Silvio Vaz Jr

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I dedicate this book to my daughter Ana Cecília T. Vaz and to my father Silvio Vaz (in memoriam).

Preface

This book deals with analytical techniques and methods for several sectors of industry related to chemistry in order to offer a concise and up-to-date reference for the practical application of analytical chemistry.

Its addressed audience can be composed by professionals (chemists, biochemists, engineers, etc.), researchers, professors, and graduate students.

The main topics covered are the fundamentals of analytical chemistry, the modern analytical technologies and their application for sectors and activities as agrochemicals and pharmaceuticals, ores and mining, polymers, biotechnology, oil & gas, and environmental issues of industry.

The book relevance is to offer a large number of analytical technologies associated with analytical methodologies for practical application to solve problems from the main industrial sectors and technological activities related to chemistry turn it in a reference in analytical chemistry. It is divided into 10 chapters that cover from the basis of analytical chemistry to market, analytes, and sample properties to advanced analytical technologies description.

Finally, problems to solve are related to quality control for raw materials, products and processes, contamination of formulation, environmental pollution, evaluation of products and processes, sustainability, research & development, among others.

Chapter 1 demonstrates that analytical chemistry is a strategic science by supply strategic knowledge (e.g., composition and concentration) for all substances and materials produced by the man or found in the nature. Analytical chemistry, as a scientific branch of chemistry, is a generator of knowledge related to the characterization, identification, and determination for several materials from several origins in all states of matter.

Chapter 2 deals with the chemical analysis—the practical branch of analytical chemistry—which can be applied in three different or complementary situations: characterization, identification, and determination of organic and inorganic analytes. These sets of information are involved in the economic and social aspects of relevant industrial and technological activities in the modern society. Furthermore, the

description of classical and instrumental techniques and examples ensures what can be obtained by industry from analytical chemistry.

Chapter 3 deals with the understanding of the application of analytical techniques for the analysis of several analytes of industrial and technological interest, for which it is essential to introduce fundamental terms of analytical chemistry. Figures of merit is the first set because they are parameters of control used in analytical chemistry for the application of a certain analytical method. Then, these terms and applications are explored in this chapter in order to supply the necessary know-how to put into practice the chemical analysis.

Chapter 4 deals with pharmaceuticals and agrochemicals, the most representative family of biologically active molecules obtained by means of industrial processing that embrace a huge diversity of compounds—mainly organic compounds—used as drugs for diseases and pesticides for pest control in modern agriculture. In this chapter, several classes of analytical techniques, as chromatographic techniques, spectroscopic and spectrometric techniques, thermal techniques, among others are presented and discussed in order to be applied in the fine chemical industry and their analytical matrices.

Chapter 5 deals with mining, which corresponds to an economic and industrial activity that consists of research, exploration, extraction, and processing of ores present in the subsoil. Aspects of physical properties of analytical matrices (e.g., powders, solids, semi-solids) and their preparation, and the most common analytical techniques (e.g., atomic emission and absorption spectrometries, X-ray fluorescence and diffractometry) are considered in order to describe relevant applications of those techniques.

Chapter 6 demonstrates that polymers are one of most relevant materials for the modern society to maintain its lifestyle and its life quality. Plastics, fibers, etc. are present in our day-to-day life from the morning (e.g., toothbrush) up to the night (e.g., lampshade). For the quality control and for the research & development of the polymeric materials, we have several advanced analytical techniques, as microscopic (SEM, TEM, AFM), spectroscopic (EDS, NMR, SAXS), chromatographic (GPC), thermal (TGA, DTA, DSC), and sorption/desorption (BET) techniques.

Chapter 7 deals with the industrial biotechnology, a multidisciplinary branch which comprises aspects of organic chemistry, analytical chemistry, biochemistry, chemical engineering, and microbiology in order to explore the biochemical routes of conversion for several classes of organic compounds. In order to guarantee the quality of these bioproducts and their bioprocess and to support the research & development in this field, this chapter discusses chromatographies (gaseous and liquid phases), spectroscopies (absorption in the UV-Vis and infrared regions, Raman, and nuclear magnetic resonance), OMICS, and process analytical chemistry/process analytical technology.

Chapter 8 deals with the oil & gas industry, which has a large usage by the modern society to provide energy, chemicals, and materials. Analytical techniques as GC, MS, AEOS/OES, XRF, and isotopic analysis are explored in this chapter for

their application from the quantification and characterization of the hydrocarbons to the determination of the presence of contaminants, as metals.

And Chap. 9 deals with environmental issues that are a hotspot for the modern industry dedicated to pursuing more sustainable products and processes. Advanced analytical techniques as LC, GC, AAS, and AES/OES to supply quantitative information about the pollutant in aqueous and gaseous effluents are discussed in this chapter. Besides, size distribution analysis to determine the size of the particulate matter and LIBS, to supply quantitative information for carbon footprint, are discussed also.

Finally, Chap. 10 summarizes the main remarks and conclusions by each chapter in order to facilitate the practical application of the book content.

Good lecture!

Brasília, Brazil Silvio Vaz Jr
2023

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I would like to thank Springer Nature's team for the opportunity to publish this book, especially to Sofia Costa for the editorial support. Besides, I would like to thank my family for the support in the manuscript writing.

Contents

Chapter 1
A Holistic View of Analytical Chemistry

Abstract Analytical chemistry is a paramount science by supply strategic knowledge (e.g., composition and concentration) for all substances and materials produced by man or found in nature. Analytical chemistry, as a scientific branch of chemistry, is a generator of knowledge related to the characterization, identification, and determination of several materials from several origins in all states of matter. Considering a broad context, analytical sciences are the experimental basis to understand the matter composition in completely different areas and economic sectors, in order to guarantee, for instance, the best uses of chemicals and related materials in the modern society. Concepts of green analytical chemistry and circular economy can be explored for industrial purposes in order to attribute a sustainable characteristic to the chemical analysis. Trends (e.g., automation, miniaturization, chemometrics, machine learning) and challenges (e.g., computational tools, rapid and automated technologies) are considered in order to pave the road for this branch of chemical science and to reach the whole potential to solve real problems.

Keywords Analytical chemistry · Green chemistry · Green analytical chemistry · Circular economy · Miniaturization · Automation

1.1 Introduction to Analytical Chemistry

Analytical chemistry is a paramount science by supply strategic knowledge (e.g., composition and concentration) for all substances and materials produced by man or found in nature.

From this short statement we can get an idea about the applicability and complexity of analytical chemistry as a branch of chemical sciences. However, the complexity can promote misconception among the people not familiarized with the chemical sciences. Indeed, we can keep in mind an essential difference: the chemical analysis (the common application) is not the analytical chemistry (the scientific and technical fundamentals that promote the application)—this mistake is a source of conceptual errors and misinterpretations.

Analytical chemistry starts its road in the middle of the eighteenth century in Europe with works related to qualitative methods of analysis with its gradual

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development in the nineteenth century. However, its relevance as a scientific field just was recognized in the first decade of the twentieth century. Some chemists should be highlighted during this period for their efforts to create the technical and scientific basis, as the Swedish scientist Torbern Bergman (1735–1784), recognized as the first to introduce a qualitative system of analysis to chemistry; the German chemists Wilhelm Ostwald (1853–1932) and Walther Nernst (1864–1941) are regarded for their work to bring physical chemistry foundations to analytical chemistry; the Danish chemist Søren Peter Lauritz Sørensen (1868–1939) for the pH concept; and the Dutch chemist Izaak Maurits Kolthoff (1894–1993) for the Treatise on Analytical Chemistry series. The latter stated that: "*Analytical chemistry will remain a scientific discipline of chemistry as long as* ***industry*** *continues to make new products, as long as there are unsolved problems in chemistry, and as long as chemistry remains an integral part of the natural sciences.*"[1]

Figure 1.1 depicts a considerable number of industrial and scientific areas directly impacted by analytical chemistry. In order to best understand these impacts we can consider:

- *Agriculture*: analyses of soil, fertilizers, pesticides, chemical residues, several products and their residues, water, effluents, among others.
- *Biotechnology*: OMICs, i.e., genomics, proteomics, metabolomics, metagenomics, phenomics, and transcriptomics for microorganisms and plants.
- *Engineering*: analysis of several materials and processes for several usages (e.g., construction, production, tests, research and development, among others).
- *Industrial chemistry*: analysis of several raw materials, processes of conversion or transformation, products, by-products, residues, and effluents.
- *Nanotechnology*: analysis of surface, composition, particle size, stability, among others.
- *Pharmaceutical chemistry*: as for industrial chemistry, analyses of several raw materials, processes of conversion or transformation, products, by-products, residues, and effluents.

The large number of applications requires a large number of analytical techniques and analytical methods to be seen and explored in this book.

1.2 The Role of Analytical Chemistry in the Modern Society

Analytical chemistry, as a highly applicable scientific branch of chemistry, is a generator of knowledge related to the characterization, identification, and determination for several materials from several origins in all physical states of matter. Considering a broad context, analytical sciences are the experimental basis to understand the matter composition in completely different areas and economic

[1] *Izaak Maurits Kolthoff, 1964 Willard Gibbs Medal Lecture.*

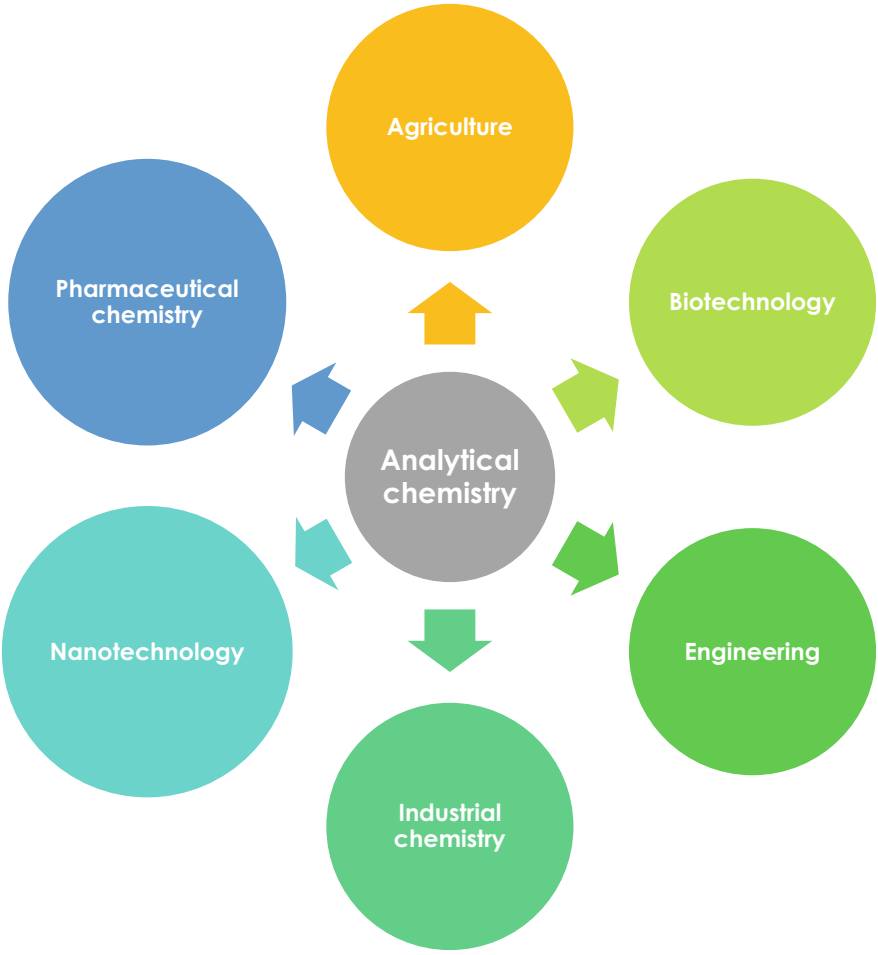

Fig. 1.1 Industrial and scientific areas directly impacted by analytical chemistry

sectors, in order to guarantee, for instance, the best uses of chemicals and related materials by the modern society. Examples of this general role and importance to our society are:

- Advanced materials: application for properties determination, for example, surface area, particle size, and morphology.
- Agriculture: application for assessment of the presence of agrochemical residues in agricultural products.
- Environment: application for control and monitoring of pollutants in air, soil and water, and related matrixes.
- Industry: application for quality control (QC) of raw materials, products, and processes.

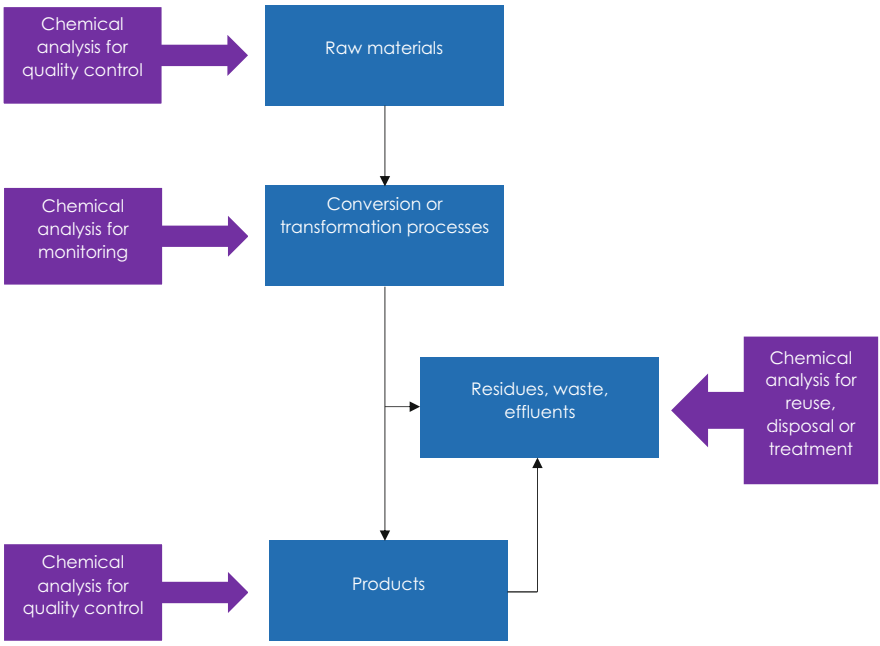

Fig. 1.2 The basic unitary operations for an industry of transformation and their relation with analytical chemistry by means of the chemical analysis Source: Author

- Life sciences: application for diagnostic methods, e.g., clinical analyses.
- Others: application for forensic, art and heritage investigation or evaluation.

And these appointments highlight those applications previously seen in Sect. 1.1.

For the case of industrial purposes in an industry of transformation—as the chemical and pharmaceutical industries—the analytical chemistry roles can be summarized as in Fig. 1.2. We can see that analytical chemistry, by means of the chemical analyses, can be applied for all components and stages of production from the raw material processing to the waste treatment.

1.3 Green Analytical Chemistry

It is an urgency the development and use of techniques and methods according sustainable approaches, wich promotes a reduction in the negative impact on the public health and on the environment from conversion or transformation processes in laboratory and in the industry. From this holistic view, the 12 fundamental principles of green chemistry can aid to achieve these premises. They comprise (ACS Green Chemistry Institute 2023):

- *Principle 1—Prevention*: it is better to prevent waste than to treat or clean up waste after it has been created.
- *Principle 2—Atom economy*: synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
- *Principle 3—Less hazardous chemical syntheses*: wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
- *Principle 4—Designing safer chemicals*: chemical products should be designed to affect their desired function while minimizing their toxicity.
- *Principle 5—Safer solvents and auxiliaries*: the use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.
- *Principle 6—Design for energy efficiency*: energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.
- *Principle 7—Use of renewable feedstocks*: a raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.
- *Principle 8—Reduce derivatives*: unnecessary derivatization (use of blocking groups, protection/deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.
- *Principle 9—Catalysis*: catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
- *Principle 10—Design for degradation*: chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.
- *Principle 11—Real-time analysis for pollution prevention*: analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.
- *Principle 12—Inherently safer chemistry for accident prevention*: substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

Regarding analytical chemistry and their chemical analyses, we can highlight principles 5, 8, 11, and 12 as those that prove to be the most interesting for application in an analytical laboratory.

Furthermore, and to facilitate the understanding, the concept of green analytical chemistry can be addressed for these purposes (Armenta et al. 2008):

- Sample treatment
- Oriented scanning methodologies
- Alternatives to toxic reagents
- Waste minimization
- Recovery of reagents

- The online decontamination of wastes
- Reagent-free methodologies

Waste prevention, safe solvents and auxiliaries, energy efficiency and inherently safer chemistry for accident prevention are obvious requirements for all chemical operations. Safer chemicals, the reduction of derivatives, and the use of catalysts should be taken into account for each analysis because each analytical process has its own technical particularities. For example, the use of real-time analysis for pollution control is a good opportunity for technological development in analytical chemistry in the use of an in-situ system for effluent analyses (gaseous and liquids). In a large number of cases, it is not possible to apply all of these principles due to the particularities of either the sample or the analytical matrix, but is very important to consider these individually in an analytical process. This exercise will ensure the "greenness" of the analysis.

As a practical guidance, De la Guardia and Garrigues (2011) established the main objectives to be considered in a green analytical method:

- Simplification
- The selection of reagents to be avoided based on toxicity, renewability, or degradability data
- The maximization of information
- The minimization of consumables, taking into consideration the number of samples, the volumes, or masses of reagents and energy consumption
- The detoxication of wastes

These objectives will define the best strategy to be applied as a result of the principles of green chemistry. A very useful application of green chemistry principles permeates toxicology for the studies of environmental pollutants, as demonstrated by Crawford et al. (2017), which can promote the reduction of animal testing.

Furthermore, considering green analytical chemistry application for industrial purposes, it is possible to observe a close relation of this "new" area with aspects of sustainability, as the decreasing in the negative environmental impacts for the analytical methods due to a change in attitudes and behavior in the chemical industry (Płotka-Wasylka et al. 2021).

Considering the circular economy context—a model of production and consumption, which involves sharing, leasing, reusing, repairing, refurbishing, and recycling existing materials and products as much as possible (European Parliament 2022)—the use of green analytical methods and techniques according to the green chemistry principles will promote more sustainable production chains.

1.4 Brief Examples of Analytical Chemistry for the Industry

Considering the content of Figs. 1.1 and 1.2, some brief and relevant examples of applications of analytical chemistry for agricultural and industrial chemistry purposes can be addressed. The applications are explored in detail from Chap. 4 to Chap. 9 of this book.

1.4.1 For Agriculture or Agroindustry

Agricultural products, inputs, raw materials, and wastes are very heterogeneous, mainly, due to the biomass chemical composition and their necessities for growth and production which demand a large number of analytical techniques and their methods—it can be seen in the book *Analytical Techniques and Methods for Biomass*, published by Springer (Vaz 2016).

Table 1.1 depicts a variety of application examples for agricultural purposes.

We can observe in Table 1.1 a strong contribution of the instrumental techniques, as spectroscopy and spectrometry (e.g., UV, AAS, and ICP-OES) and chromatography (e.g., GC, HPLC, and IC) in order to obtain quantitative data.

1.4.2 Industrial Chemistry

Industrial chemistry comprises a lot of final products—organic and inorganic—as polymers, paints, petrochemicals, various ingredients, special gases, among many others, in all physical states. These characteristics will generate several and variable analytical demands for steps as the quality control. Some analytical techniques are depicted as follows, as a general overview:

- High performance liquid chromatography (HPLC), using detectors as ultraviolet-visible light (UV-VIS), mass spectrometry (MS), diode array detector (DAD), and refractive index detector (RID)—this is the most common technique for quantification for organic chemicals.
- Gas phase chromatography (GC), using detectors as flame ionization detector (FID) and mass spectrometry (MS)—for quantification of organic chemicals, as volatiles (VOCs) and semi-volatiles (SEMIVOCs).
- Pyrolysis gas phase chromatography mass spectrometry (Py-GC-MS): can be used to characterize most materials including insoluble and complex materials at trace levels often without any sample pretreatment, e.g., polymers, plastics, rubber, paints, dyes, resins, coatings, cellulose, wood, textiles, oils, etc.

Table 1.1 Required analytical information and the corresponding analytical technique for agricultural matrices

Agricultural matrix	Required analytical information and technique	Purpose
Fertilizers	• 2-amino-4-chloro-6-methylpyrimidine (AM) by HPLC-UV (295 nm) • 1-amidino-2-thiourea (ASU) by HPLC-UV (262 nm) • Activity coefficient of nitrogen by titration • Alkalinity by chelatometric titration with EDTA • Ammoniacal nitrogen by distillation and (neutralization) titration • Ammonium thiocyanate (sulfurized cyanide) by IC-ECD or HPLC-ECD • Arsenic by hydride generation AAS or ICP-OES • Ash content by ignition and gravimetry • Biuret nitrogen by HPLC-UV (190 nm) • Cadmium by flame AAS or ICP-OES • Carbon dioxide by thermogravimetry • Carbon-nitrogen ratio • Citrate-soluble boron by azomethine-H and spectrophotometry • Citrate-soluble magnesium by flame AAS • Citrate-soluble manganese by flame AAS • Citrate-soluble phosphoric acid by ammonium vanadomolybdate solution and spectrophotometry (420 nm) analysis • Chlorine by IC-ECD • Chromium by flame AAS • Clopyralid and its degradation products by HPLC-MS tandem • Cold buffer solution soluble nitrogen (water-soluble nitrogen) by cold buffer solution and (neutralization) titration • Dicyandiamide nitrogen by HPLC-UV (215 nm) • Electrical conductivity by electrical conductivity meter • Granularity by dry-type sieving testing • Guanidine nitrogen by HPLC-UV (190 nm) • Guanylurea nitrogen by HPLC-UV (190 nm) • Heat buffer solution soluble nitrogen (hot water-soluble nitrogen) by heat buffer solution and (neutralization) titration • Humic acid (acid insoluble—alkali soluble component) by gravimetry • Initial elution rate by standing-in-water and flow rate • Lead by flame AAS or ICP-OES • Melamine and its degradation products by GC-MS • Mercury by cold vapor AAS • Moisture or moisture content by loss heating • Nickel by flame AAS or ICP-OES	Quality control of products

(continued)

Table 1.1 (continued)

Agricultural matrix	Required analytical information and technique	Purpose
	• Nitrate nitrogen by distillation and titration • Nitrous acid by HPLC-UV (210 nm) • Oil content by diethyl ether extraction (using a Soxhlet extractor) and gravimetry • Organic carbon by dichromate oxidation and titration • pH by electrochemistry (direct potentiometry) • Residue of agrochemicals by multi-component analysis by HPLC-MS • Sodium by flame AAS • Soluble lime by flame AAS • Soluble magnesium by flame AAS • Soluble manganese by flame AAS • Soluble phosphoric acid by ammonium vanadomolybdate and spectrophotometry (420 nm) • Soluble silicic acid by precipitation titration • Sulfamic acid (amidosulfuric acid) by IC-ECD or HPLC-ECD • Total copper by flame AAS or ICP-OES • Total lime by flame AAS. • Total nitrogen by Kjeldahl (neutralization) titration or combustion and gravimetry with a total nitrogen analyzer • Total phosphoric acid by spectrophotometry (420 nm) • Total potassium by flame AAS method or flame photometry • Total sulfur content by gravimetry • Total zinc by flame AAS or ICP-OES • Urea nitrogen by urease catalysis and titration • Water-soluble boron by ICP-OES • Water-soluble calcium by flame AAS or ICP-OES • Water-soluble cobalt by flame AAS or ICP-OES • Water-soluble copper by flame AAS • Water-soluble iron by flame AAS or ICP-OES • Water-soluble magnesium by flame AAS or ICP-OES • Water-soluble manganese by flame AAS or ICP-OES • Water-soluble molybdenum by spectrophotometry or ICP-OES • Water-soluble phosphoric acid by spectrophotometry or ICP-OES • Water-soluble potassium by flame AAS, ICP-OES, or flame photometry • Water-soluble silicic acid by titration • Water-soluble zinc by flame AAS or ICP-OES	
Food	• Carbohydrates by gravimetry • Component amino acids (for proteins) by IC-ECD	Food and nutritional security

(continued)

Table 1.1 (continued)

Agricultural matrix	Required analytical information and technique	Purpose
	• Fats by gravimetry • Pesticides residues by HPLC-UV • Protein content as total nitrogen content by titration	
Soil	• Calcium, copper, magnesium, iron, potassium, free and total SO_2, urea, ammonia by automated discrete photometry • Carbon content by organic elemental analysis • Effective cation exchange capacity by titration • Hydrogen content by organic elemental analysis • Nitrogen content by organic elemental analysis • Organic matter by combustion or loss on ignition • pH in $CaCl_2$ by electrochemistry (direct potentiometry) • Phosphorous (resin extractable) by spectrophotometry (625 nm) • Sulfur content by organic elemental analysis • Multi-elemental analysis (elemental nutrients, pollutants) by ICP-OES • Total exchangeable bases by distillation and titration • Trace elemental analysis (elemental nutrients, pollutants) by flame AAS or ICP-MS	Nutrient analysis, metal, and organic pollutants

Source: Adapted from Vaz (2019). Reprinted with permission from Springer Nature
AAS atomic absorption spectrometry, *FID* flame ionization detector, *DSC* differential scanning calorimetry, *ECD* electric conductivity detector, *EDTA* ethylenediaminetetraacetic acid, *GC* gas chromatography, *HPLC* high performance liquid chromatography, *IC* ion chromatography, *ICP-OES* inductively coupled plasma-optical emission spectrometry, *MS* mass spectrometry, *UV* ultraviolet

- Thermogravimetric analysis (TGA): to observe the thermal decomposition of the sample, that means the mass loss according to the temperature increase—for organic and inorganic chemicals.
- Differential scanning calorimetry (DSC): for the determination of the glass transition temperature of the sample—a physicochemical property; the difference in the amount of heat required to increase the temperature of a sample and a reference material is measured as a function of temperature—for organic and inorganic chemicals.
- Fourier transformed infrared absorption spectroscopy (FTIR): for structural elucidation by means of the infrared absorption, producing absorption bands based on vibrational modes which is characteristic for certain chemical groups according to their polarity—commonly used for organic chemicals.
- Scanning electron microscopy (SEM), combined with the identification of chemical elements present in the sample surface by energy-dispersive X-ray spectroscopy (EDS): for the observation of the morphology of the sample surface

(to determine the physicochemical property) and for the determination of its chemical composition—for organic and inorganic chemicals.
- Transmission electron microscopy (TEM): as SEM, it is a microscopy technique that permits higher magnifications than SEM and also shows the crystallographic structure and the composition of a material—for organic and inorganic chemicals.
- X-ray spectroscopy (XRS): used for the elemental, chemical, crystalline, structural, and dynamic analysis of a broad range of materials—for organic and inorganic chemicals.
- Raman spectroscopy: a non-destructive chemical analysis technique which provides detailed information about chemical structure, phase and polymorphy, crystallinity and molecular interactions. It is based upon the interaction of light with the chemical bonds within a material and is very similar to FTIR—commonly used for organic chemicals.

Spectroscopic techniques can be highlighted due to their facility for handling industrial ambience. On the other hand, chromatographic techniques can be highlighted due to their capacity to separate and enable small quantities to quantify. And microscopic and thermal techniques are very useful to determine certain physical (e.g., size, morphology) and chemical (e.g., products of degradation) properties.

1.5 Trends, Challenges, and Future

Nowadays, important trends can be appointed considering, once again, the content of Fig. 1.1—of course these trends can be changed during the years:

- Automation (e.g., use of robotized systems) and miniaturization—especially green miniaturized technologies, as microextraction techniques and lab-on-a-chip (Agrawal et al. 2021)—to optimize processes and results, for (theoretically) all analytical technologies and their applications.
- Chemometrics means, for instance, multivariate calibration methods and algorithms—to extract information especially from spectroscopic data (Wang et al. 2022).
- Machine or deep learning in order to extract qualitative and quantitative information from high-dimensional and complex chemical measurements (Debus et al. 2021), for (theoretically) all analytical technologies and their applications too.
- Microfluidic—a miniaturized technology based on the manipulation of fluids on a microscopic scale for drug discovery and delivery for pharmaceutical chemistry (Feng et al. 2023).

Indeed, Adams and Adriaens (2020) observed that there is a great number of fundamental works to do in ensuring the quality of data collection, data handling, and data reduction. Additionally, the use of chemometrics is necessary to transform data in actionable insight. Eventually, “smart metrology”—that is, artificial

intelligence (AI)—will play a role in all analytical technology. However, the potential is remarkable as is the increasing use of AI in analytical chemistry, which can be allied to advanced chemical instrumentation. Moreover, aspects of sustainability—environmental, economic, and societal impacts—related to the analytical processes should be considered when analytical chemistry and its concepts, techniques, and methods will be applied to solve real problems.

Regarding challenges, we can consider this point of view according their relevance to solve practical problems:

- Wearable sensors for remote monitoring (Kalasin and Surareungchai 2023)
- Computational tools for liquid phase chromatography (Damiani et al. 2023)
- Development of rapid and automated identification tests based-on spectroscopy (Napolitano et al. 2022)
- Rapid and reliable tests for viruses and demonstrated by the Covid-19 pandemic (Zhang et al. 2022)

Nevertheless, these challenges must be understanding as opportunities for scientific and economic development.

Regarding the future for analytical chemistry, we can expect an increase in their applications according to an increase in the demand from industries (e.g., quality control for raw materials and products) and services (e.g., analytical laboratories) for several economic sectors. However, the use of rapid, automated, and miniaturized analytical technologies should take the relevance of time-consuming laboratory-based technologies.

1.6 Conclusions

It is notorious the relevance of analytical chemistry in the modern society, especially for the transformation industries, as those related, for instance, to agroindustry and industrial chemistry, whose are a few examples of application.

Aspects of green chemistry and circular economy can promote a more sustainable approach for the chemical analyses allied to up-to-date technologies based-on trends as automation, miniaturization, chemometrics, and machine learning.

However, we can observe several challenges to overcome in analytical chemistry in the future, with the development of wearable sensors, computational tools, and rapid and automated new technologies. But they can turn into opportunities to be explored.

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Chapter 2
What We Can Obtain from Analytical Chemistry for Technological and Industrial Purposes?

Abstract Chemical analysis—the practical branch of analytical chemistry—can be applied in three different or complementary situations: characterization, identification, and determination of organic and inorganic analytes. These sets of information are involved in the economic and social aspects of relevant industrial and technological activities in the modern society e.g., agriculture, biotechnology, chemicals, oil and gas, materials, and pharmaceuticals. We can observe a considerable variation related to the families of analytical techniques available for each economic sector, which comes from the complexity of the analytical parameters involved, as concentration of a certain compound and their physicochemical properties; for instance, in the case of chemicals and pharmaceuticals they require both qualitative (e.g., structural resolution obtained by spectroscopic techniques) and quantitative information (e.g., concentration of products and by-products by means of chromatographic techniques)—this approach can be applied to all economic sectors considered here. Finally, the description of classical and instrumental techniques and examples ensure what can be obtained by industry from analytical chemistry.

Keywords Economic sectors · Qualitative information · Quantitative information · Classical techniques · Instrumental techniques

2.1 Introduction

In order to understand the application of analytical chemistry in several industrial and technologic activities with economic relevance, it is of fundamental importance to introduce some basic terms of analytical chemistry.

Initially, it should be considered that chemical analysis—the practical branch of analytical chemistry—can be applied in three different or complementary situations:

- *Characterization*: observation of some physical property attributed to the *analyte*—the species of interest in the analytical process. For example, the absorption of visible radiation in the wavelength range of 400–450 nm or the behavior of the molecule against the incidence of radiation of other wavelengths—this is the typical application of certain spectroscopic techniques

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(e.g., infrared absorption and nuclear magnetic resonance) and microscopic techniques.

- *Identification*: qualitative information from the analytical data on the presence or absence of the analyte—a good example is mass spectrometry, which identifies the compounds from the fragmentation of their molecular structure.
- *Determination*: quantitative information from the analytical data on the analyte concentration in the sample—an example is the elemental analysis of the composition and the chromatographic analyses coupled with the detection techniques.

These three terms will be applied, frequently, during this book.

2.2 Scenarios of Relevant Industrial and Technological Activities in the Modern Society

Before the exploration of the analytical chemistry potential for those situations described in Sect. 2.1 (i.e., characterization, identification, and determination), we should consider the economic and social aspects of some very relevant industrial and technological activities in the modern society, especially for agriculture, biotechnology, chemicals, oil and gas, materials, and pharmaceuticals due to their influential and large production chains.

2.2.1 Agriculture

This is a basic and fundamental activity to guarantee food and feed, among other products as fibers, chemicals, biofuels, etc. Food security is, indeed, a subject of global concern in order to establish and keep the minimum of subsistence conditions.

Table 2.1 depicts the global production of some relevant agricultural products.

Table 2.1 Global production for the year2020 for commonly used agricultural products

Agricultural product	Production (t)
Maize	1.16 billion
Oil crop	419.71 million
Fiber crop	515.53 thousand
Potatoes	359.07 million
Rice	756.74 million
Soybean	353.46 million
Sugar crops	2.12 billion
Wheat	760.93 million

Source: Adapted from United Nations Food and Agriculture Organization (2023). Reproduced with permission from UN Food and Agriculture Organization

These huge quantities of agricultural products need—unfortunately—a huge quantity of agrochemicals for their growth, production, and harvest, which are, mainly, divided into pesticides (e.g., herbicides, fungicides, insecticides, and bactericides) and fertilizers (e.g., N-, P- and K-based)—other agrochemicals are adjuvants and plant growth regulators.

According to the United Nations Food and Agriculture Organization (2023), the global consumption of NPK fertilizers for the year of 2020 was of 273.4 million metric ton. Once again according to the United Nations Food and Agriculture Organization (2023), in 2020 herbicide consumption worldwide nearly reached 1.4 million metric ton, whereas consumption of fungicides and bactericides stood at around 606 and 471 thousand metric ton, respectively.

Despite their recognized negative impacts to the environment and public health, agriculture is deeply dependent on agrochemicals to reach the desired yields demanded by the modern society.

2.2.2 Biotechnology

The global biotechnology market size was estimated at USD 1023.92 billion in 2021 (Grand View Research 2023). This market comprises several products, among others, vaccines, agricultural inputs, functional food and feed, and some chemicals and pharmaceuticals.

The interest on the biochemical (or biotechnological) processes is due to the possibility of converting raw materials (e.g., sugars) into specific products under mild conditions of temperature, pH value, and pressure when compared against chemical processes in the chemical industry; furthermore, it can be related to a sustainability approach based on the use of bio-based conversion. However, some general limitations of the biochemical processes—mainly *microbial processes*—are as follows:

- Slow rate of reaction, from hours to days
- Low conversion rate and yield
- Production of a large number of by-products with large quantities of impurities, create the necessity for more rigorous separation and purification steps
- Conversions at aqueous medium for polar compounds (e.g., organic acids), creating the necessity of additional separation (or recovery) step, e.g., by precipitation as a salt

However, some biochemical processes can be economically advantageous as the ethanol production by means of yeast fermentation using *Saccharomyces cerevisiae* with a well-established industry around the world, i.e., ethanol from sugarcane and corn.

In microbial processes, the raw material conversion is carried out by fungi, bacteria, and yeasts catalyzed by the action of many enzymes, which can be distributed inside and outside the microorganism. Generally, in addition to the

carbon source, other compounds must be added to the bioreactor to maintain cell viability. Microorganisms can consume different components of carbon-generating products through different metabolic routes comprising, for instance, aerobic and anaerobic fermentation. Biotechnological advances demonstrate that microorganisms can be engineered toward producing plant metabolites as food applications as polyphenols and terpenoids; however, precursor supply and product toxicity are key limitations during microbial production (Kallscheuer et al. 2019).

In *enzymatic processes*—the other class of bioprocesses—we have enzymes that are proteins and the natural (bio)catalysts to enhance the rate of metabolic reactions. Immobilized enzymes are the most useful; they are, by definition, enzymes retained or confined in a space and that can be used repeatedly in a given process. This approach makes use of surface chemistry, pendent functional entities, and ease in tunability of various materials to promote the best enzyme activity and use (Liu et al. 2021).

Part of the Biotechnology section has been reproduced with permission from Elsevier (Biochemical synthesis for carbon derivatives—ScienceDirect).

2.2.3 Chemicals

Chemicals can be divided into inputs and end-use products, what can include a huge number of classes and applicability, varying in terms of inorganic and organic products.

The chemical industry uses many raw materials, both organic and inorganic too. Naphtha is the basic material for a series of products, which are called *petrochemicals*, precisely because they are made from naphtha (or natural gas) and, consequently, *petroleum*.

In addition to end-use products, the chemical industry provides raw materials for almost every other industry. It is hard to imagine any consumer product in which the chemical industry is not present in some way.

We can consider the following segments in the global chemical industry definition (Brazilian Chemical Industry 2023):

- Industrial chemicals
- Pharmaceuticals
- Fertilizers
- Personal hygiene
- Perfumery and cosmetics
- Agricultural pesticides
- Soaps and detergents
- Paints and varnishes
- Artificial and synthetic fibers
- Others

For instance, the production of inorganic chemicals can involve, among others:

- Chlor-alkali manufacturing
- Manufacture of intermediates for fertilizers
- Manure and fertilizer manufacturing
- Industrial gas manufacturing

On the other hand, the production of organic chemicals can involve, among others, too:

- Manufacture of basic petrochemicals
- Manufacture of intermediates for plasticizers, resins, and fibers
- Manufacture of agricultural pesticides
- Manufacture of household disinfectants

According to the International Council of Chemical Associations (2023), the chemical industry plays a crucial role in most sectors of regional economies, which has led to innovative, life-enhancing products and technologies that not only support the global economy, but also help people live longer, healthier, and more sustainable lives. In 2017, the chemical industry contributed USD 5.7 trillion to global gross domestic product (GDP), equivalent to 7% of the world's GDP.

2.2.4 Oil and Gas

The oil and gas industry—sometimes known as petrochemical industry—is part of the chemical and energy industries. It is characterized by using a petroleum derivative (naphtha) or natural gas as basic raw materials or as source of energy (for the both substances). However, many products called petrochemicals, such as polyethylene, can be obtained both from these raw materials and from others, such as coal (as in South Africa) or alcohol (as in Brazil). Indeed, the oil industry is highlighted as one of the most powerful branches in the world economy, resulting in constant advances in upstream (extraction) and downstream (refining) activities. Natural gas highlights as a relatively cheap and sustainable energy source for industries and homes.

Figures 2.1 and 2.2 depict the world's proven gas and crude oil reserves, respectively, in order to demonstrate the capacity for demands in the near future.

From Figs. 2.1 and 2.2 we can observe a strong contribution from Middle East in both commodities (natural gas and crude oil) and from Russia for natural gas, what can impact on geopolitical behaviors and strategies in these regions and worldwide for petrochemicals and energy in the near future.

2.2.5 Materials

As materials we can consider a large classes of products, as follows:

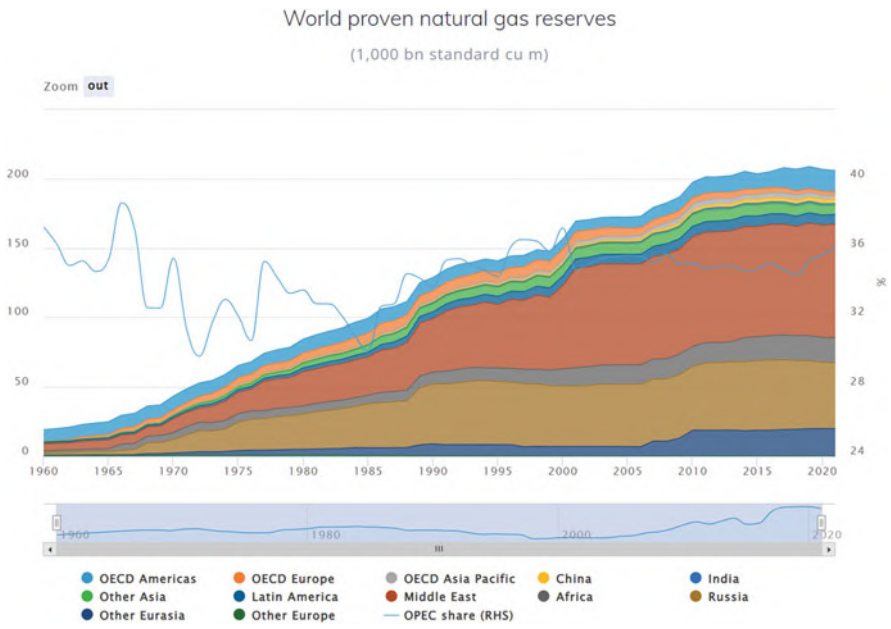

Fig. 2.1 World's proven natural gas reserves based on OPEC statistics. Source: Organization of Petroleum Export Countries (2022) Reproduced with permission from Organization of Petroleum Export Countries

- Ceramics
- Composites
- Fibers
- Glasses
- Metals and metallic alloys
- Plastics
- Polymers
- Resins
- Semiconductors

And each of them has this chemical composition and physicochemical characteristics to be considered for their functionality and use. Besides, production processes vary considerably from one to another due to raw materials and, of course, the final product.

Some applications of these materials are as follows:

- Aerospace—e.g., metallic alloys
- Biomedical—e.g., ceramics, resins, and metallic alloys
- Electronic materials—e.g., semiconductors
- Energy and environmental technologies—e.g., ceramics and polymers

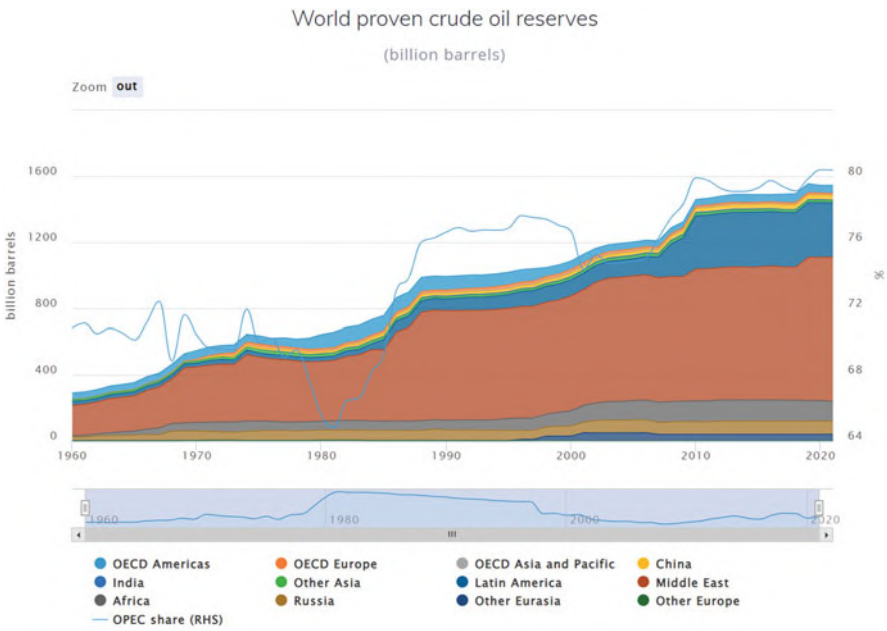

Fig. 2.2 World's proven crude oil reserves based on OPEC statistics. Source: Organization of Petroleum Export Countries (2022). Reproduced with permission from Organization of Petroleum Export Countries

Nanotechnology—the set of techniques and methods to obtain products at 10^{-9} m size—is an approach used to improve the properties and functionality of the materials, e.g., surface availability, when compared against conventional materials. Nanotechnology is generally understood as engineered structures, devices, systems, and nanomaterials that are defined as those things that have a length scale between 1 to 100 nanometers. At this size, materials begin to exhibit unique properties that affect physical, chemical, and biological behavior.

As observed by market research (Data Intelligence 2023), the market for advanced materials—in the order of billion USD—is highly fragmented, with both international and local manufacturers present. The market is extremely competitive due to the introduction of sophisticated materials and the growth of the material sector.

2.2.6 Pharmaceuticals

The pharmaceutical industry is, without any doubt, those more closely bounded to the human and animal health, producing our medicines or drugs. It can be considered a branch of the chemical industry with their own characteristics.

Before being available to patients, every drug goes through a long and costly research and development step. From the first activities related to the discovery of an active principle to the beginning of commercialization, the production of a new drug comprises different stages and can take more than ten years, with a very low success rate. For each drug commercially launched, up to 10,000 compounds can be tested in the initial phases. Costs are also very high. Estimates of total average capitalized pre-launch research and development costs vary widely, ranging from $161 million to $4.54 billion; therapeutic area-specific estimates are highest for anticancer drugs (between $944 million and $4.54 billion) (Schlander et al. 2021).

The pharmaceutical industry can comprise the following:

- Manufacture of drugs for human use
- Manufacture of drugs for veterinary use
- Manufacture of pharmaceutical preparations

The impacts of the Covid-19 pandemic were uneven across different sectors of the economy worldwide. Some sectors such as medicines and vaccines and health services in general, for instance, gained even more relevance. The race for a vaccine and the scarcity of inputs to produce it have highlighted the importance of fostering and developing an industry of products and raw materials.

Figure 2.3 depicts the use of medicines, by therapy area, in developed countries. Cardiovascular, pain, and dermatological medicines are highlighted as the most used medicines in these countries.

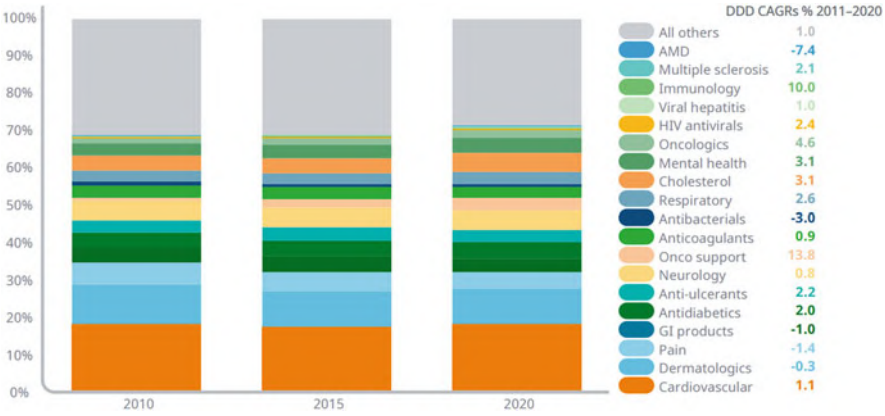

Fig. 2.3 The distribution of medicine usage across therapy areas in developed countries. The defined daily doses (DDD) per capita compared to per capita gross domestic product PPP$ *Purchasing power parity*–measure of the price of specific goods in different countries; compound annual growth rate (CAGR). *AMD* age-related molecular degeneration, *GI* gastrointestinal, *HIV* human immunodeficiency virus. Source: IQVIA Institute (2022)

2.3 Analytical Necessities for Several Industrial and Technological Sectors

Based on the examples of industrial and technological activities discussed in Sect. 2.2, Table 2.2 presents analytical necessities of several economic activities.

We can observe a considerable variation related to the families of analytical techniques available for each economic sector presented in Table 2.2. It comes

Table 2.2 Analytical necessities for several economic activities

Economic activity	Analytical necessities	Impact of analytical necessities on the activity performance[a]	Examples of techniques for application
Agriculture	Quality control of raw materials, inputs, and final products Investigation of pesticide residues	High, for both necessities	Infrared absorption spectroscopy (for qualitative information). Liquid phase chromatography with several detectors (for quantitative information)
Biotechnology	Quality control of raw materials, inputs, and final products	High	Liquid phase chromatography with several detectors (for quantitative information)
Chemicals	Quality control of raw materials, inputs, and final products	High	Liquid phase and gas phase chromatography with several detectors (for quantitative information) Several spectroscopic techniques (for qualitative and quantitative information)
Oil and gas	Quality control of raw materials, inputs, and final products	High	Gas phase chromatography with several detectors (for quantitative information) Several spectroscopic techniques (for qualitative and quantitative information)
Materials	Quality control of raw materials, inputs, and final products	High	Several microscopic techniques (for qualitative information) Several spectroscopic techniques (for qualitative and quantitative information)
Pharmaceuticals	Quality control of raw materials, inputs, and final products Investigation of by-product residues	High, for both necessities	Liquid phase and gas phase chromatography with several detectors (for quantitative information) Several spectroscopic techniques (for qualitative and quantitative information)

[a]Considering application and market dimensions

from the complexity of the analytical parameters involved, such as concentration of certain compounds and their physicochemical properties; for instance, in the case of chemicals and pharmaceuticals these products require both qualitative (e.g., structural resolution obtained by spectroscopic techniques) and quantitative information (e.g., concentration of products and by-products by means of chromatographic techniques)—this approach can be applied to all economic sectors considered here.

As expected, all analytical necessities should have a high impact on the activity performance—considering application and market dimensions—which corroborate with the relevance of analytical chemistry in the industrial (and agroindustrial) sectors.

2.4 Classic and Instrumental Techniques: Differences and Uses

The analytical techniques applied in the quantification of the analytes (the chemical species to be determined by the chemical analysis) can be divided into two classes:

- The classical techniques based on the measurement of mass, moles, and charge—which provide absolute values.
- The instrumental techniques, which work with values expressed as mg L^{-1}, mg kg^{-1}, μg m^{-3}, and so on.

Until the beginning of the twentieth century, chemists used the separation of analytes by techniques such as extraction, precipitation, or distillation as the basic approaches to start a chemical analysis. For qualitative analysis, these separated analytes were treated with appropriate reagents, yielding compounds which could be identified by properties such as solubility, color, melting point, and boiling point. The quantitative analysis was done using reasonably simple and good precision techniques that are used till the present day, such as volumetry (volume measurement) and gravimetry (mass measurement)—they are typical examples of classical techniques.

Since then, different aspects from those observed by the classical techniques have begun to be investigated and several experiments have been carried out to measure the analytes from some particular physicochemical properties, usually associated with phenomena such as the absorption and emission of radiation, which are the principle of instrumental techniques such as atomic spectrometry. These findings have boosted the development of a great diversity of instruments that are employed in this class of techniques. Instrumental techniques are generally faster than classical techniques and are employed in the determination of low concentrations of analyte as trace concentrations at or below of ng L^{-1}/ng kg^{-1} values.

The following equations express the fundamentals of these two sets of techniques:

Table 2.3 Physical properties employed in the most used analytical techniques in chemical analysis of industrial matrices

Property	Analytical techniques	Uses
Absorption of radiation	Spectrophotometry and photometry (ultraviolet and visible) Atomic spectrometry Infrared spectroscopy (near, medium, and far)	Quantification of organic compounds (e.g., UV); quantification of elements (e.g., AAS); determination of chemical groups (e.g., FTIR)
Electric current	Voltametries (cyclic, square wave, anodic, cathodic, polarography)	Quantification and speciation of inorganic species
Emission of radiation	Emission spectroscopy (X-ray, ultraviolet, and visible) Optical emission spectrometry Fluorescence (X-ray, ultraviolet, and visible)	Quantification of elements (e.g., ICP-OES)
Mass	Gravimetry	Several organic and inorganic compounds and materials
Electric potential	Potentiometry	Measurement of pH value in aqueous media and titrimetric measurements of organic compounds
Mass/charge ratio	Mass spectrometry	Determination of organic compounds (when allied to chromatography) and elements (when allied to emission spectrometry)

Source: Adapted from Vaz (2018). Reproduced with permission from Springer Nature
AAS atomic absorption spectrometry, *FTIR* Fourier transform infrared absorption spectroscopy, *ICP-OES* inductively coupled plasma-optical emission spectrometry, *UV* ultraviolet absorption spectroscopy

$$A_S = kn_A \tag{2.1}$$

$$A_S = kC_A \tag{2.2}$$

Equation 2.1 applies to classical techniques, where A_S is the measured signal—or response—of the analyte, k is the proportionality constant to be standardized, and n_A is the number of moles, charge, or grams obtained for the measurement. Equation 2.2 applies to instrumental techniques, where A_S is also the measured signal (or response) of the analyte, k is again the proportionality constant to be standardized, and C_A is the relative concentration of the measured analyte. However, some spectroscopic techniques do not obey these two equations, since they commonly provide information about the structural characteristics of the sample, e.g., infrared absorption spectroscopy.

In Table 2.3 are listed some physical properties explored by the analytical techniques in order to provide the response of the measurement.

Instrumental techniques measure a physical phenomenon resulting from a molecular or atomic property that will be qualitatively or quantitatively (the most desirable) related to the analyte; that is, the physical phenomenon will produce a signal

that will be directly correlated to the presence or concentration of the analyte in the sample.

Those techniques from Table 2.3—commonly considered *detection techniques*—can be hyphenated (or coupled) to *separation techniques*, as chromatography or electrophoresis. It will:

- minimize or eliminate matrix effects on the final result;
- improve the analyte signal;
- decrease the amount of sample and residues generated during the analytical process;
- decrease time.

Other set of techniques commonly used for advanced instrumental analysis are the thermochemical techniques (e.g., thermogravimetry and differential scanning calorimetry). Besides, a set of not well-defined in both classical and instrumental techniques is the microscopy (e.g., scanning electron microscopy and transmission electron microscopy)—the analytical techniques cited here will be deeply discussed in Chaps. 4, 5, 6, 7, 8, and 9.

As we can see, the definition of classical and instrumental techniques is relatively simple for some cases based on absorption and emission of radiation, but complex for another (e.g., microcopies). However, the exploration of their uses and application in the next chapters can contribute to clarify this issue, considering the direct contribution of both classes to the best usages of industrial products and their whole production chain.

2.5 Conclusions

Analytical chemistry can provide industry information related to characterization, identification, and determination of organic and inorganic analytes originated from several products and processes.

The considered economic and technological sectors in this chapter (i.e., agriculture, biotechnology, chemicals, oil and gas, materials, and pharmaceuticals) have different analytical necessities based on their raw materials, processes, and products; with analytical chemistry—by means of chemical analysis—having a high impact on the activity performance of products.

Then, the use of classical and instrumental techniques contributes directly to the best usages of industrial products and to the maintenance of their whole production chain.

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Chapter 3
Terminology of Analytical Chemistry

Abstract In order to understand the application of analytical techniques for the analysis of several analytes of industrial and technological interest, it is paramount to introduce fundamental terms of analytical chemistry. Figures of merit is the first set because they are parameters of control used in analytical chemistry for the application of a certain analytical method—the most representative figures are accuracy, linearity, limit of detection, limit of quantification, precision, selectivity, sensitivity, and robustness. The processes of development and validation make up the modus operandi of any analytical method in its best performance condition. As an example of the importance of these both processes, we can mention the constant and necessary evaluation of those figures of merit for the correct obtaining of an analytical result, a major procedure in an accreditation of the analytical laboratory. Then, these terms and applications will be explored in this chapter in order to supply the necessary know-how to put into practice the chemical analysis.

Keywords Figures of merit · Method development · Method validation · Chemical metrology

3.1 Introduction

Generally, chemical analysis can be considered the practical use of concepts of analytical chemistry and its techniques and methods in the investigation and solution of real problems of variable complexity in different scientific or technological areas. As previously discussed in Chap. 2, chemical analysis can generate information, from analytical data, of both qualitative and quantitative character related to a certain sample (Fig. 3.1).

