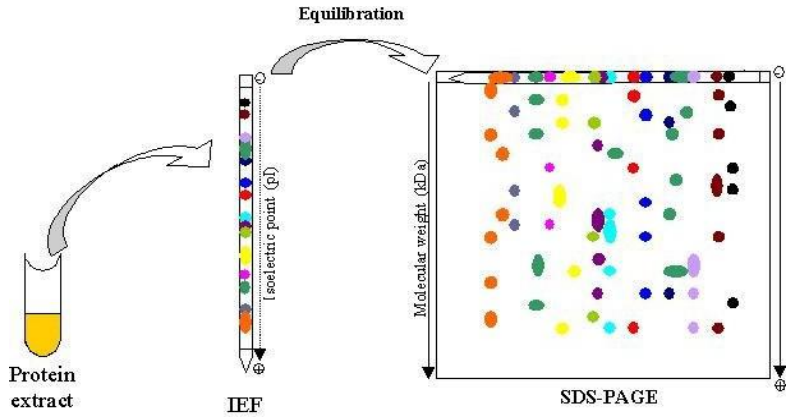


Figure 33.Two-dimensional electrophoresis

7.9.3. Immunoelectrophoresis

Immunoelectrophoresis is a general name for a number of biochemical methods for separating and characterizing proteins based on electrophoresis and reaction with antibodies. All variants of immunoelectrophoresis require immunoglobulins, also known as antibodies that react with the proteins to be separated or characterized.

This technique is used for the detection of small amounts of proteins and the study of the purity of a protein by allowing the confirmation of the presence of a single antigen. The 1% agarose gel, about 1 mm thick, buffered at a high pH (around 8.6), is traditionally preferred for electrophoresis, as well as for the reaction with antibodies.

Agarose was chosen as a matrix because it has large pores allowing for the free passage and separation of proteins, but it also provides an anchor for immunoprecipitated proteins and specific antibodies. The high pH was chosen because antibodies are practically immobile at high pH.

Immunoprecipitates can be seen in the wet agarose gel, but they are stained with protein dyes like Coomassie Brilliant Blue in the dried gel. Unlike SDS-gel electrophoresis, agarose electrophoresis allows for native conditions, preserving the native structure and activities of the proteins under investigation, so immunoelectrophoresis allows, in addition to electrophoretic separation, the characterization of enzymatic activity and ligand binding, etc.

There is actually a whole set of techniques that use antibodies associated with electrophoretic separations. We can distinguish the following techniques:

7.9.3.1. Immuno-electrophoresis of Grabar and Williams

This technique is used for the separation and identification of a mixture of antigen (Ag). The support used is agarose gel, which has two types of wells: a punctual well for the deposition of the Ag (serum to be analyzed) and a trough-shaped well for the deposition of the antibody (Ab). First, electrophoresis is performed on the gel after the serum is deposited. In a second step, the Ac is placed in the well. This Ac can be specific to a targeted protein or be a mixture of Ac. Ag and Ac diffuse into the gel. When the Ag meets its specific Ac, the Ac-Ag complex precipitates. The maximum precipitation occurs when the Ag-Ac ratio corresponds to the equivalence zone. When the Ag-Ac precipitation is completely finished, the plate is washed with a saline solution. The revelation is done

using the appropriate dye (Coomassie blue). Identification is carried out by comparison with a control serum treated under the same conditions. Fractionation methods 76 normal treated under the same conditions. With a total antiserum, one can, for example, distinguish 30-40 proteins in human serum.

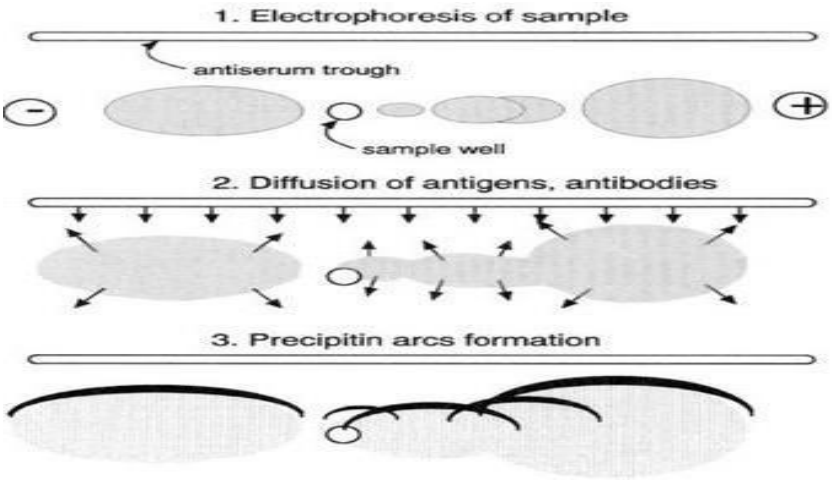


Figure 34. Immuno-electrophoresis of Grabar and Williams

7.9.3.2. Double electro-immunodiffusion (electrosyneresis)

This method actually derives from Ouchterlony's double immunodiffusion technique. It consists of accelerating the diffusion by an electric field. The electrophoretic conditions are chosen so that the antigens and antibodies migrate in opposite directions, this is possible because the pHi of Immunoglobulins is higher than that of many proteins.