In order to clarify, the analytical method can be understood as the application of a certain analytical technique (e.g., infrared absorption spectroscopy) to determine an

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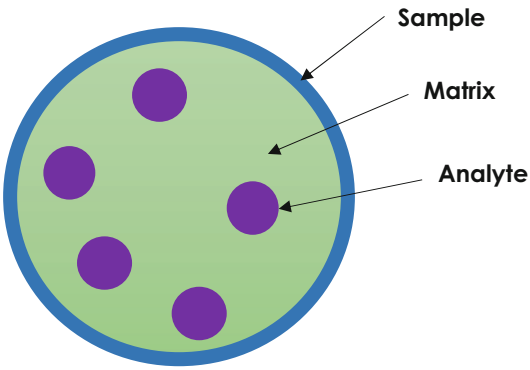

Fig. 3.1 The illustration of a sample constitution. The sample (blue circle) is composed of the matrix (the medium, green) and of the analyte (magenta balls)

analyte in a specific medium or analytical matrix. It is important to consider that we are discussing here the chemical metrology, which supports the identification and determination, i.e., quantification, of chemical species using several analytical techniques in a wide variety of materials or analytical matrices.

And in order to understand the application of analytical techniques for the analysis of several analytes of industrial and technological interest, it is paramount to introduce fundamental terms of analytical chemistry, which are the subject of this chapter.

3.2 Detailed Description of Figures of Merit

Figures of merit are parameters of control used in analytical chemistry for the application of a certain analytical method. The most representative figures are *accuracy*, *linearity*, *limit of detection*, *limit of quantification*, *precision, selectivity*, *sensitivity*, and *robustness* (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use 2005; International Standard Organization 1993).

Before validating the analytical method (to be seen in Sect. 3.4), these parameters must be defined, as well as the limits at which results can be accepted.

3.2.1 Accuracy

Represents the degree of agreement between a measured value and a value taken as a "true value." The accuracy (Eq. 3.1) expresses the relative error of the measure:

$$AC = (V_D - V_T/V_D) \times 100 \tag{3.1}$$

Where: V_T is the true value and V_D is the determined value.

3.2.2 Linearity

Expresses the agreement between the results obtained by a given analytical method for a given experimental parameter, such as the absorbance and the analyte concentration, in a given concentration range. The linear correlation coefficient (r), calculated by the linear regression equation (Eq. 3.2), is used to indicate if the mathematical model is adequate. Alternatively, we can use the coefficient of determination r^2, that the closer to 1 (one) the greater the linearity represented by the equation below:

$$y_i = a + bx_i \tag{3.2}$$

Where: a is the line intercept and b is its slope coefficient.

3.2.3 Limit of Detection and Limit of Quantification

The limit of detection (LOD) for an analytical method may vary depending on the type of sample and it is defined as the minimum concentration of a measured and declared substance with 95 or 99% of confidence that the analyte concentration is greater than zero. There are several ways to calculate LOD, but the recommendation is that at least seven replicates of the blank are made in the calculation.

The limit of quantification (LOQ) is the lowest analyte concentration that can be determined with an acceptable level of accuracy; can be considered as the mean value of the blank readings by adding 5, 6, or 10 times the *standard deviation* (see ahead). Equations 3.3 and 3.4, respectively, commonly are used to determine LOD and LOQ:

$$\text{LOD} = 3.3s/S \tag{3.3}$$

$$\text{LOQ} = 10s/S \tag{3.4}$$

Where: s is the standard deviation of the mean (Eq. 3.5 ahead) and S is the slope of the calibration curve (or b in Eq. 3.2).

3.2.4 Precision

It is the degree of agreement between indications or measured values, obtained by repeated measurements, on the same object or similar objects, under specified conditions. It is generally expressed in numerical form by means of dispersion measures such as *standard deviation*, *variance*, or *coefficient of variation*, under

specified measurement conditions (i.e., *recovery*, *repeatability*, or *reproducibility*—to be seen ahead).

The importance of precision and its modes of measurement in analytical chemistry must be highlighted, which the main measurement form is the *standard deviation*. A measure of the data precision can be obtained by the population standard deviation (σ); or, more commonly, by the *standard deviation of the mean* (*s*), depicted in Eq. 3.5:

$$s = \sqrt{\frac{\sum(xi - \overline{x})}{n - 1}} \tag{3.5}$$

Where: x_i is the value of a given measure; $\bar{x}$ is the arithmetic mean of the values of the measures ($\overline{x} = \sum xi/n$); n is the number of measurements taken.

The coefficient of variation, or *relative standard deviation*, is useful to observe the relative accuracy of the measurements (Eq. 3.6):

$$\text{CV}\ (\%) = \frac{s}{\overline{x}} \times 100 \tag{3.6}$$

The confidence interval for the mean (CI_M) (Eq. 3.7) is very useful when expressing the *confidence interval of a measure*, a relevant aspect in the elaboration of an analytical report:

$$\text{CIM} = \overline{x} \pm t_{n-1} \frac{s}{\sqrt{n}} \tag{3.7}$$

Where: $\overline{x}$ is the arithmetic mean of the values of the measures ($\overline{x} = \sum xi/n$); n is the number of measurements performed; s is the standard deviation of the mean (Eq. 3.5); t_{n-1} is the tabulated critical value of the Student's t-distribution (Eq. 3.11, Section 3.4).

3.2.5 Sensitivity or Sensibility

It is the measure of the ability to discriminate between small differences in the concentration of an analyte. Two factors limit sensitivity: the slope of the analytical curve and reproducibility. For two methods having the same precision, the one with the most inclined *analytical curve* will be the most sensitive; if the analytical curves are equal, the one that exhibits greater precision will be more sensitive. This figure can be represented by Eq. 3.8:

$$\Delta CA = \Delta S_A / k_A \tag{3.8}$$

Where: ΔS_A is the smallest increase in the signal that can be measured (the smallest difference is the analyte concentration that can be detected) and k_A is the proportionality constant to be measured.

3.2.6 Selectivity

It is a property of a measurement system, whereby the system provides measured values for one or several measurands (magnitude to be measured), such that the values of each measurand are independent of each other. If the chosen method does not exhibit selectivity, the matrix components will interfere with the measurement performance. The evaluation of the selectivity of a method involves assays with *reference standards* or *reference materials*, samples with and without the analyte, and evaluation of the efficiency in determining the analyte in the presence of interferents. Equation 3.9 defines this figure of merit, if S is equal to unity means that the method is selective for the analyte:

$$S = K_A(C_A + K_{A,I}C_I) \tag{3.9}$$

Where: K_A is the analyte's sensitivity coefficient (calculated from Eq. 3.8), C_A is the analyte concentration, $K_{A,I}$ is the selectivity coefficient, and C_I is the interfering concentration.

3.2.7 Robustness

Measures the sensitivity of a method to small variations in the conditions of analysis—there is not an equation to define it. A method is said to be robust when it is practically insensitive to such variations. Therefore, the greater the robustness, the greater the confidence of the method related to the precision—the coefficient of variation (Eq. 3.6) can express this parameter.

3.2.8 Recovery

A figure of merit to be highlighted is the percentage of recovery, which is important for determining the efficiency of an extraction method, with its value varying between 70 and 120% (Eq. 3.10):

$$\%R = \left(C_i – C_f / C_i\right) \times 100 \tag{3.10}$$

Where: C_i is the initial added concentration of the standard to the matrix, with no traces of the analyte; C_f is the final concentration determined in the sample (matrix + standard) after the addition of a known concentration of the standard, and after the application of an extraction method.

In any measure we can consider the existence of *errors* or *uncertainties* associated with the analytical process. The word *error* can be understood in two distinct ways: it can refer to the difference between a measured value and a known value or can refer to the estimated uncertainty associated with a measurement or an experiment. Thus, the error can be classified as *random or indeterminate*, *systematic or determinate*, and *rough*:

- Random errors exist in every measure, and cannot be totally eliminated, because they are caused by uncontrollable variables in the measurement process—these errors affect the precision (Eq. 3.5) of the results.
- Systematic errors have a defined cause, being of the same order of magnitude for replicates of a measure made in a similar way. They can be caused, for example, due to the lack of calibration of an equipment—these errors affect the accuracy (Eq. 3.1) of the results.
- Rough errors are usually of great magnitude, caused by human failure. These errors lead to anomalous values that differ significantly from the other replicated values, and there are several statistical tests to identify this type of error, such as the coefficient of variation (Eq. 3.6).

3.3 Development of an Analytical Method

The development and validation processes make up the modus operandi of any analytical method, based on analytical technique, for chemical metrology. As an example of the importance of these processes, we can mention the constant evaluation of the figures of merit (Sect. 3.2) for the correct obtaining of an analytical result, a fundamental procedure in an *accreditation* of a certain analytical laboratory (to be seen in Sect. 3.5).

For the development, adaptation, or implementation of a known method, an assessment process that evaluates the method efficiency in the routine of the laboratory should be applied. An essential step at this assessment is to plan the activities to be performed so that the final result of the analysis performed is as reliable and representative as possible. In this sense, some observations are relevant:

- Calibrated equipment: the equipment and materials used in the analytical process should be properly calibrated.

- Quality of the analytical reagents: the laboratory needs reagents of high purity for analysis to avoid contaminant effects on the results. It is very critical when analyzing analytes in very small quantities or trace concentrations.
- Certified standards: whenever possible, we must work with certified standards, which contain uncertainty, and which are traceable.
- Calibrated glassware: glassware to be used in quantitative analyses, such as pipettes, beakers, erlenmeyers, etc., should be checked, observing the calibration temperature, in the case of the calibration process, and estimated at a given temperature.
- Representative sampling: establish the correct method of sampling, according to the physicochemical characteristics of the material to be analyzed—a correct sampling ensures the reliability of the result.
- Statistical tools: check for correct data interpretation.
- Qualified personnel: analysts and technicians must be trained and qualified to perform the procedures, respecting their level of training.

In addition to these points addressed, it is essential that the method chosen attends in a satisfactory form to the analytical goal, i.e., the problem to be solved by means of the chemical analysis. In this respect, it is necessary to consider the sensitivity of the method, concentration, and matrix in which the analyte is present, and the presence of chemical interferers, which may mask the result obtained—it is of great importance to know the effect of the matrix on the result. Finally, once the method has been developed, it must be optimized so that it is then validated (Sect. 3.5).

3.3.1 Calibration

It is often necessary by the analyst to provide references so that it is possible to correlate the data obtained in a measurement apparatus with the real analyte concentration in the sample—this is done by means of the construction of a *calibration curve* (Figs. 3.2, 3.3, and 3.4). This procedure is called *calibration of the method*, which is nothing more than to determine the relationship between the analytical response (e.g., intensity of absorption or emission of a wavelength of the radiation) and the analyte concentration. The main methods used in the construction of the calibration curves are as follows:

- *Standard addition*: addition of known concentrations of the analyte to known quantities of the sample to be analyzed, generating plots for the construction of the calibration curve from peak area and concentration values. By means of the extrapolation of the curve on the abscissa axis, the actual concentration of analyte in the sample is obtained; the difference between the results of the sample without addition and addition of analyte must be equal to the added concentration. The method can also be used with multiple additions of standard, which allows to verify if there is a linear relationship between the response and the concentration

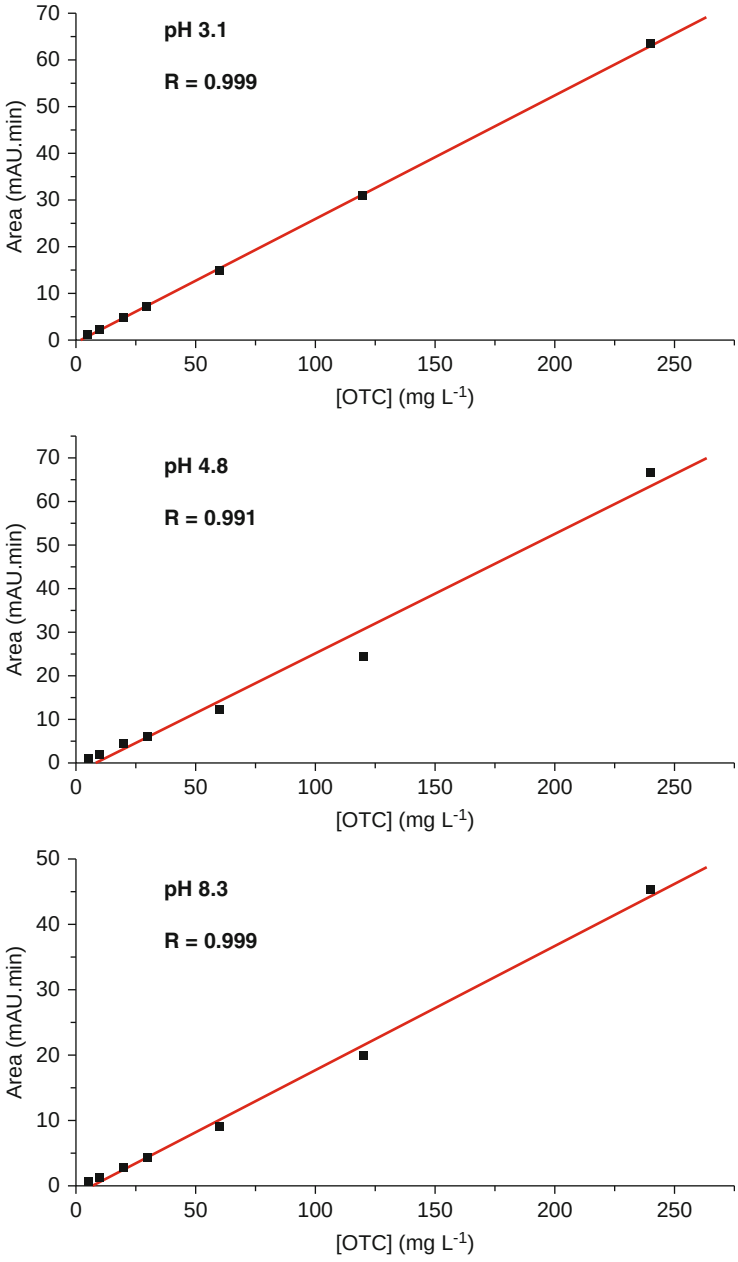

Fig. 3.2 Typical external calibration curves for a chromatographic method. *OTC* oxytetracycline (the analyte, a pharmaceutical antibiotic)

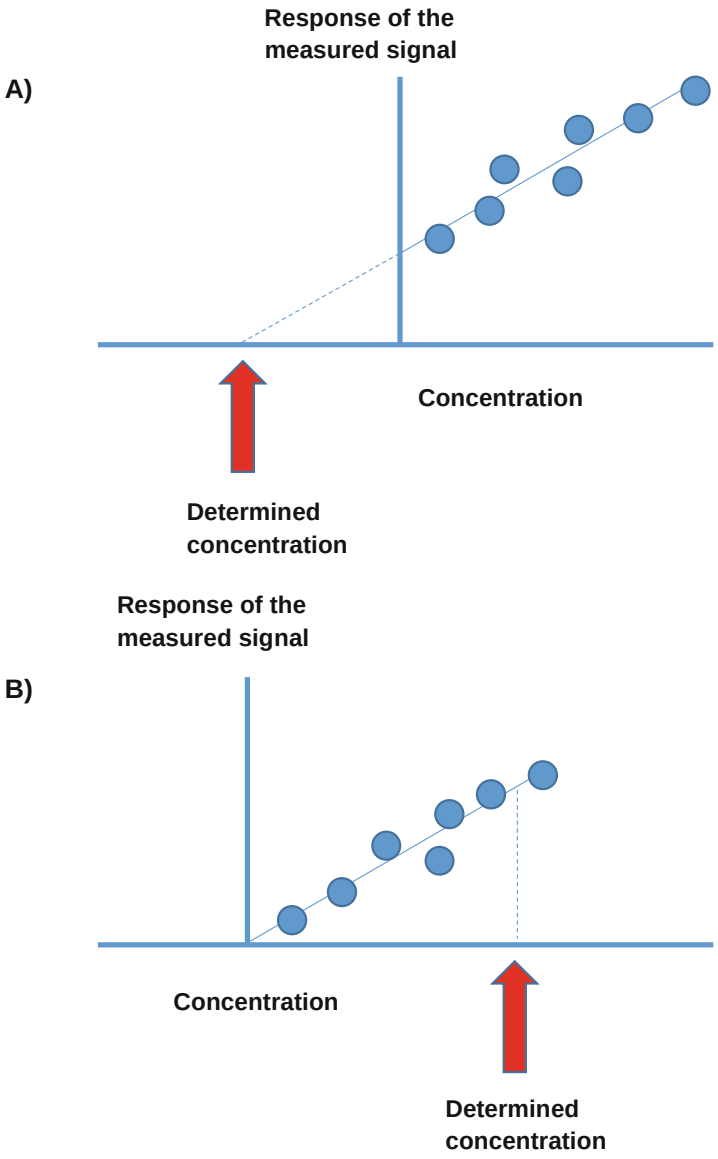

Fig. 3.3 Calibration curves: (**a**) standard addition, (**b**) external standard

of the analyte. Normally, standard addition is used when the matrix has complex composition that affects the analytical signal, or when an analyte pattern cannot be found.

- *External standard*: when it is known that the constituents of the sample do not cause interference in the analyte signal, the external standard method can be used.

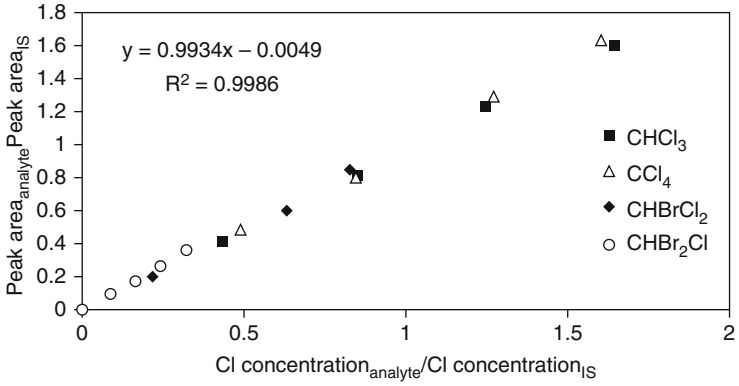

Fig. 3.4 Calibration curve constructed with internal standard. *IS* internal standard

The method consists in the construction of an analytical curve from the areas obtained with standard solutions of the analyte in known concentrations; special care must be taken with the preparation of the standard solutions, since any contamination will imply in an erroneous determination of the analyte concentration.

- *Internal standard*: addition of a standard, which is a compound with a composition different from the analyte and of known concentration, but with a similar chemical structure allowing a behavior close to that of the analyte in relation to the analytical response to a series of analyte standards with known concentrations for the construction of a calibration curve. This curve is constructed not with the analyte response, but with the ratio of the internal standard signal to the analyte signal. Any analyte of unknown concentration can then be determined with addition of the internal standard by projection of the ratio of the responses in the analytical curve. This method is especially used when small variations in the response of the equipment to each analysis performed, as in chromatographic analysis, usually occur.

3.3.2 Validation of an Analytical Method

Regarding the validation process, it is the proof by objective evidence that the requirements for a particular application or use of a method have been attended.

According to the International Standard Organization (2005), the laboratory shall validate non-standard methods, which are methods developed by the laboratory itself, or standard methods used outside the scope for which they were designed, such as extensions or modifications. The latter refer to methods developed by a standardization body or other segment whose methods are accepted by the technical sector concerned.

There are several definitions for validation in the literature. However, according to Ribani et al. (2004), the validation can proceed in two ways: *validation in the laboratory* and *full validation*. It is considered validation in the laboratory when it is used to verify the suitability of a method or when a method has been developed in the laboratory and all parameters are related to the measurements in that laboratory. Thus, validation in the laboratory is a preliminary step to the full validation, which is performed considering all performance characteristics and interlaboratory tests. Figure 3.5 illustrates the generic validation process of an analytical method.

3.3.3 Interlaboratory Studies

The participation of an analytical laboratory in interlaboratory studies can verify if its adopted methodology is consolidated for a certain type of analysis, with a well-developed and defined control system.

The International Standard Organization (2005) recommends that a technique used to determine the interlaboratory performance of a method is as follows—or a combination of them:

- *Calibration with the use of materials or reference standards*: the reference material is a sufficiently homogeneous and stable material with respect to certain physical or chemical properties and is prepared to suit an intended use in a measurement or examination of qualitative properties and shall be accompanied by documentation issued by a notified body authority, a qualification or more property values specified as uncertainties and as associated traces, called in this case a certified reference material. Already the reference standard is used for the calibration of other standards of magnitude of the same type in a laboratory.
- *Comparisons with results obtained by other methods*: the efficiency of the developing method can be verified by comparing its results with the results of a standardized method, through statistical tests (International Standard Organization 1994).

The most utilized test for the comparisons with results obtained by other methods is the Student—or *t*-test—used to compare a mean of a series of results with a reference value, as means of two sets of results, within a confidence interval. The value found is compared with the tabulated value of *t*, and the first one should be as close as possible to the validation of the proposed method, according Eq. 3.11:

$$t_{\text{calc}} = \frac{|\mu - \overline{x}|\sqrt{n}}{s} \tag{3.11}$$

Where: $\overline{x}$ is the arithmetic mean for the set values; μ is the reference value, that can be substituted by the mean from another data set; n is the number of measurements; and s is the standard deviation of the mean. Table 3.1 presents t values

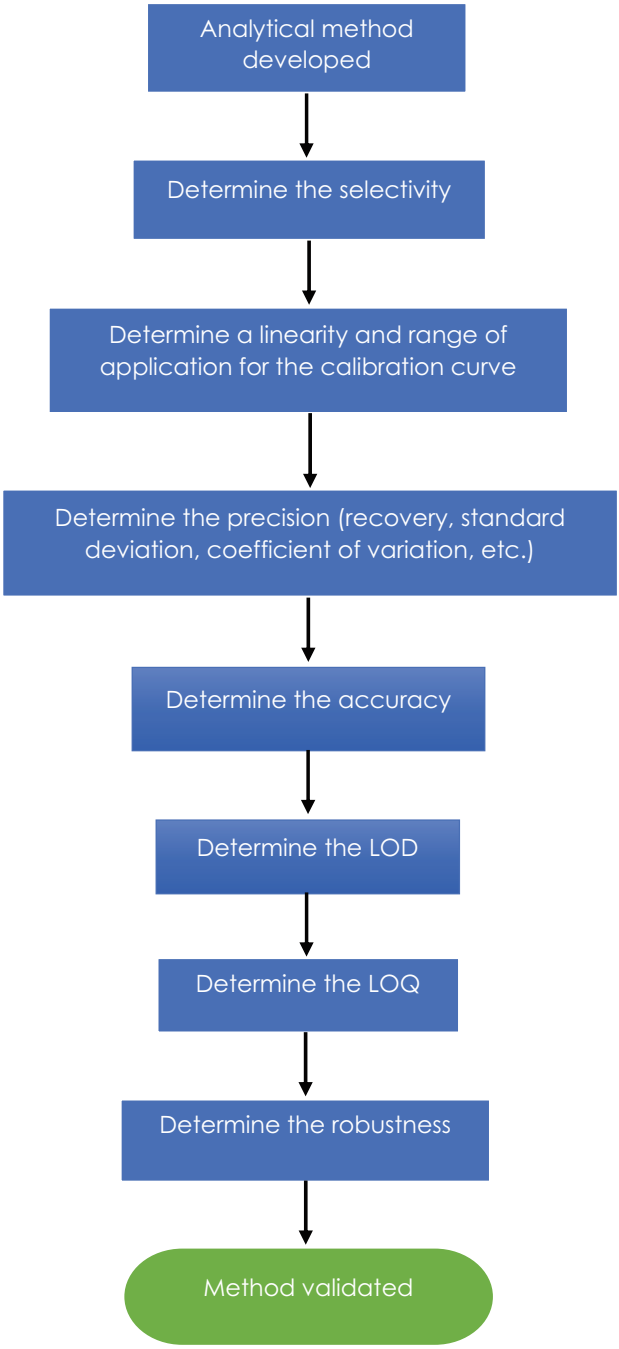

Fig. 3.5 Validation steps for a certain analytical method using figures of merit as parameters of control. *LOD* limit of detection, *LOQ* limit of quantification

Table 3.1 Tabulated value of t, according to the measurement number for 95 and 99% of confidence interval

Degrees of freedom (n—1)	Critical value of t for 95% of confidence interval	Critical value of t for 99% of confidence interval
1	12.71	63.66
2	4.30	9.93
3	3.18	5.84
4	2.78	4.60
5	2.57	4.03
6	2.45	3.71
7	2.37	3.50
8	2.31	3.36
9	2.26	3.25
10	2.23	3.17
∞	1.96	2.58

Table 3.2 Tabulated values of F for 5% of probability of significance ($p = 0.05$),[a] according to the degrees of freedom of the numerator and denominator

	Degrees of freedom (numerator)						
Degrees of freedom (denominator)	3	4	5	6	12	20	∞
3	9.28	9.12	9.01	8.94	8.74	8.64	8.53
4	6.59	6.39	6.26	6.16	5.91	5.80	5.63
5	5.41	5.19	5.05	4.95	4.68	4.56	4.36
6	4.76	4.53	4.39	4.28	4.00	3.87	3.67
12	3.49	3.26	3.11	3.00	2.69	2.54	2.30
20	3.10	2.87	2.71	2.60	2.28	2.12	1.84
∞	2.60	2.37	2.21	2.10	1.75	1.57	1.00

[a] The p-value is defined as the probability of the results of an experiment deviating from the null by as much as they did or greater if the null hypothesis is true. Traditionally, the cutoff value to reject the null hypothesis is 0.05, which means that, when there is no difference, a value as high as test statistic is expected to be less than 5% of the time

according to the measurement number and the correctness probability for those measurements.

Comparing the results of two different methods or comparing the results of two different laboratories can be done by means of F-test, according to Eq. 3.12:

$$F = \frac{Sx^2}{Sy^2} \tag{3.12}$$

Where: s is the mean standard deviation for each measurement set (x or y).

The largest value of s is always used in the numerator, so the value of F will always be greater than the unit. The value found is then compared with the tabulated value of F, considering the degrees of freedom of each set of data. To be considered

equally efficient the value found has to be less than the tabulated value. Table 3.2 presents F values for an exception probability of 5% of the cases.

3.3.4 Interlaboratory Comparisons

The analysis of the same type of sample is carried out by several laboratories, whose objective is to verify if the result obtained by the laboratory that is developing the method is *reproducible*.

3.3.5 Systematic Evaluation of Factors Influencing Results

It is important to get a good knowledge of the measurement process to evaluate the possible sources of interference in the final result and it should ideally be done continuously.

3.3.6 Evaluation of Uncertainty of Results Generated

The uncertainty of a measurement is a parameter associated with the result that characterizes the dispersion of the values obtained around the mean, since there are associated uncertainties in each measurement process (Bureau International des Poids et Mesures 2012). The combined total uncertainty or standard uncertainty, u (Eq. 3.13), is the sum of the uncertainties generated by the various components of the measurement process, each expressed as a standard deviation. Having established a confidence level, the expanded combined uncertainty, U, is determined by the confidence interval criterion, using a coverage factor, k. Most of the time, we use $k = 2$, corresponding to the confidence level of approximately 95% (Olivieri et al. 2006). The measurement of uncertainty should not be confused with the error. The error is defined as the difference between the measured value and the true value.

$$u = U/k \tag{3.13}$$

3.3.7 Repeatability and Reproducibility

These two terms are frequently observed in metrology and in analytical chemistry when we need to evaluate the reliability of analytical results for intra or

interlaboratory studies for quality control (QC) and quality assurance (Sect. 3.5 ahead).

According to the International Union of Pure and Applied Chemistry (2023), we can define:

- *Repeatability*: the closeness of agreement between independent results obtained with the same method on identical test material, under the same conditions (same operator, same apparatus, same laboratory, and after short intervals of time). The measure of repeatability is the standard deviation (Eq. 3.5, Section 3.2) qualified as *repeatability standard deviation*.
- *Reproducibility*: the closeness of agreement between independent results obtained with the same method on identical test material but under different conditions (different operators, different apparatus, different laboratories, and/or after different intervals of time). The measure of reproducibility is the standard deviation (Eq. 3.5, Sect. 3.2) qualified as *reproducibility standard deviation*.

3.4 Quality Control and Quality Insurance

After development and validation, the analytical method requires permanent control. It is of extreme relevance, since it allows verifying the need for a revalidation. In general, a revalidation must be carried out, with one of the following situations (International Standard Organization 1994):

- Introduction of a new analytical method in place of the previously validated one
- Exchange of a particular reagent for another of different brand that has lower purity and quality specifications
- Preventive or corrective maintenance in an instrument used in the methodology, altering the original technical configurations of the manufacturer
- Changes in work concentration of the analytical method, and changes not predicted in parameters of the analytical method in the original robustness test

The use of the previously described figures of merit (Sect. 3.2) is essential for such methodological control.

According to the U.S. Environmental Protection Agency (2022), quality assurance/quality control measures are those activities that are undertaken to demonstrate the accuracy (how close to the real result) and precision (how reproducible results are)—these figures of merit were seen in the Sect. 3.2.

Quality assurance (QA) generally refers to a broad plan for maintaining quality in all aspects of a program. This plan comprises the following:

- Proper documentation of all procedures
- Training of volunteers
- Study design, data management, and analysis
- And specific quality control measures

Quality control (QC) consists of the steps to determine the validity of specific sampling and analytical procedures with internal (e.g., field blank, replicates, spike samples, calibration blank, and calibration standards), and external (e.g., internal field duplicates, split samples) checks. The assessment of the overall precision and accuracy of the generated data, after the analyses, is the *quality assessment*.

A *quality management system*—which comprises QA and QC statements—is constituted by the following components (Prichard and Barwick 2007):

- Management structure and responsibility
- Third-party assessment
- Annual review (by senior management)
- Auditing (internal and external)
- Training (internal and external)
- Records (validation, calibration, quality control, complaints)
- Documentation (central and local)

It is relevant to highlight the good laboratory practices (GLPs) (Organisation for Economic Co-operation and Development 1998) as the reference of QA for chemical analysis and the norm ISO/IEC 17025 (International Standard Organization 2005) as the reference of competency requirements, according to a quality management system, for QC.

In addition to QA/QC, the *accreditation* process provides independent confirmation of competence. For an analytical laboratory, it aims to guarantee the reliability of its results issued against quality parameters established and evaluated by a recognized accrediting body. It is required for an agency or official body to accept its results.

At present, accreditation is one of the main requirements for the performance of an analytical laboratory, considering the fact that it accredits the quality, since the accredited laboratory complies with the norm ISO/IEC 17025 (International Standard Organization 2005). There are cases that GLPs (Organisation for Economic Co-operation and Development 1998) should also be considered in the accreditation process—such as for studies for environmental fate and impacts of a chemical. Therefore, accreditation according to ISO/IEC 17025 (International Standard Organization 2005) should be the motto of the quality of laboratories carrying out chemical analyses.

3.5 Conclusions

Figures of merit are paramount mathematical tools to guarantee the reliability of analytical method and, consequently, the reliability of any analytical result obtained from an analytical process.

Method of development and validation are relevant processes for the correct analytical method application and, again, to ensure the reliability and, also, the

uncertainty from a measurement. Here, the figures of merit are expanded and used coupled to other concepts (e.g., repeatability and reproducibility).

Finally, quality control and quality assurance are useful approaches to maintain and to assess the quality of the measurements in order to achieve the accreditation for the analytical laboratory.

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Chapter 4
Agrochemistry and Pharma

Abstract Pharmaceuticals and agrochemicals, the most representative family of biologically active molecules obtained by means industrial processing, embrace a huge diversity of compounds—mainly organic compounds—used as drugs for diseases, and pesticides for pest control in modern agriculture. As relevant industrial sectors for the modern society, both pharmaceuticals and agrochemicals demand advanced analytical techniques and methods for monitoring and quality control of processes, raw material, inputs, and products. However, these sectors generate a diversity of analytical matrices with their own physical characteristics. In this chapter several classes of analytical techniques, as chromatographic techniques, spectroscopic and spectrometric techniques, thermal techniques, among others are presented and discussed in order to be applied in the fine chemical industry and their analytes; furthermore, relevant auxiliary tools as artificial intelligence, quality by design, and chemometrics are introduced to obtain all potential and reliability from the analytical data.

Keywords Analytical instrumentation · Analytical matrices · Artificial intelligence · Quality by design · Chemometrics

4.1 Introduction

Pharmaceuticals and agrochemicals, the most representative family of biologically active molecules obtained by means of industrial processing, embrace a huge diversity of compounds—mainly organic compounds—used as drugs for diseases, and pesticides for pest control in modern agriculture (Fig. 4.1).

Despite the differences related to the final products' application, their synthetic routes are very similar in terms of process engineering, safety, and pollution prevention, as we can observe in reference books as the *Handbook of Industrial Chemistry: Organic Chemicals* (Ali et al. 2005). Furthermore, both sectors are part of the fine chemical industry.

Tables 4.1 and 4.2 describe examples of pharmaceuticals and agrochemicals commonly used by the modern society. And Figs. 4.2 and 4.3 present some chemical structures of their molecules.

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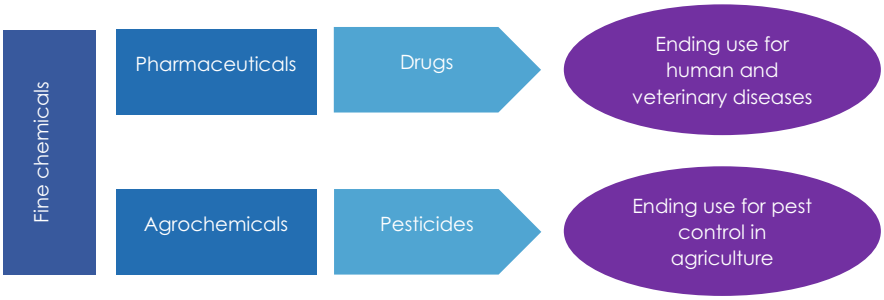


Fig. 4.1 The strategic classification of the biologically active molecules obtained by the industry

Table 4.1 Modern pharmaceuticals commonly used for several diseases, based on open public information of the pharmaceutical sector

Commercial name	Active pharmaceutical ingredient	Application(s)	Producer (laboratory)
Keytruda	Pembrolizumab, a humanized monoclonal antibody	Treatment of adult and pediatric patients with large cell lymphoma	Merck Sharp & Dohme
Comirnaty	Single-stranded mRNA embedded in lipid nanoparticles	Vaccine for COVID-19	Pfizer
Humira	Adalimumab, a humanized monoclonal antibody	Treatment of rheumatoid arthritis etc.	AbbVie
Paxlovid	Nirmatrelvir + ritonavir (antivirals for systemic use)	Treatment of COVID-19	Pfizer
Eliquis	Apixaban	Decreasing of the risk of venous thromboses, systemic embolization and stroke	Bristol-Myers Squibb
Opdivo	Nivolumab, a humanized monoclonal antibody	Classic Hodgkin's lymphoma treatment	Bristol-Myers Squibb
Dupixent	Dupilumab, a human monoclonal antibody	Treatment of atopic dermatitis, asthma, and chronic rhinosinusitis	Sanofi
Stelara	Ustekinumab, a human monoclonal antibody	Treatment of psoriatic arthritis	Johnson & Johnson
Spikevax	Elasomeran (mRNA)	Vaccine for COVID-19	Moderna
Biktarvy	Bictegravir (antiviral) + emtricitabine (antiviral) + tenofovir alafenamide (antiviral)	Treatment of HIV	Gilead

HIV human immunodeficiency virus, *mRNA* messenger RNA

We can observe in Table 4.1 a highlighted presence of monoclonal antibody drugs as a relatively new class of pharmaceuticals, mainly applied for cancer treatment but expanding to other diseases. Monoclonal antibody therapy is a passive immunotherapy consisting of in vitro-generated identical immunoglobulin clones

Table 4.2 Modern agrochemicals commonly used for several pest control

Commercial name	Active ingredient(s)	Application class	Producer
Round-up	Glyphosate	Herbicide	Bayer
Enlist, Colex-D	2,4-D	Herbicide	Corteva
Harness Plus	Acetochlor	Herbicide	Monsanto
Cantus	Boscalid	Fungicide	Basf
Constant	Tebuconazole	Fungicide	Bayer
Artea	Propiconazole	Fungicide	Syngenta
Decis 25 EC	Deltamethrin	Insecticide	Bayer
Curbix	Ethiprole	Insecticide	Bayer
NemaStrike	Tioxazafen	Nematicide	Bayer
Ethrel PA	Ethephon	Plant growth regulator	Bayer

Source: Adapted from the Codex Alimentarius (Food and Agriculture Organization of the United Nations 2023) and open public market information
2,4-D = 2,4-Dichlorophenoxyacetic acid

which specifically bind to a defined membrane-bound surface antigen and induce tumor cell-lysis or—apoptosis via different pathways (Sasse and Engert 2008). That means, it involves a biotechnological approach instead of the chemical synthesis to obtain the final product. However, this observation doesn't exclude the large production of pharmaceuticals obtained by means of the chemical synthesis, being this last approach the most common for the pharmaceutical industry.

We can observe in Table 4.2 a variety of agrochemical classes—basically organic chemicals (Fig. 4.3)—comprising herbicides, fungicides, insecticides, nematicide, and plant growth regulator. These classes and number of molecules (or active ingredients, AIs) used in a certain country will depend on the climatic conditions (i.e., tropical or temperate), the crop type (e.g., corn, soybean, fruits, etc.), and the pest incidence (e.g., insects, diseases, etc.).

Besides those agrochemical classes listed in Table 4.2, we can add semiochemicals as an alternative for pest monitoring and control—i.e., pest insects—in order to reduce the application of active ingredients (e.g., insecticides) generally obtained by chemical synthesis. These semiochemicals are compounds involved in chemical communication as pheromones, which mediate communication between members of the same species, and allelochemicals, which denote chemicals used for communication between different species (Kost 2008). For instance, the limonene molecule (a pheromone) can be used for this purpose by means of its controlled release (Vaz et al. 2022).

4.2 How These Industries Work

In a general way, the pharmaceutical industry works by means of the discovery, development, and manufacture of drugs and medications (pharmaceuticals) by public and private organizations (Dailey 2023). Generally, this industry can

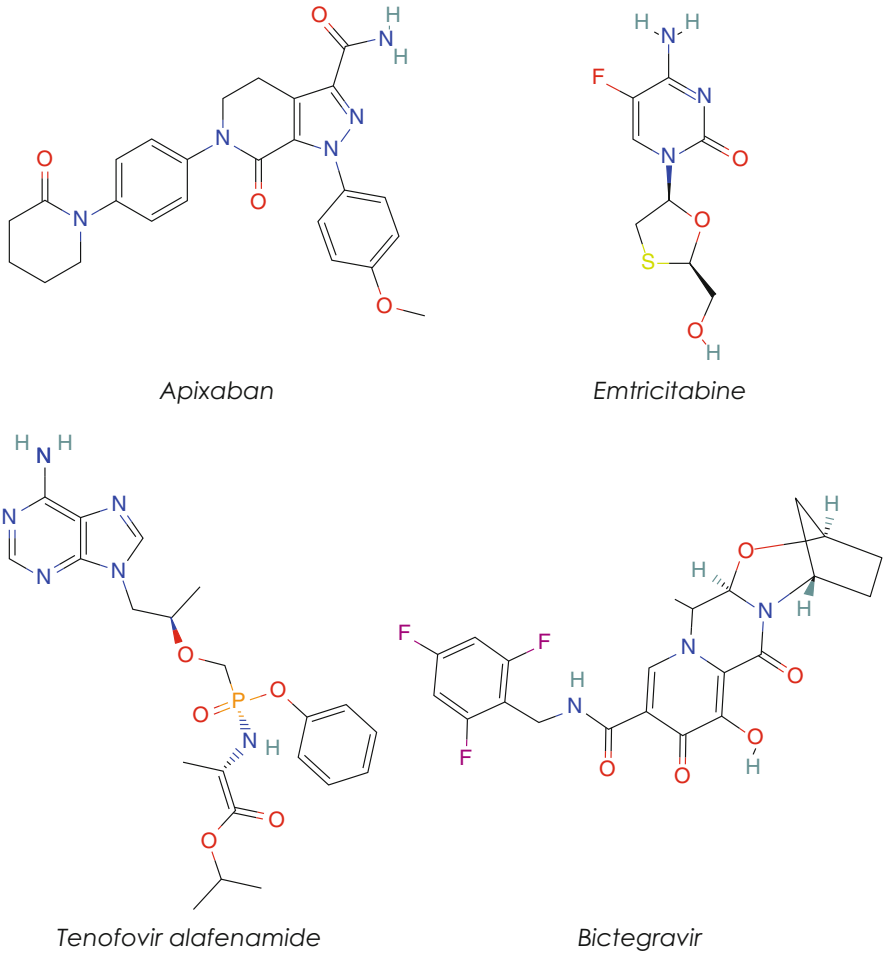


Fig. 4.2 Some molecules of pharmaceuticals listed in Table 4.1

comprise from the laboratories and industrial plants to the final consumer—a very large and complex value chain. Its relevance was depicted in Chap. 2, Sect. 2.2, remembering that the pharmaceutical industry can manufacture drugs for human and veterinary use, and pharmaceutical preparations.

Based on the statements of Ali et al. (2005), the pharmaceutical production comprises three main stages and their suitable environment:

(i) Research and development (e.g., screening by combinatorial chemistry and high-throughput screening, preclinical and clinical trials, new drug applications, etc.)—related to laboratory and pilot-plant environment.

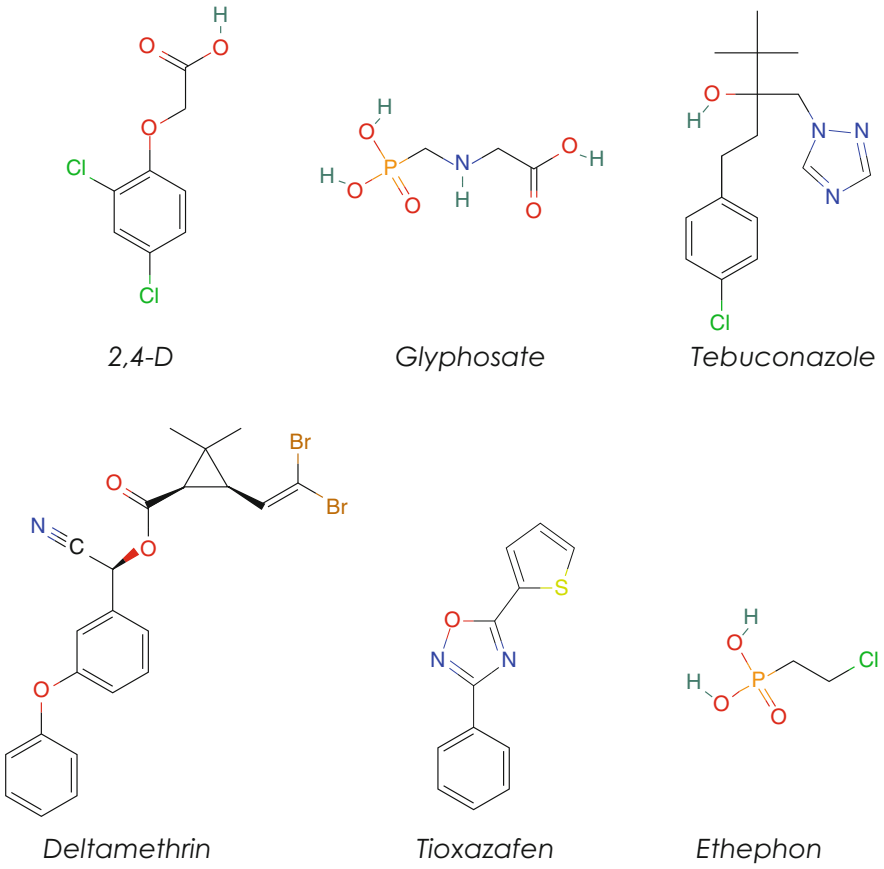


Fig. 4.3 Some molecules of agrochemicals listed in Table 4.2

(ii) The conversion of organic chemicals or natural products into bulk pharmaceutical substances or ingredients—by means of organic synthetic route, biotechnological route—related to the industrial plant environment.
(iii) The formulation of final pharmaceutical products (e.g., addition of additives as adjuvants in order to solubilize, suspend, thicken, dilute, emulsify, stabilize, preserve, color, and improve the taste of the final mixture)—related also to the industrial plant environment.

These stages will define the analytical necessity. For instance, in the research and development stage chromatographic techniques hyphenated to mass spectrometry (see in Sect. 4.4) are very useful; on the other hand, probes based on spectroscopic techniques are most useful for those demands related to the production in industrial plants.

Regarding the agrochemical industry, one of the main characteristics of this industry is that economies of scale are not very relevant, since the agrochemical

production process takes place in batches and not continuously, as occurring with other segments of the chemical industry. On the other hand, there is opportunity for economies of scope, since one can promote the differentiation of products around the same basic chemical molecule. Thus, with one or more steps of synthesis added, we can create a range of products with differentiated applications and economic potentials (De Velasco and Capanema 2006).

As well as for the pharmaceutical industry, the agrochemical industry spends funding and efforts in research and development and innovation in order to develop eco-friendly molecules compared to those earlier agrochemicals, known for their negative impacts on the environment and public health (Unsworth et al. 2019). The agrochemical industry also involves those same stages presented for the pharmaceutical industry with their own characteristics, that means the following:

(i) Research and development
(ii) Synthesis of active ingredient (the technical product)
(iii) Formulation of the final product (or commercial product) using additives for the best performance of the active ingredient

Figure 4.4 depicts a general flowchart of an organic process applied to the pharmaceutical and agrochemical industries.

From Fig. 4.4, initially, the raw material (e.g., parts of vegetables, organic or inorganic material, a natural product, etc.) must undergo a complete chemical analysis and characterization, which aims to determine its chemical constitution, in addition to some physicochemical properties that are of interest. Then, there is the separation of the precursor molecule of interest for the synthesis, and if it does not have adequate purity, a purification step is carried out. In some cases, when the raw material has the appropriate purity, it can be directly forwarded to the step of synthesis. With the obtaining of the precursor molecule, the organic synthesis step is taken, in which the search for the best catalysts may involve a catalytic screening of various catalysts, e.g., heterogeneous, and homogeneous inorganic catalysts, organometallic catalysts and enzymes, and the appropriate approach for design of the synthesis route. It is worth commenting on the development and use of catalysts for these types of organic processes, given their importance for improving yield and selectivity—considering enantioselectivity, regioselectivity, and stereoselectivity.

After the synthesis of the target product—active ingredient or active pharmaceutical ingredient—evolving, if necessary, a new step of separation and purification, it must be properly identified as to its chemical structure and purity by means of advanced analytical techniques as ^{13}C or ^{1}H nuclear magnetic resonance, mass spectrometry, and/or absorption infrared and ultraviolet spectroscopies. We can assume that the active ingredient/active pharmaceutical ingredient has its industrial potential, goes through a scale-up, and is in industrial production stage. If the final product and route are not economically viable, the search for a new raw material/precursor molecule or a new target product (i.e., an active ingredient/active pharmaceutical ingredient), or both, can be restarted.

Finally, after the obtaining the active ingredient/active pharmaceutical ingredient—in some cases known as technical product—it can be used to prepare

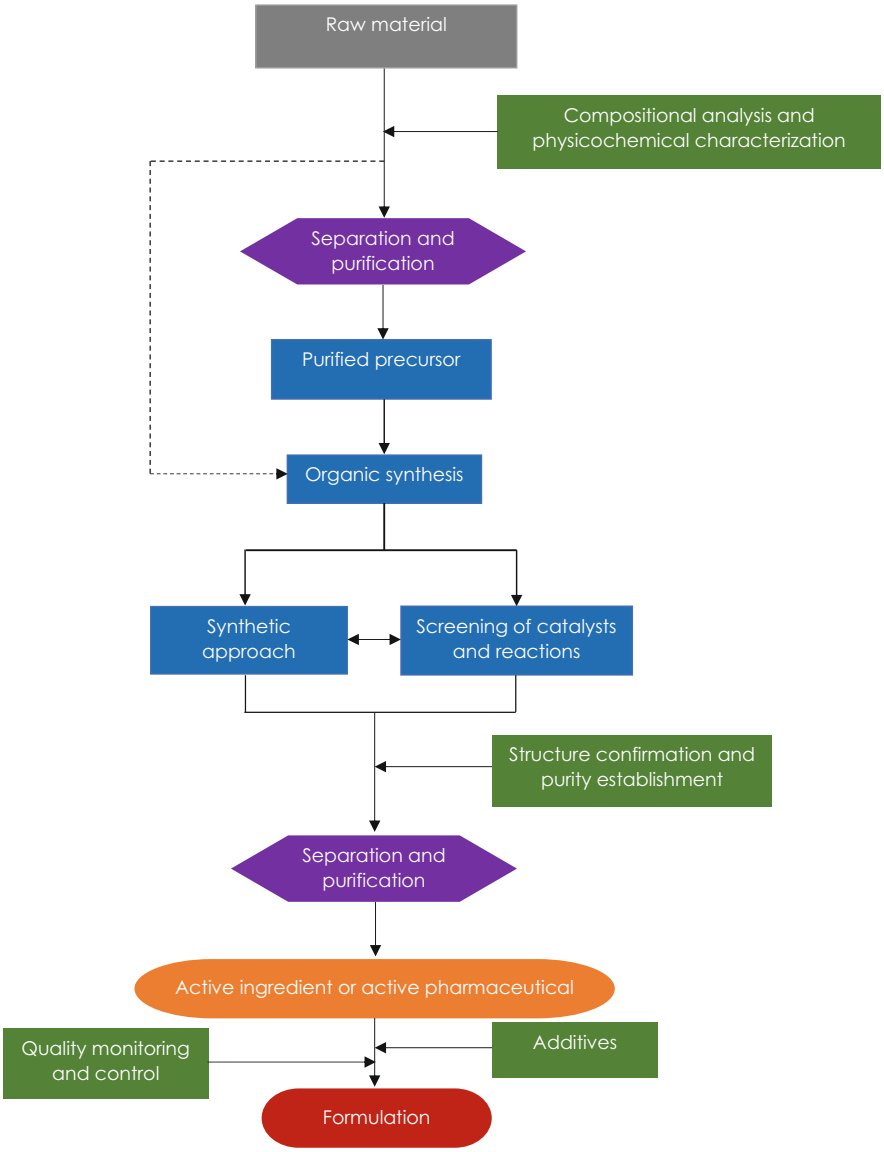


Fig. 4.4 Conceptual flowchart of a complete organic process applied to pharmaceuticals and agrochemicals

the formulation of end use by means of the addition of chemical additives, considering the quality monitoring and control by means of several analytical techniques (e.g., chromatography in liquid phase and Raman absorption spectroscopy).

4.3 Main Related Analytical Matrices

Considering that the sample is composed of the analyte and its medium (analytical matrix)—according to Sect. 3.1, Chap. 3—the consideration of the latter is paramount to define analytical techniques and methods for the most suitable applications.

As mentioned before, for pharmaceuticals and agrochemicals the end product, or formulation, is composed of the active ingredient/active pharmaceutical ingredient (generally considered the analyte) and variable quantities of additives to promote the best performance of this formulation—the sum of them constitutes the analytical matrix. These additives can turn onto analytes if we need to perform, for instance, chemical analysis to determine impurities; it is critical mainly for pharmaceuticals.

Table 4.3 depicts common additives used in pharmaceutical formulations and Table 4.4 depicts common additives used in agrochemical formulations.

Both pharmaceutical and agrochemical formulations are generally produced in liquid, solid, or a mixture of both physical states in the same end product (e.g., liquid dispersions):

- Powders: for pharmaceutical formulations
- Wettable powders: for agrochemical formulations
- Tablets: for pharmaceutical formulations

Table 4.3 Common additives for pharmaceutical formulations

Additive category	Example
Encapsulation agents	Carboxymethyl cellulose, gelatin
Emulsifying agents/solvents	Dextran, gums, egg albumin, polyoxyethylated castor oil
Synthetic sweetness	Saccharin, aspartame
Vehicles	Oils, alcohols, propylene glycol
Stabilizing agents/antioxidants	Ethylenediamine, sulfites
Dyes	Tartrazine, sunset yellow, ponceau red, xanthene dyes
Preservatives	Benzoates, parabens, thimerosal, chlorobutanol
Adjuvants	Aluminum hydroxide, zinc oxide

Source: Adapted from Ferri (2022). Reproduced with permission from Elsevier

Table 4.4 Common additives for agrochemical formulations

Additive category	Example
Adjuvants	Amine ethoxylate, polyglycerol ester
Emulsifiable concentrate	Blend of one or more anionic emulsifier with one or more non-ionic emulsifier (e.g., ethoxylates or alkoxylates)
Emulsion in water	Dimethylamide derived fatty acids
Oil dispersion	Mineral oils, vegetable oils, or esters of vegetable oils
Suspension concentrate	Nonionic wetting agents
Soluble liquids	Fatty amine ethoxylates

Source: Adapted from Clariant (2023). Reproduced with permission from Clariant

- Capsules: for pharmaceutical and agrochemical formulations
- Aerosols: for pharmaceutical formulations
- Injectable liquids: for pharmaceutical formulations
- Syrups: for pharmaceutical formulations
- Emulsions: for agrochemical formulations

In some cases, it can be found in gaseous state for pharmaceutical formulations, which could demand different approaches for the analyte extraction in order to provide the most suitable chemical species for the chemical analysis. And in some situations, independently of the physical state, it can demand a *derivatization* after the extraction step.

Thus, the considerations previously stated here give us the idea of the complexity of pharmaceutical and agrochemical matrices which require suitable preparation methods to separate the analyte from them to run the analytical process (to be seen ahead in the next sections).

According to the European Medicines Agency (2009), the physicochemical and biological properties relevant to the safety, performance, or manufacturability of the drug product should be identified and discussed. This statement opens a large demand for analytics in order to reach the best quality control proceedings, which can be spread to the agrochemicals due the inherent potential for negative impacts on the environment and public health, as in the case of residues analysis and control, according to the World Health Organization and the Food and Agriculture Organization of the United Nations (World Health Organization 2023).

4.3.1 Sample Preparation

The correct sample preparation is the first step to make available the analyte to the analytical technique and its analytical method of application.

Extraction is a highlighted technology of the analytical processing, especially in the sample preparation, and the use of solvent extraction is more recurrent in the analysis of organic molecules in several matrixes. According to the International Union of Pure and Applied Chemistry (2023), the solvent extraction can be defined as the process of transferring a substance from any matrix (e.g., water or soil) to an appropriate liquid phase (e.g., a mobile phase for HPLC). If the substance is initially present as a solute in an immiscible liquid phase, the process is synonymous with liquid-liquid extraction (Notes: if the extractable material is present in a solid (such as a tablet) the term leaching may be more appropriate). The extractable material may also be a liquid entrapped within or adsorbed on a solid phase.

However, there are other extraction techniques to be used for pharmaceuticals and agrochemical chemical analysis, for which the choice depends on the analyte and matrix physicochemical properties, number of samples, time, and costs. For instance, for the best choice we can consider that the recovery of the extraction method must be in the range of 70–120% (seen in Chap. 3).

After the extraction step, we can concentrate the analyte present in the extraction medium to promote a better analytical response. It can be made by means of the following (Mitra 2003):

- Stream of nitrogen gas flow, for non-volatile analyte and small volume to reduce
- Rotary vacuum evaporator, for large volume to reduce
- Kuderna-Danish concentrator using air-cooled condenser, for smaller volume to be reduced to less than 1 mL

After the extractive concentration, a *clean-up* step is desirable to remove interfering species previously by the chromatographic separation, the most common technique. These interfering species are very common for heterogeneous matrix composition. To overcome these difficulties, we can use:

- Gel-permeation chromatography (GPC), for the elimination of lipids, proteins, polymers, copolymers, natural resins, cellular components, viruses, steroids, and dispersed high-molecular-weight compounds from the sample. This method is appropriate for both polar and nonpolar analytes.
- Solid-phase extraction cartridges (SPE), for steroids, esters, ketones, glycerides, alkaloids, and carbohydrates. Cations, anions, metals, and inorganic compounds are interfering species, also candidates for this technique.

Regarding sample preparation for inorganic analytes—what can be seen deeply in Chap. 5—we can highlight acid digestion and microwave digestion for spectrometric techniques (see ahead).

4.4 Main Related Analytical Techniques

This section is structured in order to describe relevant aspects of analytical techniques and methods for the following:

- Quality control of raw materials, inputs, and final products
- Investigation of by-product residues and impurities

That means, common physicochemical tests (e.g., pH of an aqueous solution, melting point/range, refractive index, water content by titration, viscosity, particulate matter), and biological tests (e.g., total count of aerobic microorganisms, the total count of yeasts and molds, and the absence of specific objectionable bacteria) for quality control will not be considered because generally they don't demand advanced analytical techniques or approaches which are the subject of this chapter. For these most common chemical and biological analyses, there are free guidelines to be consulted:

- For pharmaceuticals: *ICH Q1A (R2) Stability testing of new drug substances and drug products—Scientific guideline* (European Medicines Agency 2023a).

- For agrochemicals: *OECD Test guidelines for chemicals* (Organisation for Economic Co-operation and Development 2023).

Additionally, for chemical species, we can consult some relevant books for organic and inorganic analysis, as follows:

- *Vogel's Quantitative Chemical Analysis* (Mendham et al. 2000)
- *The Systematic Identification of Organic Compounds* (Shriner et al. 2003)

Regarding the advanced analytical techniques, examples are as follows:

- For quantitative information: chromatographic techniques (gas and liquid phases) with several detectors, atomic emission spectrometry, optical emission spectrometry, atomic absorption spectrometry, and ultraviolet-visible absorption spectrophotometry
- For qualitative information: absorption of infrared spectroscopy, nuclear magnetic resonance in solid and liquid states (e.g., ^{13}C and ^{1}H nuclei), mass spectrometry, Raman spectroscopy, thermal techniques (e.g., DSC and TGA), particle size distribution, and Zeta potential

These techniques for application in pharmaceuticals and agrochemicals are introduced and discussed in the next sections.

For more detailed information about the physical fundamentals for these techniques, a reference book as *Ewing's Analytical Instrumentation Handbook* (Cazes 2005) should be consulted.

4.4.1 Chromatographic Techniques

Chromatography is, conceptually, a technique of separating components from a sample according to their *retention time*, for further identification and determination/quantification. It is the most largely used category of instrumentation technique for pharmaceutical and agrochemical analyses due to its versatility.

In most cases, chromatographic techniques are coupled with detection techniques—what is known as *hyphenated* techniques. As forms of hyphenation, we can mention the following:

- Coupling of solid-phase extraction systems, known as SPE, and SPME (solid-phase microextraction)—these systems allow increased extraction performance from equilibrium phenomena, or thermal sorption-desorption, or with organic solvents, which may help to reduce the limit of detection (LOD) and the limit of quantification (LOQ) values.
- Liquid chromatography (LC) coupled with gas chromatography (GC), or vice versa, promotes the so-called multidimensional separation techniques that allow to work with complex mixtures, such as: LC-GC, LC-GCxGC, LCxLC, etc.; however, the use of chemometrics—seen ahead—for the treatment of the generated data is required for this type of hyphenation.

Table 4.5 Description of categories of chromatographic techniques according to the stationary phase, considering only the case where the separation takes place in chromatographic columns, which is the type of separation most applied in pharmaceutical and agrochemical matrices

General classification	Category	Stationary phase	Equilibrium type
Gas chromatography	Gas-liquid	Liquid bound to solid	Gas-liquid partition
	Gas-solid	Solid	Adsorption
Liquid chromatography	Liquid-liquid partition	Liquid bound or adsorbed to solid	Liquid-liquid partition (immiscible)
	Liquid-solid or adsorption	Solid	Adsorption
	Ion exchange	Resin for ion exchange	Ion exchange
	Size exclusion	Liquid in the interstices of polymeric solid	Partition or penetration
	Affinity	Liquid bound to solid surface	Liquid-liquid partition

Source: Adapted from Vaz (2018). Reproduced with permission from Springer Nature

One way to classify the chromatographic techniques is by the physical form of the mobile and stationary phases. Thus, the first classification would be *planar* or *column*—from planar originates the thin layer chromatography and from column liquid and gas phase chromatographies. Table 4.5 provides a description of the functional division categories for GC and LC.

The division presented above is due to physicochemical equilibrium phenomena, which are those that govern the transfer of analyte mass between the mobile and stationary phases. Partitioning is emphasized here, through the chemisorption (involving covalent bonds) and physisorption (involving intra- or intermolecular interactions, usually Van der Waals forces).

The following equations conceptually define the chromatography and its application. Firstly, *resolution* is the quantitative measure of the degree of separation between two peaks A and B, referring to two different molecules, and it is defined by Eq. (4.1):

$$R = t_{R(B)} - t_{R(A)} / 0.5\ (W_B + W_A) \tag{4.1}$$

Where: $t_{R(A)}$ = retention time for the peak A; $t_{R(B)}$ = retention time for the peak B; W_A = baseline width for the peak A; W_B = baseline width for the peak B.

Separation between peaks A and B is governed by the *partition coefficient*, K_D, which measures the solute distribution—or the analyte distribution—from its concentration in the *mobile phase* (S_m) and its concentration in the *stationary phase* (S_s), in a condition of equilibrium, according to Eq. (4.2):

$$K_D = [S_m] / [S_e] \tag{4.2}$$

Thus, the higher the K_D value the lower the t_R of the analyte, and vice versa.

The *efficiency* of a *separation column* for the chromatographic analysis can be verified by the number of *theoretical plates* of the column, according to Eq. (4.3):

$$N = 16(t_R/W)^2 \tag{4.3}$$

The column efficiency increases with the increase in the value of N, which also leads to an increase in the peak resolution.

4.4.1.1 Gas Chromatography

In GC the components of a sample are separated as a function of their partition between a gaseous mobile phase, usually the helium gas, and a liquid or solid phase contained within the column. One limitation of GC is when the analyte to be analyzed is not volatile (i.e., it is thermally stable); an alternative to this limitation is the *derivatization*, when the formation is of another molecule from the analyte with lower boiling values. The *elution* of the components is done by an inert mobile phase (carrier gas) flow; that is, the mobile phase does not interact with the molecule of analyte.

The modernization of the equipment, through the development of new stationary phases and data processing software, also led to an investment in systems that provide higher speed during the chromatographic analysis. The shortest analysis time has the direct consequence of reducing the cost of the analytical process and increasing the analytical capacity of the laboratory. The increase in the speed of the chromatographic analysis can be related to the reduction of the size of the column, and reduction of its internal diameter, which compensates the loss of resolution in the determinations.

Regarding the choice of the most suitable detector to be used, the nature of the sample (analytical matrix more analyte) should be considered. Several detectors are commercially available for use in GC, with thermal conductivity (TCD), flame ionization (FID), electron capture (ECD), and mass spectrometer (MS) detectors being most commonly used. An ideal detector should meet the following characteristics:

- Adequate sensitivity
- Good stability and reproducibility
- Linear response to analytes, extending to several orders of magnitude
- Temperature range from ambient to at least 400 °C;
- Ease of use
- Similarity of response to all analytes in the sample

In practice, the detectors do not group all the features described above. Table 4.6 shows the most common detectors used in GC and their LOD.

Figure 4.5 describes a block diagram for a GC equipment. And Fig. 4.6 depicts the equipment.

Table 4.6 Most common GC detectors

Detector	LOD
Flame ionization detector (FID)	0.2 pg
Thermal conductivity detector (TCD)	500 pg
Electron capture detector (ECD)	5 fg
Thermal-ionic detector (TID) or nitrogen-phosphorus detector (NPD)	0.1 pg
Mass spectrometer (MS)	<100 pg

Source: Adapted from Vaz (2018). Reproduced with permission from Springer Nature
1 pg = 10^{-12} g; 1 fg = 10^{-15} g; *LOD* limit of quantification

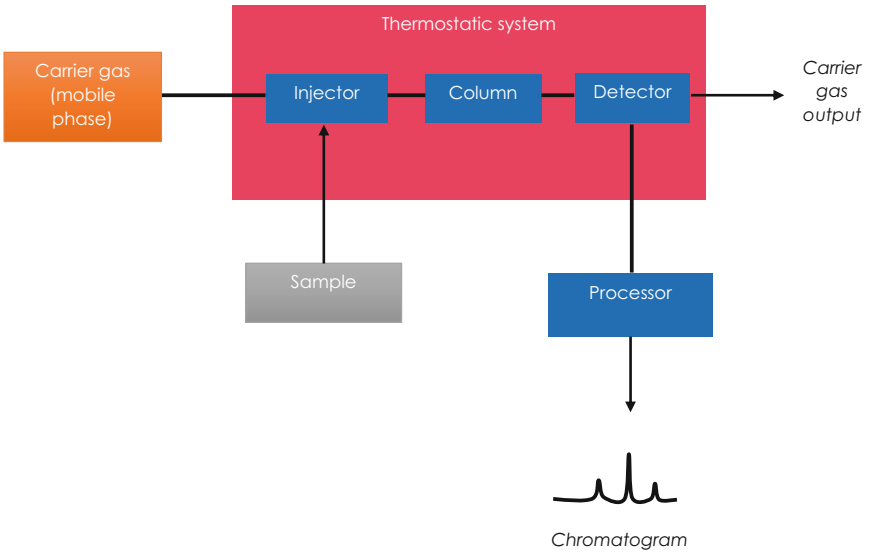


Fig. 4.5 Block diagram of a GC equipment

4.4.1.2 Liquid Chromatography

LC can be applied in a variety of operating modes, with the best mode depending on the structural characteristics of the analyte to be separated by the chosen analytical method. The most common categories are partition chromatography, adsorption chromatography, ion exchange chromatography, size exclusion chromatography, and affinity chromatography (Table 4.5).

High performance liquid chromatography (HPLC)—The use of low-pressure and high-pressure columns, called high performance liquid chromatography (HPLC), outperforms GC in the analysis of semi-volatile and non-volatile organic compounds. In its many variants, it allows the analysis of complex mixtures, difficult to separate by other techniques, especially mixtures of biomolecules.

Typically, the HPLC equipment is equipped with two or more solvent reservoirs. Elution with a single solvent or a mixture of solvents of constant composition is

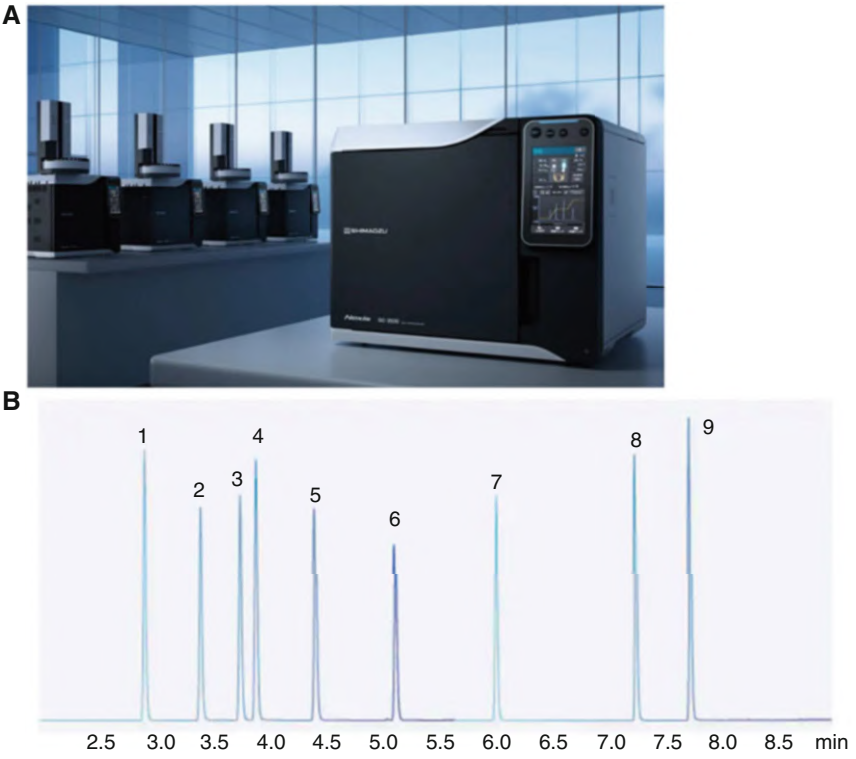


Fig. 4.6 A GC equipment (**a**), and an obtained chromatogram (**b**). Courtesy of Shimadzu

called *isocratic elution,* while the use of a mixture of solvents at different polarity, with composition varying in a programmed manner, is a *gradient elution*. Generally, gradient elution improves the efficiency of the separation process. The pumping system is an important component whose function is to ensure a constant and reproducible flow from the mobile phase to the column. They have a pressure of 0.1 to 350 bar. The columns are generally stainless steel with lengths ranging from 10 to 30 cm and internal diameters between 2 and 5 mm. The column fillings (or stationary phase) typically have particles with diameters between 3 and 10 μm. Systems with particles smaller than 2 μm and pressures in the range of 1000 bar are called *ultra-high-performance liquid chromatography* (UHPLC) or *ultra-performance liquid chromatography* (UPLC)—this mode of liquid chromatography can provide a higher resolution in a shorter retention time. Stationary phases for most chromatography modes consist of a silica material, or a polymer such as a polysaccharide or polystyrene, with functional groups of interest attached to the surface of this substrate—they may be either normal phase (polar stationary phase) type or reverse phase (non-polar stationary phase) type.

Selection of the mobile phase is critical for partitioning, adsorption, and ion exchange chromatography, and less critical for the other modes. For the solvents

Table 4.7 Characteristics of the main HPLC detectors

Detector	LOD
Ultraviolet-visible (UV-Vis) absorption or diode array detector (DAD)	10 pg
Mass spectrometer (MS)	1 pg
Fluorescence detector (FD)	1 ng

Source: Adapted Vaz (2018). Reproduced with permission from Springer Nature
1 pg = 10^{-12} g; 1 ng = 10^{-9} g; *LOD* limit of quantification

used to form this phase, properties such as the ultraviolet-visible (UV-Vis) cut-off wavelength and the refractive index are important parameters when working with UV-Vis and/or refractive index detectors. The polarity index (P′) and the eluent force (ε^0) are polarity parameters that aid in choosing the phase for partitioning and adsorption chromatography, respectively.

As for GC, there are several types of detectors available commercially, and the choice usually depends on the type of analyte and the number of analyses required. Detectors may be concentration-sensitive, when the analytical signal produced is proportional to the analyte concentration in the effluent or eluted; or mass-sensitive, when the signal produced is proportional to the mass flow rate. Table 4.7 lists the main detection systems for HPLC.

Figure 4.7 depicts a block diagram for an HPLC equipment. And Fig. 4.8 depicts the equipment.

4.4.2 Mass Spectrometry

MS is essentially a technique for detecting molecular components having the mass/charge ratio (m/z) as the unit of measurement, which are obtained by means of the original molecule fragmentation into derived chemical species (e.g., the molecular ion M^+). Depending on the ionization technique used, analytes may present with one or multiple charges. In single-charge components, the m/z ratio corresponds to the total mass of the ion in Daltons. In cases where ions with two or more charges are more frequent, the calculation of the original ion mass will depend on deconvolutions of the original signal.

Regarding the fragmentation, it is based on the removal of the electron from the molecule resulting in its ionization. Removal of electrons from either *sigma* bond, *pi* bond, or nonbonding orbitals causes the ionization. The fragmentation pattern promotes the distinction among the analytes.

The direct analysis of the sample in the mass spectrometer seldom generates results that can be considered quantitatively, even if the sample is pure. This is a consequence of the high sensitivity of the technique and the efficiency of the ionization process, besides the intrinsic characteristics of each sample that allow greater or less easiness of ionization.

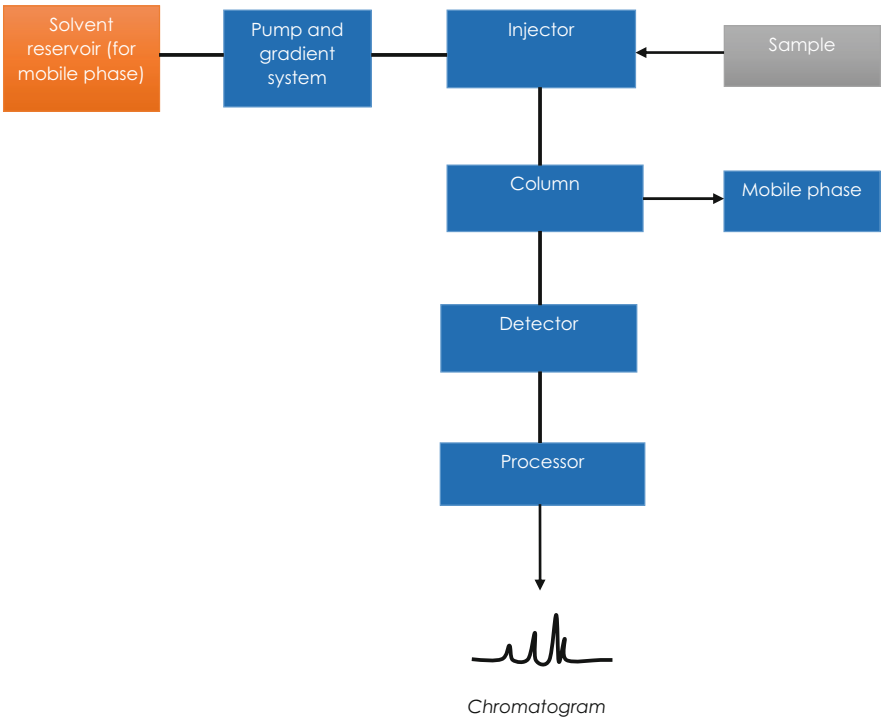
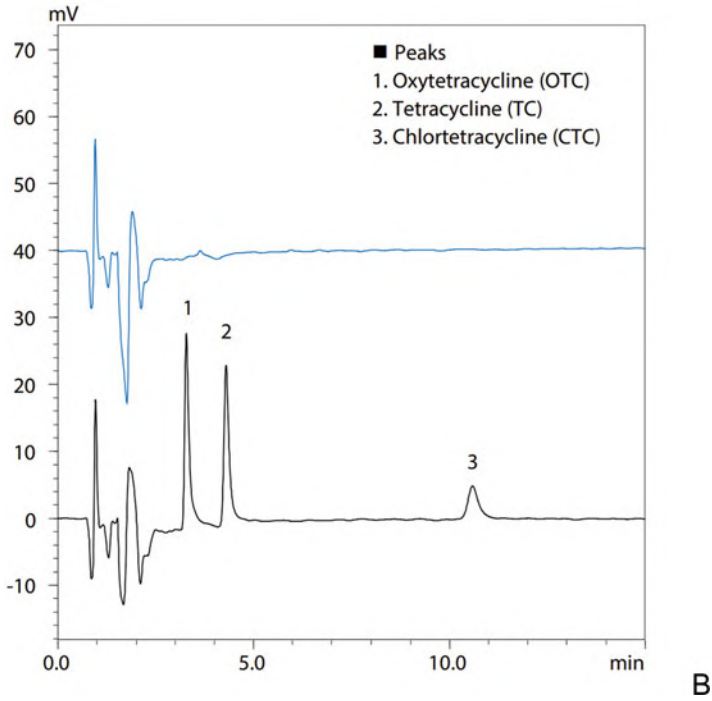


Fig. 4.7 Block diagram for an HPLC equipment

MS is often associated with a separation technique, usually gas chromatography or liquid chromatography—or the hyphenated techniques—where a separation technique coupled to a detection and quantification technique is used. In this case, the mass spectrometer functions as a detector. Such hyphenated techniques make it possible to separate complex mixtures, identify the components, and quantify them in a single operation. Almost all measurements of MS are done under high vacuum, as this allows the conversion of most of the molecules into ions, with a lifetime enough to allow their measurement. The mass spectrometer consists essentially of three components: ionization source, mass analyzer, and ion detector.

There are several commercially available ionization systems: electron impact ionization (EI), chemical ionization (CI), fast atom bombardment (FAB), particle beam bombardment (PBB), matrix-assisted laser desorption ionization (MALDI), electrospray ionization (ESI), atmospheric pressure photoionization (API), and atmospheric pressure chemical ionization (APCI). For high molecular weight, non-volatile and heat-sensitive materials, such as some pesticides, MALDI, APCI, and FAB techniques are used. The most common analyzers are quadrupole, quadrupole ion trap, and time-of-flight tube. The detection is done by electron multiplier tube.

A



B

Fig. 4.8 An HPLC equipment (**a**), and an obtained chromatogram (**b**). Courtesy of Shimadzu

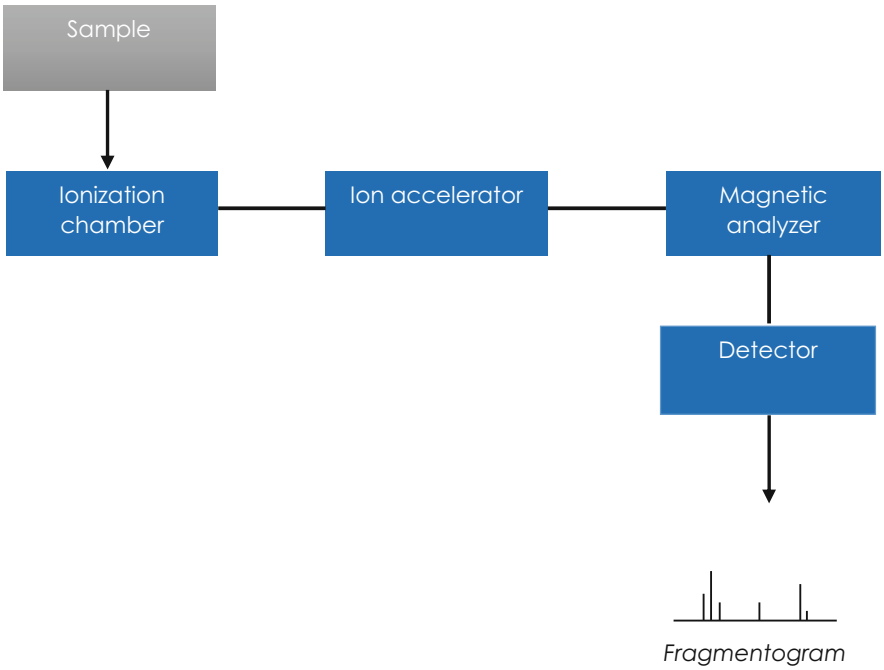


Fig. 4.9 Block diagram of a mass spectrometer

The high-resolution MS provides comprehensive accurate mass information in a single analysis by MS^n technology (tandem mode); detects more low-level components in complex samples; and is designed and well suited for large molecule analysis (Vaz 2021). However, the main limitation is that a large volume of data to process requires an experienced analyst to operate.

As Fourier transform infrared absorption spectroscopy (FTIR) and nuclear magnetic resonance (NMR), MS can be used also to study the presence of residues in the active ingredient/active pharmaceutical ingredient for regulation purposes.

Figure 4.9 depicts a block diagram of an MS instrument, and Fig. 4.10, the instrument.

4.4.3 Spectroscopic/Spectrometric Techniques

The most applied spectroscopic techniques englobe ultraviolet, visible, and infrared regions of the electromagnetic radiation—the most common mode is the absorption of radiation, followed by the emission of radiation. Additionally, we can consider the radio wave region for the nuclear magnetic resonance. Generally, techniques that provide qualitative information are known as spectroscopic techniques and those

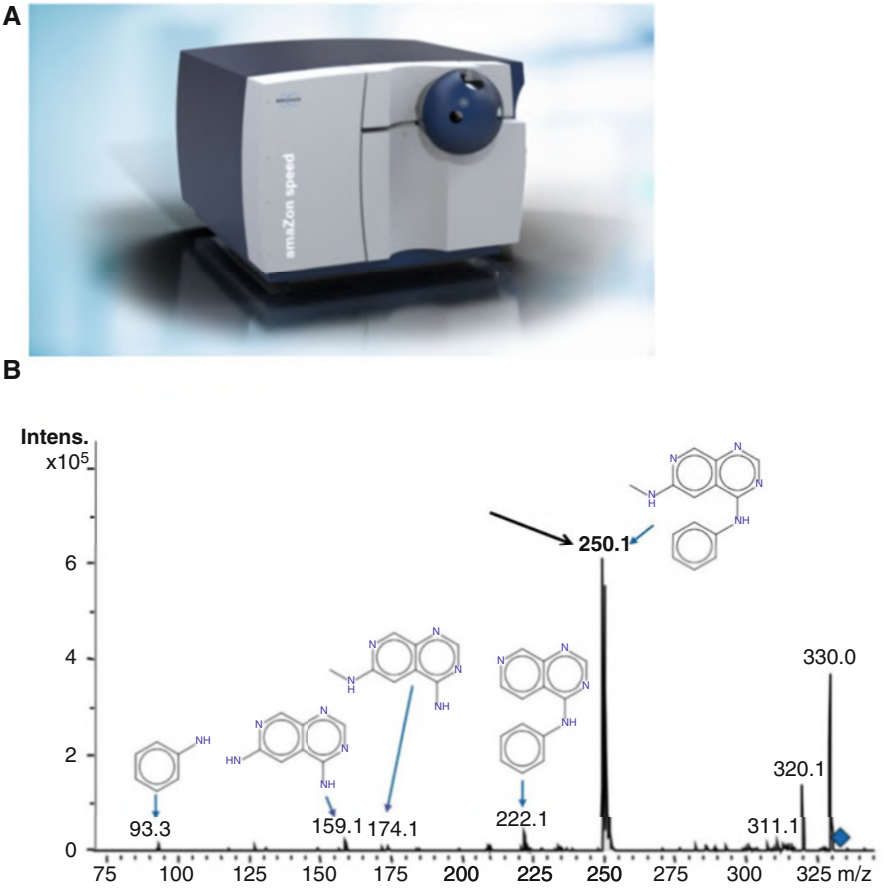


Fig. 4.10 An ion trap mass spectrometer (**a**), and the obtained fragmentogram (**b**). Courtesy of Brüker

that provide quantitative information are spectrometric techniques, but these nomenclatures can vary in some texts.

Electromagnetic radiation exhibits wave and particle properties. While wave has characteristics like speed, wavenumber, and frequency, it is quite common to use the wavenumber in cm^{-1} to describe the radiation. The wave number of the electromagnetic radiation (k) is directly proportional to its energy and, consequently, to its frequency (ν), as can be evidenced by Eqs. (4.4 and 4.5):

$$E = h\nu \tag{4.4}$$

$$E = h\,c/\lambda = h\,c\,k \tag{4.5}$$

Where: E = energy (J); h = Planck's constant (6.626×10^{-34} J s); ν = frequency (Hz); c = speed of light (2998×10 m s^{-1}); λ = wavelength (nm); k = wave number (cm^{-1}).

It should be remembered that the frequency ν is directly proportional to c/λ, while the wave number k is proportional to $1/\lambda$.

4.4.3.1 Absorption of Ultraviolet-Visible Radiation, or Molecular Spectrophotometry

This technique is widely used for the identification and determination of organic, inorganic, and biological species. Usually, molecular absorption spectra are more complex than atomic absorption spectra due to the higher number of energy states of the molecule compared to the isolated atoms (see ahead in the atomic spectrometry item).

The ultraviolet (UV) region of the electromagnetic spectrum is approximately ranges between 200 to 400 nm and the region of the visible (Vis) ranges between 400 and 750 nm. The absorption of radiation by molecules in these regions results from the interactions between photons and electrons that participate in a chemical bond, or between electrons that are not bound in atoms like oxygen, sulfur, nitrogen, and halogens. The wavelength where absorption occurs depends on the type of bond that these electrons participate. Electrons shared in single C-C or H-H bonds are so tightly bound that they require high energy at wavelengths below 180 nm and are not observed by the most common methods of analysis. Due to experimental difficulties in working in this region, single-bond spectra are poorly explored. The electrons involved in double and triple bonds are not so strongly trapped and, consequently, they are excited more easily and produce more useful absorption peaks.

Absorption spectroscopy in UV-Vis is mainly used in quantitative analysis of several organic compounds containing mainly C=O and C=C bonds, as the intensity of the absorption peaks can be directly correlated to the concentration of the analyte, now called spectrophotometry—it is widely used as a detector after separation by liquid chromatography. The signal intensity at a given wavelength value can be directly correlated with the analyte concentration, which allows quantitative data to be obtained—it is worth noting that it is necessary to have the respective curve with linear behavior (as seen in the Chap. 3).

The Lambert-Beer Law (Eq. 4.6) correlates the signal intensity at a given wavelength value directly with the analyte concentration, which allows quantitative data to be obtained—it is worth noting, again, that it is necessary to have the respective curve with linear behavior.

$$A = \varepsilon bc \tag{4.6}$$

Where: A = absorbed radiation (arbitrary units); ε = molar absorptivity of the medium (cm^{-1} L mol^{-1}); b = cell length (cm); c = concentration (mol^{-1} L).

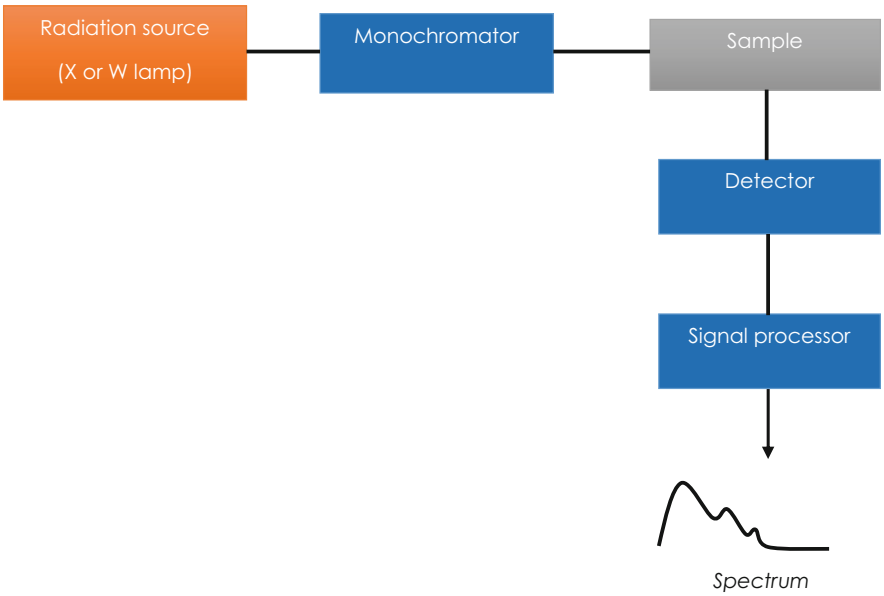


Fig. 4.11 Block diagram for a UV-Vis absorption spectrophotometer

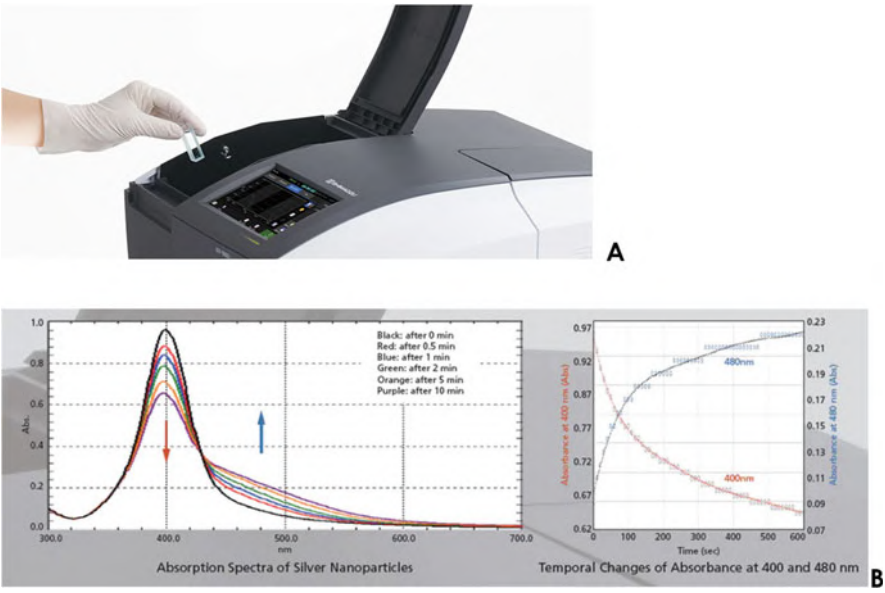


Fig. 4.12 A UV-Vis absorption spectrophotometer (**a**), and the obtained spectrum (**b**). Courtesy of Shimadzu

Table 4.8 Examples of chemical groups, which absorb UV radiation, and their associated electronic transitions

Chemical group	Structure	Electronic transitions	λ_{max} (nm), nearly
Carbonyl (ketone)	RR'C=O	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	180 271
Carbonyl (aldehyde)	RHC=O	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	190 293
Carboxyl	RCOOH	$n \rightarrow \pi^*$	204
Amide	$RC=ONH_2$	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	208 210
Conjugated diene	RCH-CH=CH-CHR	$\pi \rightarrow \pi^*$	250
Aromatic	C_6H_6	$\pi \rightarrow \pi^*$	256

Source: Adapted from Vaz (2018). Reproduced with permission from Springer Nature

Figure 4.11 describes the block diagram for a UV-Vis absorption instrument. Figure 4.12 depicts the instrument.

Table 4.8 describes information on electronic transitions and wavelengths of UV absorption of some chemical groups present in several organic compounds.

According to the EAG Laboratories (2023), advantages of UV-Vis absorption spectrophotometry are as follows: a fast sample analysis; suitability for a wide variety of analytes; user-friendly interface; and little maintenance required. And limitations are as follows: effects of fluctuations from scattered light and temperature changes; relatively low sensitivity; other sample components may cause interferences; not as specific as chromatography; and requires a relatively large sample volume, > 0.2 mL.

4.4.3.2 Absorption Infrared Molecular Spectroscopy

Vibrational spectroscopy refers to a type of interaction of the radiation with vibrational states of the chemical bonds. Therefore, there is no electronic transition. Here we can highlight infrared (IR) absorption spectroscopy in its three wavelength ranges: near, medium, and far. Polarity has a direct influence on the IR spectrum, modifying its form.

The electromagnetic region of the IR is located between the visible region and the microwaves, that is, from 12,800 to 10 cm^{-1}, remembering that the unit cm^{-1} refers to the wavenumber. As previously introduced, the IR spectrum is subdivided into three regions: near-infrared (NIR), mid-infrared (MIR), and far-infrared (FIR). MIR, which is the most used technique in organic analysis, is subdivided into two regions: frequency groups, from 4000 up to 1300 cm^{-1}; and absorption of functional groups of two atoms, or vibration, of 1300 to approximately 700 cm^{-1}, also called *fingerprint*. In NIR the radiation is comprised between 12,800 cm^{-1} and 4000 cm^{-1}. The absorption bands in this region are harmonic or combinations of fundamental stretching bands, often associated with hydrogen atoms, due to the ease of handling of the sample.

Table 4.9 Characteristic bands of deformations and vibrational stretches, which may be present in pharmaceutical and agrochemical products

Band position (cm^{-1})	Assignment	Intensity
3500–3000	Intramolecular stretching of O-H and N-H	Medium absorption
2940–2900	Asymmetric stretching of aliphatic C-H	Strong absorption
1725–1720	Stretching of C = O in COOH and ketones	Strong absorption
1660–1630	Stretching of amide groups (amide band I) and quinone; C = O stretching of hydrogen bonded to conjugated ketones; Stretching of COO^-	Strong absorption
1620–1600	Stretching of aromatic C = C; stretching of COO^-	Medium to weak absorption
1460–1450	Stretching of aromatic C-H	Medium absorption
1400–1390	Deformation of O-H and stretching of C-O and OH phenolic; deformation of C-H in CH_2 and CH_3; asymmetric stretching of COO^-	Medium absorption
1170–950	Stretching of C-O in polysaccharides or polysaccharides-like compounds	Strong absorption

Source: Adapted from Vaz (2018). Reproduced with permission from Springer Nature

Absorbance of IR radiation is determined by Eq. (4.7):

$$A = \log_{10}(1/T) = \log_{10}(I_0/I) \tag{4.7}$$

Where T is the radiation transmission, I_0 is the measured intensity of the source radiation (the background) incident on the sample, and I the radiation transmitted through the sample. Normally the output is expressed as percent transmittance. However, in some cases the absorbance is considered as the output.

IR spectra are typically employed to identify pure organic compounds or impurities, interactions, and binding formation. It is important to consider that a Fourier transform converts the intensity vs. time signal into the intensity vs. frequency spectrum—from this we have the most used MIR technique FTIR (Fourier transform infrared absorption spectroscopy).

Table 4.9 describes the main possible correlations for the assignment of the absorption bands in the MIR—generally, the FTIR approach—as a function of the type of bound.

Again, according to the EAG Laboratories (2023), advantages of FTIR are as follows: capacity to identifying organic functional groups and often specific organic compounds; availability of extensive spectral libraries for compound and mixture identifications; ambient conditions (vacuum is not necessary) for operation with applicability for semi-volatile compounds; minimum analysis area (~15 μm);

Fig. 4.13 Block diagram of an MIR spectrometer with Fourier transform (FTIR)

rule-of-thumb: if you can see the sample by eye, it most likely can be analyzed; can be quantitative with appropriate standards and uniform sample thicknesses. On the other hand, limitations are as follows: limited surface sensitivity (typical LOD is a film thickness of 25 nm); only specific inorganic species exhibit an FTIR spectrum (for example: silicates, carbonates, nitrates, and sulfates); sample quantitation requires the use of standards; glass absorbs infrared light and is not an appropriate substrate for FTIR analysis; water strongly absorbs IR radiation and may interfere with the analysis of dissolved, suspended, or wet samples; simple cations and anions, e.g., Na^+ and Cl^-, do not absorb IR radiation and hence cannot be detected by FTIR; identification of mixtures/multiple sample components may require additional laboratory preparations and analyses; metals reflect light and cannot be analyzed by FTIR.

The Fig. 4.13 describes a block diagram of an FTIR instrument. And Fig. 4.14 depicts the commercial instrument.

The Raman spectroscopy is a branch of the vibrational spectroscopy. But differently from the FTIR, this technique is based on the radiation diffraction in a wavelength from 3000 to 100 cm^{-1}.

Raman spectroscopy results in information about intra- and intermolecular vibrations and enables an additional understanding of a given reaction. Raman spectroscopy and FTIR provide a characteristic spectrum of the specific vibrations of a molecule—the *molecular identity* or fingerprint (seen in the chapter beginning)—and are important for the identification of a substance. However, Raman spectroscopy can provide additional information about low-frequency modes and vibrations that increase understanding about the crystal lattice and fundamental molecular structure.

Unlike FTIR spectroscopy, which investigates changes in dipole moments, Raman investigates changes in the polarizability of molecular bonds. The interaction of light with a molecule can induce deformation of its electron cloud. This

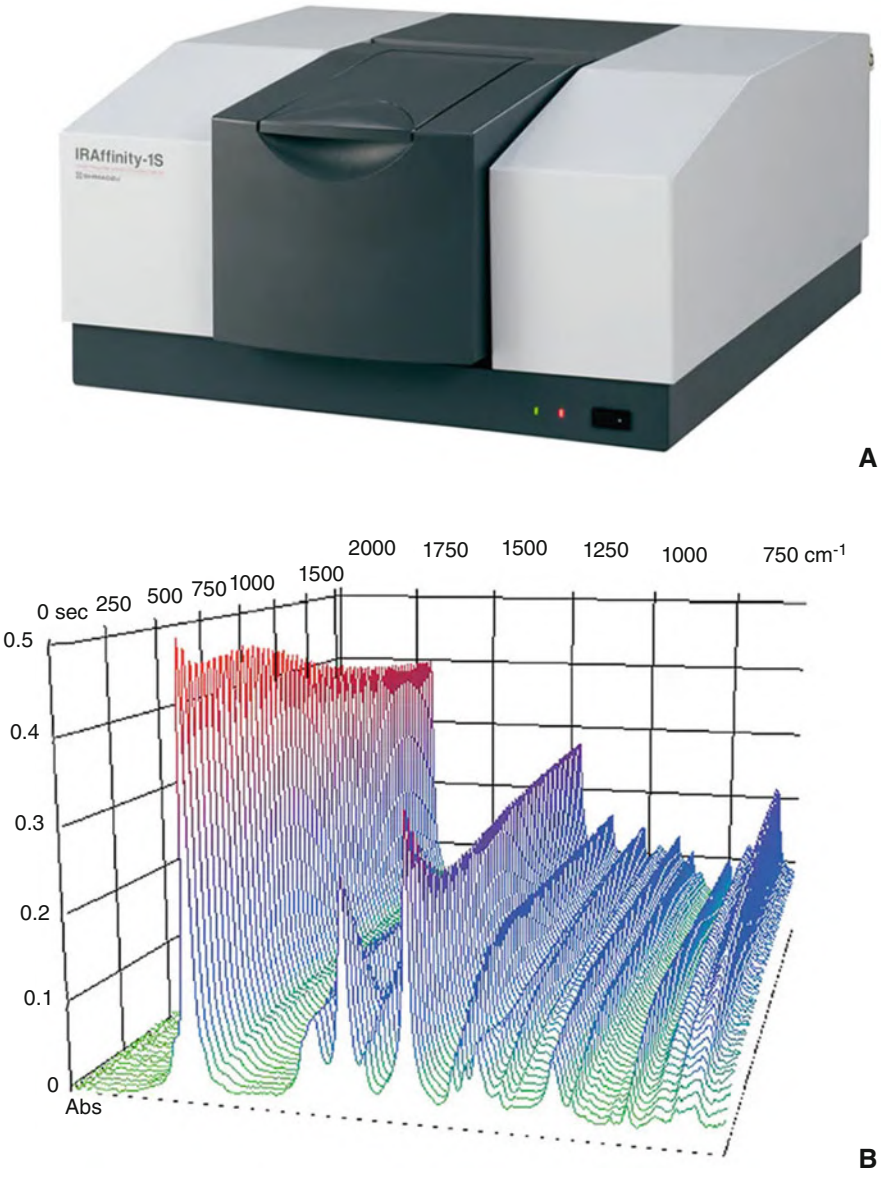


Fig. 4.14 An MIR-FTIR spectrometer (**a**), and the obtained spectrum (**b**). Courtesy of Shimadzu

deformation is known to be a change in polarizability. Molecular bonds have specific energy transitions in which a change in polarizability occurs, giving rise to Raman-active modes. As an example, molecules that contain bonds between homonuclear atoms, such as C-C, S-S, and N-N bonds, undergo a change in polarizability when

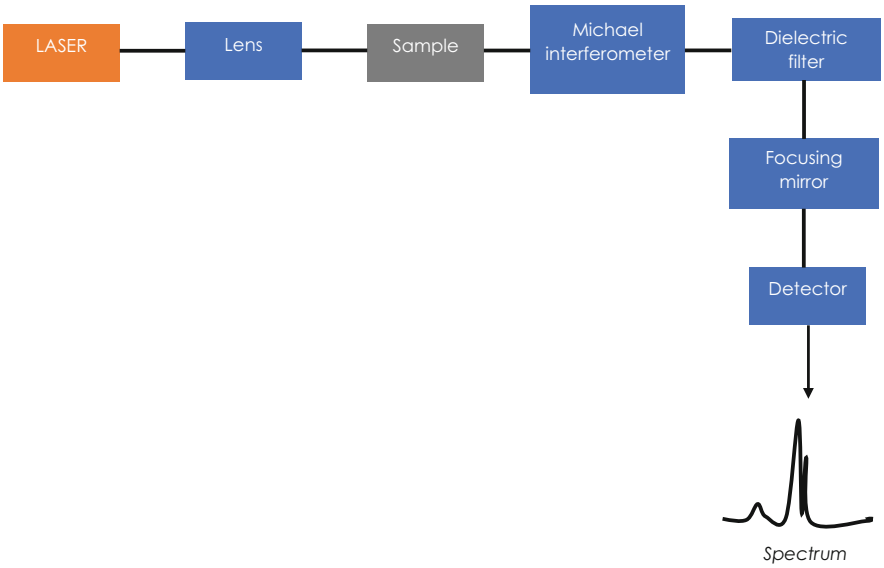


Fig. 4.15 Block diagram of a Raman instrument. *LASER* light amplification by stimulated emission of radiation

photons interact with them. These are examples of bonds that give rise to spectral bands that are active in Raman but would be difficult to see or not visible in FTIR.

Figure 4.15 depicts a block diagram of a Raman instrument, and Fig. 4.16 depicts the instrument.

4.4.3.3 Nuclear Magnetic Resonance

Unlike other types of spectroscopies, in nuclear magnetic resonance (NMR) it is the nuclei of atoms that absorb radiation and not their electrons. The absorption of radiation by the nuclei occurs when they are subjected to an external magnetic field produced by low energy waves (radio frequency; from 10^{-3} to 10^{1} m).

In some cases, the nuclear charge can rotate around the nuclear axis, generating a magnetic dipole. The angular momentum of the moving load can be described in terms of the spin I moment (m). The most explored nuclei in NMR for pharmaceuticals and agrochemicals are the ^{1}H and ^{13}C nuclei that have I equal to 1/2; ^{31}P can be explored in some cases, as in the biomass composition study. The absorption of radio frequency by these nuclei is characteristic and influenced by neighboring nuclei. This allows the molecular structure of a series of chemical compounds to be determined as a function of the chemical shift (δ) produced according to the electronic density of the atoms present in the molecule.

In the presence of an applied magnetic field, the nuclei are either aligned with the magnetic field with spins of $m = +1/2$ or aligned against the magnetic field with

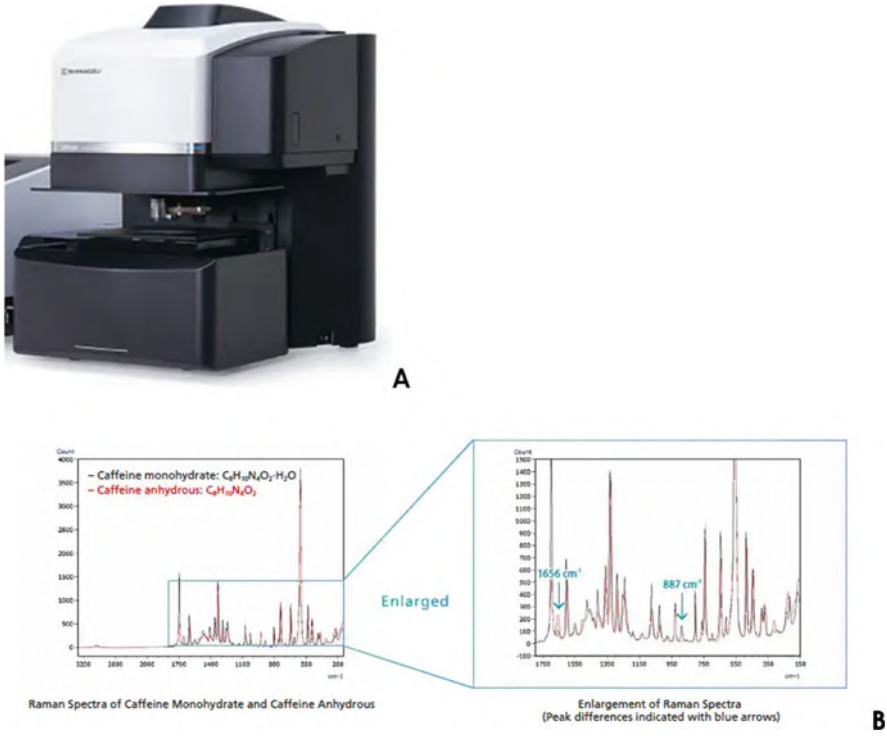


Fig. 4.16 A Raman spectrometer (**a**), and the obtained spectrum (**b**). Courtesy of Shimadzu

spins of $m = -1/2$. The energies in these two spin states, E_{lower} and E_{upper}, are given by Eqs. (4.8 and 4.9):

$$E_{\text{lower}} = -\gamma h 4\pi B_0 \tag{4.8}$$

$$E_{\text{upper}} = +\gamma h 4\pi B_0 \tag{4.9}$$

Where γ is the magnetogyric ratio for the nucleus, ? is Planck's constant, and B_0 the strength of the applied magnetic field. The difference in energy, ΔE, between the two states is given by Eq. 4.10:

$$\Delta E = E_{\text{upper}} - E_{\text{lower}} = +\gamma h 4\pi B_0 - (-\gamma h 4\pi B_0) = \gamma h 2\pi B_0 \tag{4.10}$$

From Eq. 4.4 previously seen and combining it with Eq. 4.10, we can obtain Eq. 4.11 for the electromagnetic radiation need to effect a change in spin state:

$$\nu = \gamma B_0 2\pi \tag{4.11}$$

And this is called the Larmor frequency for the nucleus, where ν is the frequency.

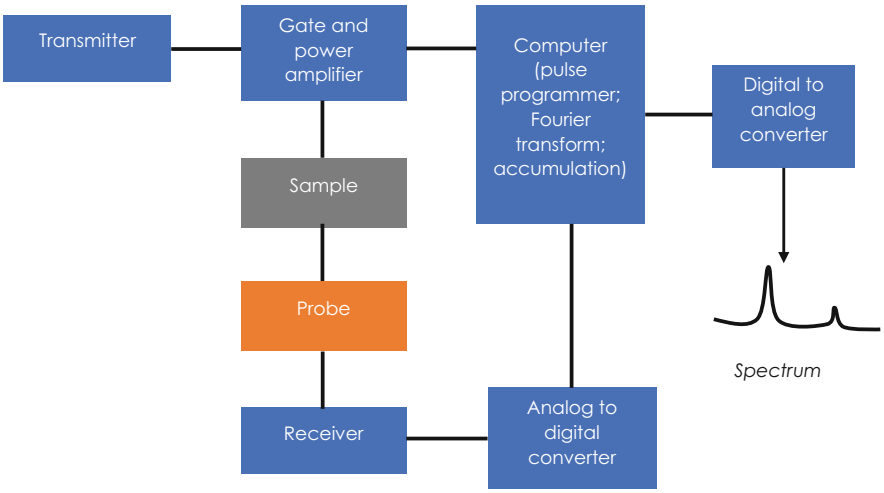


Fig. 4.17 Block diagram for a pulsed Fourier transformed-NMR instrument

Figure 4.17 depicts the block diagram of an NMR instrument, and Fig. 4.18 the instrument.

As FTIR, NMR can be used to study the presence of residues in the active ingredient/active pharmaceutical ingredient for regulatory purposes. NMR is most largely used to generate qualitative data; however, it can be used to produce quantitative data with limitations.