7.9.3.3. One-dimensional electro-immunodiffusion (Laurell)

This is a quantitative method that allows for the determination of a particular antigen present in a mixture. The proteins are deposited on gels containing the antiserum. We place ourselves at a pH where the Ig migrate little. The proteins move and encounter the antibodies, which then form rocket-shaped precipitates called "rockets" whose height is proportional to the protein concentration. We use some of the wells to create a calibration.

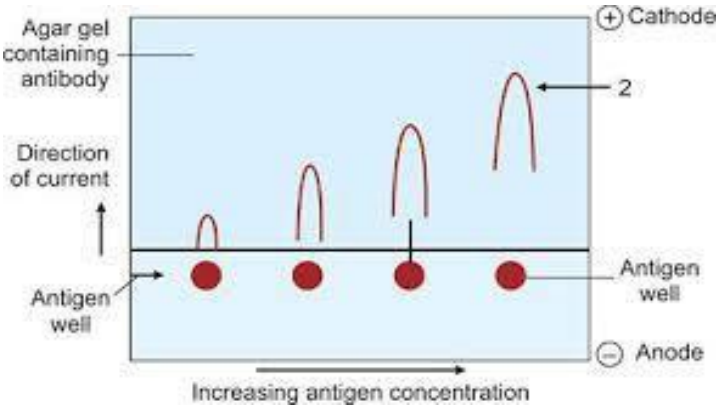


Figure 35. One-dimensional electro-immunodiffusion

7.9.3.4. Two-dimensional electro-immunodiffusion (Laurell)

We separate the proteins in a first dimension in agarose gel. We then pour a gel containing the polyspecific antiserum, and then we perform the second dimension.

7.9.3.5. Pulsed-field electrophoresis

This technique is used for the electrophoresis of high molecular weight DNA molecules (15 -100 kb). In this size range (beyond 20 kb), the molecules are no longer separated by classical methods, as migration becomes independent of size. The use of two orthogonal fields used alternately causes the DNA molecules, which take some time to orient themselves in the direction of the electric field, to migrate only when this orientation is achieved. The time required for orientation is all the greater as the DNA molecule is longer.

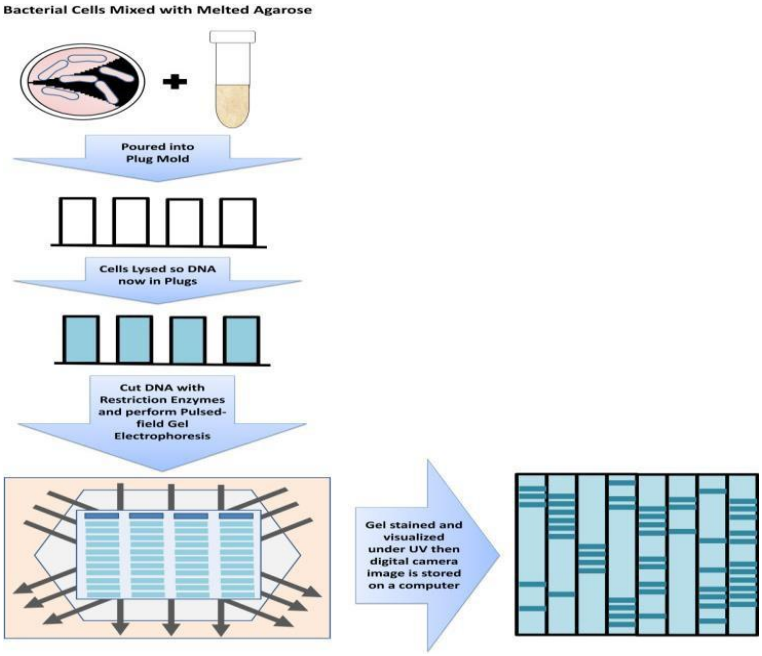


Figure 36. Pulsed-field electrophoresis

7.9.3.6. Capillary electrophoresis

It is a recent technique that is beginning to develop and essentially offers the advantages of speed, very high resolution, and, consequently, very high detection sensitivity. Electrophoresis uses a silica capillary with a diameter of about 50 µm and a length of 1 m (filled with buffer or gel), and high voltages (15 -30 kV). This results in very rapid migration speeds of the compounds in the capillaries, and these are detected by UV absorption, fluorimetry, or conductimetry directly on the capillary, thus in a very small volume. This therefore provides particularly high sensitivity (only a few nanoliters of the sample are injected).