In a general way, the following assignments of groups as a function of chemical shift can be made for the ^{13}C-NMR spectrum:

- 0–45 ppm: unsubstituted aliphatic C, as in alkanes and fatty acids, due to methyl-terminal groups
- 45–65 ppm: C associated with N-alkyl, as in amino acids, peptides and proteins, and C methoxy
- 60–110 ppm: C associated with aliphatic O
- 110–140 ppm: unsubstituted and alkyl substituted aromatic C
- 110–160 ppm: total aromatic C related to unsubstituted, alkyl substituted, and phenolic group
- 140–160 ppm: C phenolic
- 160–185 ppm: C in carboxylate
- 185–230 ppm: ketone C in esters and amides

For ^{1}H-NMR spectrum:

- 10–9 ppm: H in aldehyde
- 9–6 ppm: H in aromatic and heteroaromatic
- 7.5–4.5 ppm: H in alkene
- 7–2 ppm: H in α-disubstituted aliphatic
- 1.5 ppm: H in α-monosubstituted aliphatic

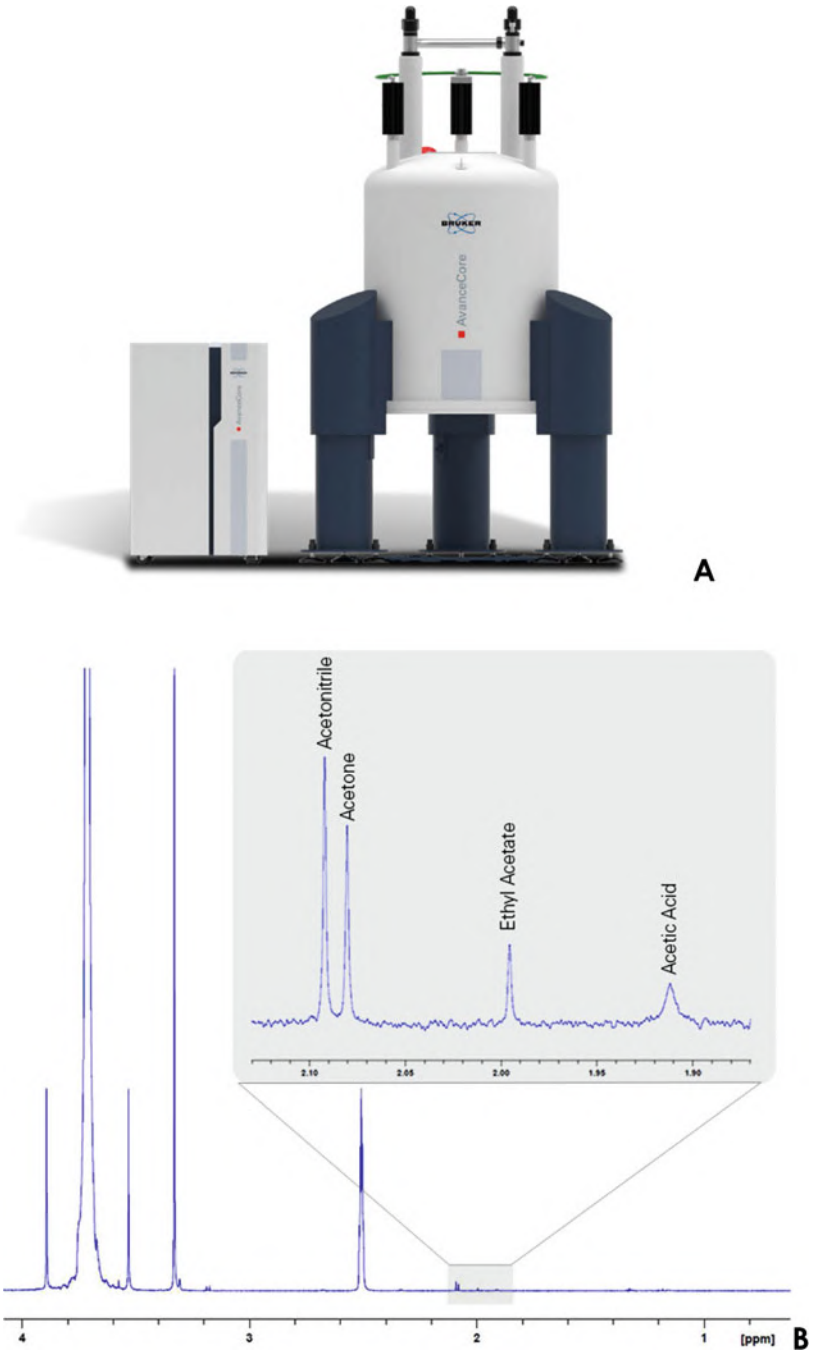


Fig. 4.18 A 400 MHz NMR spectrometer (**a**), and the obtained spectrum for the ^{1}H nuclei (**b**). Courtesy of Brüker

Table 4.10 Methods for 2D-NMR

Method	Information obtained from cross peaks
Correlation spectroscopy (COSY)	Coupling between two protons (^{1}H–^{1}H) that are within three chemical bonds of each other
Total correlation spectroscopy (TOCSY)	Coupling between all protons (^{1}H) in the molecule
Heteronuclear correlation spectroscopy (HETCOR)	Coupling between a proton (^{1}H) and another nucleus, such as carbon (^{1}H–^{13}C) or nitrogen (^{1}H–^{15}N)
Nuclear Overhauser and exchange spectroscopy (NOSEY)	Coupling between two protons (^{1}H–^{1}H) that are within approximately 5 Å of each other
Heteronuclear single quantum correlation (HSQC)	Coupling between a proton (^{1}H) and another nucleus, such as carbon (^{1}H–^{13}C) or nitrogen (^{1}H–^{15}N)
Heteronuclear multiple bond coherence spectroscopy (HMBC)	Coupling between a proton and a carbon (^{1}H–^{13}C) that are two or three bonds apart
Incredible natural abundance double-quantum transfer (INADEQUATE)	Coupling between adjacent carbon atoms (^{13}C–^{13}C)
Double quantum filtered correlation spectroscopy (DQF–COSY)	Suppresses signals from water

Source: Adapted from Harvey (2022). Reproduced with permission from the author

- 3–1.5 ppm: H in alkyne
- 1.5–0.5 ppm: H in β-substituted aliphatic
- 2–0 ppm: H in aliphatic alicyclic

The related assignments for ^{1}H and ^{13}C shifts were dedicated to one-dimension (1D) spectra, which are related to the frequency absorbed by the analyte's nuclei expressed in *ppm*. These spectra were acquired by applying a brief radio frequency (RF) pulse to the sample, recording the resulting *free induction decay* (FID), and then using a Fourier transform to obtain the NMR spectrum. In addition to 1D experiments, there are a host of 2D experiments in which we apply a sequence of two or more pulses, recording the resulting FID after applying the last pulse. Table 4.10 describes the most common methods for 2D-NMR; the main advantage of 2D when compared to 1D is that the first can distinguish between the overlapping signals that exist in larger molecules, which is not possible by means of 1D-NMR.

4.4.3.4 Atomic Absorption Spectrometry

When electromagnetic radiation is applied to atoms in the gaseous state, some of these atoms can be brought to a level of energy that allows the emission of the characteristic radiation of that atom. However, most can remain in the ground state and absorb energy, which in general would correspond to the energy in the gaseous state at the wavelength they would emit if they were excited from the ground state. Thus, when atoms absorb energy, an attenuation of the intensity of the radiation beam occurs. Thereby, atomic absorption spectrometry (AAS) is based on the

absorption of the electromagnetic radiation by gaseous atoms in the ground state. The Maxwell-Boltzmann expression (Eq. 4.12) defines this physical phenomenon:

$$N_e/N_0 = (g_e/g_0)e^{-(E_e - E_0)Kt} \tag{4.12}$$

Where N_e is the relative population of the excited state, N_0 the relative population in the ground state (measure in AAS), g_e and g_0 are the statistical weights of the excited and ground states, respectively; E_0 is the energy in excited state; E_e is the energy in ground state; k is the Boltzmann constant; and t the absolute temperature.

AAS is widely used in the inorganic analysis of metals, semi-metals, and non-metals in a huge variety of analytical matrices. Initially, we have three different types of atomizers: combustion flame of different gases (hydrogen, acetylene, or natural gas), graphite furnace (or electrothermal), and cold mercury vapor (for determination of the mercury present by reduction to elemental mercury), with the application of each of them depending mainly on the analyte to be determined and the LOD required by the method—the flame AAS is the most used technique. The radiation absorbed has a direct relation with the analyte concentration that turns this technique very useful in quantitative analysis of metallic species.

In general, the spectra obtained by AAS are simpler than those obtained by atomic or optical emission (see ahead). A particular chemical element absorbs energy at certain wavelengths. Typically, for analysis of an element, the highest absorption wavelength is chosen if there is no interference due to the absorption of the radiation by another element at that wavelength. Due to its simplicity and cost, AAS is the most widely used atomic spectrometric technique.

Figure 4.19 depicts a block diagram for an AAS instrument, and Fig. 4.20 the instrument.

Elements frequently detected by AAS are metals and non-metals, except: H, Fr, Ra, Ac, La, Hf, Tc, Os, C, N, O, F, P, S, halogens, and noble gases.

4.4.3.5 Atomic Emission Spectrometry or Optical Emission Spectrometry

Atomic emission spectrometry (AES), or optical emission spectrometry (OES), is based on the measurement of the emission of the electromagnetic radiation in the ultraviolet-visible (UV-Vis) region by neutral and ionized atoms, not in excited state, being widely used in elemental analysis. The most common AES/OES system uses an argon plasma torch that can reach up to 9000 K (as the hyphenated inductively coupled plasma, ICP) for the electrons excitation in gaseous state. ICP can also be coupled to a quadrupole mass analyzer (ICP-OES-MS), which is extremely high sensitivity to a wide range of elements.

The technique has high stability, sensitivity, low noise—S/N or signal/noise ratio—and low background emission intensity. However, because it involves relatively expensive methods that require extensive operator training, it is not applied as

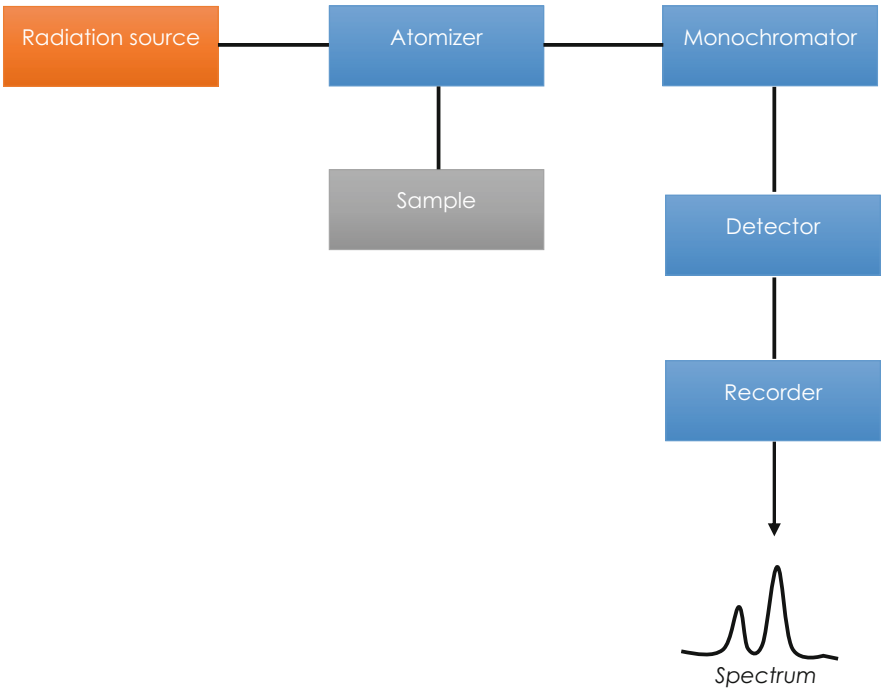


Fig. 4.19 Block diagram for an AAS instrument

AAS. All metals or non-metals of pharmaceutical and agrochemical interest, determined by AAS, can be determined by AES/OES—the latter can favor, for some elements, the achievement of lower values of LOD and LOQ.

AES/OES also obey the Maxwell-Boltzmann expression (Eq. 4.12) for the physical phenomenon definition.

According to the EAG Laboratories (2023), advantages of OES are as follows: bulk chemical analysis technique that can determine simultaneously up to 70 elements in a single sample analysis; the linear dynamic range is over several orders of magnitude; instrumentation is suitable to automation, thus enhancing accuracy, precision, and throughput. Limitations are as follows: the emission spectra are complex and inter-element interferences are possible if the wavelength of the element of interest is very close to that of another element; in MS mode, determination and quantification of certain elements can be affected by interference from polyatomic species, matrix elements, and atmospheric elements; the sample to be analyzed must be completely digested, or dissolved prior to analysis in order to determine the element(s) of interest.

Figure 4.21 depicts a block diagram for an ICP-OES instrument, and Fig. 4.22, the instrument.

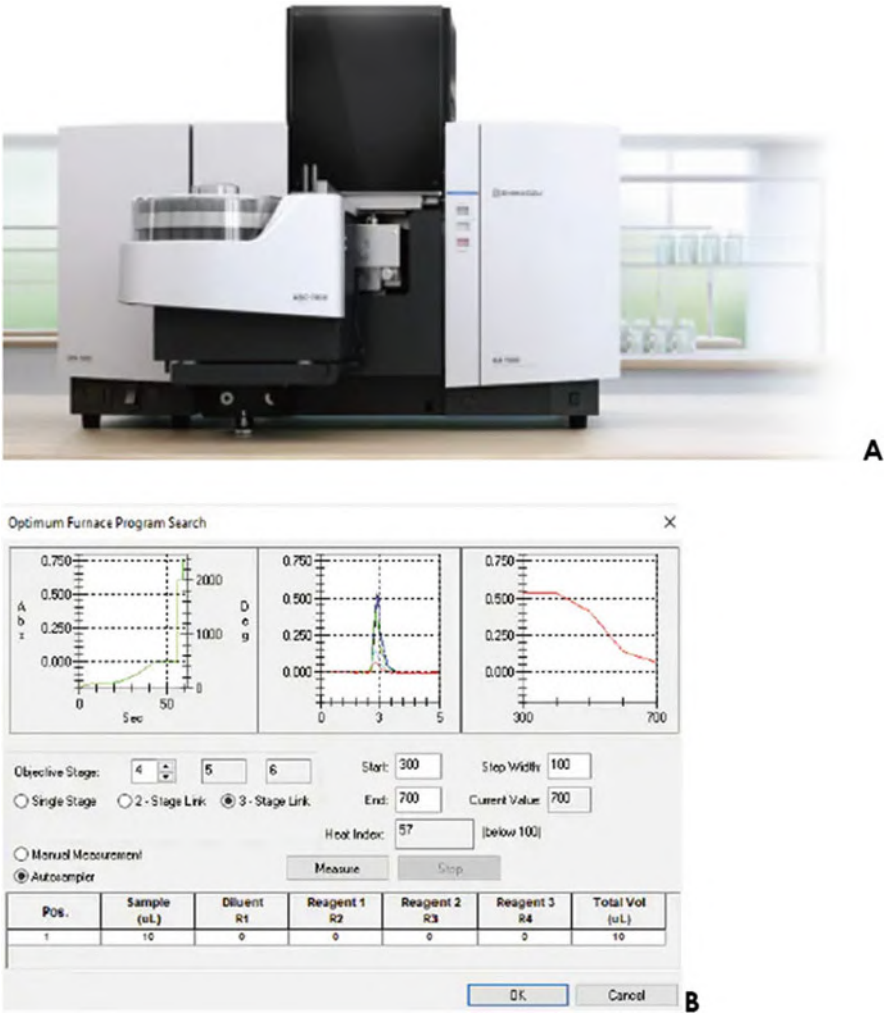


Fig. 4.20 An AAS spectrometer (**a**), and the obtained measurement (**b**). Courtesy of Shimadzu

4.4.3.6 X-Ray Atomic Emission or Fluorescence Spectrometry

This technique allows a rapid and non-destructive multi-element analysis for solid and liquid samples (identification and quantification). When an atom is excited by the removal of an electron from its inner layer, it emits X-rays (from 10^{-12} to 10^{-8} m) when returning to its ground state; such radiation has a typical signal intensity for each element, which is used in the analysis. Here, we can use the Lambert-Beer law (Eq. 4.6) to operationalize the physical phenomenon.

There are two X-ray fluorescence (XRF) systems available: the wavelength dispersive spectrometer (WD-XRF) and the energy dispersive spectrometer

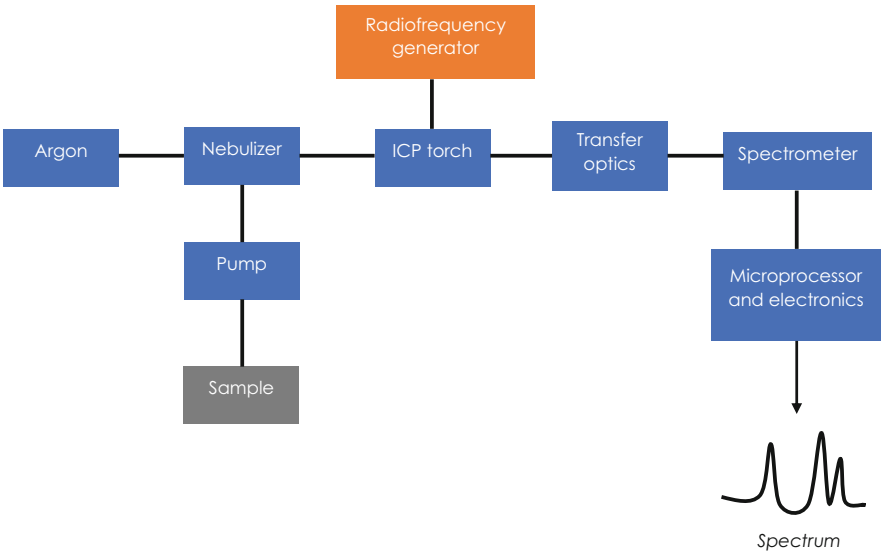


Fig. 4.21 Block diagram for an ICP-OES (or ICP-AES) instrument

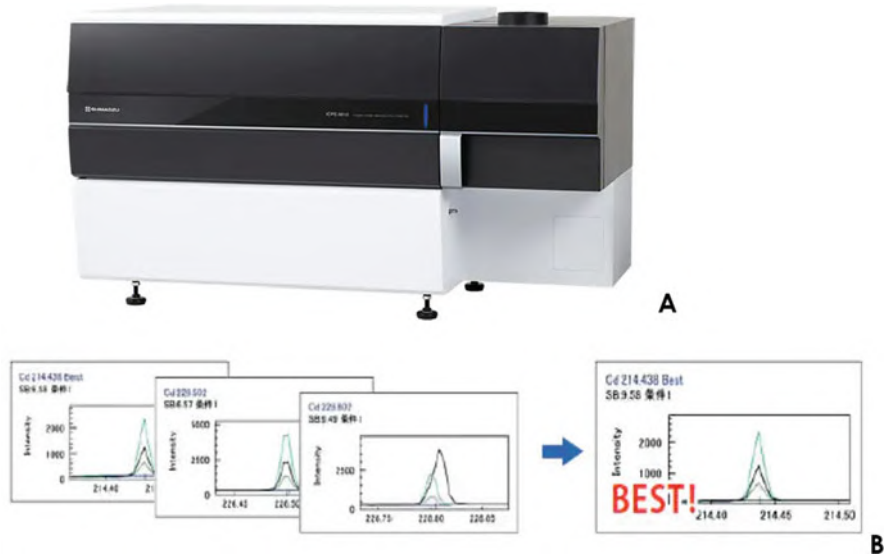


Fig. 4.22 An ICP-OES (or ICP-AES) instrument (**a**), and the obtained spectra (**b**). Courtesy of Shimadzu

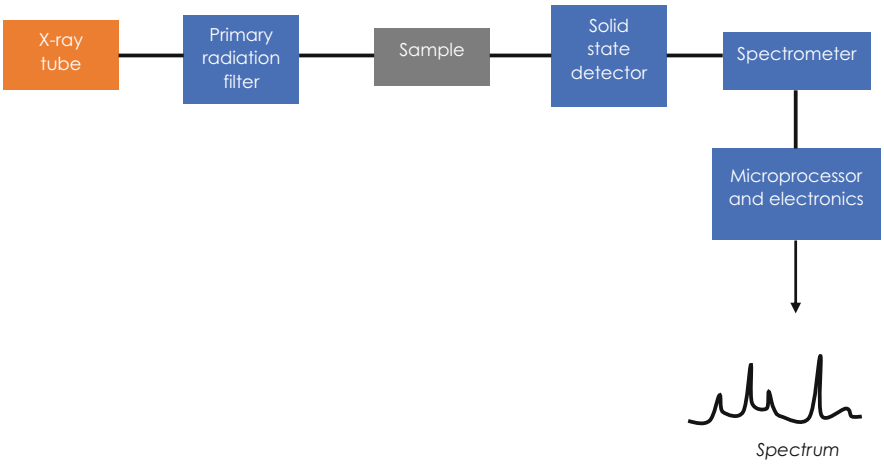


Fig. 4.23 Block diagram of an XRF instrument

(ED-XRF)—the latter has higher signal throughput, which enables small area analysis or mapping.

According to the EAG Laboratories (2023), advantages of XRF are as follows: non-destructive technique; can analyze areas as small as ~150 μm; can analyze any solid material; and sampling depth ranging from a few micrometers to several millimeters depending on the material. Limitations are as follows: cannot detect elements lighter than aluminum using small spot ED-XRF; and highest accuracy measurements require reference standards similar in composition and/or thickness to the test sample.

Figure 4.23 presents a block diagram for an XRF instrument, and Fig. 4.24, the instrument.

4.4.4 Thermal Techniques

Thermal analysis uses thermochemical processes, such as combustion and pyrolysis, to determine the percentage of mass loss or compound formation and the atomic percentage, in addition to allowing the observation of thermodynamic properties of materials. Such a class of analytical techniques is fundamentally used for the thermal characterization of organic and inorganic compounds and various materials, although it also provides quantitative percentage information. One can cite the great utility of thermogravimetric analysis and elemental analysis for the characterization of solid formulations and for determining its chemical composition.

Thermogravimetric analysis (TGA) provides data on mass loss as a function of temperature, which is especially interesting when one wants to observe the thermal behavior of a material during processing steps. The differential scanning calorimetry

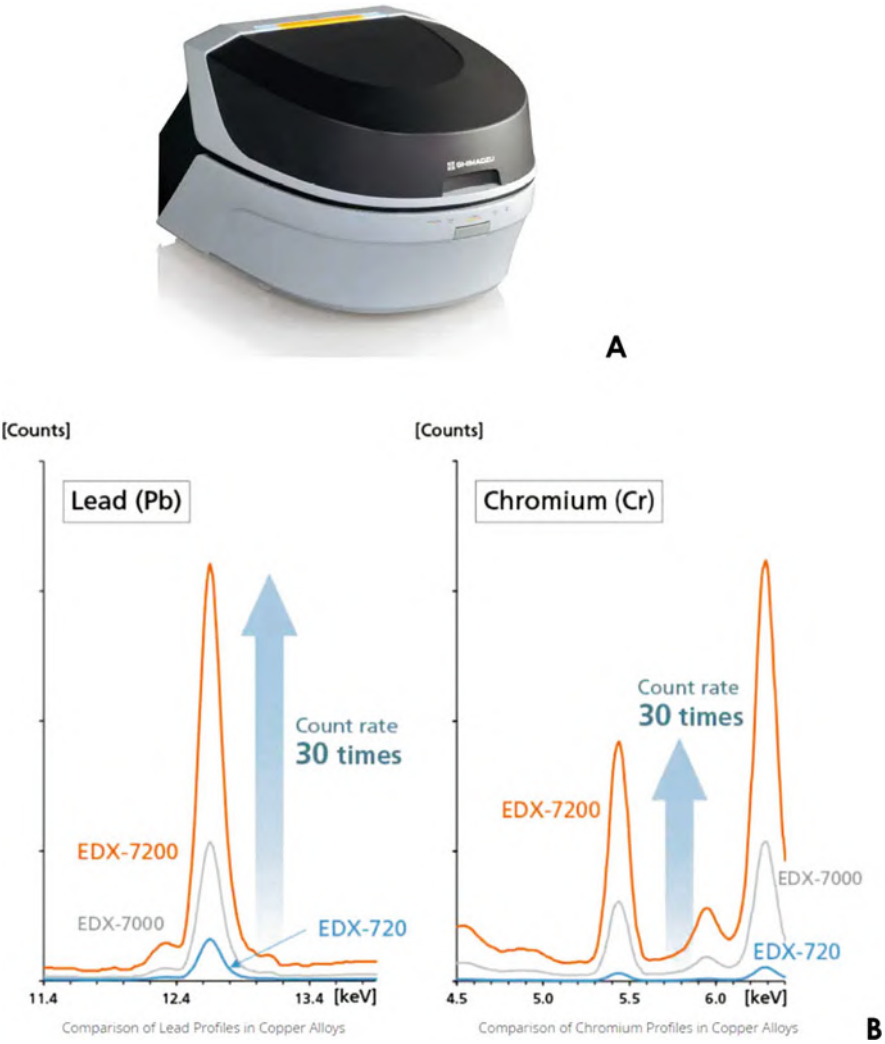


Fig. 4.24 An ED-XRF instrument (**a**), and the obtained spectra (**b**). Courtesy of Shimadzu

(DSC) provides thermodynamic data, which is of great help for its thermal processing; by measuring the energy exchanges in predetermined heating cycles, the endothermic and exothermic behavior of the sample is obtained, in addition to calculating the specific heat (Cp). The differential thermal analysis (DTA) is based on the difference in temperature between the sample and a reference material is monitored against time and temperature, while the temperature of the sample, in a specific atmosphere, is programmed.

Elemental analysis or microanalysis is an analytical technique that makes it possible to determine what are the constituent elements of a molecule, mainly

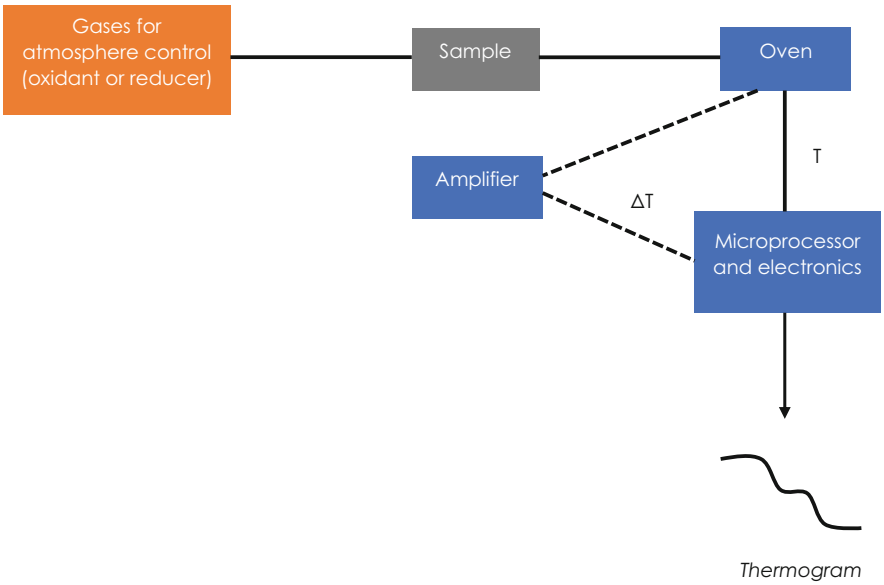


Fig. 4.25 Block diagram of a thermogravimetric analyzer instrument

organic, through the pyrolysis of a sample that contains oxygen, carbon, sulfur, nitrogen, and hydrogen, and the analysis of the gases resulting from its decomposition (e.g., NxOx, SO_2, CO_2, and H_2O). Thus, it can be inferred about the percentage composition by mass of the elements present in the sample.

Figure 4.25 depicts a block diagram of a thermogravimetric analyzer, and Fig. 4.26, the instrument.

4.4.5 Other Relevant Techniques

4.4.5.1 Particle Size Distribution

Sample particles are distinguished by their properties. The most common property of particles is the equivalent sphere diameter (also called the equivalent diameter). Particles are ordered by their property on the *x*-axis of a coordinate system.

Also, particles are assigned to different classes (gap between two nodes on *x*-axis). If the amounts of all grades relative to the total sample amount were determined, these amounts can be plotted as *particle size distributions*.

The measurement technique or focus of interest often implies the use of a certain evaluation criterion (DP Union 2023). Weighing the particles of each class gives mass or volume distributions. Alternatively, counting the particles gives numerical distributions. Both types of quantity are most frequently used in practice. In addition, the particles can be ordered in a row, i.e., "length" is an evaluation criterion. In some

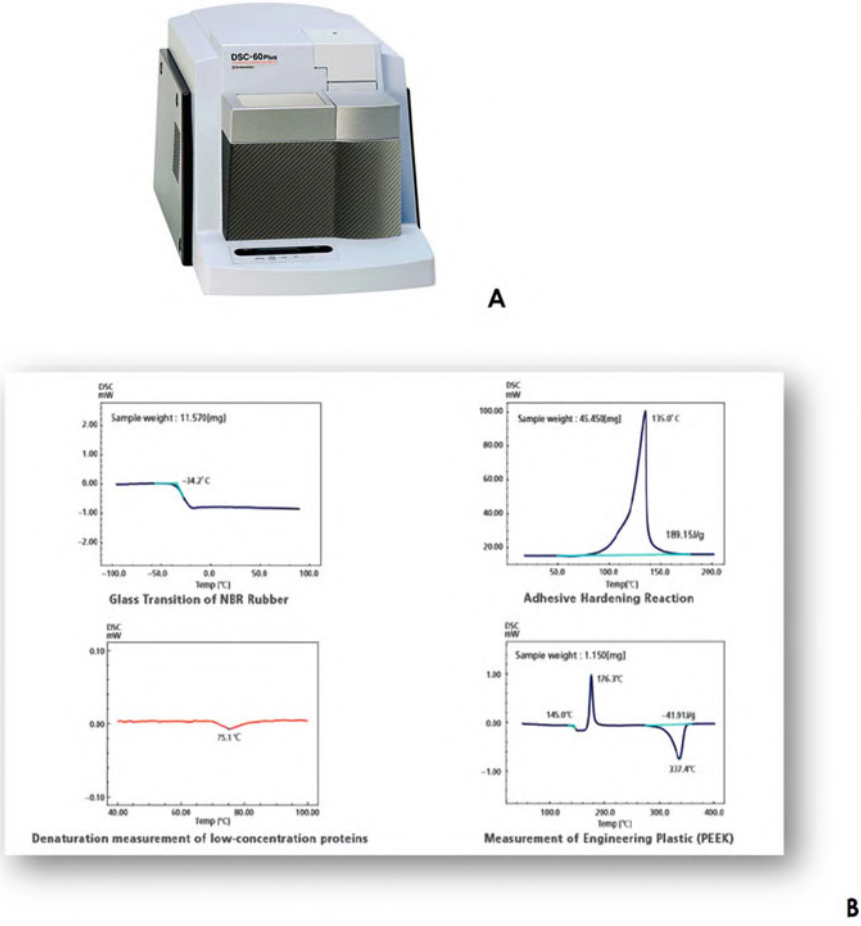


Fig. 4.26 A DSC instrument (**a**), and the obtained thermograms (**b**). Courtesy of Shimadzu

cases, projection areas are measured. As a logical consequence, "area" is also an evaluation criterion.

Frequently, the particle size of grains can be measured by means of dynamic light scattering (DLS) where particles experience Brownian motion, and smaller particles move faster than larger ones. On the other hand, the size of a small particle can be measured at an angle of θ by the Rayleigh scattering, which is defined in Eq. 4.13:

$$R_\theta = I/Io = Kr^6 \tag{4.13}$$

Where I is the ratio of the intensity of the scattered light, I_0 the intensity of the light source, r is the radius of the particle, and K is a constant that is a function of the angle of scattering, the wavelength of radiation used, the refractive index of the particle, and the distance to the particle.

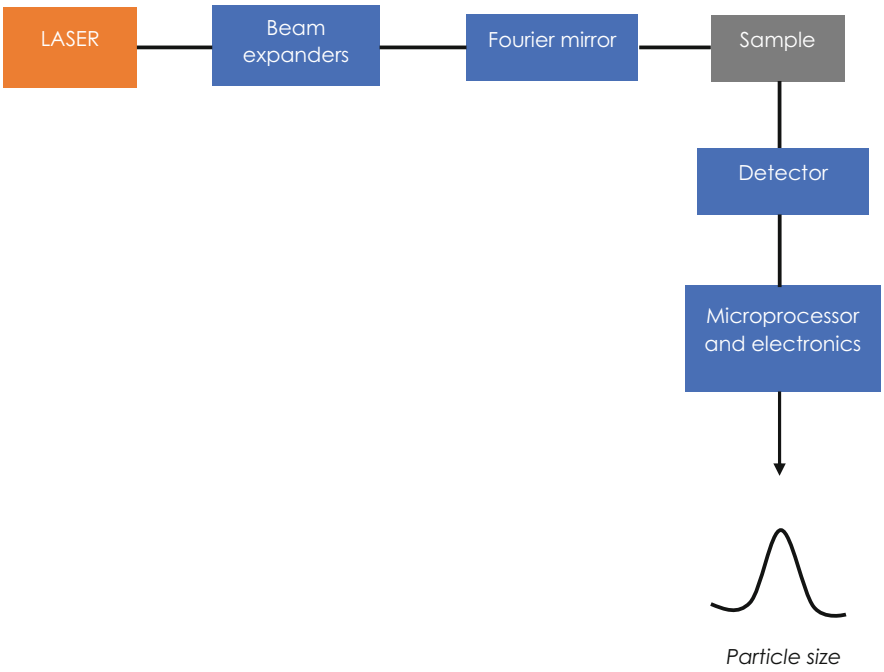


Fig. 4.27 Block diagram of a particle size distribution instrument. *LASER* light amplification by stimulated emission of radiation

The particle size is very useful to determine if a certain formulation is in nanoscale or submicron-scale, for instance.

Figure 4.27 depicts the block diagram for a particle size distribution instrument, and Fig. 4.28, the instrument.

4.4.5.2 Zeta Potential

The zeta potential (ζ) can be described as the electric potential in the hydrodynamic shear plane between the flowing particle and the solvent. Classically it is determined through electrokinetic measurements, experiments in which the relationship between the current or voltage and the relative flux of the phases in colloidal dispersion or suspension are measured. The potential is determined by the surface potential of the particles and by the electric double layer formed by counter-ions and ions, which form the Stern layer, counter-ions strongly bound to the surface of the particle, and part of the diffuse layer. The distribution of ions in the diffuse layer is dependent on the electrolyte concentration, the formal charge of the ions and the solvent, and thus, the potential ζ is due to the contributions of the particle and the solvent in which the particle was inserted. This potential can be determined by Eq. 4.14:

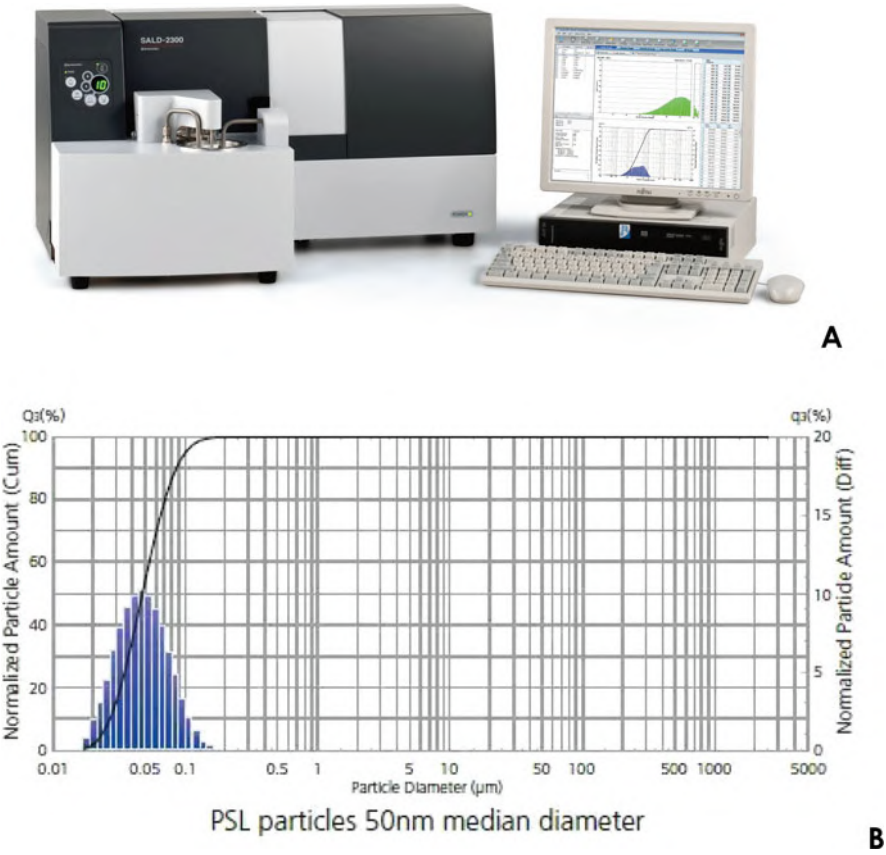


Fig. 4.28 A particle size analyzer (**a**), and a plot of particle size distribution (**b**). Courtesy of Shimadzu

$$\zeta = 4\pi\eta/\varepsilon \times U \times 300 \times 300 \times 1000 \tag{4.14}$$

Where η is the viscosity of solution, U is the electrophoretic mobility, and ε the dielectric constant.

The zeta potential of a sample can determine the behavior of particles in a liquid and the tendency to aggregate and/or flocculate. It is usually applied in the characterization of materials such as molecules and particles, including nanoparticles, micelles, proteins, polymers, emulsions and vesicles, clay minerals, silica, and pigments.

For pharmaceuticals and agrochemicals, it is especially useful to determine the stability of formulations, where a zeta potential value of ±61 mV determines an excellent stability of the particles (Raja and Barron 2022).

Figure 4.29 depicts the block diagram for a zeta potential instrument, and Fig. 4.30, the instrument.

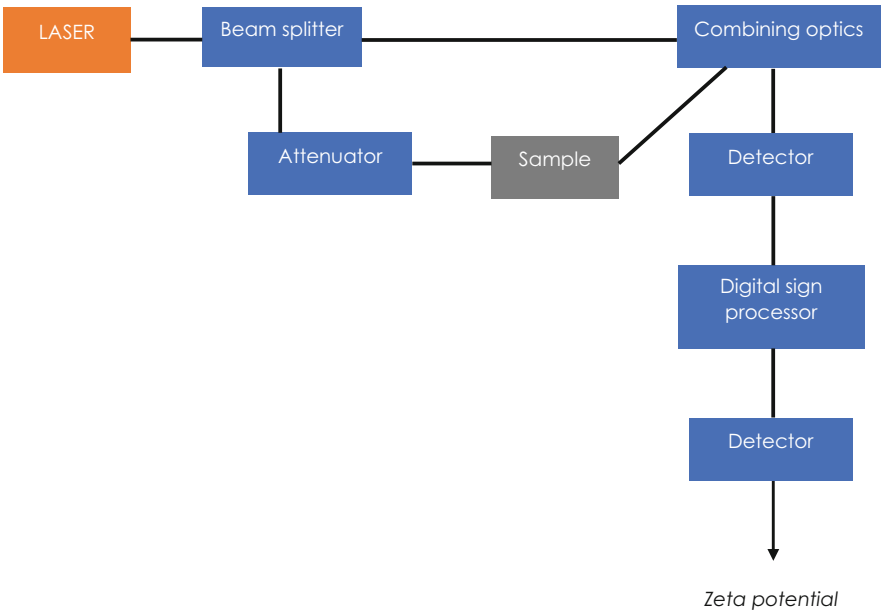


Fig. 4.29 Block diagram of a zeta potential instrument. *LASER* light amplification by stimulated emission of radiation

Fig. 4.30 A Zetasizer based on electrophoretic light scattering phenomenon. Courtesy of Malvern Panalytical

4.4.6 Process Analytical Technology

The need for quality control of chemical and biochemical processes of production has leveraged the use of process analytical chemistry (PAC), often also known as PAT (process analytical technology).

For the industry based on process of synthesis, the use of robust techniques and methods is privileged, preferably in real time, with the analyses being carried out directly in the reactor, instead of analyses carried out in the laboratory—it follows

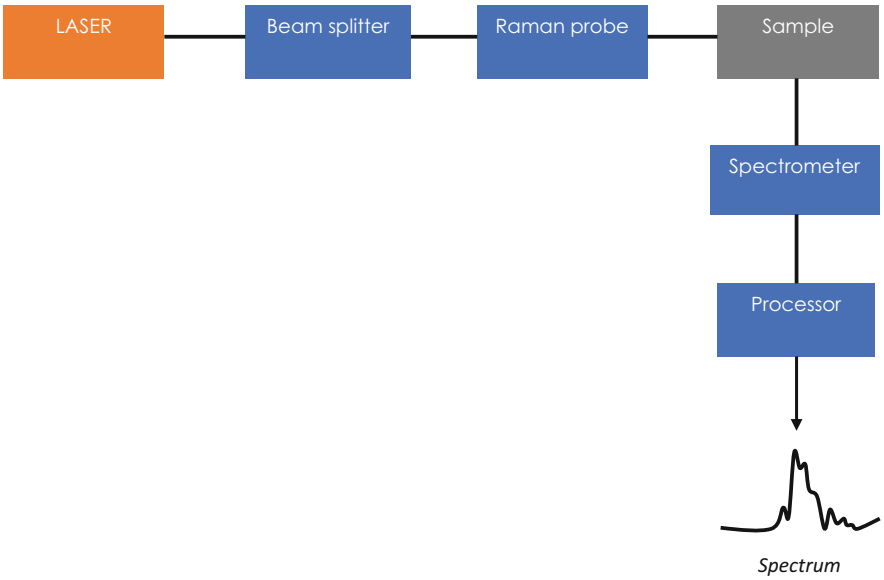


Fig. 4.31 Block diagram for a Raman probe. *LASER* light amplification by stimulated emission of radiation

the green chemistry principle 11, seen in Chap. 1. The main advantage of this type of analytical approach in relation to the traditional one, where manual sampling is carried out followed by sample transport and subsequent analysis in the laboratory, is that analyses carried out in situ provide greater speed for taking corrective actions and consequent adjustment of the production process. On the other hand, the need to have robust and automated analytical instrumentation, such as electrochemical sensors and simple-to-use spectroscopic probes, ends up limiting the number of analytical parameters that can be analyzed, in addition to compromising the limit of detection (LOD) and the limit of quantification (LOQ).

However, the continuous development of new analytical technologies and new materials will certainly increase the possibilities of obtaining more results, both by accepting greater variation in the physical and chemical conditions of the medium, and by allowing a better identification of chemical compounds. In the latter case, mainly using ultraviolet-visible (UV-Vis), mid- and near-infrared (MIR and NIR) and Raman absorption detectors or probes used as PAT. The block diagram for a Raman probe is illustrated in Fig. 4.31, and the instrument in Fig. 4.32.

The main aspects to be carefully considered in the methodological planning of measures in dynamic systems were listed by van Staden (1999) and should be considered here:

- Selection of process variables, such as temperature, pressure, and pH, or definition of process quality parameters to be measured.

Fig. 4.32 A Raman probe for manufacturing process monitoring. Courtesy of Thermo Fisher Scientific

- Establishment of a quantitative relationship between controllable properties because there is not always a direct relationship between them.
- Definition of sampling or analysis locations.
- Definition of intervals and number of measurements, in addition to the process correlation time, considering that for continuous measurements the system time constant must be defined.
- Determining the duration of the measurements, which will comprise sampling, measurement, and data calculation; that means: $t_{\text{elapsed}} = t_{\text{sampling}} + t_{\text{measurement}} + t_{\text{calculation}}$.
- Definition of tolerance limits (lower and upper) for the measured variables, which will determine the quality of the process.
- Selection of appropriate instrumentation.
- Establishment of instrument costs and their maintenance.
- Definition of instrument calibration frequency.
- Elaboration and evaluation of the costs of the measures, also considering regulatory issues.
- Establishing the reliability of measurements and the ease of obtaining them.

Once these considerations are made, the PAC/PAT becomes a very helpful tool for controlling processes in the pharmaceutical and agrochemical industries (see Table 4.11 in Sect. 4.5 ahead), leading to a very positive impact on the quality of the final product.

Figure 4.33 depicts an on-line analysis scheme for a certain process of synthesis, using mainly spectroscopic probes such as Raman, NIR, and MIR.

4.5 Commented Examples of Applications

The analytical techniques introduced and discussed in this chapter can be applied for a large variety of analytes and analytical matrices for pharmaceuticals and agrochemical chemical analysis, according to the application:

- For quality control
- For investigation of impurities

Table 4.11 Applications of analytical techniques for pharmaceutical analysis

Analytical technique	Application for pharmaceuticals	References
HS-GC with TCD, FID, and MS detectors	Water determination is solid pharmaceutical bulk products	Aspromonte et al. (2022)
HPLC-PD	Quantification of febuxostat in formulations	Haque et al. (2023)
LC-MS	Screening of 57 pharmaceutical and illicit drugs	Dos Santos et al. (2023)
Multinuclear NMR	Quantitative determination of organic and inorganic contaminants in heparin	Monakhova and Diehl (2022)
DSC and DTA	Thermal study of APIs used as analgesics	Alencar et al. (2022)
ICP-OES	Determination of inorganic impurities in liquid formulations	Pinheiro et al. (2019)
AAS	Simultaneous determination of inorganic impurities in solid formulations	Adolfo et al. (2019)
UV-Vis spectrophotometry	Colorimetric analysis of doxycycline hyclate and oxymetazoline hydrochloride in liquid formulations	Abdulsattar et al. (2020)
FTIR	Determination of sclerosis drugs in plasma and formulations	Oraby et al. (2021)
Raman spectroscopy	Several applications in pharmaceutical industry	Silge et al. (2022)
Particle size distribution	Use with ML for quality control of tablet formulations	Mäki-Lohiluoma et al. (2021)
Zeta potential	Development of self-emulsifying drug delivery system	Nazir et al. (2019)

The analytical method for each one can be accessed in the cited publication
AAS atomic absorption spectrometry, *APIs* active pharmaceutical ingredients, *DTA* differential thermal analysis, *DSC* differential scanning calorimetry, *FID* flame ionization detector, *FTIR* Fourier transformed infrared, *HPLC-PAD* high-performance liquid chromatography-photodiode array detector, *HS-GC* headspace-gas chromatography, *ICP-OES* inductively coupled plasma-optical emission spectrometry, *ML* machine learning, *LC-MS* liquid chromatography, *MS* mass spectrometer detector, *NMR* nuclear magnetic resonance, *TCD* thermal conductivity detector, *UV-Vis* ultraviolet-visible

- For waste monitoring
- For research and development for the three first applications above

Remembering that the necessity is generally determined by the official guideline's requirements for the methods (e.g., LOD, LOQ, etc.), besides issues related to costs, infrastructure, and availability of skilled labor.

Tables 4.11 and 4.12 describe relevant examples of applications for both pharmaceutical and agrochemical sectors.

It is highly recommended to use the concept of green analytical chemistry (seen in Chap. 1) for the reduction of possible negative impacts on the environment from the analytical methods.

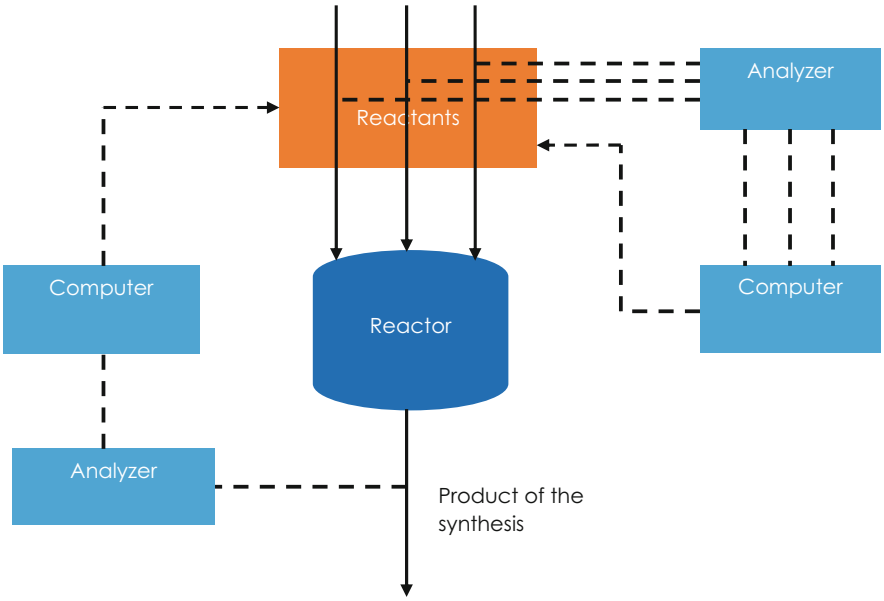


Fig. 4.33 A flowchart for on-line PAC/PAT application in a generic process of synthesis in pharmaceutical or agrochemical industries. Source: Adapted from Rouessac and Rouessac (2007). Reproduced with permission from Wiley

4.6 Auxiliary Tools

4.6.1 Artificial Intelligence

The use of artificial intelligence (AI) tools as machine learning (ML) (seen in Table 4.11) can expand the potential to obtain information from an analytical method application.

AI can be defined as "*It is the science and engineering of making intelligent machines, especially intelligent computer programs. It is related to the similar task of using computers to understand human intelligence, but AI does not have to confine itself to methods that are biologically observable*" (McCarthy 2007).

For analytical chemistry, AI is useful for extracting insights from large intractable data sets, as well as aiding in the automation of repetitive tasks (Baum et al. 2021), which are intrinsic challenges to be overcome related to analytical instrumentation. AI can also help to turn the PAC/PAT approach (seen in the Sect. 4.5) more scalable and applicable.

For instance, the primary function of ML in an automated lab is that it cycles and directs data from synthesis and validation, melding them with data from available published literature (Mullin 2021). This approach improves the data sets from the analytical processes that generate huge quantities of results, as for high-throughput screening and combinatorial chemistry for drug and pesticides discovery.

Table 4.12 Applications of analytical techniques for agrochemical analysis

Analytical technique	Application for agrochemicals	Reference
GC-MS	Semi-quantitative multianalysis of organochloride pesticides in soil	Huang et al. (2023)
HPLC-UV	Determination of encapsulation efficiency of formulations of cellulose-based nanocarriers loaded with hydrophobic fungicides) (captan and pyraclostrobin)	Machado et al. (2021)
^{1}H-, ^{19}F- and ^{31}P-NMR	Chiral discrimination for enantiomers ratio of fipronil and malathion	Iarocz and Silva (2021)
DSC	PAT for monitoring co-crystallization process in the agrochemical industry	Powell et al. (2016)
FTIR	Study of photodegradation of several agrochemical AIs	Saravanan et al. (2022)
Raman spectroscopy	Quantitative analysis of active components in commercial pesticide formulations	Armenta et al. (2005)
Particle size distribution	Analysis of aggregates for photosensitive agrochemical stabilizer	Wang et al. (2022)
Zeta potential	Stability measurements of chitosan NPs carrier loaded with spinosad and permethrin	Sharma et al. (2019)

The analytical method for each one can be accessed in the cited publication
AIs active ingredients; *DSC* differential scanning calorimetry, *FTIR* Fourier transformed infrared, *HPLC-UV* high-performance liquid chromatography-ultraviolet detector, *GC-MS* gas chromatography-mass spectrometer detector, *NMR* nuclear magnetic resonance, *NPs* nanoparticles, *PAT* process analytical technology

Despite its applicability in analytical instrumentation and analytical processes, AI is more accessible for the instrumentation suppliers in order to develop more advanced software and systems for laboratory automation and control. However, chemometrics for data treatment (seen ahead) can be allied to AI to several spectroscopic techniques (e.g., Raman spectroscopy, NMR, XRF) in order to reach the better understanding of generated data (Houhou and Bocklitz 2021).

4.6.2 Quality by Design

It is an especially useful mathematical tool for the pharmaceutical industry. According to the European Medicines Agency (2023b), "*quality by design is an approach that aims to ensure the quality of medicines by employing statistical, analytical and risk-management methodology in the design, development and manufacturing of medicines.*" One of the goals of quality by design is to ensure that all sources of variability affecting a process are identified, explained, and managed by appropriate measures. This enables the final product (drug) to consistently meet its predefined characteristics from the start—so that it is "right first time."

Quality by design (QbD) centers on the use of multivariate analysis often in combination with modern process analytical chemistry (PAC)/process analytical

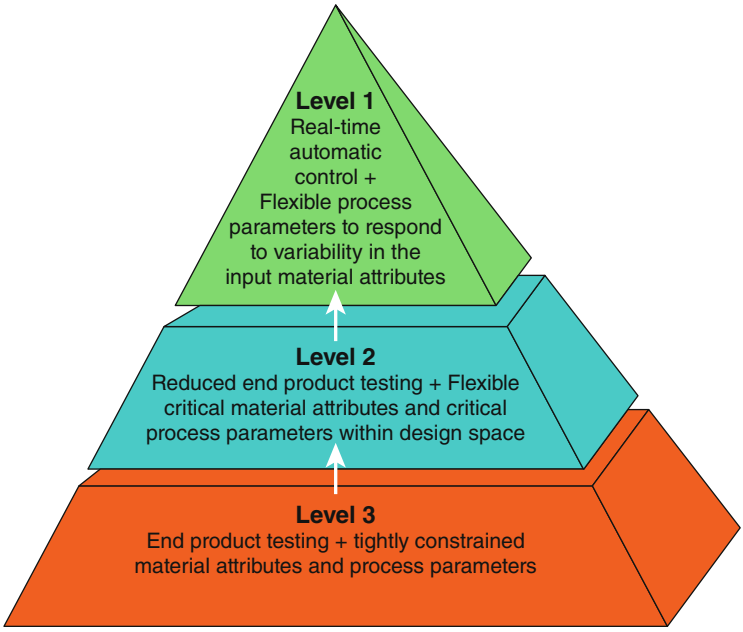


Fig. 4.34 Levels for the implementation of control strategy based on the application of quality by design in pharmaceutical processes. Source: Yu et al. (2014). Reproduced with permission from Springer Nature

technology (PAT) previously seen in Sect. 4.4—methods and knowledge-management tools to enhance the identification and understanding of critical attributes of materials and critical parameters of the manufacturing process. This enhanced understanding of product and process is used to build quality into manufacturing and provide the basis for continuous improvement of products and processes. In this way, an internationally recognized guideline for the implementation of the QbD in the pharmaceutical industry is the *ICH Guideline Q9 (R1) on Quality Risk Management* (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use 2023).

Figure 4.34 depicts a control strategy for pharmaceutical process based on QbD application.

4.6.3 Chemometrics

In some applications, an analytical methodology alone is not sufficient to provide qualitative or quantitative information of the sample, using only data such as the intensity of absorption or emission, and/or the region of absorption of the

electromagnetic spectrum—called univariate analysis. Often, the analysis is associated with chemometric tools to provide the best information.

Chemometrics can be understood as an area of knowledge of analytical chemistry that uses mathematic models, along with formal logic to interpret and predict data, thus extracting the maximum of relevant information. It is largely used for spectroscopic and chromatographic data—in the case of spectroscopic data, each wavelength is a variable. Spectra or complete chromatograms, parts of them or selection of variables can be used. Since several variables are treated at the same time, the data analysis is called *multivariate analysis*. In order to carry out the multivariate analysis, the data are first organized in matrix form, called matrix X of original data, where the columns correspond to the predictor variables (such as absorbance) and the lines correspond, for example, to the concentration of an analyte (Martens and Naes 1989).

After organizing the data in the matrix, sometimes it is necessary to pre-process, eliminating irrelevant information or standardizing the data. The objective of the multivariate analysis can be from an exploratory analysis to the quantification of an analyte (Brereton 2003). The exploratory analysis is performed with the objective of obtaining initial information from a set of samples, such as the formation of clusters according to a certain chemical property. The main chemometric tool used in the exploratory analysis is the PCA (*principal component analysis*). When it is desired to verify similarities between samples of a certain class, samples are classified, with the most common methods being KNN (k-nearest neighbor), LDA (linear discriminant analysis), HCA (hierarchical cluster analysis), and SIMCA (soft independent modeling of class analogy). When it is intended to predict analyte concentration, calibration models are constructed, with patterns of known concentration and working range that contemplate the analyte concentration. The most widely used method for this purpose is PLS (partial least squares). For instance, Tables 4.13 and 4.14 illustrate the application of PCA on analytical data. Initially, Table 4.13 presents data for a spectroscopic hypothetical analysis.

From the data of Table 4.13 we obtain the covariance matrix—a joint variance[1] of two variables—in Table 4.14.

This shows that, for example, the covariance for the fluorescence intensities at 350 and 400 nm is −1.15909. The table also gives the variances of the fluorescence intensities at each wavelength along the leading diagonal of the matrix: for the fluorescence intensities at 350 nm the variance is 2.75. We can consider this kind of information in a practical way to understand the propagation of errors, and consequent reliability, for an analysis.

According Szymánka et al. (2015), chemometrics application for quantitative and qualitative purposes can be generated as follows:

- For qualitative results: compound identification, compound classification, and sample classification.

[1] Variance is the square of the standard deviation (S); covariance is the sum of variance for a certain measurement.

Table 4.13 Relative intensities of fluorescence emission at four different wavelengths (300, 350, 400 and 450 nm) for 12 compounds, A–L

	Wavelength (nm)			
Compound	300	350	400	450
A	16	62	67	27
B	15	60	69	31
C	14	59	68	31
D	15	61	71	31
E	14	60	70	30
F	14	59	69	30
G	17	63	68	29
H	16	62	69	28
I	15	60	72	30
J	17	63	69	27
K	18	62	68	28
L	18	64	67	29
Mean	15.75	61.25	68.92	29.25
Standard deviation	1.485	1.658	1.505	1.485

Adapted from Miller and Miller (2005). Reproduced with permission from Pearson

Table 4.14 Covariance matrix for the data from Table 4.13

	λ (nm)			
λ (nm)	300	350	400	450
300	2.20455			
350	2.25000	2.75000		
400	−1.11364	−1.15909	2.26515	
450	−1.47727	−1.70455	1.02273	2.20455

Adapted from Miller and Miller (2005). Reproduced with permission from Pearson

- For quantitative results: sample calibration and QSAR (quantitative structure—activity relationship); the latter especially for pharmaceuticals.

Chemometrics does not only apply to measurements, but also to the extraction step. Because it is based on multiparametric analyses, it allows to evaluate the effect of the variation of the operational parameters on the recovery percentage values of the extraction method. It is possible, for example, to verify among several extraction methods the most suitable for a group of analytes, or the effect of the matrix on the analyte group against more than one extraction method.

Figure 4.35 depicts the application of chemometrics and machine learning (ML), as an artificial intelligence (AI) tool, for analytical techniques for synthetic processes.

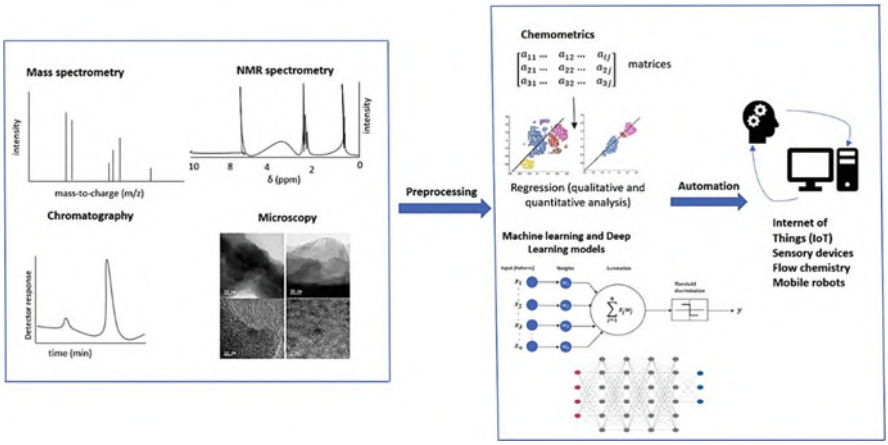


Fig. 4.35 Overview of chemometrics and machine learning (ML) methods applied to analytical techniques. Spectroscopy, chromatography, and microscopy are depicted on the left panel (not drawn to scale, not representative of any data). The right panel depicts chemometrics and ML models applied on analytical data after pre-processing. Finally, it depicts navigation toward automation that utilizes Internet of Things (IoT), sensory devices, flow chemistry, and mobile robots. Source: Joshi (2023). Reproduced with permission from Springer Nature

4.7 Conclusions

Chemical analysis applied to pharmaceuticals and agrochemicals is a fascinating branch of the analytical chemistry involving a large family of analytical techniques for several analytical matrices and analytical methods.

Analytical techniques as GC, HPLC, NMR, DSC, AAS, ICP-OES, etc. give us the deep chemical knowledge of raw materials, inputs, and end products supplying constitution and properties in order to guarantee the quality and safety to those who make use of drugs and pesticides, highlighting the health and environmental aspects. Besides, PAC/PAT is a useful approach to monitoring and control the processing steps in industries.

Allied to the analytical techniques and their methods we have relevant tools as AI and chemometrics to automate the laboratory and to extract all data potential. Furthermore, and especially for pharmaceutical industry, QbD can promote a reliable and robust quality system for pharmaceuticals.

Thus, these two classes of biologically active molecules can be well-monitored and controlled by the analytical instrumentation in order to offer reliable industrial end products to the modern society.

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Chapter 5
Ores and Mine

Abstract Mining corresponds to an economic and industrial activity that consists of research, exploration, extraction, and processing of ores present in the subsoil. And it is possible to say that mining is indispensable to socioeconomic development. This chapter considers the global outlook of mineral exploration and how this industry works regarding their most common processes. Aspects of physical properties of analytical matrices (e.g., powders, solids, semi-solids) and their preparation, and the most common analytical techniques (e.g., atomic emission and absorption spectrometries, X-ray fluorescence, and diffractometry) are considered in order to describe relevant applications of those techniques. Furthermore, the process analytical technology approach is also considered.

Keywords Analytical matrices · Advanced analytical techniques · Sample preparation · Spectroscopic techniques

5.1 Introduction

Mining corresponds to an economic and industrial activity that consists of research, exploration, extraction, and processing of ores present in the subsoil. This activity is one of the main factors responsible for the current configuration of the modern society in which we live, since several products and resources used by us come from this activity, such as computers, cosmetics, roads, metallic structures, among others.

Thus, it is possible to say that mining is indispensable to socioeconomic development.

Considering the diversity of mineral substances and their global demands—as the example illustrated in Table 5.1—the degree of difficulty in their use, the destination of the production obtained, in addition to social aspects, mineral exploration is carried out by legal modalities or regimes for the use of mineral resources. An example of an exploration regime is that of authorizations and concessions for mineral substances, applied by several countries with some variations (U.-S. Department of Interior/Bureau of Land Management 2023; Augusto and Vilhena 1997).

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Table 5.1 Estimated critical minerals statistics in 2022; production in metric tons. Source: Modified from U.S. Geological Survey (2023). Reprinted with permission from U.S. Geological Survey

Critical mineral	Applications	Leading producing country	Production in leading country	Percentage of world total	World production total
Aluminum (bauxite)	Metallurgy and many sectors of the economy	Australia	100,000,000	26	380,000,000
Antimony	Flame retardants and lead-acid batteries	China	60,000	55	110,000
Arsenic	Semiconductors	Peru	28,000	46	61,000
Barite	Hydrocarbon production	India	2,600,000	33	7,900,000
Beryllium	Aerospace and defense	United States	180	64	280
Bismuth	Medical, metallurgy, and atomic research	China	16,000	80	20,000
Chromium	Metallurgy	South Africa	18,000,000	44	41,000,000
Cobalt	Batteries and metallurgy	Congo (Kinshasa)	130,000	68	190,000
Fluorspar	Cement, industrial chemical, and metallurgy	China	5,700,000	69	8,300,000
Gallium	Integrated circuits and optical devices	China	540	98	550
Germanium	Defense and fiber optics	China	NA	NA	NA
Graphite (natural)	Batteries, fuel cells, and lubricants	China	850,000	65	1,300,000
Indium	Liquid crystal displays	China	530	59	900
Lithium	Batteries	Australia	61,000	47	130,000
Magnesium	Metallurgy	China	900,000	90	1,000,000
Manganese	Batteries and metallurgy	South Africa	7,200,000	36	20,000,000
Nickel	Batteries and metallurgy	Indonesia	1,600,000	48	3,300,000
Niobium	Metallurgy	Brazil	71,000	90	79,000
Palladium	Catalytic converters and catalysts	Russia	88	42	210
Platinum	Catalytic converters and catalysts	South Africa	140	74	190

(continued)

Table 5.1 (continued)

Critical mineral	Applications	Leading producing country	Production in leading country	Percentage of world total	World production total
Rare earths (compounds and metals)	Aerospace alloys, batteries, ceramics, colorants, and permanent magnets; cancer treatments, nuclear; metallurgy, solar cells, and thermoelectric devices; fiber optics, lasers, and solid-state devices; scintillators	China	210,000	70	300,000
Scandium	Ceramics, fuel cells, and metallurgy	China	NA	NA	NA
Tantalum	Capacitors and metallurgy	Congo (Kinshasa)	860	43	2000
Tellurium	Metallurgy, solar cells, and thermoelectric devices	China	340	53	640
Tin	Metallurgy	China	95,000	31	310,000
Titanium (metal)	Metallurgy and pigments	China	150,000	58	260,000
Tungsten	Metallurgy	China	71,000	85	84,000
Vanadium	Batteries, catalysts, and metallurgy	China	70,000	70	100,000
Yttrium	Catalysts, ceramics, lasers, metallurgy, and phosphors	China	NA	NA	NA
Zinc	Metallurgy	NA	NA	NA	NA
Zirconium (ores and concentrates)	Metallurgy and nuclear	Australia	500,000	36	1,400,000

From Table 5.1, we can highlight the world production of aluminum (380 million of metric tons), chromium (41 million of metric tons), and manganese (20 million of metric tons). They are, indeed, one of the most demanded metals for mining and processing for several uses, e.g., for metallurgical application.

Certainly, ores and mining involve a profitable value chain which generated 34,700 million dollars only in the United States in the year of 2022 (U.S. Geological Survey 2023). When we consider it in a global scale—the 464.2 million metric tons of the total world mineral production in Table 5.1—the values are gigantic.

5.2 How This Industry Works

Figure 5.1 depicts a flowchart for zinc mining in order to describe the main unitary operations related to this processing. It is a brief example of the mineral processing.

From Fig. 5.1, we can observe the main unitary operations such as roasting, gas treatment, leaching, purification, electrolysis, and melting addressed to obtain a special high-grade zinc.

The mineral processing technology for ores can involve three main families of processes (U.S. Department of Energy 2000):

1. Mineral preparation: comminution; makedown; classification; and blasting and drilling.
2. Physical separations: flotation; dewatering; thickening or settling; filtering; drying; flocculation; screening or sieving; magnetic separation; classification by sizes, densities, and shapes; and washing.
3. Chemical separations: solvent extraction; leaching; bioleaching; melting; refining; electrowinning; and pelletizing or briquetting.

Regarding the analytical technology for these kinds of processes, in-line characterization of key chemical and physical properties, such as composition, density, surface characteristics, particle size, hardness, impurity level, pH, etc., are all

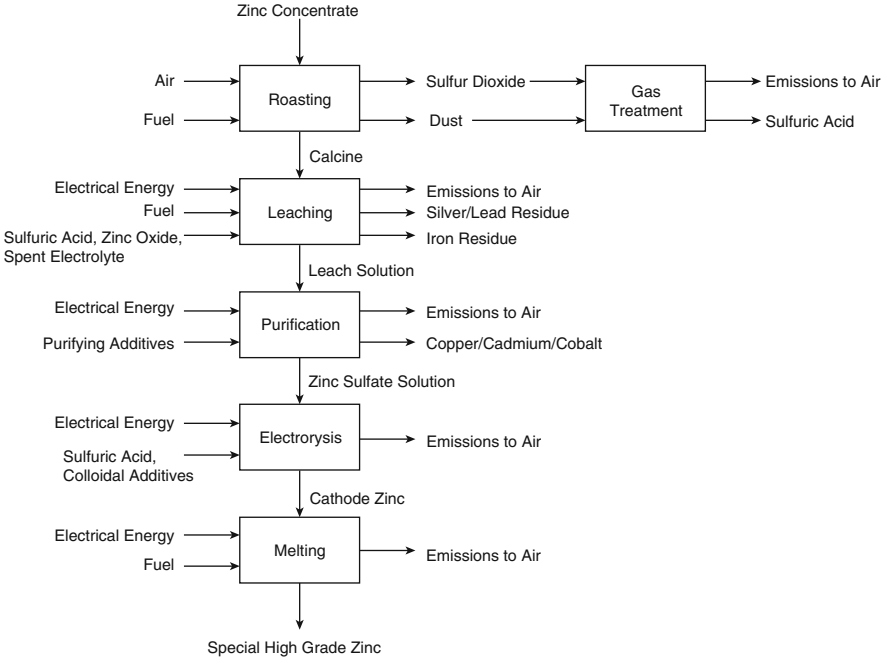

Fig. 5.1 Flowchart of special high-grade zinc production via electrometallurgical smelting. Source: van Genderen et al. (2016). Reprinted with permission from Springer Nature

needed, specially to utilize sophisticated process modeling. Sensors and instrumentation are useful to improve the characterization of the ore to determine if more or less crushing is needed to greatly enhance the process.

On-line systems to characterize the mineral going into a mill or coal preparation plant will enable a better understanding of the feed in terms of ore type and oxide content. Imaging sensors for use in classification devices, especially cyclones, can assist in optimizing feed to comminution processes.

Sensing devices are critical in all aspects of the mining process. Advances in real-time sensing, data collection, and data analysis and interpretation can help to understand the characteristics of materials prior to processing and improve the efficiency and processing activities.

These statements can be seen as premises to be met by the analytical techniques described ahead.

5.3 Main Related Analytical Matrices

In a general way, analytical matrices related to ores and mining are available in three physical states:

- Solids, e.g., metallic alloys, rocks, carbonaceous materials
- Powders, e.g., particles with size in the order of micrometers
- Semi-solids, e.g., sediments

In some cases, we can observe matrices in liquid state, e.g., metallic species present in low concentration in water, smelting materials, among others.

For the sample preparation for the chemical analysis, generally some techniques should be applied:

- Acid digestion of all kinds of samples for elemental analysis by atomic absorption spectrometry (AAS) and inductively coupled plasma–atomic emission spectrometry/inductively coupled plasma–optical emission spectrometry (ICP-AES/ICP-OES) (Balaram and Subramanyam 2022).
- Microwave digestion of all kinds of samples, also for elemental analysis by AAS, ICP-AES/ICP-OES, and inductively coupled plasma–optical emission-spectrometry-mass spectrometry (ICP-OES-MS) (Balaram and Subramanyam 2022).
- Fused beads or pressed pellets preparation of solid samples for elemental analysis by X-ray fluorescence (XRF) (Azo Materials 2023);
- Suspended powder of solid samples also for XRF (Pashkova et al. 2022).

Each preparation technique can be accessed by the cited reference.

Adaptation of techniques and methods can be necessary according to the sample characteristics, which will depend on the analyst's know-how and training.

5.4 Main Related Analytical Techniques

This section is structured in order to descript relevant aspects of analytical techniques for quality control of raw materials, inputs, and final products. That means, common physicochemical tests (e.g., pH value of an aqueous solution, water content by titration, particulate matter, etc.) for quality control will not be considered because generally they don't demand advanced analytical techniques or advanced analytical approaches, which are the subject of this chapter.

As deeply discussed in Chap. 4, auxiliary tools as chemometrics and artificial intelligence can be applied here to obtain all potential from the analytical data generated by means of the measure.

5.4.1 Atomic Absorption Spectrometry

When electromagnetic radiation is applied to atoms in the gaseous state, some of these atoms can be brought to a level of energy that allows the emission of the characteristic radiation of that atom. However, most can remain in the ground state and absorb energy, which in general would correspond to the energy in the gaseous state at the wavelength they would emit if they were excited from the ground state. Thus, when atoms absorb energy in the ultraviolet-visible (UV-Vis) region (from 10^{-8} to 10^{-6} m), an attenuation of the intensity of the radiation beam occurs. Thereby, atomic absorption spectrometry (AAS) is based on the absorption of the electromagnetic radiation by gaseous atoms in the ground state. The Maxwell-Boltzmann expression (Eq. 5.1 ahead) defines this physical phenomenon.

$$N_e/N_0 = (g_e/g_0)e^{-(E_e - E_0)Kt} \tag{5.1}$$

Where N_e is the relative population of the excited state, N_0 the relative population in the ground state (measured by AAS), g_e and g_0 are the statistical weights of the excited and ground states, respectively; E_0 is the energy in ground state; E_e is the energy in excited state k is the Boltzmann constant; and t the absolute temperature.

AAS is widely used in the inorganic analysis of metals, semi-metals, and non-metals in a huge variety of matrices. Initially, we have three different types of atomizers: combustion flame of different gases (hydrogen, acetylene, or natural gas), graphite furnace (or electrothermal), and cold mercury vapor (for determination of the mercury present by reduction to elemental mercury), with the application of each of them depending mainly on the analyte to be determined and the LOD required by the method—the flame AAS is the most used technique. The radiation absorbed has a direct relation with the analyte concentration that turns this technique very useful in quantitative analysis of metallic species for mineral chemical analysis.

In general, the spectra obtained by AAS are simpler than those obtained by atomic or optical emission (see ahead). A particular chemical element absorbs

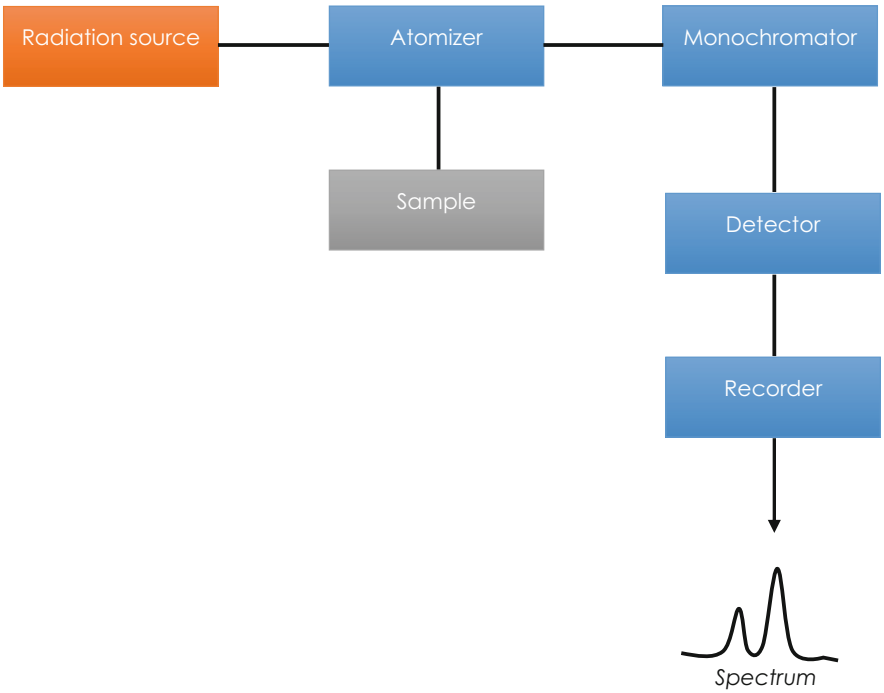

Fig. 5.2 Block diagram for an AAS instrument

energy at certain wavelengths. Typically, for analysis of an element, the highest absorption wavelength is chosen if there is no interference due to the absorption of the radiation by another element at that wavelength. Due to its simplicity and cost, AAS is the most widely used atomic spectrometric technique.

Figure 5.2 depicts a block diagram for an AAS instrument, and Fig. 5.3, the instrument.

Elements frequently detected by AAS are metals and non-metals, except: H, Fr, Ra, Ac, La, Hf, Tc, Os, C, N, O, F, P, S, halogens, and noble gases. That means, its capacity covers most analytical needs for minerals.

5.4.2 Atomic Emission Spectrometry

Atomic emission spectrometry (AES), or optical emission spectrometry (OES), is based on the measurement of the emission of the electromagnetic radiation in the ultraviolet-visible (UV-Vis) region—from 10^{-8} to 10^{-6} m—by neutral and ionized atoms, not in excited state, being widely used in elemental analysis. The most common OES system uses an argon plasma torch that can reach up to 9000 K (as the hyphenated inductively coupled plasma, ICP) for the electrons excitation in

Fig. 5.3 An AAS spectrometer (**a**), and the obtained measurement (**b**). Courtesy of Shimadzu

gaseous state. ICP can also be coupled to a quadrupole mass analyzer (ICP-AES-MS/ICP-OES-MS); it offers extremely high sensitivity to a wide range of elements.

The technique has high stability, sensitivity, low noise—S/N or signal/noise ratio—and low background emission intensity. However, because it involves relatively expensive methods that require extensive operator training, it is not applied as AAS. All metals or non-metals of mining interest, determined by AAS, can be determined by OES—the latter can favor, for some elements, the achievement of lower values of LOD and LOQ.

AES/OES also follow the Maxwell-Boltzmann expression (Eq. 5.1) for its physical phenomenon.

This technique is, without doubt, a strong ally to the mining industry.

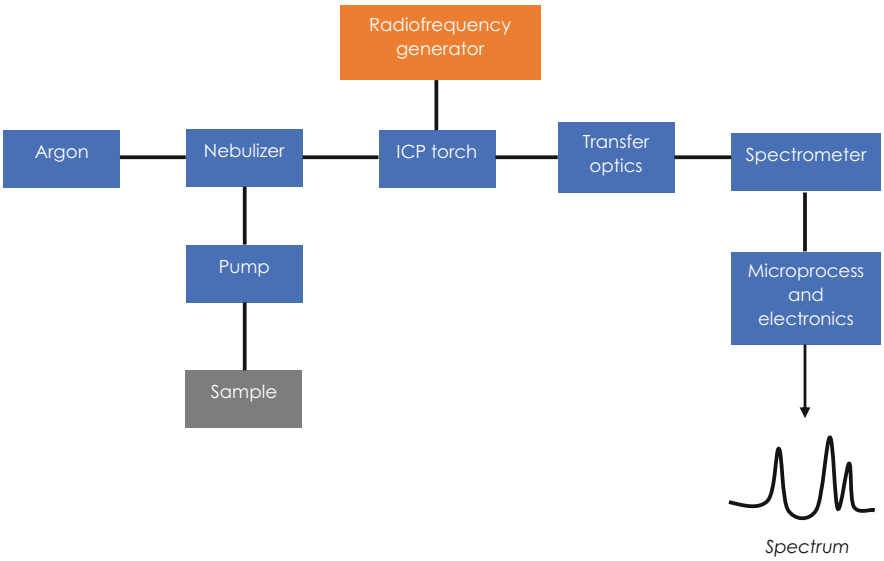

Fig. 5.4 Block diagram for an ICP-OES (or ICP-AES) instrument

According the EAG Laboratories (2023), advantages of OES are as follows: bulk chemical analysis technique that can determine simultaneously up to 70 elements in a single sample analysis; the linear dynamic range is over several orders of magnitude; instrumentation is suitable to automation, thus enhancing accuracy, precision, and throughput. Limitations are as follows: the emission spectra complexity and inter-element interference when the wavelength of the element of interest is very close to that of another element; in MS mode, determination and quantification of certain elements can be affected by interference from polyatomic species, matrix elements, and atmospheric elements; the sample to be analyzed must be completely digested, or dissolved prior to analysis in order to determine the element(s) of interest.

Figure 5.4 depicts a block diagram for an ICP-OES instrument, and Fig. 5.5, the instrument.

5.4.3 Inductively Coupled Plasma Mass Spectrometry

ICP-MS is a variation of the atomic emission spectrometry (AES) (or optical emission spectrometry, OES), where the excitation source for atoms and ions is an inductively coupled plasma (ICP) hyphenated with a mass spectrometer (MS). As ICP-AES/ICP-OES, this technique can be applied for elemental analysis of minerals.

The high electronic density of argon plasmas in the ICP device decreases the occurrence of ionization interference. However, in some cases, the addition of an

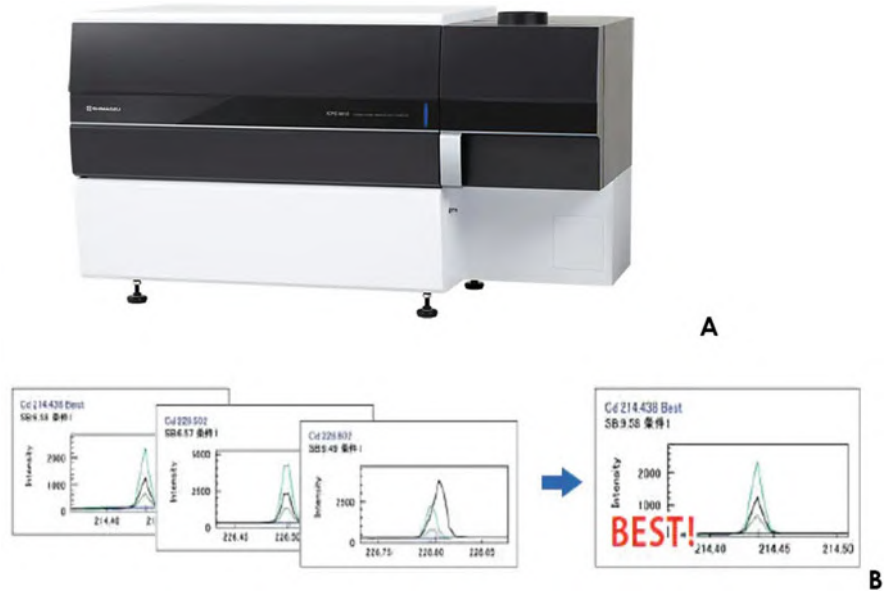

Fig. 5.5 An ICP-OES (or ICP-AES) instrument (**a**), and the obtained spectrum (**b**). Courtesy of Shimadzu

easily ionizable element, such as Na or K, can cause changes in the spatial emission profile of atoms and ions.

The plasma is formed in an argon stream, usually 8–20 L min^{-1}, which flows through three concentric quartz tubes (15 to 30 mm in diameter) which combine to form the torch. The quartz torch is positioned concentrically to the induction coil, which in turn is coupled to a radio frequency generator. The generator operates at frequencies ranging from 4 to 50 MHz (the most common frequencies are 27 and 40 MHz) and powers of 1 to 2 kW. Argon flowing through the torch maintains the plasma, cools the walls, and transports the sample aerosol.

Mass spectrometry (MS) is based on the separation of ions using the mass/charge (m/z) ratio. Gaseous ions are generated and introduced into the mass spectrometer which operates under a vacuum ranging from 10^{-4} to 10^{-9} torr. Due to the action of electric fields and magnetic elements on the ions with different m/z ratios, each ion describes a trajectory and there is a selection of the species that will reach the detector at each moment.

Argon-induced plasma in the ICP device has temperatures of up to 8000 K and this high thermal energy is sufficient to cause excitation and ionization of most chemical elements. Considering a temperature of 7500 K and an electron concentration of 10^{15} electrons cm^{-3} it can be shown that more than 50% of the chemical elements usually determined are predominantly ($> 90\%$) present in the monovalent

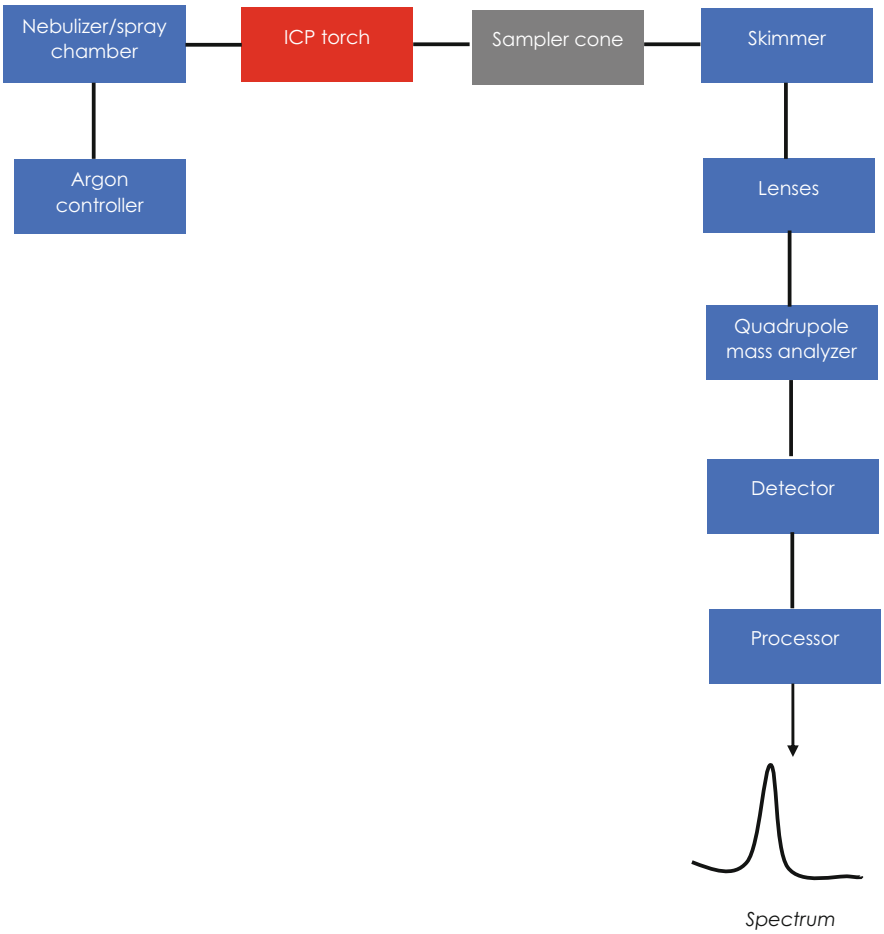

Fig. 5.6 Block diagram for an ICP-MS instrument

ionic form ($M^{+}_{(g)}$). Furthermore, with the exception of the lanthanides, the formation of divalent ions ($M^{2+}_{(g)}$) is low and, consequently, the mass spectrum is simplified.

From these statements, all metallic, semi-metallic, and certain non-metallic elements can be determined by ICP-MS, in a work range[1] of μg L^{-1}.

Figure 5.6 describes a block diagram of an ICP-MS, and Fig. 5.7, the instrument.

[1] According to the International Union of Pure and Applied Chemistry, *working range* is a set of quantity values over which a measuring instrument or measuring system provides results with acceptable measurement uncertainty, under defined conditions.

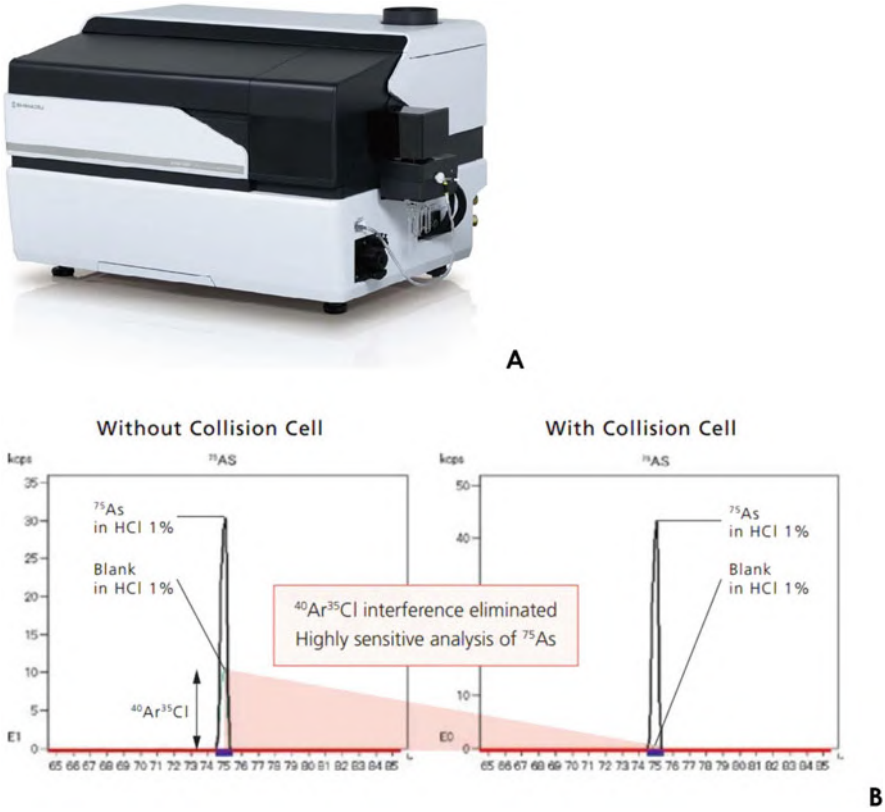

Fig. 5.7 An ICP-MS instrument (**a**), and the obtained spectrum (**b**). Courtesy of Shimadzu

5.4.4 Particle Size Distribution

Sample particles are distinguished by their properties. The most common property of particles is the equivalent sphere diameter (also called the equivalent diameter). Particles are ordered by their property on the *x*-axis of a coordinate system.

Also, particles are assigned to different classes (gap between two nodes on *x*-axis). If the amounts of all grades relative to the total sample amount were determined, these amounts can be plotted as *particle size distributions*.

The measurement technique or focus of interest often implies the use of a certain evaluation criterion (DP Union 2023). Weighing the particles of each class gives mass or volume distributions. Alternatively, counting the particles gives numerical distributions. Both types of quantity are most frequently used in practice. In addition, the particles can be ordered in a row, i.e., "length" is an evaluation criterion. In some cases, projection areas are measured. As a logical consequence, "area" is also an evaluation criterion.

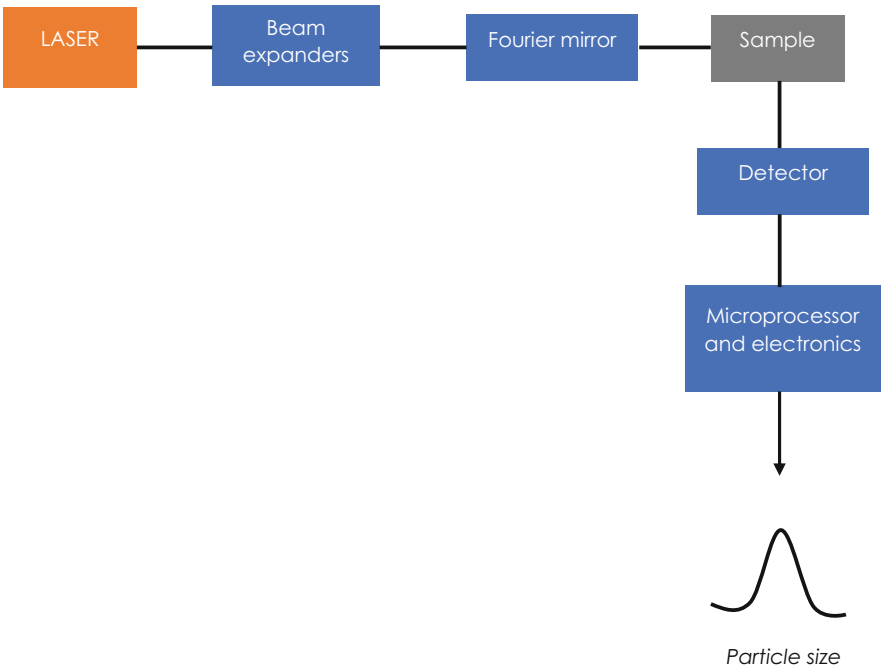

Fig. 5.8 Block diagram of a particle size distribution instrument. *LASER* light amplification by stimulated emission of radiation

Frequently, the particle size of grains can be measured by means of dynamic light scattering (DLS) where particles experience Brownian motion, and smaller particles move faster than larger ones. On the other hand, the size of a small particle can be measured at an angle of θ by the Rayleigh scattering, which is defined in Eq. (5.2):

$$R_\theta = I/Io = Kr^6 \tag{5.2}$$

Where I is the ratio of the intensity of the scattered light, I_0 the intensity of the light source, r is the radius of the particle, and K is a constant that is a function of the angle of scattering, the wavelength of radiation used, the refractive index of the particle, and the distance to the particle.

As we can see ahead in Sect. 5.5, this technique can be applied for process control.

Figure 5.8 depicts the block diagram for a particle size distribution instrument, and Fig. 5.9, the instrument.

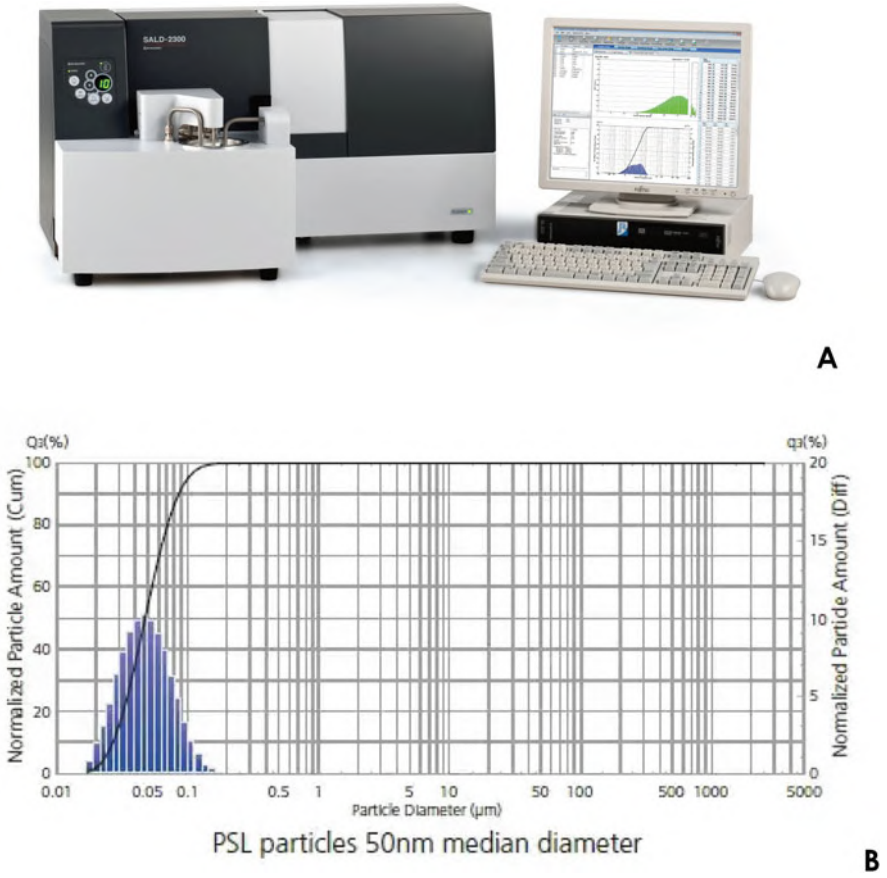

Fig. 5.9 A particle size analyzer (**a**), and a plot of particle size distribution (**b**). Courtesy of Shimadzu

5.4.5 X-ray Atomic Emission or Fluorescence Spectrometry

This technique allows a rapid and non-destructive multi-element analysis for solid and liquid samples (identification and quantification). When an atom is excited by the removal of an electron from its inner layer, it emits X-rays (from 10^{-12} to 10^{-8} m) when returning to its ground state; such radiation has a typical signal intensity for each element, which is used in the analysis. Here, we can use the Lambert-Beer law (Eq. 5.3) to operationalize the physical phenomenon.

$$A = \varepsilon bc \tag{5.3}$$

Where: A = absorbed radiation (arbitrary units); ε = molar absorptivity of the medium (cm^{-1} L mol^{-1}); b = cell length (cm); c = concentration (mol^{-1} L).

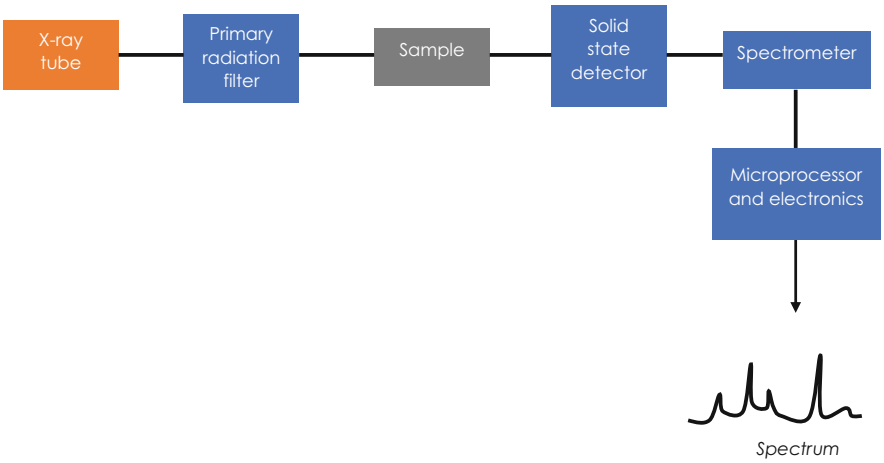

Fig. 5.10 Block diagram of an XRF instrument

There are two X-ray fluorescence (XRF) systems available: the wavelength dispersive spectrometer (WD-XRF) and the energy dispersive spectrometer (ED-XRF)—the latter has higher signal throughput, which enables small area analysis or mapping.

According the EAG Laboratories (2023), advantages of XRF are as follows: non-destructive technique; can analyze areas as small as ~150 μm; can analyze any solid material; and sampling depth ranging from a few micrometers to several millimeters depending on the material. Limitations are as follows: cannot detect elements lighter than aluminum using small spot ED-XRF; and highest accuracy measurements require reference standards similar in composition and/or thickness to the test sample.

Fig. 5.10 presents a block diagram for an XRF instrument, and Fig. 5.11, the instrument.

5.4.6 X-ray Diffraction Spectrometry

X-ray diffraction (XRD) is a popular technique to determine the structure of inorganic crystals. It is also used to determine the degree of long-range order and symmetry present in a crystal, or lacking in a glass. And, in a general way, XRD is usually well known for qualitative and quantitative analyses of crystalline phases in materials—e.g., the determination of percentage of amorphous and crystalline species in a certain material—characterization of solid solutions, crystallite size and shape, crystal orientation, internal elastic strains/stresses at different levels, effect of temperature, and close surface characterization (Gonon 2021).

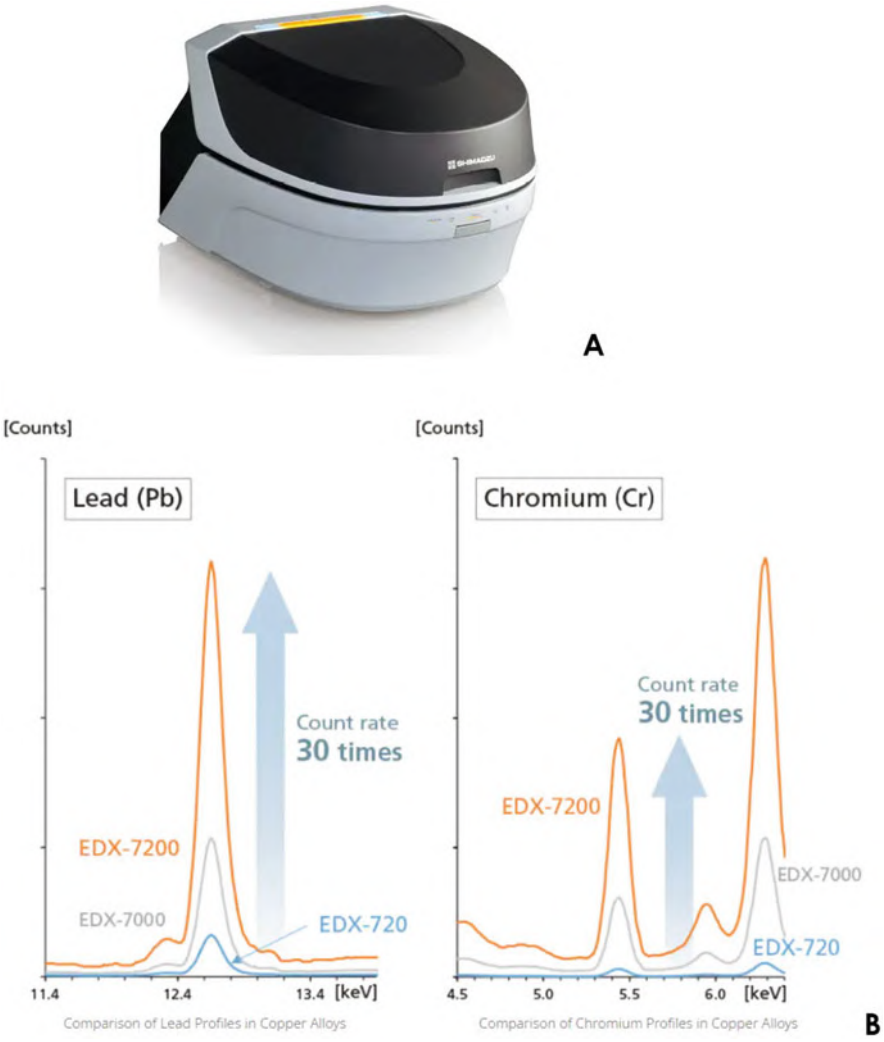

Fig. 5.11 An ED-XRF instrument (**a**), and the obtained spectrum (**b**). Courtesy of Shimadzu

The sample, preferably a powder, is bombarded with an electron beam, causing the X-rays (from 10^{-12} to 10^{-8} m) to diffract from the surface as a function of its crystallinity. The diffraction intensity is associated with the double of its diffraction angle, according to Bragg's law (Eq. 5.4).

$$n\lambda = 2\mathrm{d} \times \sin\theta \tag{5.4}$$

Where θ is the incidence angle, d is the distance among the atomic layers in a crystal, and λ is the wavelength of the X-ray incident beam on the sample.

X-rays reflect off each atomic plane in a crystal, producing patterns of destructive and constructive interference according to Braggs' law. Specifically, for XRD, it involves monochromatic X-rays bouncing off a rotating target; the resulting peaks indicate the identity and spacing of the close-packed planes (Massachusetts Institute of Technology 2023).

This technique is very useful for mineral characterization.

Figure 5.12 describes the block diagram of an XRD spectrometer, and Fig. 5.13, the instrument.

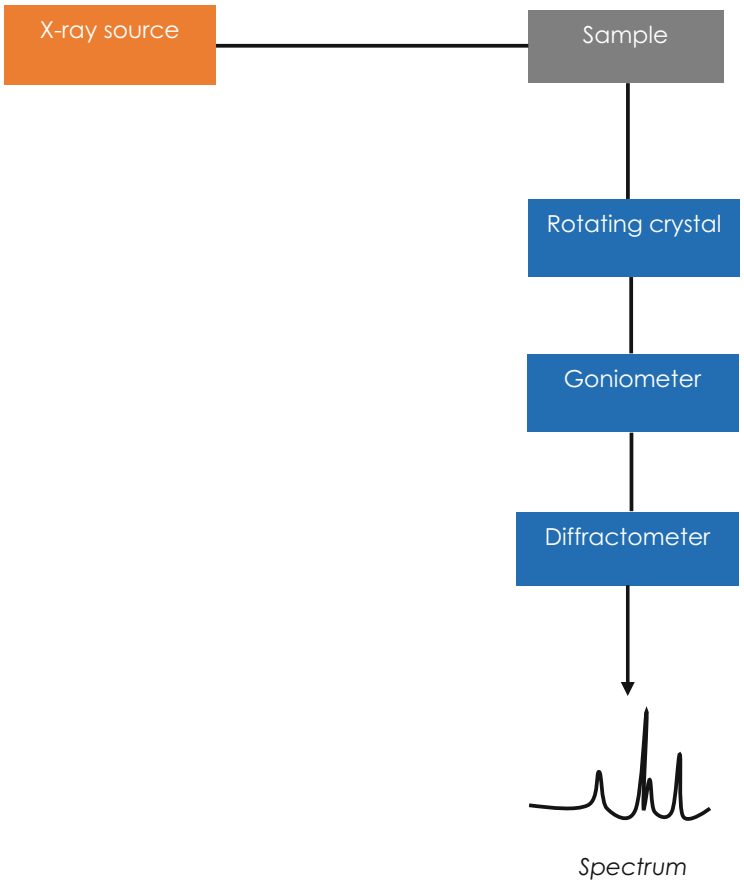

Fig. 5.12 Block diagram of an XRD equipment

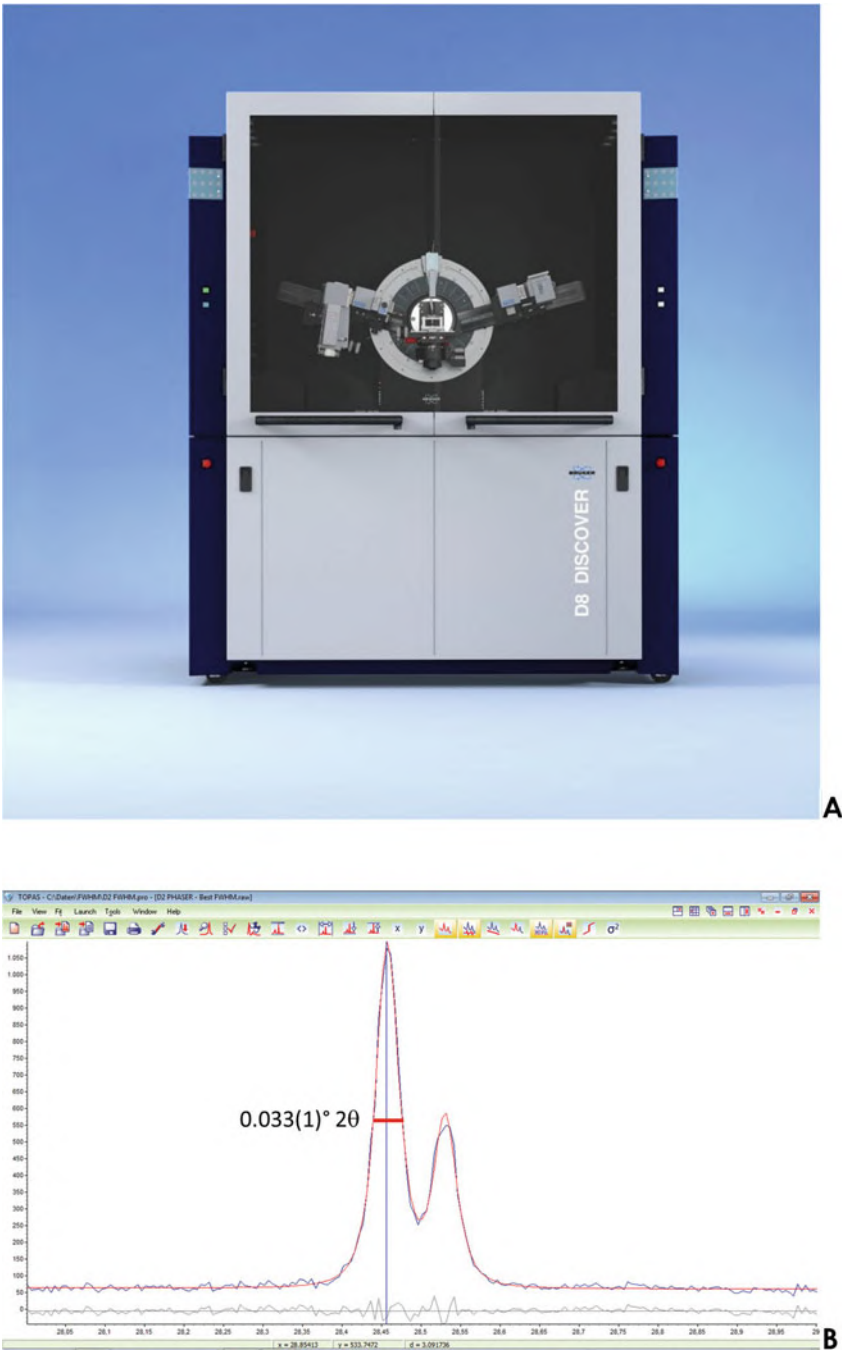

Fig. 5.13 An XRD instrument (**a**), and the obtained spectrum (**b**). Courtesy of Brüker

5.4.7 *Process Analytical Technology*

The need for quality control of transformation processes of production has leveraged the use of process analytical chemistry (PAC), often also known as PAT (process analytical technology).

For the mineral industry, the use of robust techniques and methods is privileged, preferably in real time, with the analyses being carried out directly in the reactor or in the mixer, instead of analyses carried out in the laboratory. The main advantage of this type of analytical approach in relation to the traditional one, where manual sampling is carried out followed by sample transport and subsequent analysis in the laboratory, is that analyses carried out in situ provide greater speed for taking corrective actions and consequent adjustment of the production process. On the other hand, the need to have robust and automated analytical instrumentation, such as simple-to-use spectroscopic probes, ends up limiting the number of analytical parameters that can be analyzed, in addition to compromising the LOD and LOQ.

However, the continuous development of new analytical technologies and new materials will certainly increase the possibilities of obtaining more results, both by accepting greater variation in the physical and chemical conditions of the medium, and by allowing a better identification of chemical species.

The main aspects to be carefully considered in the methodological planning of measures in dynamic systems were listed by Van Staden (1999) and should be considered here:

- Selection of process variables, such as temperature, pressure and pH, or definition of process quality parameters to be measured.
- Establishment of a quantitative relationship between controllable properties, because there is not always a direct relationship between them.
- Definition of sampling or analysis locations.
- Definition of intervals and number of measurements, in addition to the process correlation time, considering that for continuous measurements the system time constant must be defined.
- Determining the duration of the measurements, which will comprise sampling, measurement, and data calculation; that means: $t_{elapsed} = t_{sampling} + t_{measurement} + t_{calculation}$.
- Definition of tolerance limits (lower and upper) for the measured variables, which will determine the quality of the process.
- Selection of appropriate instrumentation.
- Establishment of instrument costs and their maintenance.
- Definition of instrument calibration frequency.
- Elaboration and evaluation of the costs of the measures, also considering regulatory issues.
- Establishing the reliability of measurements and the ease of obtaining them.

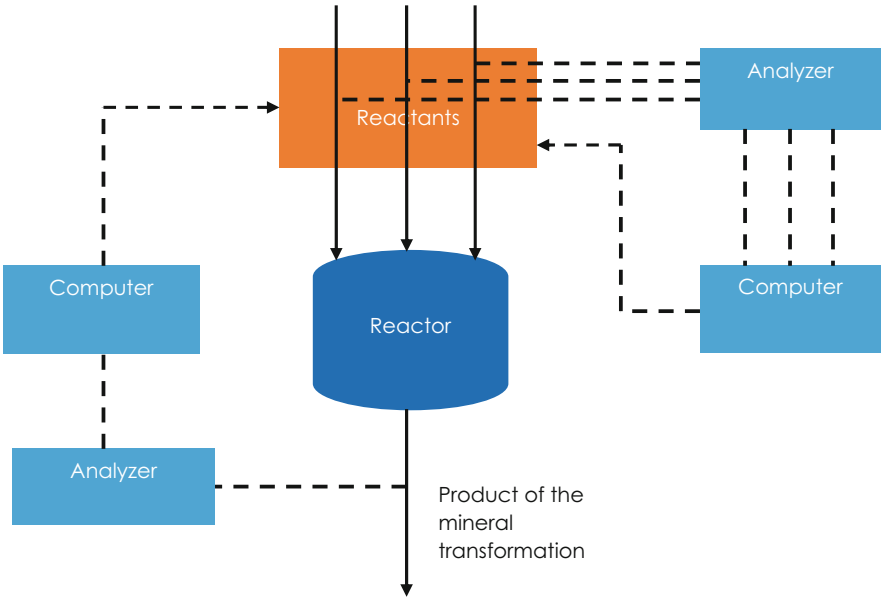

Fig. 5.14 A flowchart for on-line PAT application in a mineral generic processing, e.g., metallurgic process. Source: Adapted from Rouessac and Rouessac (2007). Reproduced with permission from Wiley

Fig. 5.15 A particle size analyzer for a mineral processing plant, fully automated real-time (or manual at-line) size measurement of particulates. Courtesy of Malvern Panalytical

Once these considerations are made, the PAC/PAT becomes a very helpful tool for controlling processes in the mineral industry, leading to a very positive impact on the quality of the final product.

Figure 5.14 depicts a flowchart for on-line PAT application, and Fig. 5.15, a particle size analyzer.