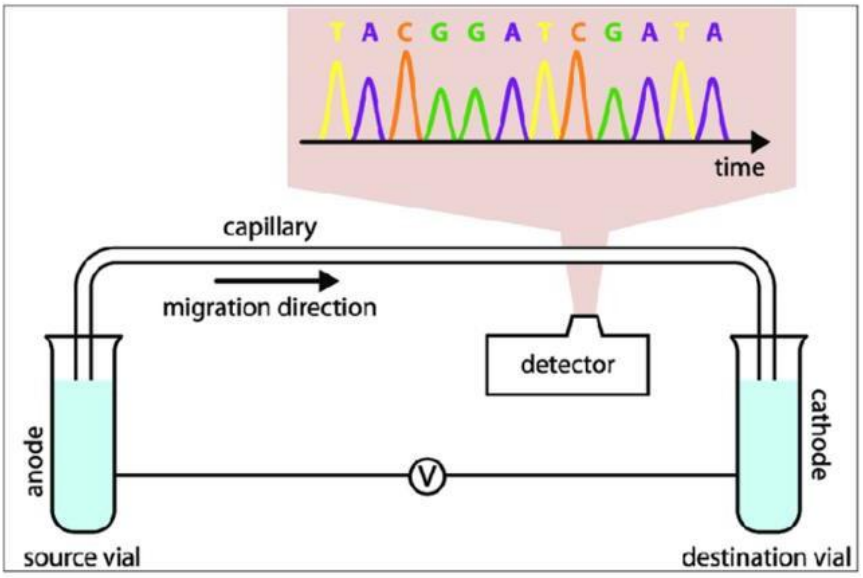


Figure 37. Capillary electrophoresis

The fields of application are a priori numerous: analysis of peptides, amino acids, oligonucleotides,… the number of plates is on the order of 500,000 per meter, which provides remarkable resolution (one can separate peptides differing by one amino acid, complex mixtures of oligonucleotides, tryptic fragments of peptides; the method is used to test the purity of synthetic peptides or oligonucleotides,…). The technique can also be applied to non-ionized molecules in the presence of appropriate detergent micelles.

BIBLIOGRAPHIC REFERENCES

Afnor. Various standards for water analysis (e.g., NF T90-105 for Suspended Solids). Association Française de Normalisation.

Audigié, D., Dupont, G., & Zonszain, F. (1995). Principles of biochemical analysis methods. Volume 1. Ed Doin; Paris, Pp: 4-81.

Bamforth, C. W. (2005). Food, Fermentation and Micro-organisms. Blackwell Science Ltd.

Berg, J. M., Tymoczko, J. L., Gatto, G. J., & Stryer, L. (2019). Biochemistry (9th ed.). W.H. Freeman.

Betts, G. D., et al. (Eds.). (2019). Microbiological Risk Assessment in Food Processing. Woodhead Publishing.

Bonaventura, C., & Henkens, R. (2014). Spectrophotometry and the Lambert-Beer Law: An Essential Analytical Tool. Journal of Chemical Education, 91(12), 2111-2114.

Bottrill, M., Kwok, L., & Long, N.J. (2006). Lanthanides in magnetic resonance imaging. Chem. Soc. Rev., 35(6), 557.

Bourdichon, F., et al. (2012). Food fermentations: Microorganisms with technological beneficial use. International Journal of Food Microbiology, 154(3), 87-97.

Brereton, R. G. (2018). Applied Chemometrics for Scientists. John Wiley & Sons.

Christie, W. W. (2003). Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids (3rd ed.). The Oily Press, PJ Barnes & Associates.

Cserhati, T. (2008). Multivariate Methods in Chromatography: A Practical Guide . John Wiley and Sons Ltd. The Atrium, Southern Gate, Chichester, England.

De Hoffmann, E., & Stroobant, V. (2007). Mass Spectrometry: Principles and Applications (3rd ed.). John Wiley & Sons.

Eaton, A. D., Clesceri, L. S., Rice, E. W., Greenberg, A. E., & Franson, M. A. H. (Eds.). (2017). Standard Methods for the Examination of Water and Wastewater (23rd ed.). American Public Health Association (APHA).

Fellows, P. J. (2009). Food Processing Technology: Principles and Practice (3rd ed.). Woodhead Publishing Series in Food Science, Technology and Nutrition.

Fox, P. F., McSweeney, P. L. H., Cogan, T. M., & Guinee, T. P. (Eds.). (2017). Fundamentals of Cheese Science (2nd ed.). Springer.

Funk, W., Dammann, V., & Donnevert, G. (2007). Quality Assurance in Analytical Chemistry: Applications in Environmental, Food and Materials Analysis, Biotechnology, and Medical Engineering (2nd ed.). Wiley-VCH.