5.5 Commented Examples of Applications

The most common advanced technique applications can comprise the following:

- Non-invasive mineral characterization: XRD (non-destructive technique for characterization of crystalline materials); XRF (non-destructive elemental analysis for composition measurements).
- Common mineral compositional analysis in several physical states: ICP-AES/ICP-OES, ICP-MS and AAS (destructive techniques for elemental analysis of metals and semi-metals); they can be expanded to sediment analysis.

Table 5.2 describes several examples of these applications for miscellaneous purposes for ores and mining.

The detailed analytical method for each example in Table 5.2 can be accessed by the cited reference.

The ASTM International (the former American Society for Testing and Materials) provides several standardized analytical methods for ores and mining for laboratory applications (ASTM International 2023). For instance:

- ASTM E3061-17: Standard test method for analysis of aluminum and aluminum alloys by inductively coupled plasma atomic emission spectrometry (performance-based method); this test method describes the application of ICP-AES (or ICP-OES) to determine Si, Fe, Cu, Mn, Mg, Cr, Ni, Zn, Ti, Ag, As, B, Ba, Be, Bi, Ca, Cd, Co, Ga, Li, Mo, Na, P, Pb, Sb, Sc, Sn, Sr, Ti, Tl, V, and Zr in aluminum materials with a variable application range for specified compositional limits.
- ASTM E536-16: Standard test methods for chemical analysis of zinc and zinc alloys; this test method describes the application of AAS to determine Al, Cd, Cu, Fe, Pb, Mg, and Sn in zinc materials for compliance with compositional specifications.
- ASTM E1446-13: Standard test method for chemical analysis of refined gold by direct current plasma atomic emission spectrometry; this test method describes the application of ICP-AES (or ICP-OES) to determine Cu, Fe, Pb, Pd, and Ag in gold materials for compliance with compositional specifications.
- E1621-22 Standard guide for elemental analysis by wavelength dispersive X-ray fluorescence spectrometry; this standard provides guidelines for developing and describing analytical procedures using a wavelength dispersive X-ray spectrometer for elemental analysis of solid metals, ores, and related materials. Material forms discussed herein include solids, powders, and solid forms prepared by chemical and physical processes such as borate fusion and pressing of briquettes.

All chemical analyses presented by these cited methods must be performed accompanied by the respective *reference material*[2]—or reference standard—for

[2] According to the National Institute of Standards and Technology (NIST), a *reference material* is a material, sufficiently homogeneous and stable with respect to one or more specified properties,

Table 5.2 Examples of application of advanced analytical techniques in the mineral industry

Analytical technique	Application	Observation	Reference
AAS	Determination of Cu in lixiviant solution produced by the extraction of this metal through hydrometallurgical route on low grade Cu ore sample	The aliquot for analysis passed suffered a simple dilution in distilled water	Mohanraj et al. (2022)
ICP-OES/ ICP-AES	Determination of rare earth elements (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu) in uranium-based nuclear grade materials	Selective extraction of uranium matrix using tri (2-ethylhexyl)phosphate (TEHP), eliminating several matrix interferences	Baghaliannejad et al. (2021)
ICP-MS	Trace element analysis (^{34}S, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{71}Ga, ^{72}Ge, ^{75}As, ^{107}Ag, ^{111}Cd, ^{115}In, ^{118}Sn, ^{121}Sb, ^{197}Au, ^{205}Tl, ^{208}Pb, and ^{209}Bi) of sphalerite and pyrite samples from carbonate-hosted Pb-Zn ore	Use of LASER ablation (wavelength of 193 nm and maximum energy of 200 mJ)	Zhou et al. (2022)
Particle size distribution	Investigation of the relationship between transportable moisture limit and particle size distribution for an iron ore	Basis for the understanding of the moisture-related solid bulk cargo failure in the ore transportation by sea	Ferreira and Lima (2023)
Process analytical technology	Process monitoring in a cement plant	Use of fast X-ray detectors	Workman et al. (2005)
XRF	Elemental analysis (geochemical analysis) of bauxite ore samples	Determination of average percentages of SiO_2, Al_2O_3, Fe_2O_3, TiO_2, CaO, MgO, Na_2O, and K_2O compounds	Altikulaç (2022)
XRD	Mineralogical characterization of a sample of roasted calcine of a copper-rich pyritic refractory gold ore	Determination of quartz as the main phase in the roasted ore sample	Msumange et al. (2023)

AAS atomic emission spectrometry, *ICP-AES* inductively coupled plasma-atomic emission spectrometry, *ICP-OES* inductively coupled plasma-optical emission spectrometry, *ICP-MS* inductively coupled plasma-mass spectrometry, *LASER* light amplification by stimulated emission of radiation, *XRF* X-ray fluorescence, *XRD* X-ray diffraction

which has been established to be fit for its intended use in a measurement process; additionally, a *certified reference material* (CRM) is a reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability.

the correct method calibration, in order to guarantee the uncertainty and the *metrological traceability*[3] of the measurements.

5.6 Conclusions

Analytics for ores and mining is an exciting branch of analytical chemistry dedicated to inorganic species with the highlighted use of spectroscopy-based analytical techniques, e.g., AAS, ICP-OES/ICP-AES, XRF, XRD, and particle size distribution. Furthermore, process analytical technology—based on spectroscopy also—supplies a strategic knowledge of material composition for quality control of products and their processes for mineral processing.

The heterogeneous compositional characteristic of ores requires special attention with the sample preparation in order to avoid the effect of interferents on the chemical analysis. Additionally, the use of reference materials (or references standards) is essential to guarantee uncertainty and traceability of the analytical result.

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[3] According to the International Union of Pure and Applied Chemistry (IUPAC), the *metrological traceability* is a property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty.

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Chapter 6
Natural and Synthetic Polymers

Abstract Polymers are one of the most relevant materials for the modern society to maintain its lifestyle and its life quality. Plastics, fibers, etc., are present in our day-to-day life from the morning (e.g., toothbrush) up to the night (e.g., lampshade). For the quality control and for the research and development of the polymeric materials we have several advanced analytical techniques, as microscopic (SEM, TEM, AFM), spectroscopic (EDS, NMR, SAXS), chromatographic (GPC), thermal (TGA, DTA, DSC), and sorption/desorption (BET) techniques. These techniques and their analytical methods are treated in this chapter. Furthermore, physical-chemical properties of natural and synthetic polymers, and aspects of market are also presented.

Keywords Macromolecules · Homopolymers · Copolymers · Additives · Physicochemical properties · Advanced analytical techniques

6.1 Introduction

Synthetic and natural polymers, especially the first, are one of the most relevant materials for the modern society in order to maintain its lifestyle and its life quality. Plastics, fibers, etc., are present in our day-to-day life from the morning (e.g., toothbrush) up to the night (e.g., lampshade). Indeed, with the boom of the petrochemical industry in the middle of twentieth century to convert oil into plastic utensils, we are surrounded by synthetic polymers on all sides—both for good (i.e., convenience) and for bad (i.e., negative environmental impacts).

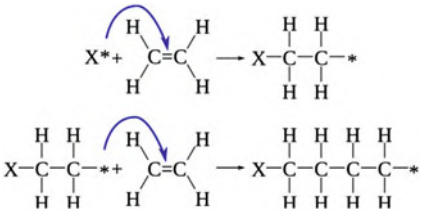
To show the polymer relevance, for the year 2021 the global production of plastics reached 367 million metric tons, with China highlighted as the major producer responding with 32% of this production, followed by North America (18%), and European Union (15%) (Statista 2023). It is worth highlighting the expectation of an increase in the participation of bio-based plastics (e.g., plastics derived from starch and from ethanol) as an alternative to substitute petrochemical plastics. However, this huge global production can promote negative impacts on the environment and health by means of, for instance, the microplastics release (Vaz Jr 2018).

S. Vaz Jr, *Applications of Analytical Chemistry in Industry*,
https://doi.org/10.1007/978-3-031-38952-8_6

$$C_6H_{16}N_2H_4 + HOC_4H_8CO_2H \rightarrow [C_{12}H_{22}O_2]n + H_2O$$

Scheme 6.1 The reaction of condensation for the synthesis of Nylon polymer from hexamethylenediamine and adipic acid as reactants, and water as by-product

Scheme 6.2 The polyethylene synthesis by reaction of addiction. X^* is free radical initiator



According to the International Union of Pure and Applied Chemistry (2023), a polymer can be defined as a substance composed of *macromolecules* which are understood as molecules of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass—for synthetic and natural polymers, the units of repetition are monomers. The polymer formation—or polymerization—is the process of converting a monomer mixture into a polymer. Polymers can be classified into *homopolymers* and *copolymers*. The first are derived from one species of monomer, while the second are derived from more than one species of monomer.

Synthetic polymers can be obtained by reactions of *condensation* between an ester and an acid (e.g., polyamides as nylon) (Scheme 6.1); and by reactions of *addiction* (Scheme 6.2) using monomers with one or more double bonds (e.g., polyvinyl chloride).

Natural polymers comprise a large number of materials occurring in plants and animals, with the metabolic routes of synthesis, for some of them, not totally understood. Examples of these biopolymers are lignin and cellulose from plant cell wall, and chitosan from exoskeleton of crustaceans (e.g., shrimp shells).

From selected physical properties we can propose to classify the high molecular weight polymers—the most relevant polymer family for industrial purposes—in *elastomers*, *plastics,* and *fibers* as follows:

- Elastomers are cross-linked amorphous synthetic polymers, and are used in the automobile industry to make tires, braking systems, chassis, interior parts, etc. (Neitzel et al. 2012). Examples are natural rubber, polyisobutylene (PIB), and polychloroprene (synthetic rubber).
- Plastics are a mixture of a synthetic resin obtained from oil (generally) and natural gas and additives (e.g., functional additives (stabilizers, antistatic agents, flame retardants, plasticizers, lubricants, slip agents, curing agents, foaming agents, biocides), colorants (pigments, soluble azocolorants), fillers (mica, talc, kaolin, clay, calcium carbonate, barium sulfate), reinforcements (glass fibers, carbon fibers)), with thermoplastics highlighting for their production and use

Table 6.1 Common physical properties of polymers

Properties	Elastomers	Plastics	Fibers
Capacity of stretching	Approximately 1000%	Approximately 100–200%	Approximately 10–20%
Nature of deformation	Completely reversible and instantly elastic	Low reversible elasticity and some permanent deformation	Some instant elasticity, some delayed elasticity, and some permanent elasticity
Dependence of the mechanical properties of the temperature	Elasticity increases with the temperature increasing; brittle and inextensible at low temperature	Elasticity dependent on the temperature	Elasticity: almost independent of the temperature between −50 °C and +150 °C
Tendency to crystallize	Very low	Moderate	Very high

(Hahladakis et al. 2018). Examples are polystyrene (PS), polyvinyl acetate (PVA), and polyvinyl chloride (PVC).

- Fibers are filiform elements constituted by polymers (natural or synthetic, organic, or inorganic), which have a high length in relation to the maximum transverse dimension, being characterized by their flexibility and fineness. Examples are cellulose (natural fiber), silk fibroin (natural fiber), and polyamides (synthetic).

Table 6.1 describes general physical properties for application for elastomers, plastics, and fibers.

Figures 6.1 and 6.2 depict chemical structures of synthetic and natural polymers with industrial usages.

Table 6.2 provides some physicochemical properties of those polymers presented in Figs. 6.1 and 6.2.

6.2 How This Industry Works

As expected, the main raw material source of the polymer industry is the oil, followed by plants and a small contribution of animal sources (e.g., chitosan).

Regarding the processing, we can note several polymerization techniques to be applied in industry which can be divided into two main families (Al-Muallem 2005):

1. Free radical polymerization, in bulk, suspension, solution, or emulsion, has been the first and the most common.
2. Ionic and other non-radical polymerizations, produced in solution polymerizations.

Figure 6.3 depicts a flowchart of the green polyethylene (PE) of Braskem (Braskem 2023) as an example of industrial polymerization to produce a polymer from renewable source, i.e., a biopolymer.

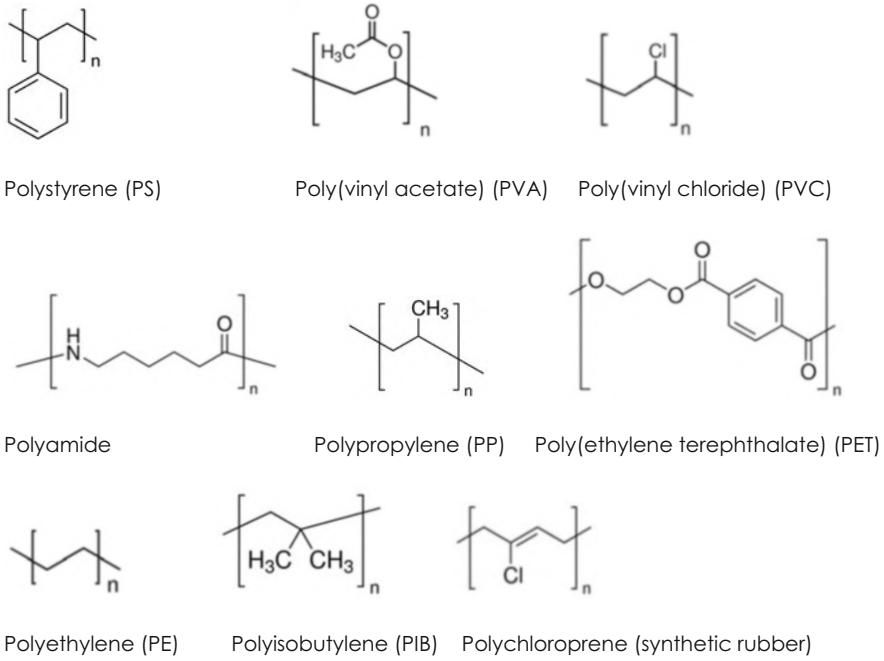


Fig. 6.1 Chemical structure of synthetic polymers of industrial relevance

It is worth to mention that the green PE in the Fig. 6.3—a biopolymer—is obtained from a renewable monomer source, i.e., ethylene produced from ethanol produced from sugarcane. The ethanol molecule should be previously dehydrated in presence of an acid (e.g., sulfuric acid) to generate the monomer unities of ethylene before the addition polymerization step (polymerization in solution) in the presence of a catalyst (e.g., alumina) (Scheme 6.3).

According to Kumar et al. (2022), PE has the ability to alter easily during processing, thus gives relatively longer chain length, density, and crystallinity, allowing PE products to have tailored properties for a variety of applications. High-density polyethylene (HDPE) and low-density polyethylene (LDPE) are some types of PE plastic.

6.3 Main Related Analytical Matrices

In a general way and according to Bartenev et al. (1981), polymers can exist in four physical states:

- One crystalline state
- Three amorphous states (glassy, rubbery, and viscous flow)

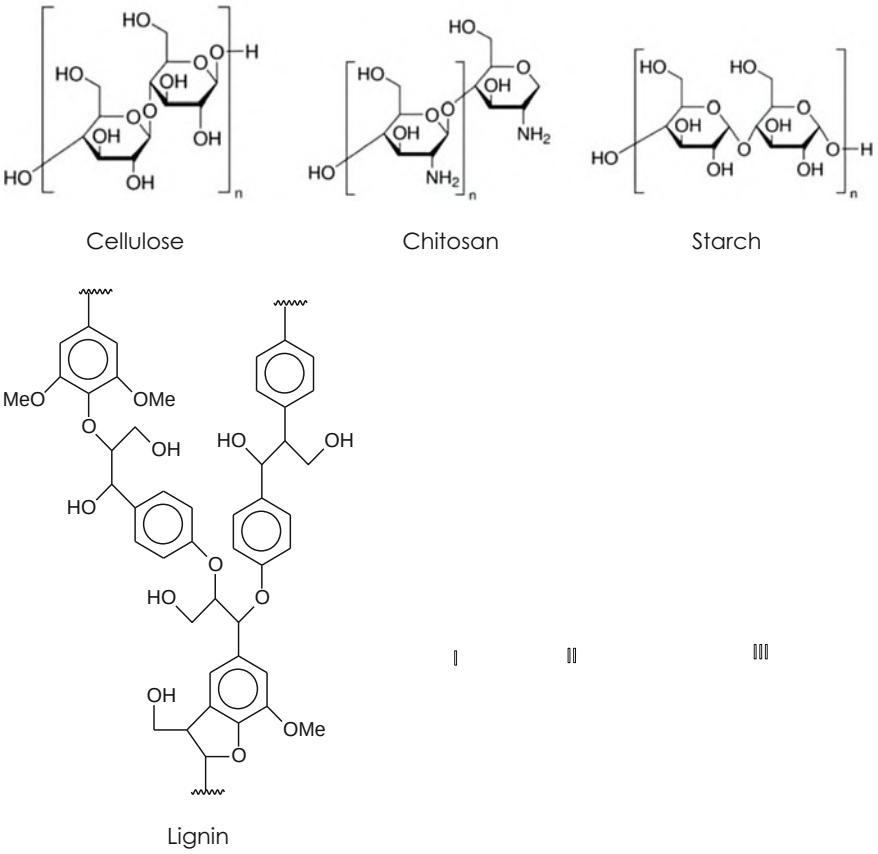


Fig. 6.2 Chemical structure of natural polymers of industrial relevance

And those polymers that exist in the glassy or crystalline state are sometimes called *rigid polymers*. The distribution of these states in a polymeric structure can be visualized in Fig. 6.4.

For analytical purposes, polymers can be present as:

- Solids, e.g., a part of a car dashboard
- Semi-solids, e.g., a plastic bag or a polymeric film
- Powders, e.g., a dried Kraft lignin
- Liquid samples, e.g., a certain polymer solubilized in an organic solvent

However, as a considerable number of additives for the polymer formulation are required, we should consider this chemical class as a part because it can act as analyte or as interfering in the chemical analysis.

Table 6.2 Physicochemical properties of synthetic and natural polymers of industrial relevance. Source: Elaborated from free information available on Merck webpage[a]

Polymer	CAS number	Formula	Melting point (°C)	Density (g cm^{-3})
Polystyrene (PS)	9003-53-6	$[CH_2CH(CH_6H_5)]_n$	240	1.047
Polypropylene (PP)	9003-07-0	$C_{22}H_{42}O_3$	210–290	0.905
Poly(vinyl acetate) (PVA)	9003-20-7	$[(CH_2CH(O_2CCH_3)]_n$	*Not available*	1.191
Poly(vinyl chloride) (PVC)	9002-86-2	$(C_2H_3Cl)_n$	92	1.38
Polyamide (Nylon 6)	25038–54-4	$[-NH(CH_2)_5CO-]_n$	220	1.084
Polypropylene (PP)	9003-07-0	$[CH_2CH(CH_3)]_n$	158–170	0.92
Poly(ethylene terephthalate) (PET)	25038–59-9	$(C_{10}H_8O_4)_n$	250–255	1.68
Polyethylene (PE)	9002-88-4	$H(CH_2CH_2)_nH$	92	0.92
Polyisobutylene (PIB)	9003-27-4	$[(CH_2C(CH_3)_2]n$	*Not available*	0.92
Polychloroprene (synthetic rubber)	9010-98-4	$[-CH_2CH=C(Cl)CH_2-]_n$	*Not available*	1.23
Lignin (Kraft or alkali)	8068-05-1	*Not available*	*Not available*	1.3
Cellulose	9004-34-6	*Not available*	*Not available*	*Not available*
Chitosan	9012-76-4	$C_{12}H_{24}N_2O_9$	102.5	*Not available*
Starch	9005-25-8	$(C_6H_{10}O_5)_n$	256–258	0.14

[a]www.sigmaaldrich.com

6.3.1 Additives

Additives for polymers are materials added as auxiliary components of plastics and/or rubbers, and their adding in the formulations aims at one or more specific applications such as, for instance, lowering the cost, modifying and/or improving various properties, facilitating processing, coloring, etc.

The main additives in plastics and rubbers are described in Table 6.3.

As the additives presented in Table 6.3—produced by companies as BASF and BAYER, among others—can act as modifiers of physical-chemical and physical properties, they can produce a direct influence on the analytical results, which demands special attention to their presence and concentration in the sample.

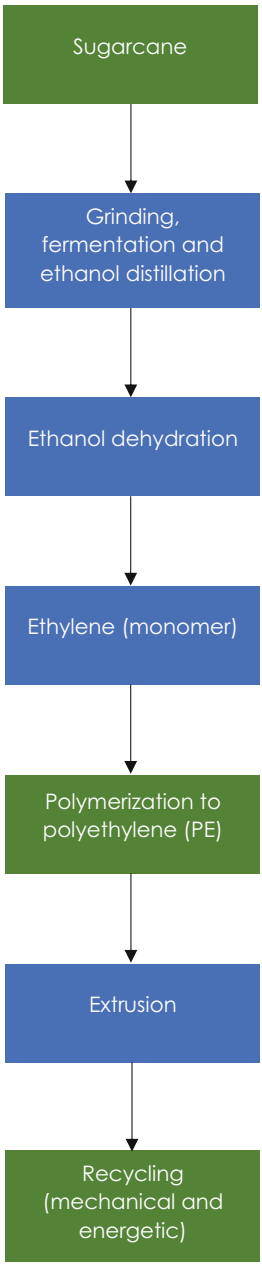


Fig. 6.3 Process production of the green polyethylene (PE). Source: Elaborated according to Braskem's public information

$$\text{Step } a\text{: } CH_3CH_2OH + H^+ \rightarrow H_2C{=}CH_2 + H_2O$$

$$\text{Step } b\text{: } nH_2C{=}CH_2 \rightarrow {-}(CH_2CH_2)_n{-}$$

Scheme 6.3 The two-step production of green PE. In the step *a* the monomer (ethylene) is obtained by the acid dehydration of ethanol, followed by the step *b* of polymerization

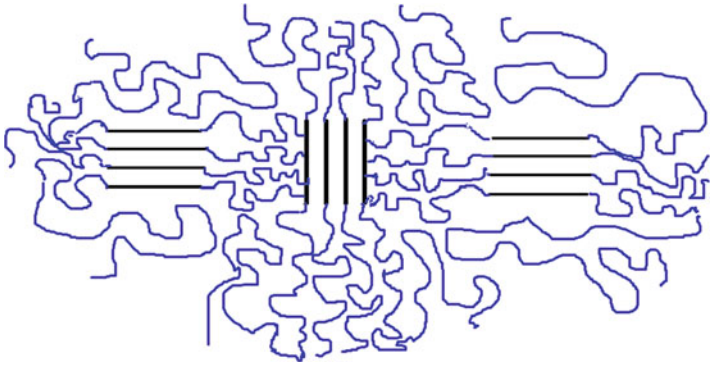


Fig. 6.4 An illustration of polymeric structure distribution, with crystalline regions (crystallites) in black—straight lines—and amorphous regions in blue—tortuous lines. The structure is stabilized by means of interactions as Wan der Waals forces

6.4 Main Related Analytical Techniques

This chapter is dedicated to advanced analytical techniques for polymer analysis in order to obtain more sophisticated information, and doesn't contain simple analytical techniques which involve the absence of specialized knowledge in analytical chemistry—e.g., fatigue, tensile, impact, hardness, and viscosity tests related to rheological analysis.

The techniques introduced here should be applied to quality control of products (mainly) and processes, besides research and development in several scales, i.e., from laboratory to industrial plants.

The families of techniques comprise electron microscopies, spectroscopies, chromatography, and sorption.

6.4.1 Electron Microscopies

Despite not providing quantitative information, fleeing the conceptual classification of instrumental techniques, and being little used in hyphenation, electron microscopic techniques end up having a strong similarity with instrumental techniques due

Table 6.3 Commercial additives for polymers used in the industry

Additive class	Examples of additives
Reinforcing fibers or fibrous reinforcement	Glass, carbon, graphite, aramid
Inert fillers	Starch, calcium carbonate, magnesium oxides, aluminum phosphates
Reinforcing or reinforcing fillers	Precipitated calcium carbonate, fumed silica, talc, carbon black, kaolin
Plasticizers	Di(2-ethylhexyl) phthalate (DEHP), di(isononyl phthalate) (DNIP)
Lubricants	Stearic acid, butyl stearate, oleamide, formamide
Pigments	Metal oxides and sulfides, carbon black
Colorants	Chromium oxide, cobalt aluminate, iron oxide, phthalocyanines, tetrachloroisoindolinones, benzimidazolones
Thermal stabilizers	Organophosphites
Antioxidants	Phenols (sterically hindered), secondary aromatic amines, alkyl radical scavengers
Antiozonants	Hydrocarbon waxes, *p*-phenylenediamine derivatives
Ultraviolet absorbers	Lignin derivatives, benzophenone, benzotriazide, hydroxyphenylatrazine
Flame retardants	Halogenated compounds, organophosphorous compounds, melamines, metal hydroxides
Blowing agents	Azodicarbonamide, *p,p'*-oxybisbenzenesulfonylhydrazide
Antistatic agents	Imidazolium, pyridinium, piperidinium, and morpholizium salts
Anti-fungals	Azoles derivatives
Impact modifiers	Acrylics, styrenics, copolymers

to their technological apparatus used in the examination of modifications occurring on the surfaces of materials, such as natural and synthetic polymers.

For the analysis of polymers, three microscopic techniques are commonly used: scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM). The first two provide detailed information about the size and distribution of components, while the latter detects the presence of large molecular agglomerates and the polymeric structure in atomic level. The main characteristics of the three techniques are described as follows.

Scanning electron microscopy (SEM)—is used for surface imaging; its principle is based on the scanning of the surface of the sample by a narrow beam of 10 nm of primary electrons with energy in the order of 10 keV, which leads to the construction of the image; this is probably the most widely used microscopic technique for examining polymers and materials in general. A relevant SEM characteristic is that it can be coupled to an EDS equipment (to be seen ahead) to produce quantitative information about the elemental constitution of the material surface.

Transmission electron microscopy (TEM)—is used to determine the volume of the structure of materials; its principle is based on the transmission of high energy electrons through an ultrathin section of the sample, with the image formed after the

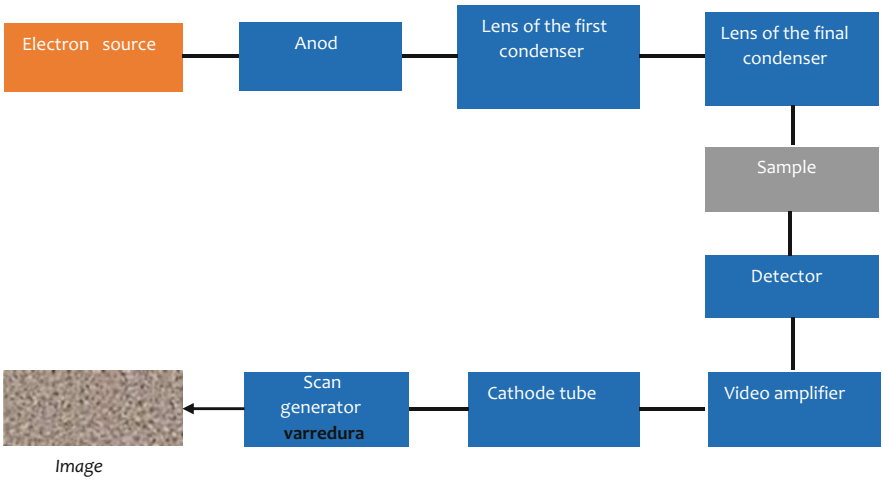


Fig. 6.5 Block diagram for a scanning electron microscope

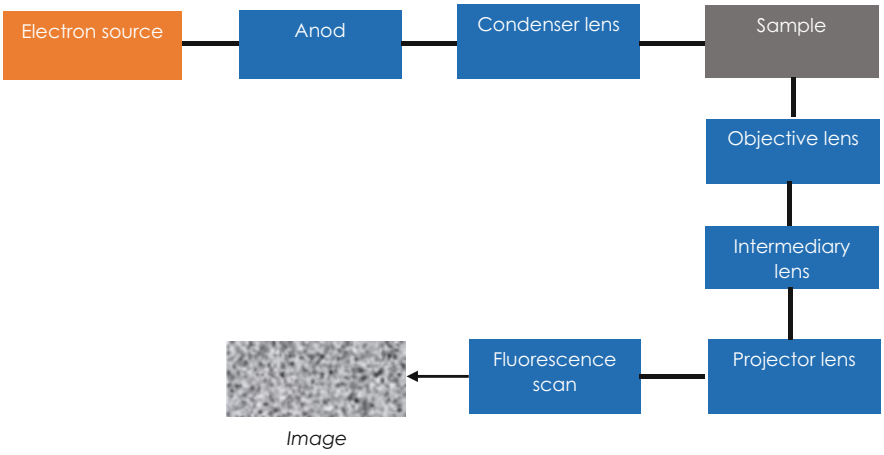


Fig. 6.6 Block diagram for a transmission electron microscope

electron hits a photographic film kept below the sample, which produces the electron scattering.

Atomic force microscopy (AFM)—is used for topographic imaging, being able to resolve structures in the directions of the *x*, *y*, and *z* axes, and providing images in atomic resolution; its principle is based on the incidence of radiation on the sample, whose source is a LASER (light amplification by stimulated emission of radiation), considering the forces involved in the interaction at the atomic level.

Figures 6.5, 6.6, and 6.7 show the block diagrams of the three types of microscopes based on the techniques presented here (SEM, TEM, and AFM).

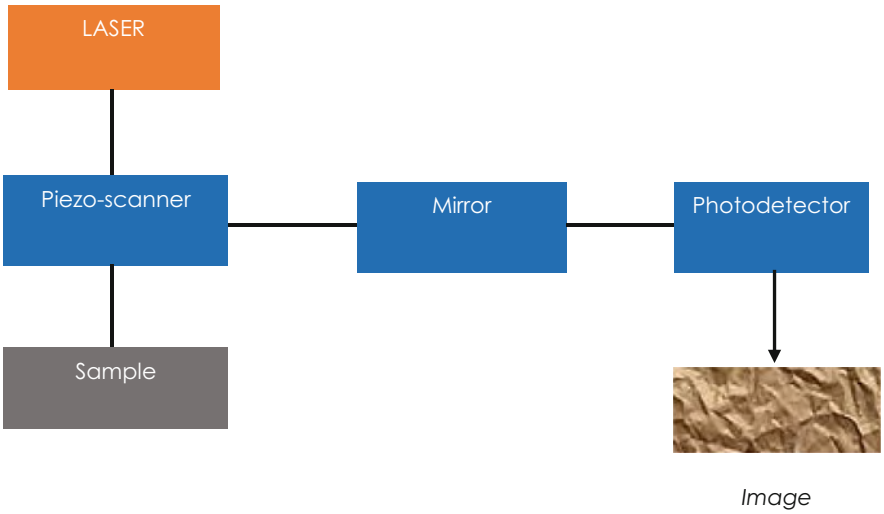


Fig. 6.7 Block diagram for an atomic force microscope. *LASER* light amplification by stimulated emission of radiation

Figures 6.8, 6.9, and 6.10 depict commercial equipment for SEM, TEM, and AFM.

6.4.2 Energy Dispersive X-ray Spectroscopy

Energy dispersive X-ray spectroscopy (EDX) or just energy dispersive spectroscopy (EDS)—the most common abbreviation—coupled with scanning electron microscopy (SEM, previously seen) allows the amount of the main elements to be quantified (from 0.5 wt%) with atomic number > 5 (Piccardo et al. 2013), using the X-ray emission after a beam radiation incidence that has a characteristic spectrum based on its atom of origin.

This analytical technique enables the chemical characterization and the elemental analysis of materials, as synthetic and natural polymers, by means of the following (Thermo Fisher Scientific 2023):

- EDS elemental mapping—supplies compositional information at the atomic level and can be obtained with the addition of an EDS detector to an electron microscope (i.e., an SEM instrument).
- EDS materials analysis: sensitive to low concentrations (LOD <0.1), high degree of relative precision (typically 2–4%), non-destructive (in most situations), minimal sample preparation, and delivers complete analyses of complex samples quickly (<1 min).

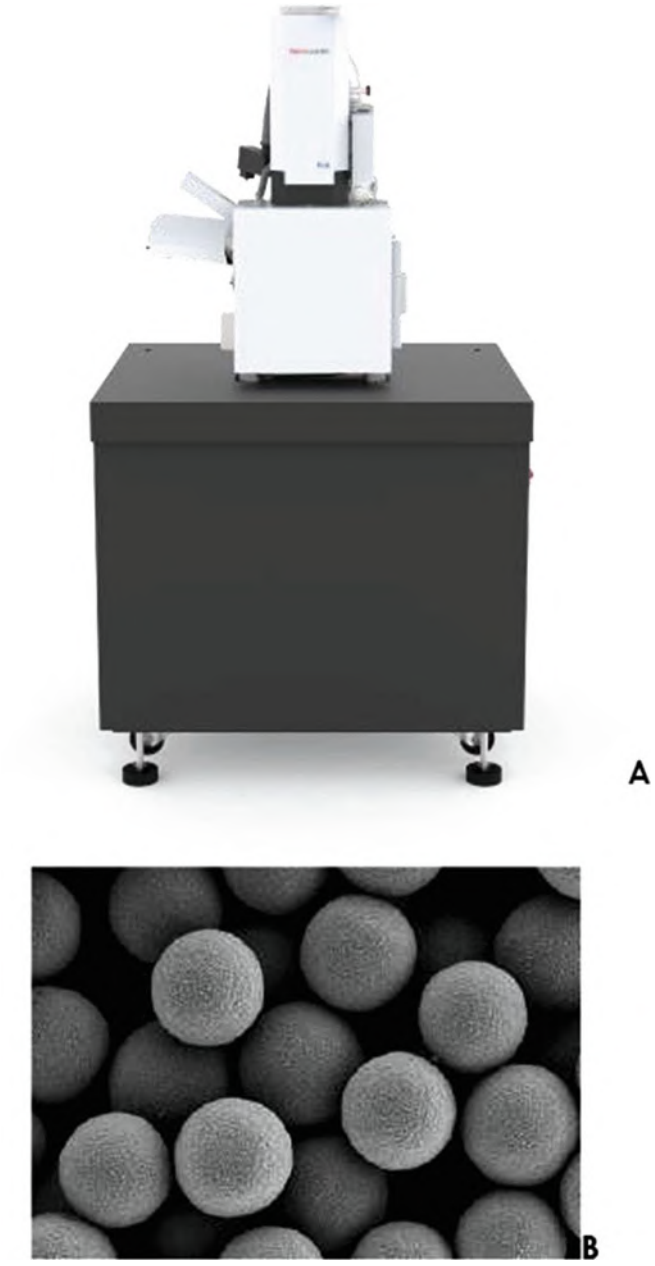


Fig. 6.8 A scanning electron microscope (**a**), and a low keV image of polymer beads with metallic nanoparticles (**b**) generated by the instrument—the secondary electron image shows the morphology of the polymer beads. Courtesy of Thermo Fisher Scientific

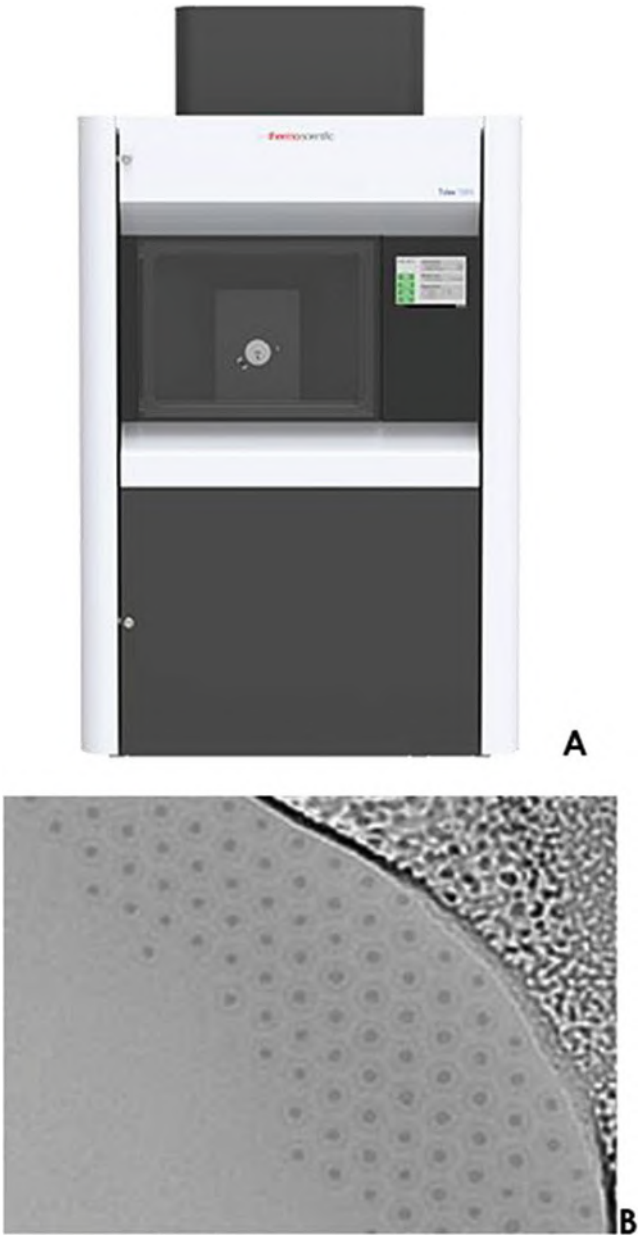


Fig. 6.9 A transmission electron microscope (**a**), and a TEM low-dose imaging of block copolymer under cryogenic condition (**b**) generated by the instrument—the copolymer micelle is in water, with sphere-sphere packing assembled from blends of PAA-PI-PS and PAA-PS. *PAA* poly(acrylic acid), *PI* polyimide, *PS* polystyrene. Courtesy of Thermo Fisher Scientific

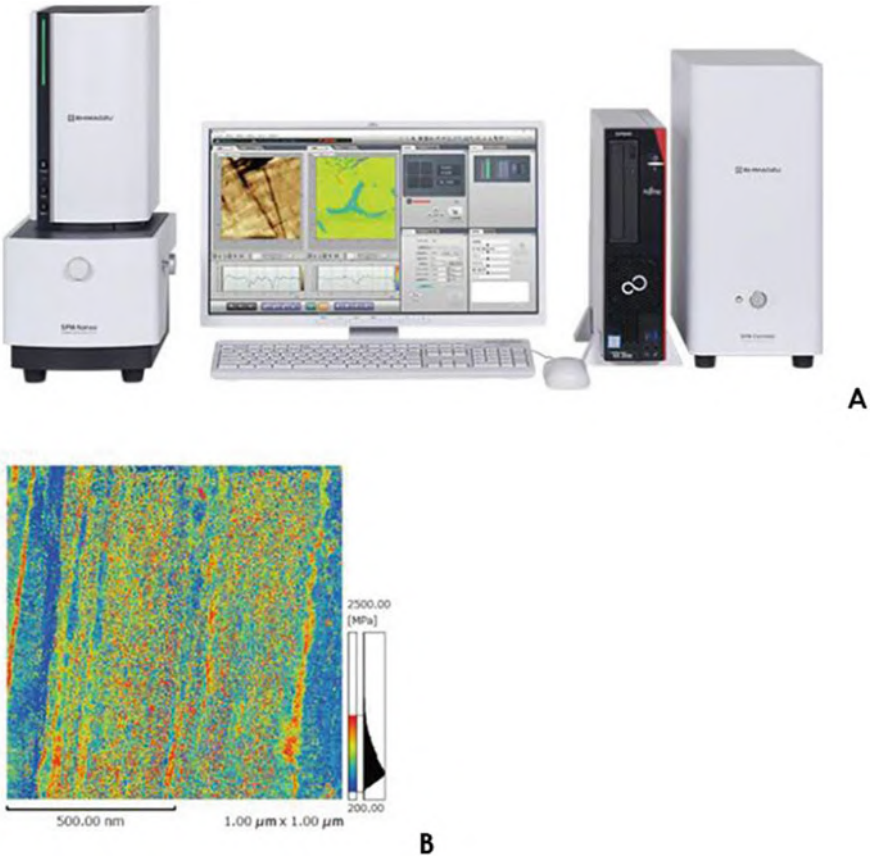


Fig. 6.10 An atomic force microscope (**a**), and a mapping elastic modulus of HDPE (**b**) generated by the instrument. *HDPE* high density polyethylene. Courtesy of Shimadzu

However, and according to experimental observations, the elemental analysis is limited to the surface of sample that means this is not applicable for the sample "core"—what is a common limitation of non-destructive spectroscopic techniques.

Figure 6.11 depicts a block diagram for an EDS instrument, while Fig. 6.12 depicts the instrument.

6.4.3 Gel Permeation Chromatography

The introduction to chromatographic techniques—especially chromatography in liquid phase—can be seen in detail in Chap. 4.

Gel permeation chromatography (GPC) or size exclusion chromatography (SEC), a branch of liquid phase chromatography, with evaporative light scattering detector

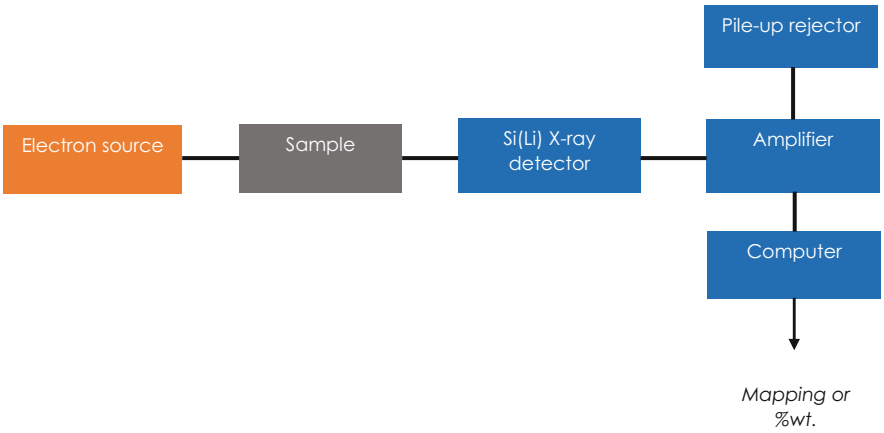


Fig. 6.11 Block diagram for an EDS detector

(ELSD), photoarray detector (PAD), or refractive index detector (RID) is used to observe the size distribution of the macromolecule in order to determine this physicochemical property.

Regarding the separation mechanism, the separation of solutes (analytes) depends upon their ability to enter into the pores of the stationary phase (column). Smaller solutes spend proportionally more time within the pores and take longer to elute from the column, with this behavior influencing on their retention time.

Light scattering detector is particularly suited to higher molecular weight polymers and combinations of polymer and solvent. It is most often applied to the analysis of homopolymers, e.g., polyvinyl chloride (PVC) and polymethacrylates; however, it can be applied to not well-defined macromolecule structure as Kraft lignin. The largest benefit of light scattering is that it provides molecular weights independent of a column constitution and the method calibration, and so obtaining data for analysis can be quick compared to other techniques (Agilent Technologies 2023). And, generally, a calibration curve of the analytical method is constructed with a high-purity reference material, as polystyrene (PS).

Three analytical parameters can be obtained by GPC-ELSD or SEC-ELSD:

- M_n: average molecular mass, for molecular weight distribution.
- M_w: molecular mass, also for molecular weight distribution.
- Dispersity, to describe the degree of non-uniformity of a molecular weight distribution.

These parameters are useful to characterize, for instance, branched polymers (e.g., polyolefins and polyacrylates) and polysaccharides (e.g., starch) (Gaborieau and Castignolles 2011).

Values for M_n and M_w for several polymers (homopolymers and copolymers) can be accessed in reference books as *CRC Handbook of Chemistry and Physics* (Rumble 2022).

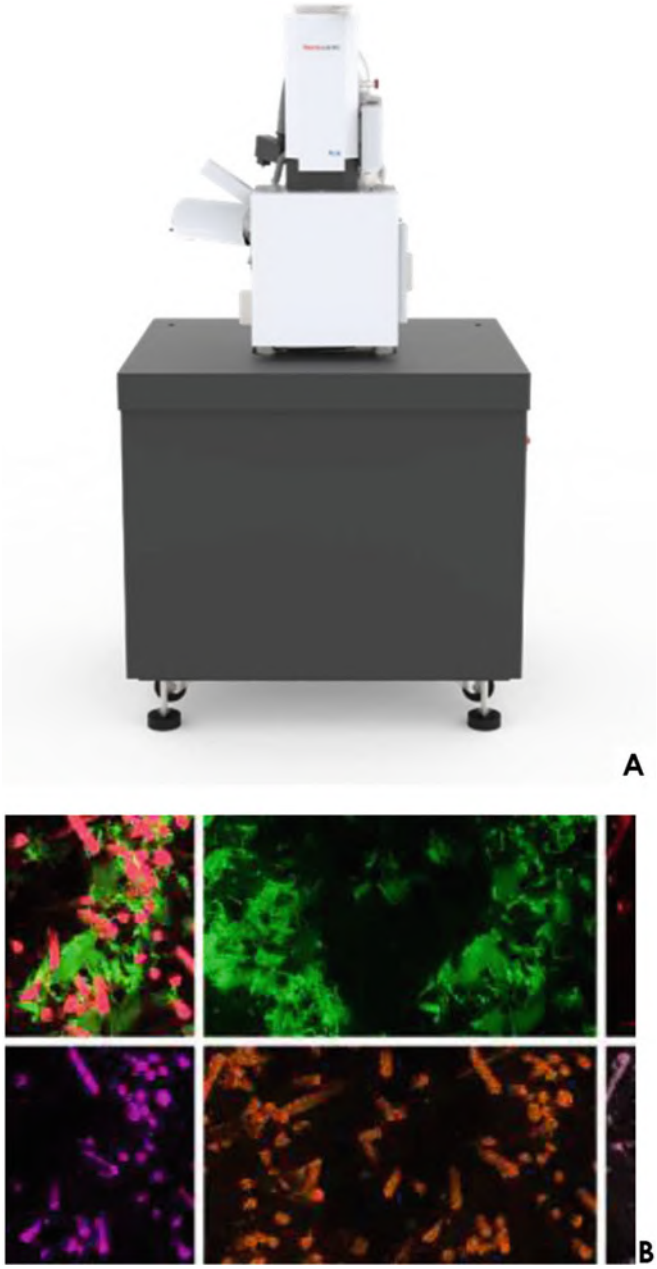


Fig. 6.12 A scanning electron microscope with a coupled EDS detector (**a**), and an EDS mapping of an uncoated glass fiber reinforced polymer (GFRP) fracture surface, obtained in a low vacuum mode (**b**). Elemental mapping shows that the matrix is carbon rich, and the glass fibers are a mixture of silicon, aluminum, and calcium oxides. Courtesy of Thermo Fisher Scientific

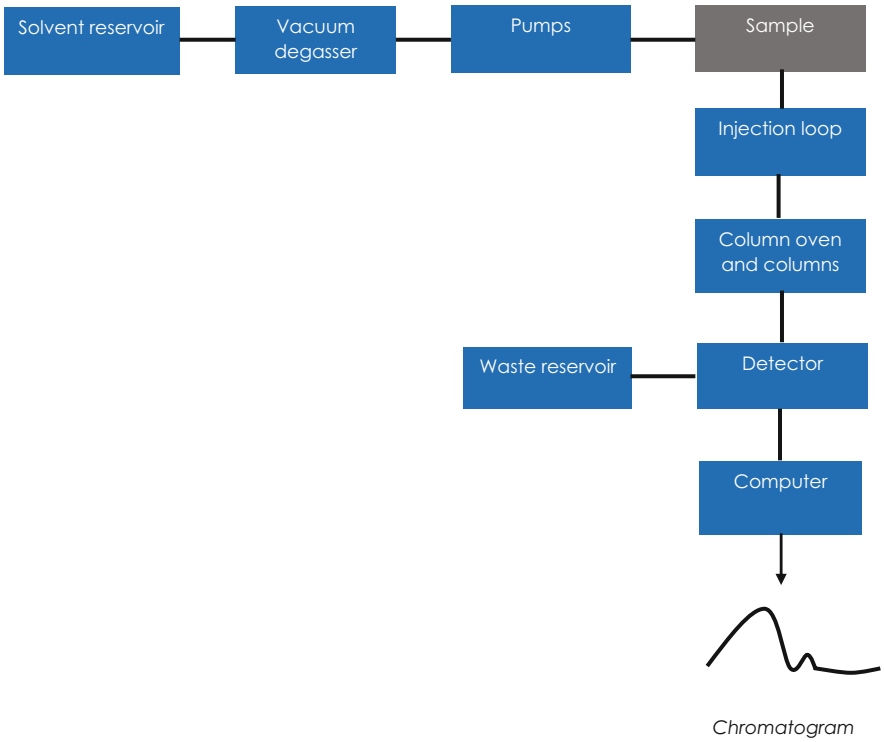


Fig. 6.13 Block diagram for an SEC-ELSD system

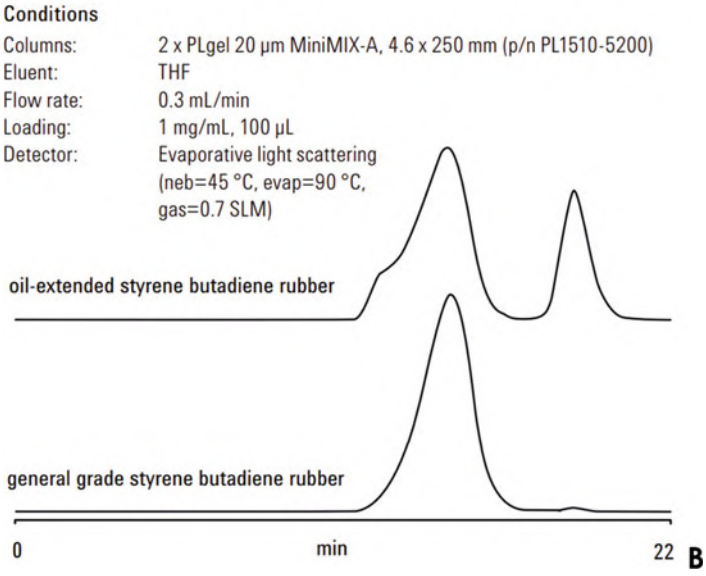
Figure 6.13 depicts a block diagram for an SEC-ELSD instrument and Fig. 6.14 depicts the instrument.

6.4.4 Nuclear Magnetic Resonance

In nuclear magnetic resonance (NMR) it is the nuclei of atoms that absorb radiation. The absorption of radiation by the nuclei occurs when they are subjected to an external magnetic field produced by low energy waves (radiofrequency; from 10^{-3} to 10^{1} m).

In some cases, the nuclear charge can rotate around the nuclear axis, generating a magnetic dipole. The angular momentum of the moving load can be described in terms of the spin I moment (m); the most explored nuclei in NMR for polymers are the ^{1}H and ^{13}C nuclei that have I equal to ½; ^{31}P can be explored in some cases (e.g., for lignin). The absorption of radio frequency by these nuclei is characteristic and influenced by neighboring nuclei. This allows the molecular structure of a series of chemical compounds to be determined as a function of the chemical shift (δ) produced according to the electronic density of the atoms present in the molecule.

A



B

Fig. 6.14 A SEC equipment (left) with a multi-detector device (right) (**a**), and a chromatogram obtained for polymers using an ELSD detector (**b**). Courtesy of Agilent Technologies

In the presence of an applied magnetic field, the nuclei are either aligned with the magnetic field with spins of $m = +1/2$ or aligned against the magnetic field with spins of $m = -1/2$. The energies in these two spin states, E_{lower} and E_{upper}, are given by the Eqs. (6.1) and (6.2):

$$E_{\text{lower}} = -\gamma h 4\pi B_0 \tag{6.1}$$

$$E_{\text{upper}} = +\gamma h 4\pi B_0 \tag{6.2}$$

Where γ is the magnetogyric ratio for the nucleus, ⍰ is Planck's constant, and B_0 the strength of the applied magnetic field. The difference in energy, ΔE, between the two states is given by the Eq. (6.3):

$$\Delta E = E_{\text{upper}} - E_{\text{lower}} = +\gamma h 4\pi B_0 - (-\gamma h 4\pi B_0) = \gamma h 2\pi B_0 \tag{6.3}$$

From the definition of energy (Eq. 6.4) and combining it with the Eq. (6.3), we can obtain Eq. (6.5) for the electromagnetic need to effect a change in spin state.

$$E = h\,\nu \tag{6.4}$$

Where: E = energy (J); h = Planck's constant (6.626×10^{-34} J s); ν = frequency (Hz).

$$\nu = \gamma B_0 2\pi \tag{6.5}$$

And this is called the Larmor frequency for the nucleus, where ν is the frequency.

Figure 6.15 depicts the block diagram of an NMR instrument, and Fig. 6.16, the instrument.

In a general way, the following assignments of groups as a function of chemical shift can be made for the ^{13}C-NMR spectrum:

- 0–45 ppm: unsubstituted aliphatic C, as in alkanes and fatty acids, due to methyl-terminal groups
- 45–65 ppm: C associated with N-alkyl, as in amino acids, peptides and proteins, and C methoxy
- 60–110 ppm: C associated with aliphatic O
- 110–140 ppm: unsubstituted and alkyl substituted aromatic C
- 110–160 ppm: total aromatic C related to unsubstituted, alkyl substituted and phenolic group
- 140–160 ppm: C phenolic
- 160–185 ppm: C in carboxylate
- 185–230 ppm: ketone C in esters and amides

For ^{1}H-NMR spectrum:

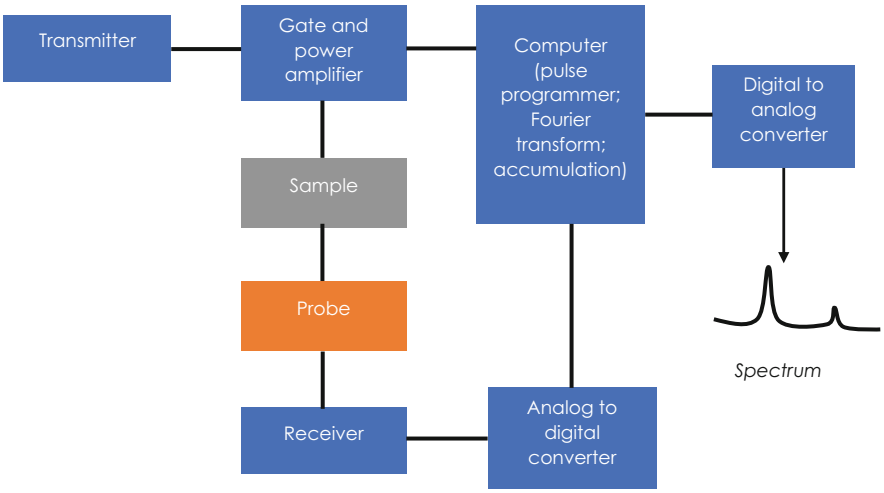


Fig. 6.15 Block diagram for a pulsed Fourier transform-NMR instrument

- 10–9 ppm: H in aldehyde
- 9–6 ppm: H in aromatic and heteroaromatic
- 7.5–4.5 ppm: H in alkene
- 7–2 ppm: H in α-disubstituted aliphatic
- 1.5 ppm: H in α-monosubstituted aliphatic
- 3–1.5 ppm: H in alkyne
- 1.5–0.5 ppm: H in β-substituted aliphatic
- 2–0 ppm: H in aliphatic alicyclic

The related assignments for ^{1}H and ^{13}C shifts were dedicated to one-dimension (1D) spectra, which are related to the frequency absorbed by the analyte's nuclei expressed in *ppm*. These spectra were acquired by applying a brief radiofrequency (RF) pulse to the sample, recording the resulting free induction decay (FID), and then using a Fourier transform to obtain the NMR spectrum. In addition to 1D experiments, there are a host of 2D experiments in which we apply a sequence of two or more pulses, recording the resulting FID after applying the last pulse. Table 6.4 describes the most common methods for 2D-NMR; the main advantage of 2D against 1D is that the first can distinguish between the overlapping signals that exist in larger molecules, which is not possible by means of 1D-NMR.

6.4.5 Surface Area and Pore Volume Analysis

Technique based on the Brunauer-Emmett-Teller theory of multilayer gas adsorption (Brunauer et al. 1938), and known as BET. The polymer sample is observed after a

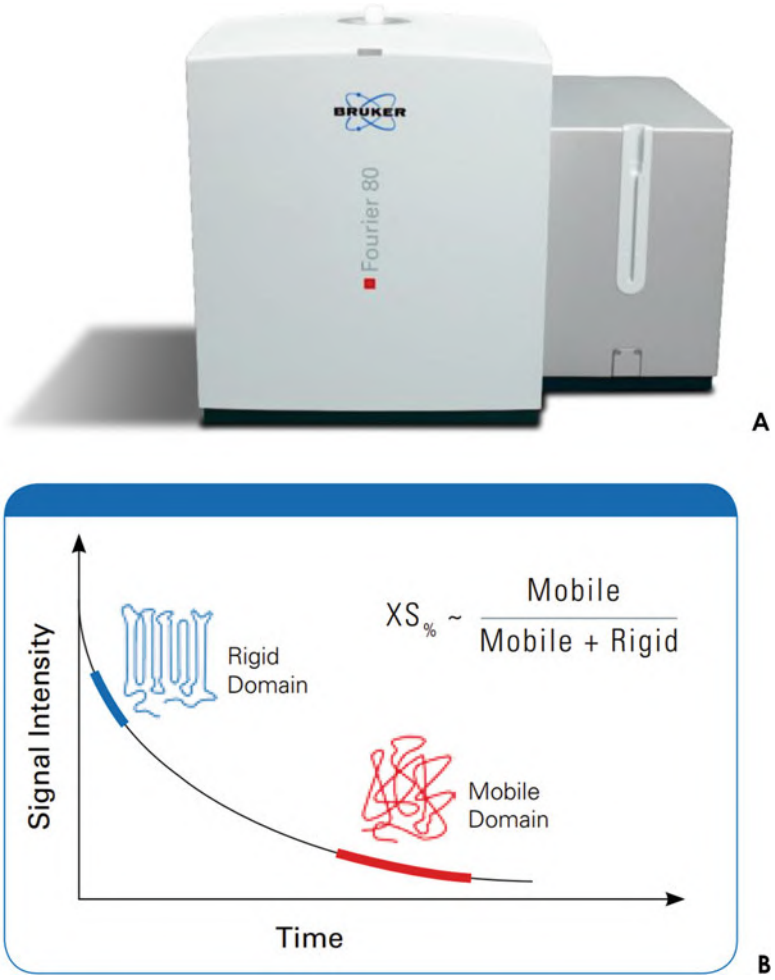


Fig. 6.16 A compact 80 MHz NMR spectrometer (**a**), and an NMR analysis for quality control of a polymer (**b**). Courtesy of Brüker

previous temperature treatment of particles to eliminate contaminations (e.g., CO_2) acquired by exposure to the ambient atmosphere.

After this treatment, the sample is cooled with liquid ingestion under vacuum until the equilibrium temperature (−200 °C). The adsorbate (high purity nitrogen gas) is inserted in a test chamber in a controlled manner, with the adsorption being measured until the equilibrium pressure was reached. From this equilibrium pressure it is possible to calculate the amount of adsorbent adsorbed on the surface of the sample and, therefore, the surface area of the latter.

These measures produce *isotherms* of volume adsorbed versus pressure of saturation, and from them we can apply the BET equation (Eq. 6.6):

Table 6.4 Methods for 2D-NMR. Source: Adapted from Harvey (2022). Reproduced with permission from the Author

Method	Information obtained from cross peaks
Correlation spectroscopy (COSY)	Coupling between two protons (^{1}H–^{1}H) that are within three chemical bonds of each other
Total correlation spectroscopy (TOCSY)	Coupling between all protons (^{1}H) in the molecule
Heteronuclear correlation spectroscopy (HETCOR)	Coupling between a proton (^{1}H) and another nucleus, such as carbon (^{1}H–^{13}C) or nitrogen (^{1}H–^{15}N)
Nuclear Overhauser and exchange spectroscopy (NOSEY)	Coupling between two protons (^{1}H–^{1}H) that are within approximately 5 Å of each other
Heteronuclear single quantum correlation (HSQC)	Coupling between a proton (^{1}H) and another nucleus, such as carbon (^{1}H–^{13}C) or nitrogen (^{1}H–^{15}N)
Heteronuclear multiple bond coherence spectroscopy (HMBC)	Coupling between a proton and a carbon (^{1}H–^{13}C) that are two or three bonds apart
Incredible natural abundance double-quantum transfer (INADEQUATE)	Coupling between adjacent carbon atoms (^{13}C–^{13}C)
Double quantum filtered correlation spectroscopy (DQF–COSY)	Suppresses signals from water

$$1/X[(P_o/P) - 1] = 1/X_m C + C - 1/X_m C(P/P_o) \tag{6.6}$$

Where X is the weight of nitrogen adsorbed at a given relative pressure (P/P_o), X_m is monolayer capacity, which is the volume of gas adsorbed at standard temperature and pressure (STP), and C is constant. STP is defined as 273 K and 1 atm.

We can also verify the gas desorption by the surface, which is valuable for polymers applied to controlled release of active molecules (e.g., agrochemicals).

Surface area and pore volume (also determined by BET) are of interest in many industries and processes that involve surfaces interacting with gas or liquids.

The rate or volume of gas adsorption and the capacity of a material to adsorb gases can have a large effect on its functional usefulness. Investigating those factors can be extremely important during research and development step for a product, or later troubleshooting and failure analysis.

Figure 6.17 depicts a block diagram of a BET instrument and Fig. 6.18, the instrument.

6.4.6 Thermal Analysis

Thermal analysis uses thermochemical processes, such as combustion and pyrolysis, to determine the percentage of mass loss or compound formation and the atomic percentage, in addition to allowing the observation of thermodynamic properties of materials. Such a class of analytical techniques is fundamentally used for the thermal characterization of organic and inorganic compounds and various materials,

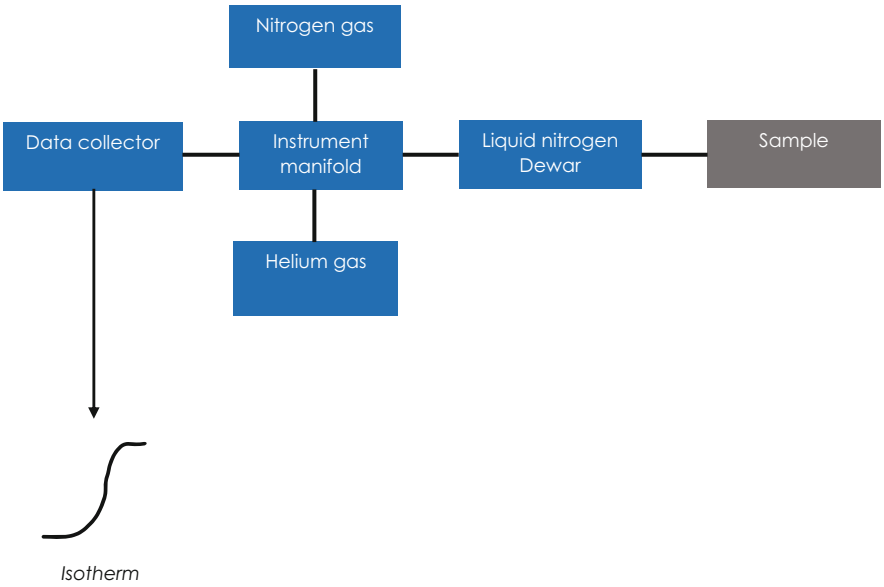


Fig. 6.17 Block diagram for a BET instrument

although it also provides quantitative percentage information. One can cite the great utility of thermogravimetric analysis and elemental analysis for the compositional analysis and for the property characterization.

Thermogravimetric analysis (TGA) provides data on mass loss as a function of temperature, which is especially interesting when one wants to observe the thermal behavior of a material during processing steps. The differential scanning calorimetry (DSC) provides thermodynamic data, of great help for its thermal processing; by measuring the energy exchanges in predetermined heating cycles, the endothermic and exothermic behavior of the sample is obtained, in addition to calculating the specific heat (Cp). The differential thermal analysis (DTA) is based on the difference in temperature between the sample and a reference material is monitored against time and temperature while the temperature of the sample, in a specific atmosphere, is programmed.

It is very important in polymer characterization the determination of, by means of DSC, the temperature-dependent properties T_g and T_m:

- T_g (glass transition temperature): the approximate temperature below which glasslike behavior is apparent.
- T_m (melt transition temperature): the temperature for the crystallites melting in a polymer chain.

Table 6.5 describes several values of T_g and T_m for common polymers.

Figure 6.19 depicts a block diagram of a thermogravimetric analyzer, and Fig. 6.20, the instrument.

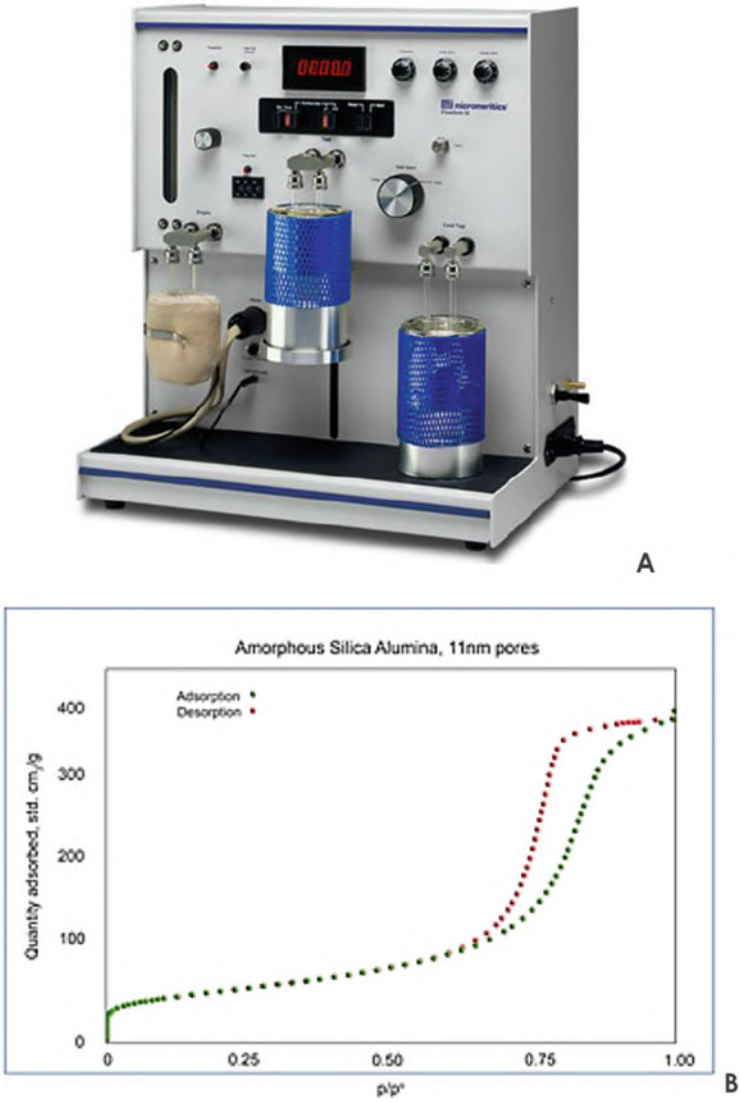


Fig. 6.18 A BET instrument (**a**), and the obtained adsorption and desorption curves (**b**). Courtesy of Micromeritics

6.4.7 X-ray Scattering

According to Mitchell (1989), X-ray scattering (XRS) is a powerful tool in the identification of the type of liquid crystal phase present for an unknown sample (e.g., a polymer). Relatively simple observation and measurement procedures allow a firm classification of *nematic* (one-dimensional orientational order of the molecules by

Table 6.5 T_m and T_g values for some common addition polymers. Source: Modified from LibreTexts Chemistry (2023). Reproduced with permission from LibreTexts Chemistry

Polymer	T_m (°C)	T_g (°C)
Low density polyethylene (LDPE)	110	−110
High density polyethylene (HDPE)	130	−100
Polypropylene (PP)	175	−10
Polyvinyl chloride (PVC)	180	80
Polystyrene (PS)	175	90
Polyacrylonitrile (PAN)	>200	95
Polytetrafluoroethylene (PTFE)	330	−110
Polymethylmethacrylate (PMMA)	180	105
Rubber	30	−70

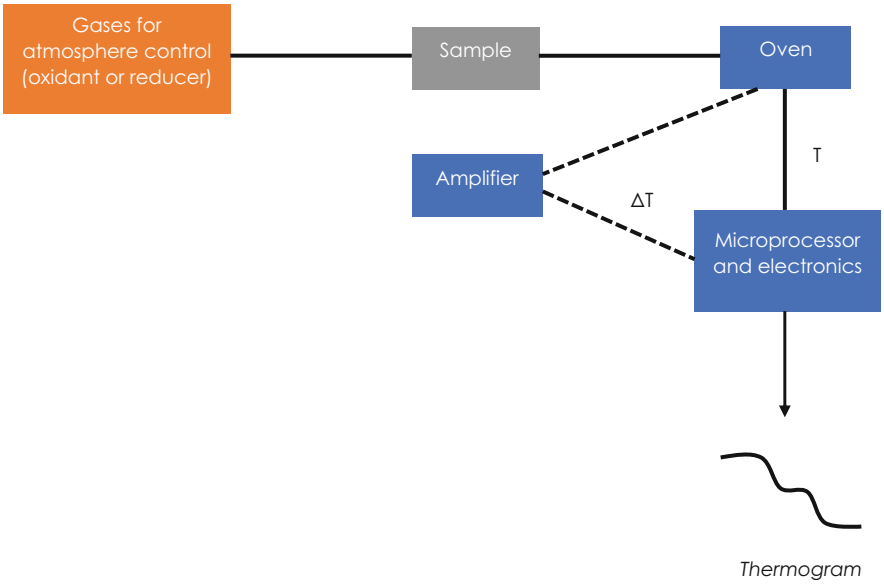


Fig. 6.19 Block diagram of a thermogravimetric analyzer instrument

virtue of correlations of the long molecular axes, although the orientational order is not polar), *smectic* (the long axes of the molecules are oriented on average in the same direction), or *cholesteric* (continuous change in the direction of the long axes of the molecules in adjacent layers within the sample) *mesophases* to be made.

In the last years was developed equipment using synchrotron as the source of X-rays improving the technique application for polymer science and technology, as the case of the small-angle X-ray scattering (SAXS) mode. Furthermore, it can be used on-line with the polymer production equipment to supply real-time analysis, e.g., in the observation of polymer crystallization process (Chu and Hsiao 2001), as preconized by process analytical chemistry/process analytical technology (PAC/PAT) (seen in Chap. 4).

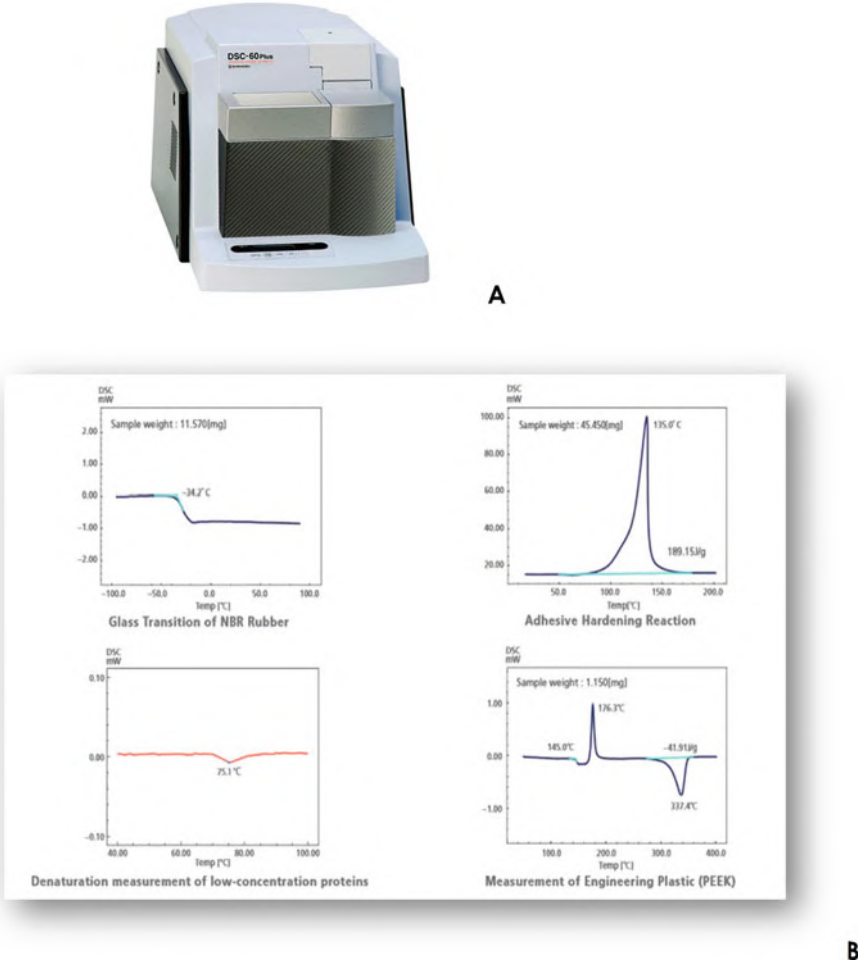


Fig. 6.20 A DSC instrument (**a**), and the obtained thermograms (**b**). Courtesy of Shimadzu

Figure 6.21 depicts the block diagram of an SAXS instrument and Fig. 6.22 depicts the instrument.

6.5 Commented Examples of Applications

It is important to consider aspects of sample preparation. The microscopic techniques (SEM, TEM, AFM) require more complex preparation involving—in certain cases—a film preparation by means of the sample cutdown. The chromatographic technique (GPC) demand sample dilution in organic solvents or aqueous solution.

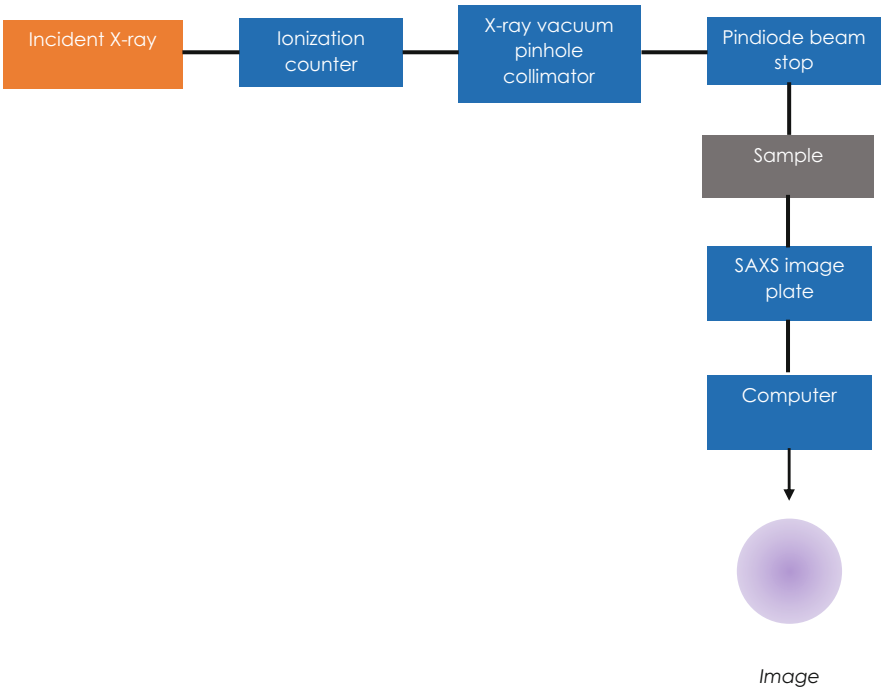


Fig. 6.21 Block diagram for an SAXS instrument

The spectroscopic techniques (EDS, NMR, XRS) can involve simple handling, except for NMR. The thermal techniques (TGA, DTA, DSC) and the sorption technique (BET) are relatively easy to handle.

Table 6.6 describes several examples of application for each technique discussed in this chapter.

The detailed analytical method for each example listed in Table 6.6 can be accessed in the respective cited reference.

Additionally, some examples of ASTM International (2023) standard methods for polymers and plastics can be highlighted:

- ASTM D3418-21: to determine transition temperatures and enthalpies of fusion and crystallization of polymers by DSC, for granular and other shapes of processed polymers; this method works from the cryogenic region to 600 °C.
- ASTM D5296-19: to determine molecular weight averages and molecular weight distribution of polystyrene by SEC; this method works for elution range defined by polystyrene standards with molecular weights from 2000 to 2,000,000 g mol^{-1}.

Again, each detailed method can be accessed on the ASTM International standard methods catalog.

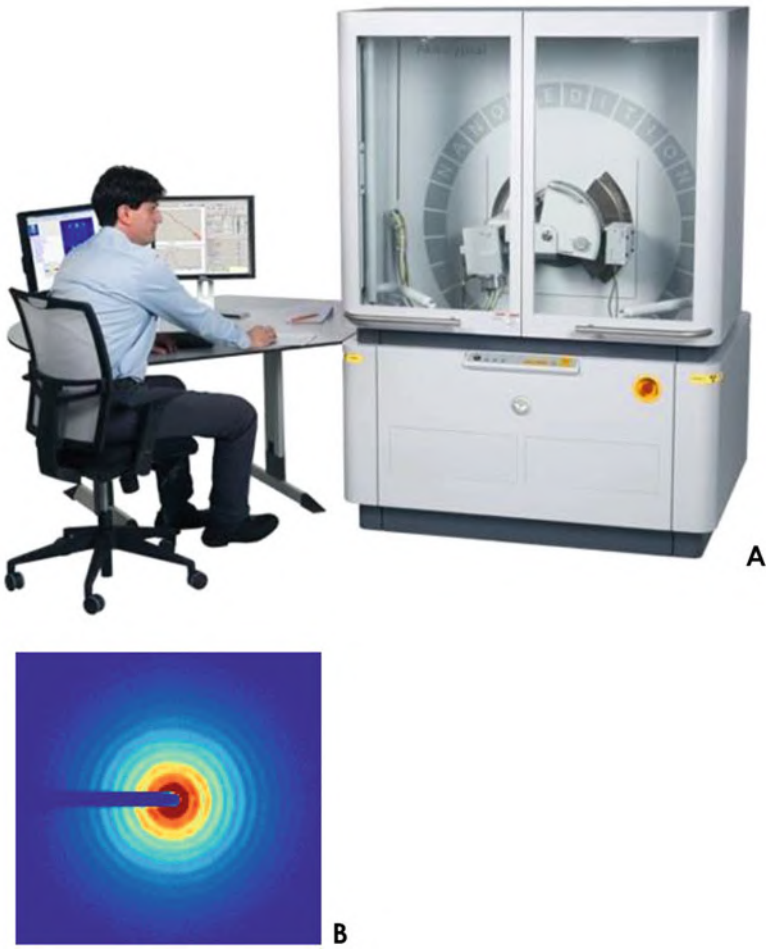


Fig. 6.22 An SAXS instrument based-on an X-ray diffraction platform (**a**), and a 2D image of a colloidal crystal generated by the instrument (**b**). Courtesy of Malvern Panalytical

6.6 Conclusions

Polymers are one of the most important family of industrial products for the modern society with the production of millions of tons each year. Certainly the modern way of life would not be possible without the polymeric materials—of course this huge production can promote negative impacts on the environment and health.

The chemical and physical analyses of synthetic and natural polymers have a considerable arsenal of advanced analytical techniques available, comprising microscopic (SEM, TEM, AFM), spectroscopic (EDS, NMR, SAXS), chromatographic (GPC), thermal (TGA, DTA, DSC), and sorption/desorption (BET) techniques. And

Table 6.6 Application examples of advanced analytical techniques for polymers

Analytical technique	Application	Brief method description	Reference
SEM	Determination of structural factors of PAN and PES polymeric filtration membranes	3D FIB/SEM measurements using 2 KeV and 200 pA for energy and current, respectively	Roberge et al. (2022)
TEM	Analysis of metal-polymer interfaces for special applications	TEM analysis performed on the cross-sections with image side Cs-corrected and acceleration voltage of 200 kV	Putz et al. (2017)
AFM	Characterization of polymer viscoelastic surfaces and nanoscale physical properties (e.g., adhesion)	Experimental data obtained by means of the dynamic atomic force mode	Rajabifar et al. (2018)
EDS	Construction of elemental mapping to verify the presence of salts on modified polymeric films of PE/carbon black	The distribution of Fe, Cu, Mn, S, and O was analyzed through elemental mappings in several parts of films surface	Linares et al. (2021)
GPC	Molecular characterization of styrene-butadiene-styrene block copolymers	Use of tetrahydrofuran (THF) solvent and a calibration curve based on mono-dispersed polystyrene standards	Canto et al. (2006)
NMR	Molecular characterization of styrene-butadiene-styrene block copolymers	Solid state ^{13}C-NMR	Canto et al. (2006)
BET	Determination of surface area and pore volume of polymer on surface modified carbon nanotubes	Nitrogen sorption/desorption isotherms measured at 77 K	Khan et al. (2016)
TGA	Determination of char residues in lignin-based polyamide blend as carbon fiber precursors	Samples of approximately 2 mm length with 5–10 mg analyzed under a nitrogen flowing atmosphere at 100 mL min^{-1}	Muthuraj et al. (2020)
DSC	Observation of melting and crystallization behaviors of lignin-based polyamide blend as carbon fiber precursors	Samples of approximately 10 mg and approximately 2 mm of length under a flowing nitrogen atmosphere (50 mL min^{-1}) using heating cycle variations	Muthuraj et al. (2020)
DTA	Study of the thermal behavior of a net resin of polymer matrix reinforced by glass fiber	Heating rate of 10 °C min^{-1} from 25 to 600 °C, and sample weight of 10–15 mg placed in alumina pans under an inert atmosphere using nitrogen	Degnah et al. (2023)
SAXS	Investigation of microfibril interval periodicity accompanying the swelling of natural cellulose fibers	Small-angle two-dimensional scattering images obtained by high-intensity X-ray from synchrotron radiation	Okugawa et al. (2023)

PAN polyacrylonitrile, *PES* polyethersulfone, *FIB* focused ion beam, *SEM* scanning electron microscopy, *TEM* transmission electron microscopy, *AFM* atomic force microscopy, *GPC* gel permeation chromatography, *EDS* energy-dispersive spectroscopy, *NMR* nuclear magnetic resonance, *BET* Brunauer-Emmett-Teller, *TGA* thermogravimetric analysis, *DSC* differential scanning calorimetry, *DTA* differential thermal analysis, *SAXS* small-angle X-ray scattering

these large number of analytical technologies can be applied to quality control and to research and development activities.

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Chapter 7
Biotechnology

Abstract Industrial biotechnology is a multidisciplinary branch which comprises aspects of organic chemistry, analytical chemistry, biochemistry, chemical engineering, and microbiology in order to explore the biochemical routes of conversion for several classes of organic compounds. This industry comprises bioproducts (e.g., alcohols, organic acids, vitamins, antibiotics, vaccines, etc.) obtained by means of bioprocesses using microbes and enzymes. In order to guarantee the quality of these bioproducts and their bioprocess and to support the research and development in this field, this chapter discusses chromatographies (gaseous and liquid phases), spectroscopies (absorption in the UV-Vis and infrared regions, Raman, and nuclear magnetic resonance), OMICS, and process analytical chemistry/process analytical technology. These analytical techniques promote a high-quality level of the biotechnological industry.

Keywords Enzymes · Microbes · Bioproducts · Bioprocesses · Analytical technologies

7.1 Introduction

Industrial biotechnology is a multidisciplinary branch which comprises aspects of organic chemistry, analytical chemistry, biochemistry, chemical engineering, and microbiology in order to explore the biochemical routes of conversion for several classes of organic compounds.

From the search for renewable processes and products, industrial biotechnology is expected to achieve prominence when compared to other technology classes for some products, as organic acids, and alcohols.

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S. Vaz Jr, *Applications of Analytical Chemistry in Industry*,
https://doi.org/10.1007/978-3-031-38952-8_7

Table 7.1 Relevant industrial chemicals obtained by biotechnological processes

Chemical	CAS number	Biotechnological process	Common uses
Ethanol	64-17-5	Microbial fermentation by the yeast *Saccharomices cerevisae*	Biofuel, reactant, solvent, disinfection agent
Citric acid	77-92-9	Microbial fermentation by the fungus *Aspergillus niger*	Food additive (e.g., acidifier, antioxidant), pharmaceutical additive (e.g., antioxidant, pH corrector), foaming agent, phosphate substitute for detergents, hardening agent for cements
Amino acids	56-87-7 (L-lysine), 61006-04-3 (sodium L-glutamate)	Microbial fermentation by the bacteria *Corynebacterium glutamicum* and *Escherichia coli*	Animal feed (e.g., L-lysine), flavor-enhancer (e.g., glutamate)
Ascorbic acid	50-81-7	Chemical reduction followed by microbial oxidation by the bacterium *Acetobacter xylinum*	Reducing agent, antioxidant agent, food additive (antioxidant and stabilizer), nutrient supplement (vitamin C), pesticide, personal care, pet care

The biochemical routes and their derived processes—or bioprocesses—are divided into two categories:

- Enzymatic routes, using free or immobilized enzymes
- Microbial routes, using microorganisms as bacteria, fungi and yeast

Generally, the industrial application for the conversion of raw materials comprises the following:

- Fermentation technologies for the conversion step, e.g., yeasts as the microorganism
- Biocatalysis technologies for the conversion step, e.g., immobilized enzymes on polymeric supports
- Separation technologies for the downstream step, e.g., activated carbon, centrifugation, and chromatography.

The biotechnological systems work, generally, under mild conditions (i.e., pressure, temperature, and acidity/basicity) in aqueous medium, reducing costs. Moreover, these aspects can be in line with the green chemistry principles, as those related to renewable feedstock (e.g., sugars) and reducing of residues generation (e.g., waste reutilization), as preconized by the green chemistry concept (ACS Green Chemistry Institute 2023). Nevertheless, as separation technologies are necessary to reach high purity of products, it can elevate the costs.

Table 7.1 depicts some relevant chemicals obtained by biotechnological processes.

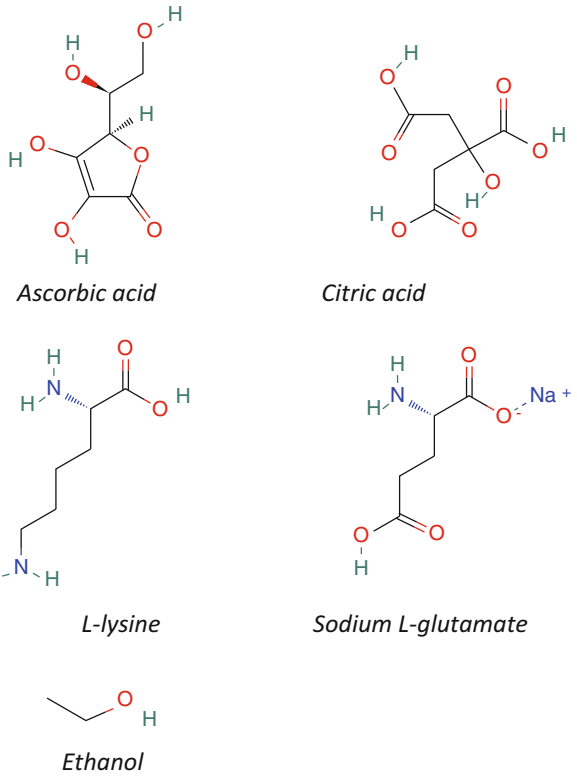

Fig. 7.1 Molecular structures of chemicals produced by biotechnological processes

For the four chemicals presented in Table 7.1, we have the following global production:

- Ethanol: 28,160 million gallons (for biofuel use) (Statista 2023)
- Citric acid: 2.39 million tons (EMR 2023)
- Amino acids: above 7 million tons (Ikeda and Takeno 2020)
- Ascorbic acid: approximately 80,000 metric tons (S&P Global 2022)

These four bioproducts are strategic chemical commodities.
Figure 7.1 depicts the molecular structures for those chemicals in Table 7.1.

7.2 How This Industry Works

As previously introduced, we can apply a microbial or enzymatic process according to interest and necessity (raw material, product, costs, purity, etc.). The biotechnology industry can comprise—but not limited to—a large variety of products for final application, highlighting:

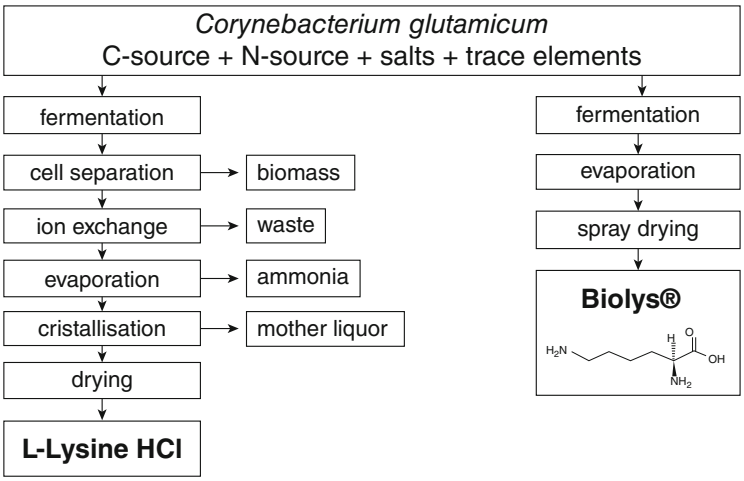

Fig. 7.2 The industrial production of amino acids by microbial fermentation. Source: Leuchtenberger et al. (2005). Reprinted with permission from Springer Nature

- Ethanol and other industrial alcohols
- Organic acids
- Vitamins
- Industrial enzymes
- Proteins and amino acids

After the conversion step—except for industrial enzymes, which are extracted and purified—we have a mixture with product(s) and by-product(s) to separate (downstream step using industrial chromatography, microfiltration, among other technologies) the components and to purify them (e.g., by crystallization) considering chemical analysis—chromatographic and/or spectroscopic—to identify and quantify the obtained compound(s), by-product(s), and residue(s). Generally, immobilized enzymes can be recycled while microorganisms cannot, except in a few cases as the yeast *Saccharomyces cerevisiae* for ethanol production.

Figures 7.2 and 7.3 depict industrial biotechnological processes—or simply bioprocesses—for amino acids and ascorbic acid production, respectively.

Other very important biotechnological products for the modern society are as follows:

- Antibiotics produced by microbial fermentation, either for use directly in human therapy (e.g., tetracyclines and macrolides) or to act as feedstock for the synthesis of chemically modified derivatives of the core antibiotic structure (e.g., sulfonamides and the fluoroquinolones), still make up the majority contribution of antibiotic agents in the treatment of human disease (Hook 2012).
- Vaccines produced from inactivated or modified (attenuated live) whole microbes, or from inactivated or recombinant parts of microbes that are responsible for disease (such as toxins or surface proteins) (Microbiology Society 2023).

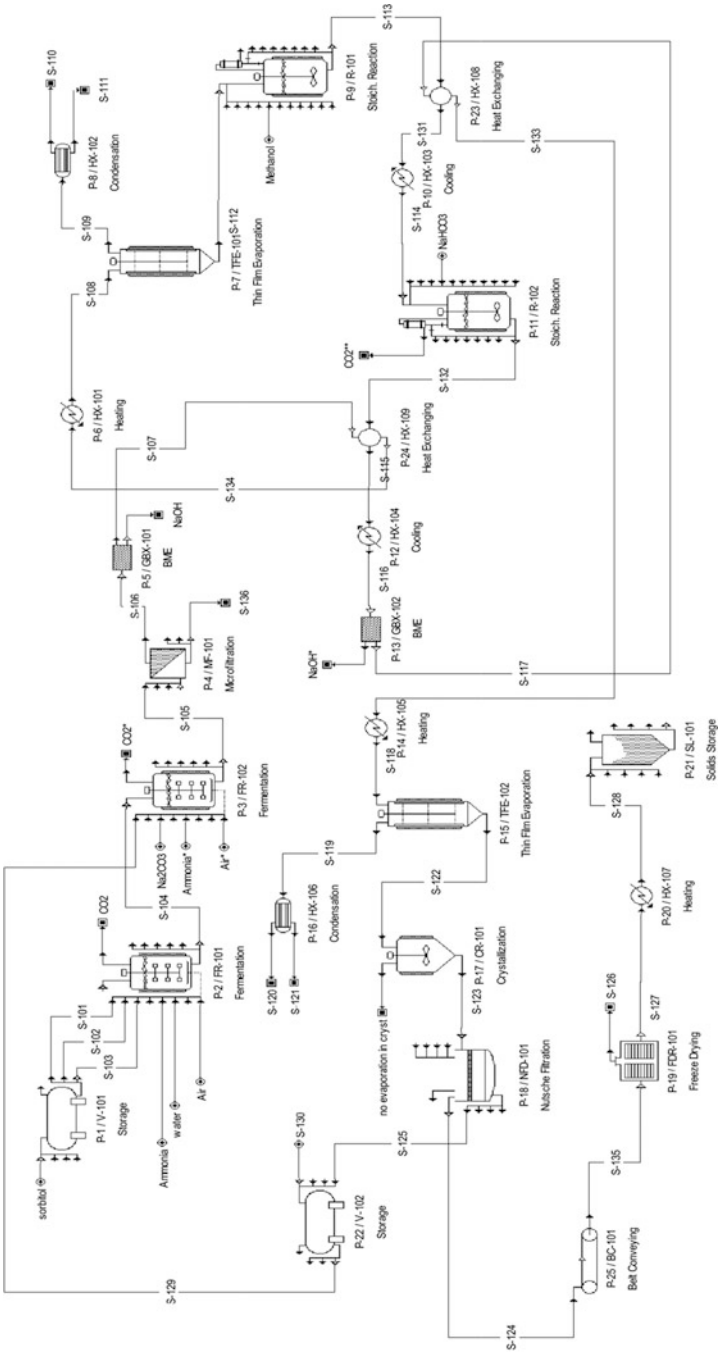

Fig. 7.3 Flowchart of a production process for ascorbic acid by fermentation. Source: Lim et al. (2020). Reproduced with permission from Springer Nature

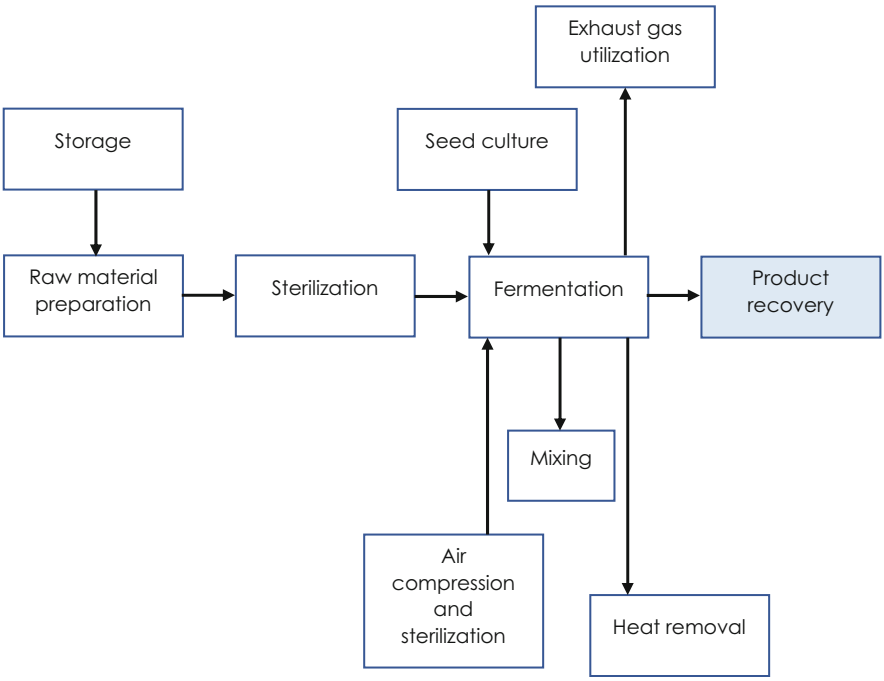

Fig. 7.4 Unit operations in an antibiotic process production by microbial fermentation by fungi (e.g., penicillin). Source: Adapted from DeTilley et al. (1983). Reprinted with permission from Taylor & Francis

Figure 7.4 depicts an industrial process of antibiotic production by fermentation. And Fig. 7.5 depicts a process for vaccine production.

7.3 Main Related Analytical Matrices

Generally, the biotechnological matrices are related to aqueous medium because microbials and enzymes suffer limitation in organic solvent medium, comprising from the activity decreasing (for enzymes) to the death (for microbials).

From this premise, the application of analytical techniques should be considering the fact that water is present when the product is not purified and well-dried. It is not a problem for liquid chromatography application but should be avoided for gas chromatography which will require a step of pre-extraction (e.g., by *headspace*) or *derivatization*, both for volatile acids. Water is also a limitation for infrared absorption spectroscopy due to the enlargement of O-H band absorption in approximately 3000 cm^{-1} that coincides with N-H.

Purified final products can be observed as follows:

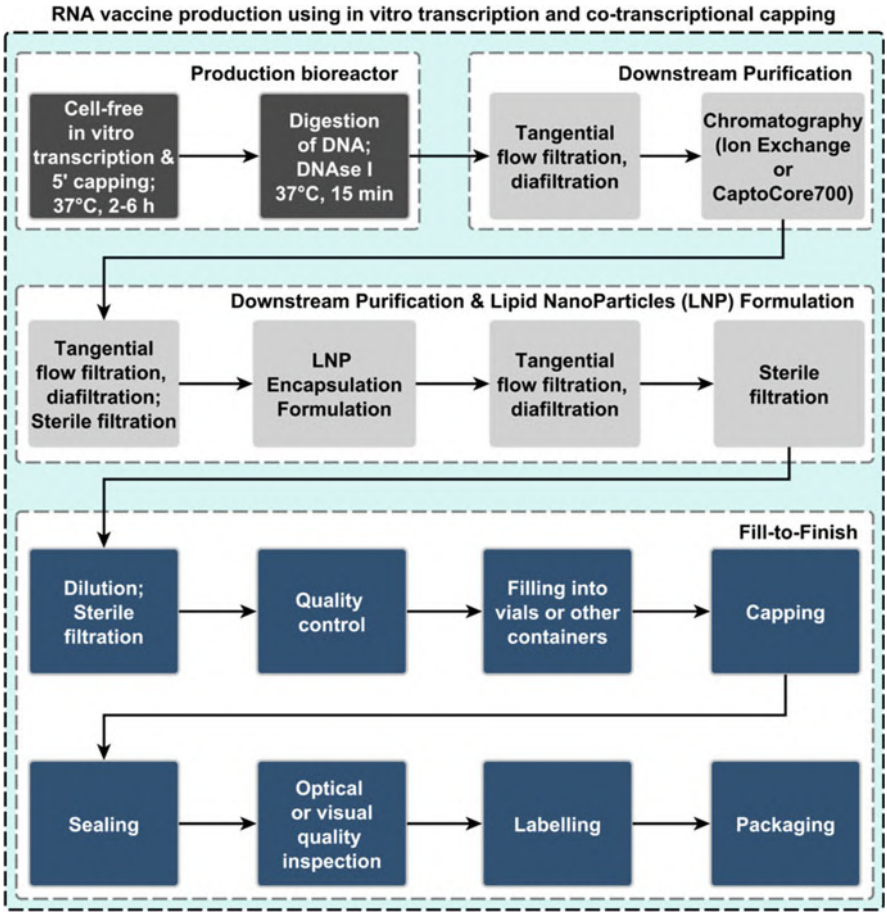

Fig. 7.5 Flowchart bioprocess for RNA vaccine production. Source: Kis and Rizvi (2001). Reprinted with permission from Public Citizen

- Powders, after drying by temperature increasing or by lyophilization.
- Aqueous solutions, to maintain the product stable or its solubilization for use.
- Other forms not specified here, according to the demand.

Regarding the sample preparation, it can follow those methods discussed in Chap. 4 for pharmaceuticals and agrochemicals, except when specified here.

7.4 Main Related Analytical Techniques

The quality control of biotechnological products and processes is conducted using some of the following analytical techniques, divided into quantitative and qualitative information, characterization, and structure resolution:

- High performance liquid chromatography (HPLC), using detectors as ultraviolet-visible absorption (UV-Vis), mass spectrometry (MS), diode array detector (DAD), and refractive index detector (RID)—this is the most common technique for quantification.
- Gas phase chromatography (GC), using detectors as flame ionization detector (FID) and mass spectrometry (MS)—for quantification.
- Spectrophotometry in the UV-Vis—for qualification (preferably) and quantification.
- Fourier transformed infrared (FTIR) and Raman, by means of probes—for characterization and qualification.
- Mass spectrometry (MS) and nuclear magnetic resonance (NMR)—for general structure identification and structure resolution.
- OMICS: refers to a field of study in biological sciences that ends with *-omics*, such as genomics, transcriptomics, proteomics, or metabolomics; the ending *-ome* is used to address the objects of study of such fields, such as the genome, proteome, transcriptome, or metabolome, respectively (Vailati-Riboni et al. 2017). This field comprises a set of technologies based on MS and NMR—for structure resolution of biological systems.

These techniques are treated in detail as follows. Additionally, process analytical chemistry/process analytical technology (PAC/PAT) is considered as an analytical approach for real-time analyses and information.

7.4.1 Chromatographic Techniques

The basis of chromatographic separations is described in Chap. 4. Here we will focus our attention on the gas phase chromatography and on the liquid phase chromatography techniques, which are most common for quality control, and for research and development of bioproducts and bioprocesses.

7.4.1.1 Gas Chromatography

In GC the components of a sample are separated as a function of their partition between a gaseous mobile phase, usually the helium gas, and a liquid or solid phase contained within the column. One limitation of GC is when the analyte to be analyzed is not volatile (i.e., it is thermally stable); an alternative is the

Table 7.2 Most common GC detectors. Source: Adapted from Vaz Jr (2018). Reproduced with permission from Springer Nature

Detector	LOD
Flame ionization detector (FID)	0.2 pg
Thermal conductivity detector (TCD)	500 pg
Electron capture detector (ECD)	5 fg
Thermal-ionic detector (TID) or nitrogen-phosphorus detector (NPD)	0.1 pg
Mass spectrometer (MS)	<100 pg

1 pg = 10^{-12} g; 1 fg = 10^{-15} g; *LOD* limit of detection

derivatization, when the formation of another molecule from the analyte with lower boiling values. The elution of the components is done by an inert mobile phase (carrier gas) flow; that is, the mobile phase does not interact with the molecule of analyte.

The modernization of the equipment, through the development of new stationary phases and data processing software, also led to an investment in systems that provide higher speed during the chromatographic analysis. The shortest analysis time has the direct consequence of reducing the cost of the analytical process and increasing the analytical capacity of the laboratory. The increase in the speed of the chromatographic analysis can be related to the reduction of the size of the column, and reduction of its internal diameter, which compensates the loss of resolution in the determinations.

Regarding the choice of the most suitable detector to be used, the nature of the sample (analytical matrix more analyte) should be considered. Several detectors are commercially available for use in GC, with thermal conductivity (TCD), flame ionization (FID), electron capture (ECD), and mass spectrometer (MS) detectors being most commonly used. An ideal detector should meet the following characteristics:

- Adequate sensitivity
- Good stability and reproducibility
- Linear response to analytes, extending to several orders of magnitude
- Temperature ranges from ambient to at least 400 °C
- Ease of use
- Similarity of response to all analytes in the sample

In practice, the detectors will rarely meet the features described above. Table 7.2 shows the most common detectors used in GC and their limit of detection (LOD) values.

Figure 7.6 describes a block diagram for a GC equipment. And Fig. 7.7 depicts the equipment.

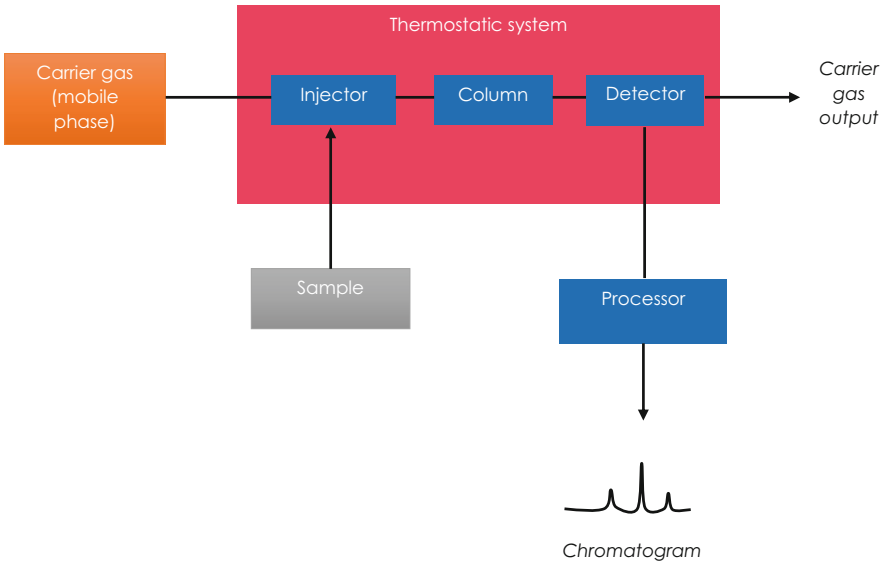

Fig. 7.6 Block diagram of a GC equipment

7.4.1.2 Liquid Chromatography

LC can be applied in a variety of operating modes, with the best mode depending on the structural characteristics of the analyte to be separated by the chosen analytical method. The most common categories are partition chromatography—or ion chromatography—adsorption chromatography, ion exchange chromatography, size exclusion chromatography, and affinity chromatography.

High performance liquid chromatography (HPLC)—The use of low-pressure and high-pressure columns, called high performance liquid chromatography (HPLC), outperforms GC in the analysis of semi-volatile and non-volatile organic compounds. In its many variants, it allows the analysis of complex mixtures, difficult to separate by other techniques, especially mixtures of biomolecules.

Typically, the HPLC equipment is equipped with two or more solvent reservoirs. Elution with a single solvent or a mixture of solvents of constant composition is called *isocratic elution*; while the use of a mixture of solvents at different polarity, with composition varying in a programmed manner, is a *gradient elution*. Generally, gradient elution improves the efficiency of the separation process. The pumping system is an important component whose function is to ensure a constant and reproducible flow from the mobile phase to the column. They have a pressure of 0.1 to 350 bar. The columns are generally stainless steel with lengths ranging from 10 to 30 cm and internal diameters between 2 and 5 mm. The column fillings (or stationary phase) typically have particles with diameters between 3 and 10 μm. Systems with particles smaller than 2 μm and pressures in the range of 1000 bar are called ultra-high-performance liquid chromatography (UHPLC) or

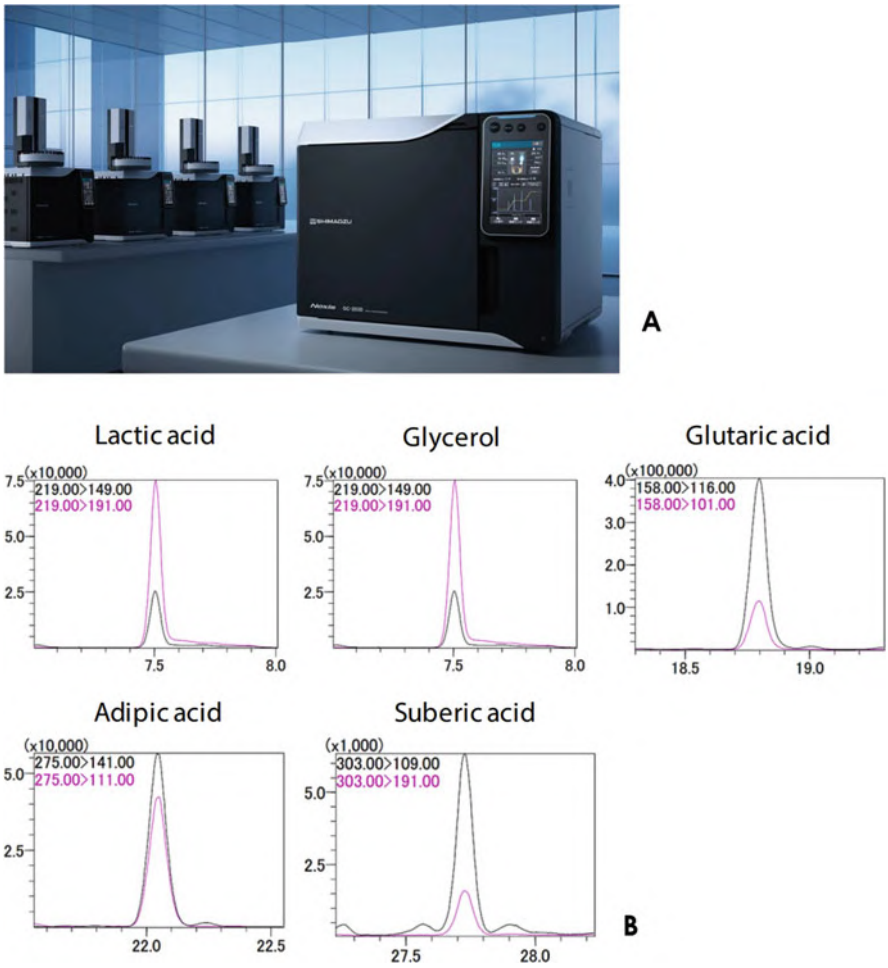

Fig. 7.7 A GC equipment (**a**), and an obtained chromatograms of organic acids and alcohol with an MS/MS detector after analytes derivatization (**b**). Courtesy of Shimadzu

ultra-performance liquid chromatography (UPLC)—this mode of liquid chromatography can provide a higher resolution in a shorter retention time. Stationary phases for most chromatography modes consist of a silica material, or a polymer such as a polysaccharide or polystyrene, with functional groups of interest attached to the surface of this substrate—they may be either *normal phase* (polar stationary phase) type or *reverse phase* (non-polar stationary phase) type.