Gänzle, M., Gobbetti, M., & Minervini, F. (2020). The Sourdough Microbiota: Diversity, Metabolism and Functional Role. Comprehensive Reviews in Food Science and Food Safety, 19(5), 2567-2609.

Geankoplis, C. J. (2003). Transport Processes and Separation Process Principles (4th ed.). Prentice Hall.

Grushka, E., & Grinberg, N. (Eds.). (2010). Advances in Chromatography (Vol. 48). CRC Press.

Hames, B. D. (Ed.). (1998). Gel Electrophoresis of Proteins: A Practical Approach (3rd ed.). Oxford University Press.

Harris, D. C. (2010). Quantitative Chemical Analysis (8th ed.). W.H. Freeman and Company.

Hutkins, R. W. (2019). Microbiology and Technology of Fermented Foods (2nd ed.). Wiley-Blackwell.

Kazakevich, Y., & Lobrutto, R. (2007). HPLC for Pharmaceutical Scientists. John Wiley and Sons, Canada.

Kellner, R., Mermet, J.-M., Otto, M., & Widmer, H. M. (Eds.). (2004). Analytical Chemistry: A Modern Approach to Analytical Science (2nd ed.). Wiley-VCH.

Koehler, J., & Meiler, J. (2011). Expanding the utility of NMR restraints with paramagnetic compounds: Background and practical aspects. Prog. Nucl. Magn. Res. Spectr., 59, 360-389.

Larkin, P. (2011). Infrared and Raman Spectroscopy: Principles and Spectral Interpretation. Elsevier.

McNair, H. M., & Miller, J. M. (2009). Basic Gas Chromatography (2nd ed.). John Wiley & Sons.

Mendham, J., Denny, R.C., Barnes, J.D., & Thomas, M. (2008). Quantitative chemical analysis of Vogel. Ed De boek. Paris, Pp: 30-338.

Metcalf & Eddy, Inc., Tchobanoglous, G., Stensel, H. D., Tsuchihashi, R., & Burton, F. L. (2014). Wastewater Engineering: Treatment and Resource Recovery (5th ed.). McGraw-Hill Education.

Meyer, V. R. (2010). Practical High-Performance Liquid Chromatography (5th ed.). John Wiley & Sons.

Miller, J. N., & Miller, J. C. (2010). Statistics and Chemometrics for Analytical Chemistry (6th ed.). Pearson Education Limited.

OECD (1997). OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Organisation for Economic Co-operation and Development. [No. 1: OECD Principles of Good Laboratory Practice].

Pavia, D. L., Lampman, G. M., Kriz, G. S., & Vyvyan, J. R. (2014). Introduction to Spectroscopy (5th ed.). Cengage Learning.

Plummer, D.T. (1987). An Introduction to Practical Biochemistry (third edition). Ed McGraw-Hill, London. Pp: 35-51.

Righetti, P. G. (2005). Isoelectric Focusing: Theory, Methodology and Applications. Elsevier.

Rouessac, F., & Rouessac, A. (2004). Chemical Analysis: Modern Methods and Instrumental Techniques. Ed Dunod, Paris, Pp: 1-309.

Sawyer, C. N., McCarty, P. L., & Parkin, G. F. (2003). Chemistry for Environmental Engineering and Science (5th ed.). McGraw-Hill.

Skoog, D. A., Holler, F. J., & Crouch, S. R. (2017). Principles of Instrumental Analysis (7th ed.). Cengage Learning.

Skoog, D. A., West, D. M., Holler, F. J., & Crouch, S. R. (2013). Fundamentals of Analytical Chemistry (9th ed.). Brooks/Cole, Cengage Learning.

Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2010). Introduction to Modern Liquid Chromatography (3rd ed.). John Wiley & Sons.

Spillane, W. J. (2006). Understanding the Science of Food: From Molecules to Mouthfeel. CSIRO Publishing.

Tamime, A. Y. (Ed.). (2009). Dairy Powders and Concentrated Products. Wiley-Blackwell.

Tchobanoglous, G., & Schroeder, E. D. (1985). Water Quality: Characteristics, Modeling, Modification. Addison-Wesley.

U.S. Food and Drug Administration (FDA). Code of Federal Regulations (CFR) Title 21, Part 58: Good Laboratory Practice for Nonclinical Laboratory Studies.

Waksmundzka-Hajnos, M., Sherma, J., & Kowalska, T. (2008). Thin layer Chromatography in phytochemistry. Taylor and Francis Group.

Westermeier, R. (2016). Electrophoresis in Practice: A Guide to Methods and Applications of DNA and Protein Separations (5th ed.). Wiley-VCH.

Wilson, K., & Walker, J. (Eds.). (2010). Principles and Techniques of Biochemistry and Molecular Biology (7th ed.). Cambridge University Press.