Selection of the mobile phase is critical for partitioning, adsorption, and ion exchange chromatography, and less critical for the other modes. For the solvents used to form this phase, properties such as the UV-Vis cut-off wavelength and the refractive index are important parameters when working with ultraviolet-visible (UV-Vis) and/or refractive index detectors. The polarity index (P′) and the eluent

Table 7.3 Characteristics of the main HPLC detectors. Source: Adapted Vaz Jr (2018). Reproduced with permission from Springer Nature

Detector	LOD
Ultraviolet-visible (UV-Vis) absorption or diode array detector (DAD)	10 pg
Mass spectrometer (MS)	1 pg
Fluorescence detector (FD)	1 ng

1 pg $= 10^{-12}$ g; 1 ng $= 10^{-9}$ g; *LOD* limit of detection

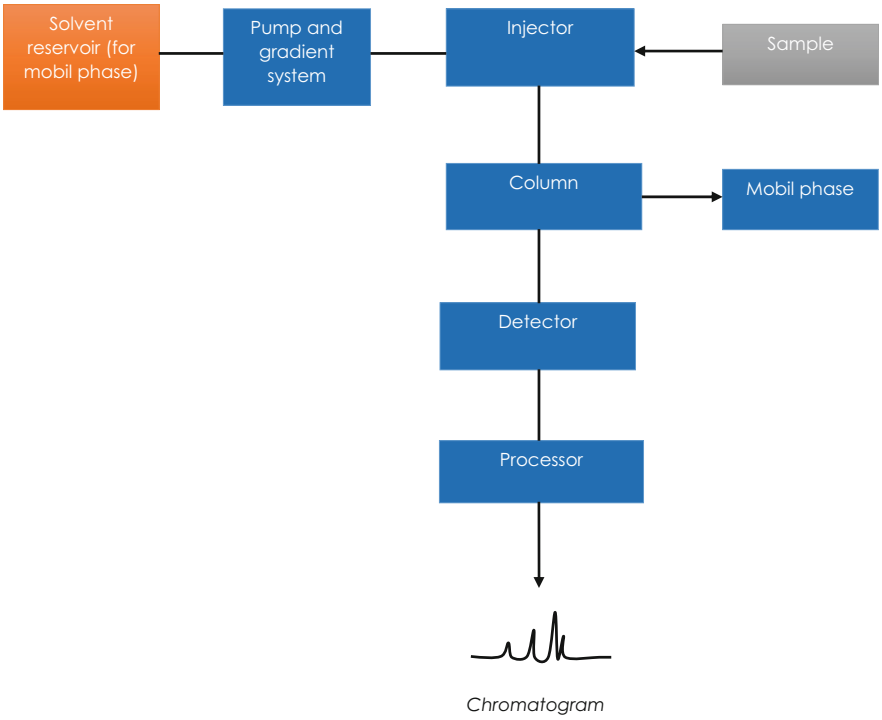

Fig. 7.8 Block diagram for an HPLC equipment

force (ε^0) are polarity parameters that aid in choosing the phase for partitioning and adsorption chromatography, respectively.

As for GC, there are several types of detectors available commercially, and the choice usually depends on the type of analyte and the number of analyses required. Detectors may be concentration sensitive, when the analytical signal produced is proportional to the analyte concentration in the effluent or eluted; or mass sensitive, when the signal produced is proportional to the mass flow rate. Table 7.3 lists the main detection systems for HPLC.

Figure 7.8 depicts a block diagram for an HPLC equipment. And Fig. 7.9 depicts the equipment.

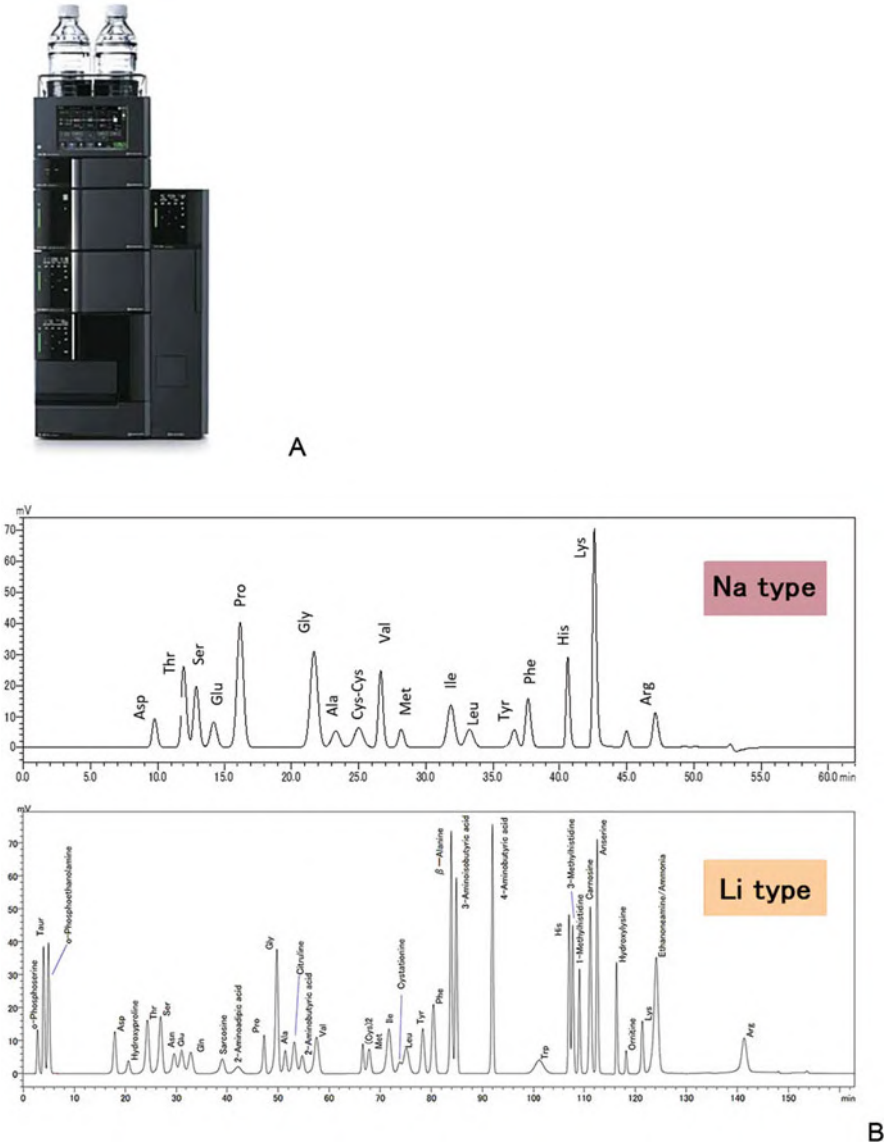

Fig. 7.9 An HPLC equipment (**a**), and an obtained chromatogram of amino acids using a fluorescence detector (**b**). Courtesy of Shimadzu

7.4.2 Mass Spectrometry

MS is essentially a technique for detecting molecular components having the mass/charge ratio (m/z) as the unit of measurement, which are obtained by means the

original molecule fragmentation into derived chemical species (e.g., the molecular ion M^+). Depending on the ionization technique used, analytes may present with one or multiple charges. In single-charge components, the m/z ratio corresponds to the total mass of the ion in Daltons. In cases where ions with two or more charges are more frequent, the calculation of the original ion mass will depend on deconvolutions of the original signal.

Regarding the fragmentation, it is based on the removal of the electron from the molecule resulting in its ionization. Removal of electrons from either *sigma* bond, *pi* bond, or nonbonding orbitals causes the ionization. The fragmentation pattern promotes the distinction among the analytes.

The direct analysis of the sample in the mass spectrometer seldom generates results that can be considered quantitatively, even if the sample is pure. This is a consequence of the high sensitivity of the technique and the efficiency of the ionization process, besides the intrinsic characteristics of each sample that allow greater or less easiness of ionization.

MS is often associated with a separation technique, usually gas chromatography or liquid chromatography—are the *hyphenated techniques*—where a separation technique coupled to a detection and quantification technique is used. In this case, the mass spectrometer functions as a detector. Such hyphenated techniques make it possible to separate complex mixtures, identify the components, and quantify them in a single operation. Almost all measurements of MS are done under high vacuum, as this allows the conversion of most of the molecules into ions, with a lifetime enough to allow their measurement. The mass spectrometer consists essentially of three components: ionization source, mass analyzer, and ion detector.

There are several commercially available ionization systems: electron impact ionization (EI), chemical ionization (CI), fast atom bombardment (FAB), particle beam bombardment (PBB), matrix-assisted laser desorption ionization (MALDI), electrospray ionization (ESI), atmospheric pressure photoionization (API), and atmospheric pressure chemical ionization (APCI). For high molecular weight, non-volatile and heat-sensitive materials, such as some pesticides, MALDI, APCI, and FAB techniques are used. The most common analyzers are quadrupole, quadrupole ion trap, and time-of-flight tube. The detection is done by electron multiplier tube.

The high-resolution MS provides comprehensive accurate mass information in a single analysis by MS^n technology (tandem mode); detects more low-level components in complex samples; and is designed and well suited for large molecule analysis (Vaz Jr 2021). However, a main limitation is that a large volume of data to process requires an experienced analyst to operate.

Figure 7.10 depicts a block diagram of an MS instrument, and Fig. 7.11, the instrument.

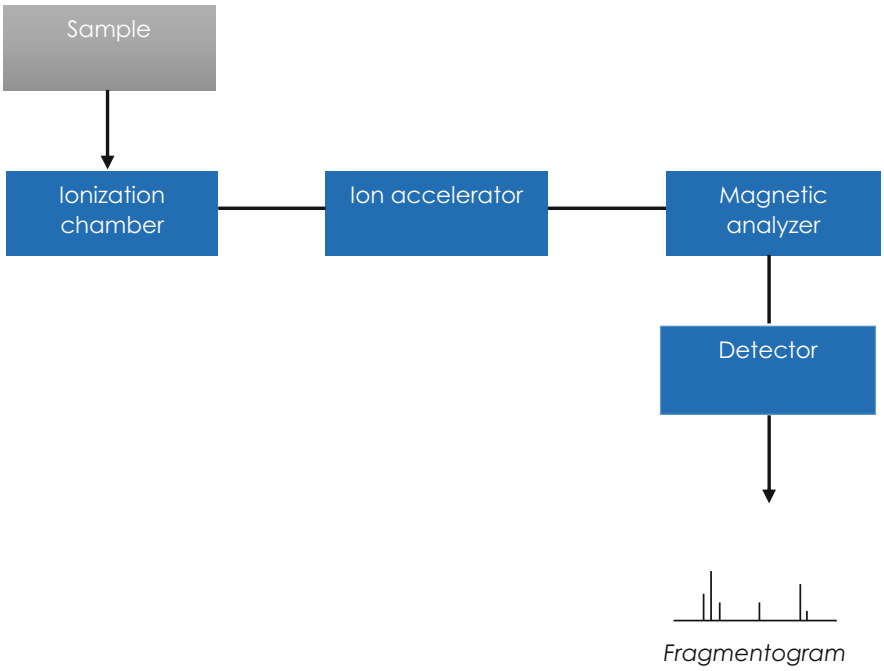

Fig. 7.10 Block diagram of a mass spectrometer

7.4.3 Spectroscopy

The theoretical basis of spectroscopy is detailed in Chap. 4. Here, UV-Vis absorption, FTIR, Raman, and NMR will be explored for chemical analysis of biotechnological products and processes.

7.4.3.1 Absorption of Ultraviolet-Visible Radiation, or Molecular Spectrophotometry

This technique is widely used for the identification and determination of organic, inorganic, and biological species. Usually, molecular absorption spectra are more complex than atomic absorption spectra due to the higher number of energy states of the molecule compared to the isolated atoms (see ahead in the atomic spectrometry item).

The ultraviolet (UV) region of the electromagnetic spectrum approximately ranges between 200 to 400 nm and the region of the visible (Vis) ranges between 400 and 750 nm. The absorption of radiation by molecules in these regions results from the interactions between photons and electrons that participate in a chemical bond, or between electrons that are not bound in atoms like oxygen, sulfur, nitrogen,

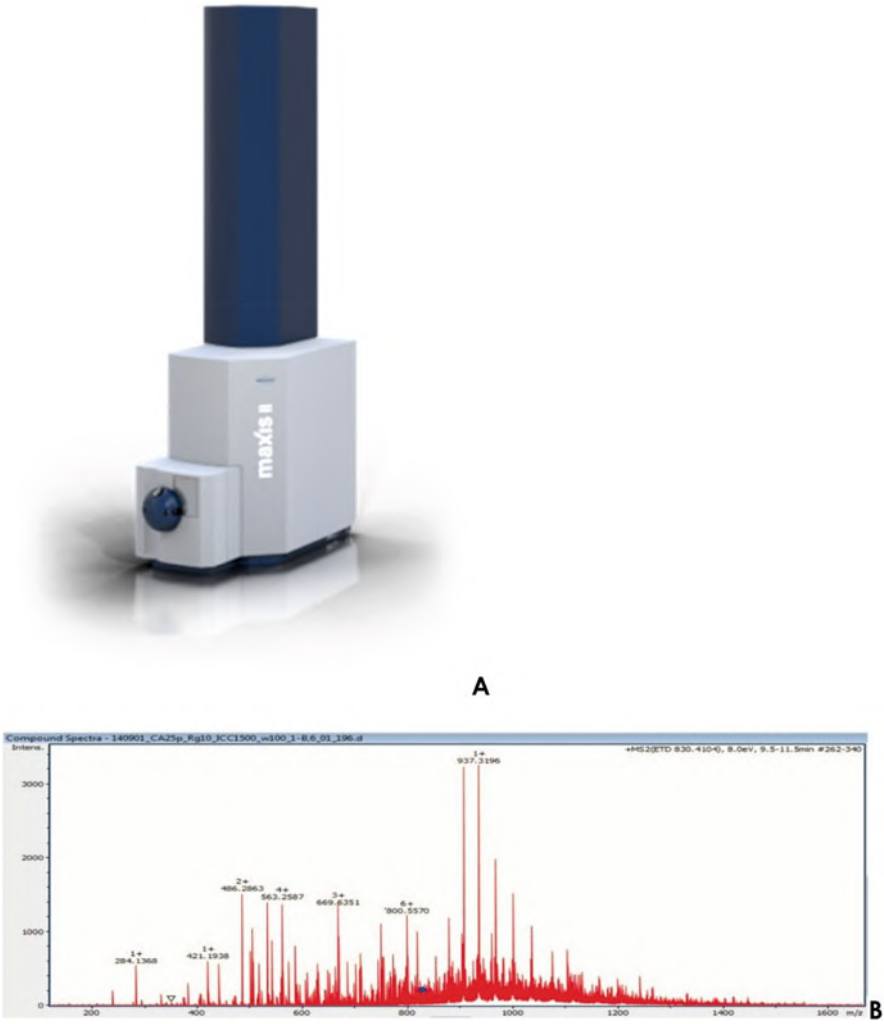

Fig. 7.11 A time-of-flight mass spectrometer (**a**), and the obtained fragmentogram (**b**). Courtesy of Brüker

and halogens. The wavelength where absorption occurs depends on the type of bond that these electrons participate. Electrons shared in single C-C or H-H bonds are so tightly bound that they require high energy at wavelengths below 180 nm and are not observed by the most common methods of analysis. Due to experimental difficulties in working in this region, single-bond spectra are poorly explored. The electrons involved in double and triple bonds are not so strongly trapped and, consequently, they are excited more easily and produce more useful absorption peaks.

Absorption spectroscopy in UV-Vis is mainly used in quantitative analysis of several organic compounds containing mainly C=O and C=C bonds, as the

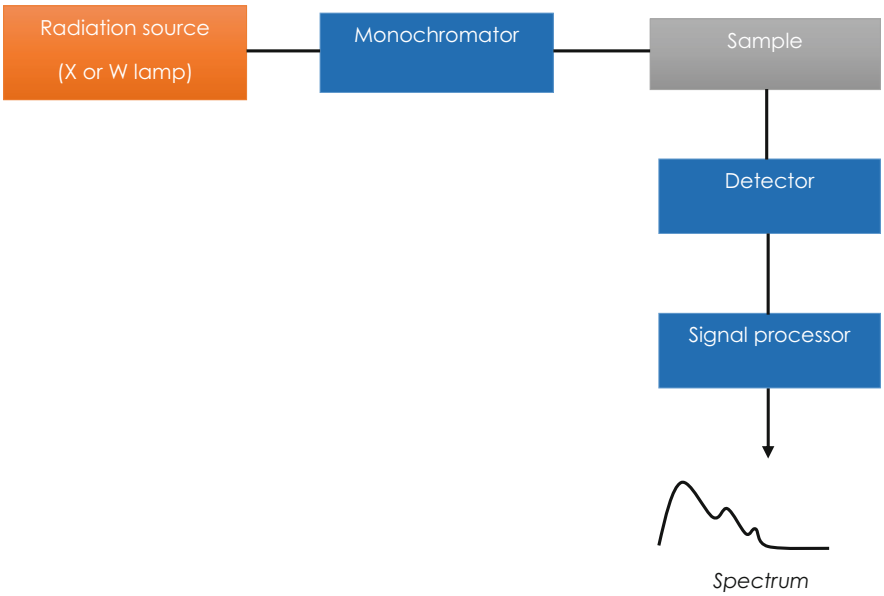

Fig. 7.12 Block diagram for an UV-Vis absorption spectrophotometer

intensity of the absorption peaks can be directly correlated to the concentration of the analyte, now called spectrophotometry—it is widely used as a detector after separation by liquid chromatography. The signal intensity at a given wavelength value can be directly correlated with the analyte concentration, which allows quantitative data to be obtained—it is worth noting that it is necessary to have the respective curve with linear behavior (as seen in Chap. 3).

The Lambert-Beer Law (Eq. 7.1) correlates the signal intensity at a given wavelength value directly with the analyte concentration, which allows quantitative data to be obtained—again, it is worth noting that it is necessary to have the respective curve with linear behavior.

$$A = \varepsilon bc \qquad ((7.1),)$$

Where: A = absorbed radiation (arbitrary units); ε = molar absorptivity of the medium (cm^{-1} L mol^{-1}); b = cell length (cm); c = concentration (mol L^{-1}).

Figure 7.12 describes the block diagram for an UV-Vis absorption instrument. And Fig. 7.13 depicts the instrument.

Table 7.4 describes information on electronic transitions and wavelengths of UV absorption of some chemical groups present in several organic compounds.

According to the EAG Laboratories (2023), advantages of UV-Vis absorption spectrophotometry are as follows: a fast sample analysis; suitability for a wide variety of analytes; user-friendly interface; and little maintenance required. And limitations are as follows: effects of fluctuations from scattered light and temperature

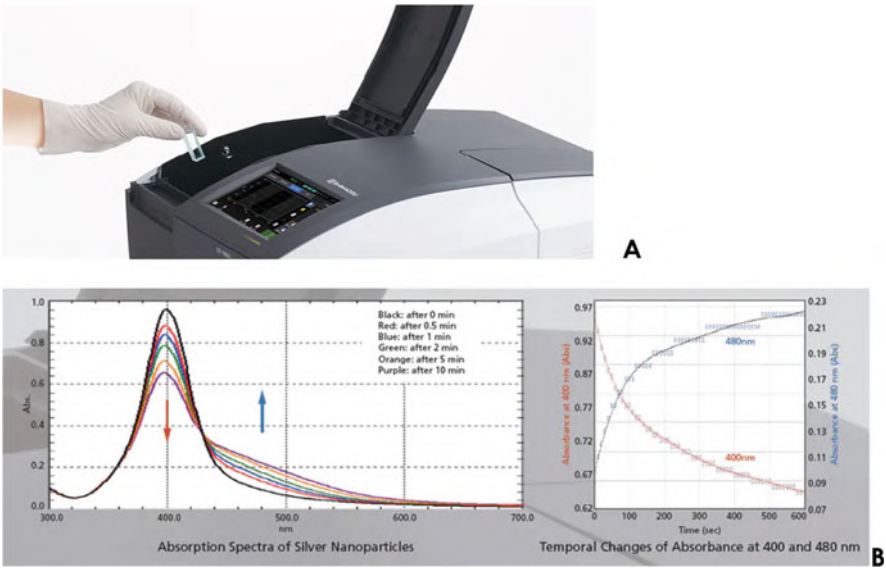

Fig. 7.13 An UV-Vis absorption spectrophotometer (**a**), and the obtained spectrum (**b**). Courtesy of Shimadzu

Table 7.4 Examples of chemical groups, which absorb UV radiation, and their associated electronic transitions. Source: Adapted from Vaz Jr (2018). Reproduced with permission from Springer Nature

Chemical group	Structure	Electronic transitions	λ_{max} (nm), nearly
Carbonyl (ketone)	RR'C=O	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	180 271
Carbonyl (aldehyde)	RHC=O	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	190 293
Carboxyl	RCOOH	$n \rightarrow \pi^*$	204
Amide	$RC{=}ONH_2$	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	208 210
Conjugated diene	RCH-CH=CH-CHR	$\pi \rightarrow \pi^*$	250
Aromatic	C_6H_6	$\pi \rightarrow \pi^*$	256

changes; relatively low sensitivity; other sample components may cause interferences; not as specific as chromatography; and requires a relatively large sample volume, >0.2 mL.

7.4.3.2 Absorption Infrared Molecular Spectroscopy

Vibrational spectroscopy refers to a type of interaction of the radiation with vibrational states of the chemical bonds. Therefore, there is no electronic transition. Here

we can highlight infrared (IR) absorption spectroscopy in its three wavelength ranges: near, mid, and far. Polarity has a direct influence on the IR spectrum, modifying its form.

The electromagnetic region of the IR is located between the visible region and the microwaves, that is, from 12,800 to 10 cm^{-1}, remembering that the unit cm^{-1} refers to the wavenumber. As previously introduced, the IR spectrum is subdivided into three regions: near-infrared (NIR), mid-infrared (MIR), and far-infrared (FIR). MIR is the most used technique in organic analysis, and it is subdivided into two regions: frequency groups, from 4000 up to 1300 cm^{-1}; and absorption of functional groups of two atoms, or vibration, of 1300 to approximately 700 cm^{-1}, also called *fingerprint*. In NIR the radiation ranges between 12,800 cm^{-1} and 4000 cm^{-1}. The absorption bands in this region are harmonic or combinations of fundamental stretching bands, often associated with hydrogen atoms, due to the ease of handling of the sample.

Absorbance of IR radiation is determined by Eq. (7.2):

$$A = \log_{10}(I/T) = \log_{10}(I_0/I) \tag{7.2}$$

Where T is the radiation transmission, I_0 is the measured intensity of the source radiation (the background) incident on the sample, and I the radiation transmitted through the sample. Normally, the output is expressed as percent transmittance. However, in some cases the absorbance is considered as the output.

IR spectra are typically employed to identify pure organic compounds or impurities, interactions, and binding formation. It is important to consider that a Fourier transform converts the intensity vs. time signal into the intensity vs. frequency spectrum—from this we have the most used MIR technique FTIR (Fourier transform infrared absorption spectroscopy).

Table 7.5 describes the main possible correlations for the assignment of the absorption bands in the MIR—generally, using the FTIR mode—as a function of the type of bound.

Again, according the EAG Laboratories (2023), advantages of FTIR are as follows: capacity to identifying organic functional groups and often specific organic compounds; availability of extensive spectral libraries for compound and mixture identifications; ambient conditions (vacuum is not necessary) for operation with applicability for semi-volatile compounds; minimum analysis area (~15 μm); r*ule-of-thumb: if you can see the sample by eye, it most likely can be analyzed*; can be quantitative with appropriate standards and uniform sample thicknesses. On the other hand, limitations are as follows: limited surface sensitivity (typical LOD is a film thickness of 25 nm); only specific inorganic species exhibit an FTIR spectrum (for example: silicates, carbonates, nitrates, and sulfates); sample quantitation requires the use of standards; glass absorbs infrared light and is not an appropriate substrate for FTIR analysis; water strongly absorbs IR radiation and may interfere with the analysis of dissolved, suspended, or wet samples; simple cations and anions, e.g., Na^+ and Cl^-, do not absorb IR radiation and hence cannot be detected by FTIR; identification of mixtures/multiple sample components may require additional

Table 7.5 Characteristic bands of deformations and vibrational stretches, which may be present in bioproducts. Source: Adapted from Vaz Jr (2018). Reproduced with permission from Springer Nature

Band position (cm^{-1})	Assignment	Intensity
3500–3000	Intramolecular stretching of O–H and N–H	Medium absorption
2940–2900	Asymmetric stretching of aliphatic C–H	Strong absorption
1725–1720	Stretching of C = O in COOH and ketones	Strong absorption
1660–1630	Stretching of amide groups (amide band I) and quinone; C = O stretching of hydrogen bonded to conjugated ketones; stretching of COO^-	Strong absorption
1620–1600	Stretching of aromatic C = C; stretching of COO^-	Medium to weak absorption
1460–1450	Stretching of aromatic C–H	Medium absorption
1400–1390	Deformation of O–H and stretching of C–O and OH phenolic; deformation of C-H in CH_2 and CH_3; asymmetric stretching of COO^-	Medium absorption
1170–950	Stretching of C–O in polysaccharides or polysaccharides-like compounds	Strong absorption

Fig. 7.14 Block diagram of an MIR spectrometer with Fourier transform (FTIR)

laboratory preparations and analyses; metals reflect light and cannot be analyzed by FTIR.

Fig. 7.14 describes a block diagram of an FTIR instrument. And Fig. 7.15 depicts the commercial instrument.

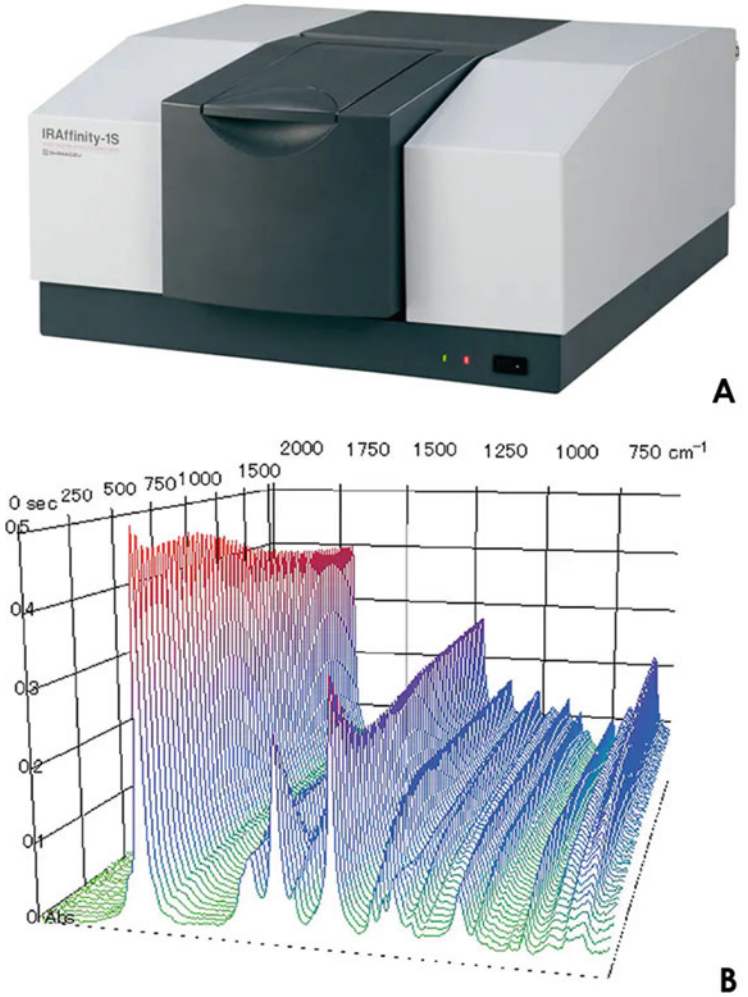

Fig. 7.15 An MIR-FTIR spectrometer (**a**), and the obtained spectrum in the absorption mode (**b**). Courtesy of Shimadzu

The Raman spectroscopy is a branch of the vibrational spectroscopy. But differently from the FTIR, this technique is based on the radiation diffraction in a wavelength from 3000 to 100 cm^{-1}.

Raman spectroscopy results in information about intra- and intermolecular vibrations and enables an additional understanding of a given reaction. Raman spectroscopy and FTIR provide a characteristic spectrum of the specific vibrations of a molecule—the *molecular identity* or fingerprint—and are important for the identification of a substance. However, Raman spectroscopy can provide additional information about low-frequency modes and vibrations that increase understanding about the crystal lattice and fundamental molecular structure.

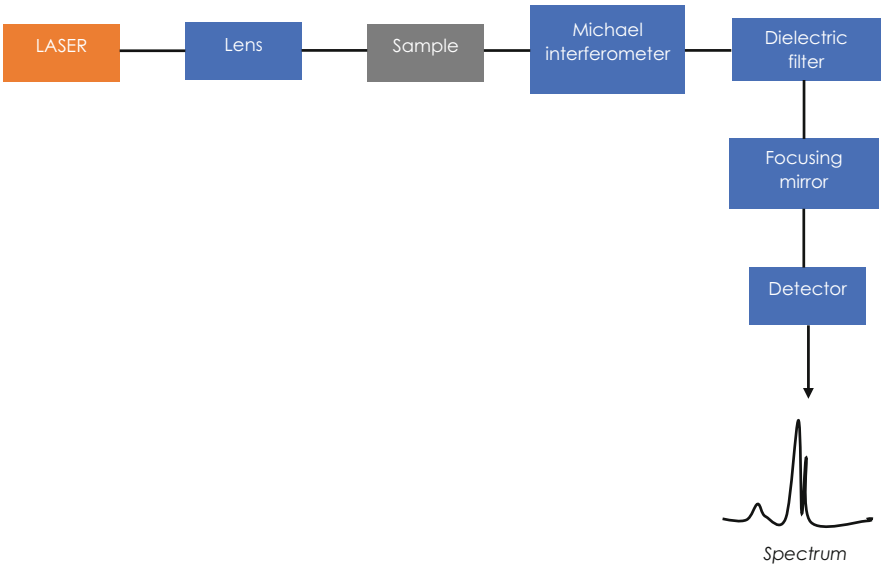

Fig. 7.16 Block diagram of a Raman instrument. *LASER* light amplification by stimulated emission of radiation

Unlike FTIR spectroscopy, which investigates changes in dipole moments, Raman investigates changes in the polarizability of molecular bonds. The interaction of radiation with a molecule can induce deformation of its electron cloud. This deformation is known to be a change in polarizability. Molecular bonds have specific energy transitions in which a change in polarizability occurs, giving rise to Raman-active modes. As an example, molecules that contain bonds between homonuclear atoms, such as C-C, S-S, and N-N bonds, undergo a change in polarizability when photons interact with them. These are examples of bonds that give rise to spectral bands that are active in Raman but would be difficult to see or not visible in FTIR.

Figure 7.16 depicts a block diagram of a Raman instrument, and Fig. 7.17 depicts the instrument.

7.4.3.3 Nuclear Magnetic Resonance

Unlike other types of spectroscopies, in nuclear magnetic resonance (NMR) it is the nuclei of atoms that absorb radiation and not their electrons. The absorption of radiation by the nuclei occurs when they are subjected to an external magnetic field produced by low energy waves (radio frequency; from 10^{-3} to 10^{1} m).

In some cases, the nuclear charge can rotate around the nuclear axis, generating a magnetic dipole. The angular momentum of the moving load can be described in terms of the spin I moment (m); the most explored nuclei in NMR for bioproducts and their raw materials or by-products are the ^{1}H and ^{13}C nuclei that have I equal to

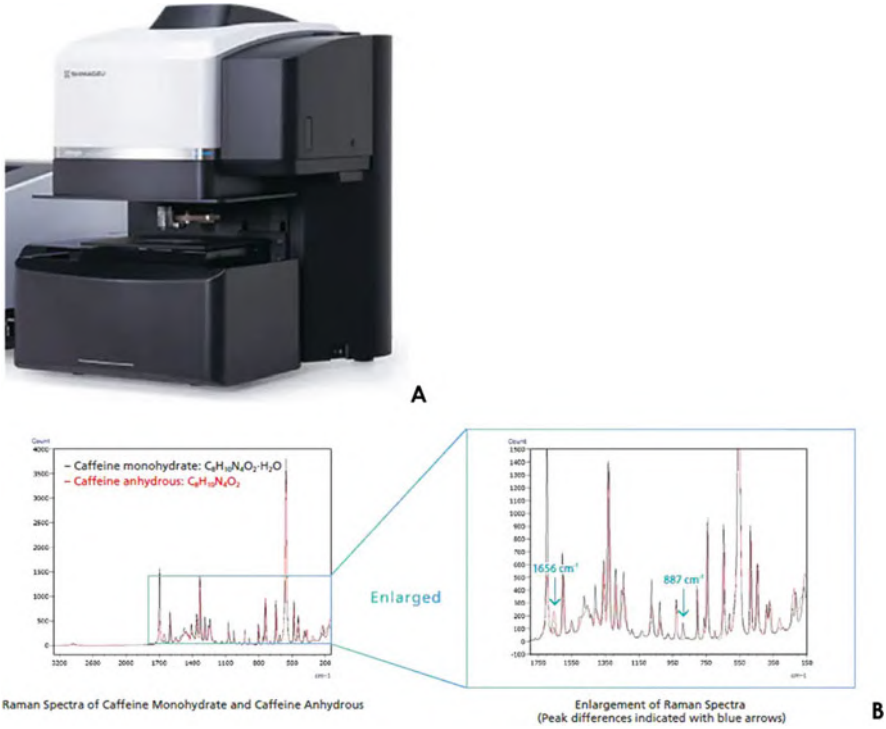

Fig. 7.17 A Raman spectrometer (**a**), and the obtained spectrum (**b**). Courtesy of Shimadzu

½; ^{31}P can be explored in some cases, as in the biomass composition study. The absorption of radio frequency by these nuclei is characteristic and influenced by neighboring nuclei. This allows the molecular structure of a series of chemical compounds to be determined as a function of the chemical shift (δ) produced according to the electronic density of the atoms present in the molecule.

In the presence of an applied magnetic field, the nuclei are either aligned with the magnetic field with spins of $m = +1/2$ or aligned against the magnetic field with spins of $m = -1/2$. The energies in these two spin states, E_{lower} and E_{upper}, are given by the Eqs. (7.3) and (7.4):

$$E_{\text{lower}} = -\gamma h 4\pi B_0 \tag{7.3}$$

$$E_{\text{upper}} = +\gamma h 4\pi B_0 \tag{7.4}$$

Where γ is the magnetogyric ratio for the nucleus, □ is Planck's constant, and B_0 the strength of the applied magnetic field. The difference in energy, ΔE, between the two states is given by the Eq. (7.5):

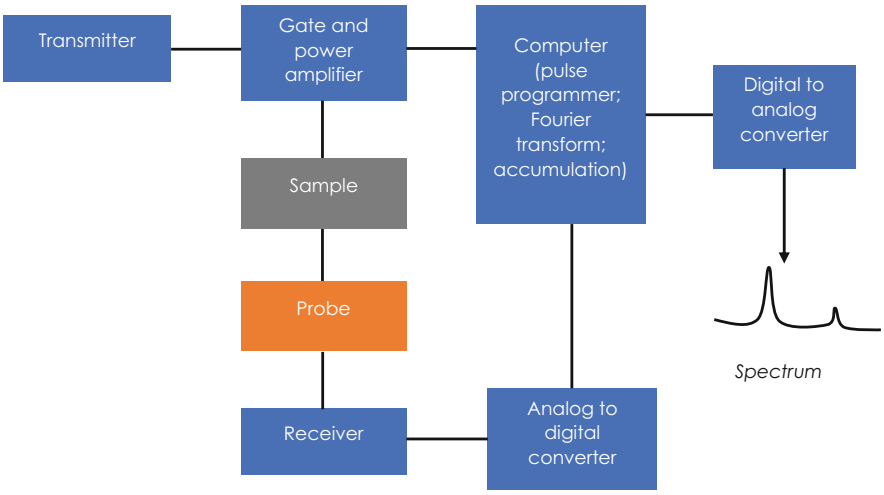

Fig. 7.18 Block diagram for a pulsed FT-NMR instrument

$$\Delta E = E_{\text{upper}} - E_{\text{lower}} = +\gamma h 4\pi B_0 - (-\gamma h 4\pi B_0) = \gamma h 2\pi B_0 \tag{7.5}$$

Considering that energy (E) is equal to the Planck's constant by the frequency ν (in Hz) combining it with the Eq. (7.5), we can obtain the Eq. (7.6) for the electromagnetic need to effect a change in spin state:

$$\nu = \gamma B_0 2\pi \tag{7.6}$$

And this is called the Larmor frequency for the nucleus, where ν is the frequency.

Figure 7.18 depicts the block diagram of an NMR instrument, and Fig. 7.19, the instrument.

NMR is most largely used to generate qualitative data; however, it can be used to produce quantitative data with limitations.

In a general way, the following assignments of groups as a function of chemical shift can be made for the ^{13}C-NMR spectrum:

- 0–45 ppm: unsubstituted aliphatic C, as in alkanes and fatty acids, due to methyl-terminal groups
- 45–65 ppm: C associated with N-alkyl, as in amino acids, peptides and proteins and C methoxy
- 60–110 ppm: C associated with aliphatic O
- 110–140 ppm: unsubstituted and alkyl substituted aromatic C
- 110–160 ppm: total aromatic C related to unsubstituted, alkyl substituted, and phenolic group
- 140–160 ppm: C phenolic
- 160–185 ppm: C in carboxylate
- 185–230 ppm: ketone C in esters and amides

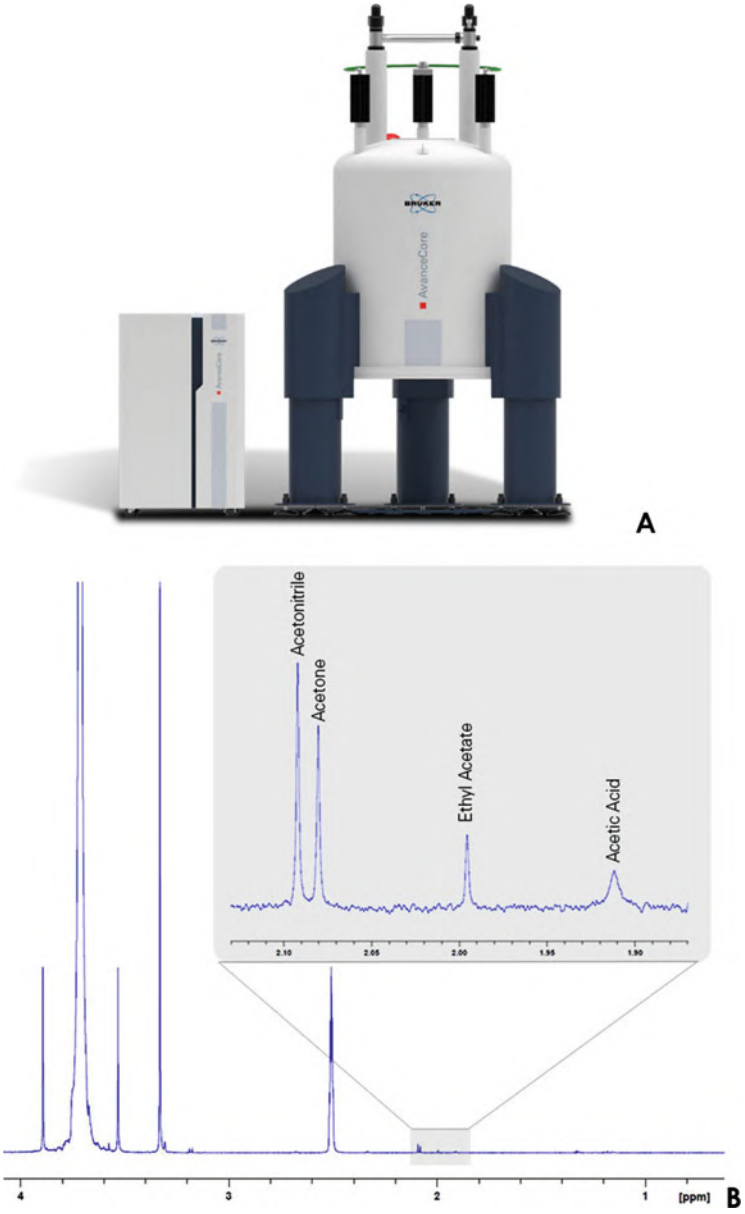

Fig. 7.19 A 400 MHz NMR spectrometer (**a**), and the obtained spectrum for the ^{1}H nuclei (**b**). Courtesy of Brüker

Table 7.6 Methods for 2D-NMR. Source: Adapted from Harvey (2022). Reproduced with permission from the Author

Method	Information obtained from cross peaks
Correlation spectroscopy (COSY)	Coupling between two protons (^{1}H–^{1}H) that are within three chemical bonds of each other
Total correlation spectroscopy (TOCSY)	Coupling between all protons (^{1}H) in the molecule
Heteronuclear correlation spectroscopy (HETCOR)	Coupling between a proton (^{1}H) and another nucleus, such as carbon (^{1}H–^{13}C) or nitrogen (^{1}H–^{15}N)
Nuclear Overhauser and exchange spectroscopy (NOSEY)	Coupling between two protons (^{1}H–^{1}H) that are within approximately 5 Å of each other
Heteronuclear single quantum correlation (HSQC)	Coupling between a proton (^{1}H) and another nucleus, such as carbon (^{1}H–^{13}C) or nitrogen (^{1}H–^{15}N)
Heteronuclear multiple bond coherence spectroscopy (HMBC)	Coupling between a proton and a carbon (^{1}H–^{13}C) that are two or three bonds apart
Incredible natural abundance double-quantum transfer (INADEQUATE)	Coupling between adjacent carbon atoms (^{13}C–^{13}C)
Double quantum filtered correlation spectroscopy (DQF–COSY)	Suppresses signals from water

For ^{1}H-NMR spectrum:

- 10–9 ppm: H in aldehyde
- 9–6 ppm: H in aromatic and heteroaromatic
- 7.5–4.5 ppm: H in alkene
- 7–2 ppm: H in α-disubstituted aliphatic
- 1.5 ppm: H in α-monosubstituted aliphatic
- 3–1.5 ppm: H in alkyne
- 1.5–0.5 ppm: H in β-substituted aliphatic
- 2–0 ppm: H in aliphatic alicyclic

The related assignments for ^{1}H and ^{13}C shifts were dedicated to one-dimension (1D) spectra, which are related to the frequency absorbed by the analyte's nuclei expressed in *ppm*. These spectra were acquired by applying a brief radio frequency (RF) pulse to the sample, recording the resulting free induction decay (FID), and then using a Fourier transform to obtain the NMR spectrum. In addition to 1D experiments, there are a host of 2D experiments in which we apply a sequence of two or more pulses, recording the resulting FID after applying the last pulse.

Table 7.6 describes the most common methods for 2D-NMR; the main advantage of 2D against 1D is that the first can distinguish between the overlapping signals that exist in larger molecules, which is not possible by means of 1D-NMR.

7.4.4 OMICS

According to Conesa and Beck (2019), OMICS technologies are defined as high-throughput biochemical assays that measure comprehensively and simultaneously molecules of the same type from a biological sample. For example:

- Genomics profile DNA
- Transcriptomics measure transcripts
- Proteomics and metabolomics quantify proteins and metabolites, respectively

The "omics" notion refers to the fact that all or nearly all instances of the targeted molecular space are measured in the assay, and therefore they provide holistic views of the biological system.

Considering OMICS from the point of view of the chemical analysis for biotechnology, we can consider two relevant analytical technologies—both previously treated here—for this analytical approach:

- Mass spectrometry (generally coupled to liquid chromatography), for metabolome, lipidome, and proteome chemical analysis and imaging (Zhao et al. 2023; Dewez et al. 2020).
- Nuclear magnetic resonance, using 1D or 2 D TOCSY/HSQC for metabolomics research by means the metabolites identification in macromolecules, e.g., proteins, lipids, and membranes (Chandra et al. 2021).

7.4.5 Process Analytical Chemistry/Process Analytical Technology

The need for quality control of chemical and biochemical processes of production has leveraged the use of process analytical chemistry (PAC), often also known as PAT (process analytical technology).

For the industry based on process of transformation or biotransformation, the use of robust techniques and methods is privileged, preferably in real time, with the analyses being carried out directly in the reactor/bioreactor, instead of analyses carried out in the laboratory. The main advantage of this type of analytical approach in relation to the traditional one, where manual sampling is carried out followed by sample transport and subsequent analysis in the laboratory, is that analyses carried out in situ provide greater speed for taking corrective actions and consequent adjustment of the production process. On the other hand, the need to have robust and automated analytical instrumentation, such as electrochemical sensors and simple-to-use spectroscopic probes, ends up limiting the number of analytical parameters that can be analyzed, in addition to compromising the limit of detection (LOD) and limit of quantification (LOQ).

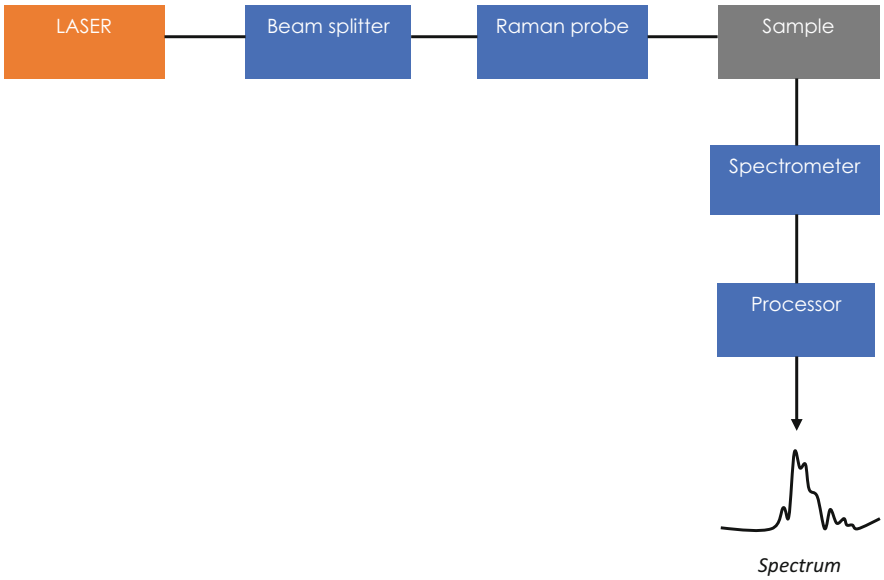

Fig. 7.20 Block diagram for a Raman probe. LASER = light amplification by stimulated emission of radiation

Fig. 7.21 A Raman probe for manufacturing process monitoring. Courtesy of Thermo Fisher Scientific

However, the continuous development of new analytical technologies and new materials will certainly increase the possibilities of obtaining more results, both by accepting greater variation in the physical and chemical conditions of the medium, and by allowing a better identification of chemical compounds. In the latter case, mainly using ultraviolet-visible (UV-Vis), mid- and near-infrared (MIR and NIR), and Raman absorption detectors or probes used as PAT.

The block diagram for a Raman probe is illustrated in Fig. 7.20, and the instrument in the Fig. 7.21.

The main aspects to be carefully considered in the methodological planning of measures in dynamic systems were listed by van Staden (1999) and should be considered here:

- Selection of process variables, such as temperature, pressure, and pH, or definition of process quality parameters to be measured.
- Establishment of a quantitative relationship between controllable properties because there is not always a direct relationship between them.
- Definition of sampling or analysis locations.
- Definition of intervals and number of measurements, in addition to the process correlation time, considering that for continuous measurements the system time constant must be defined.
- Determining the duration of the measurements, which will comprise sampling, measurement, and data calculation; that means: $t_{elapsed} = t_{sampling} + t_{measurement} + t_{calculation}$.
- Definition of tolerance limits (lower and upper) for the measured variables, which will determine the quality of the process.
- Selection of appropriate instrumentation.
- Establishment of instrument costs and their maintenance.
- Definition of instrument calibration frequency.
- Elaboration and evaluation of the costs of the measures, also considering regulatory issues.
- Establishing the reliability of measurements and the ease of obtaining them.

Once these considerations are made, the PAC/PAT becomes a very helpful tool for controlling bioprocesses, leading to a very positive impact on the quality of the final product.

Figure 7.22 depicts an on-line analysis scheme for a certain bioprocess, using mainly spectroscopic probes such as Raman, NIR, and MIR.

7.5 Commented Examples of Applications

The examples of applications treated here are addressed to quality control and to research and development for bioproducts and bioprocesses using advanced analytical techniques.

In this way, Table 7.7 presents applications for GC, LC, MS, UV-Vis, FTIR, Raman, NMR, OMICS, and PAC/PAT.

The detailed method for each example presented in Table 7.7 can be accessed inside the respective reference.

7.6 Conclusions

The biotechnological industry comprises bioproducts and bioprocess. The first one comprises ethanol and other industrial alcohols, organic acids, vitamins, antibiotics, vaccines, industrial enzymes, and proteins and amino acids; and the last one, the use

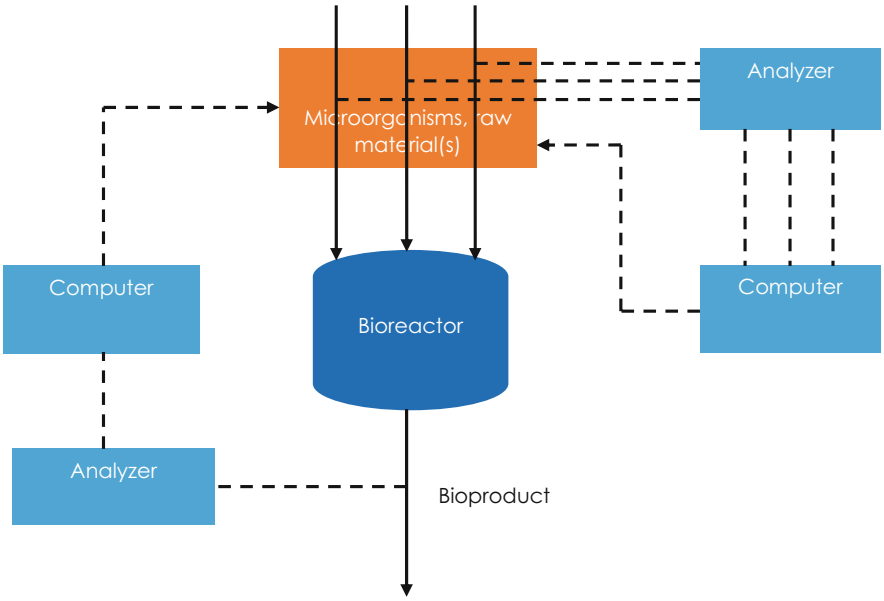

Fig. 7.22 A flowchart for on-line PAT application in a generic bioprocess in biotechnological industries. Source: Adapted from Rouessac and Rouessac (2007). Reproduced with permission from Wiley

of microbes (e.g., bacteria, fungi) and enzymes (free or immobilized). These products are used from pharmaceutical purposes to food and feed purposes.

Regarding the available analytical technologies for quality control and research and development of bioproducts and bioprocesses, we have chromatographies (gaseous and liquid phases), spectroscopies (absorption in the UV-Vis and infrared regions, Raman, and nuclear magnetic resonance), OMICS, and process analytical chemistry/process analytical technology for industrial application.

These analytical techniques promote a high-quality level of the biotechnological industry.

Table 7.7 Application of advanced analytical techniques in biotechnology

Analytical technique	Application	Brief method description	Reference
GC	Quantification of methanol and ethanol from bioreactor	GC-FID method using salting-out-assisted liquid-liquid extraction	Joseph et al. (2022)
LC	Simultaneous quantification of 2,3-butanediol, glycerol, acetoin, and ethanol in microbial cultivations	HPLC-RID method; validation was performed according to the ICH	De Souza et al. (2021)
MS	Development, manufacturing, and regulatory approval of biotherapeutics (e.g., proteins)	Use of ESI, EI, and MALDI as MS ionization techniques in industrial laboratories for biopharmaceutical	Apostol et al. (2021)
UV-Vis	Analysis of by-products (catechins and phenolic compounds) from *Cypressus lusitanica* Mill. and *Cistus ladanifer* L. distilled wastes	Absorbances measured at 290, 420, and 560 nm	Tavares et al. (2020)
FTIR	Studies of high-pressure processing-induced structural changes in pork meat	High-throughput screening approach (frequency range of 4000–600 cm^{-1}, with the spectral resolution of 4 cm^{-1}, and 64 scans collected)	Sazonova et al. (2019)
Raman	Monitoring and control of downstream bioprocesses	PAT approach using chemometrics to enrich the sensitivity of *the* in situ measurements	Lin et al. (2021)
NMR	Non-invasive on-line in vivo lipid sensor for microalgae cultivated in photobioreactor	Benchtop NMR $^{1}H/^{19}F/^{13}C$ with a magnetic field of 1.02 T and proton frequency of 43.5 MHz	Bouillaud et al. (2020)
OMICS	Scale-up to 5000-L for CHO cells bioprocess for therapeutic protein production	Combined metabolomics and proteomics analyses	Gao et al. (2016)
PAC/PAT	Batch and continuous industrial chromatography control systems for protein purification	Control strategies using in-line UV spectroscopy and on-line HPLC, and novel control strategies using combined rapid in-line data capture (e.g., NIR)	Armstrong et al. (2021)

GC gas chromatography, *GC-FID* gas chromatography-flame ionization detector, *LC* liquid chromatography, *HPLC-RID* high-performance liquid chromatography-refractive index detector, *ICH* The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, *MS* mass spectrometry, *ESI* electrospray ionization, *EI* electron impact ionization, *MALDI* matrix-assisted laser desorption ionization, *PAT* process analytical chemistry, *NMR* nuclear magnetic resonance, *CHO* Chinese hamster ovary, *PAC* process analytical chemistry, *UV* ultraviolet, *NIR* near-infrared spectroscopy

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Chapter 8
Oil and Gas

Abstract Oil and gas are fossil mixtures with a large usage by the modern society to provide energy, chemicals, and materials. And this industry is a versatile branch of global economy, involving crude oil and natural gas extraction, processing, and usage.

In order to conduct the quality control and the research and development experiments, the use of advanced analytical techniques is paramount. Analytical techniques as GC, MS, AEOS/OES, XRF, and isotopic analysis are explored in this chapter for their application from the quantification and characterization of the hydrocarbons to the determination of the presence of contaminants, as metals.

Furthermore, issues related to market, industrial processing technologies, and natural resources are addressed too.

Keywords Petrochemicals · Geochemistry · Physical-chemical properties · Petroleomics · Advanced analytical techniques

8.1 Introduction

Oil and gas are fossil mixtures with a large usage by the modern society to provide energy, chemicals, and materials. They are extracted from geological formations (or underground reservoirs) and generally are associated in the "petroleum traps"—gas above and oil below. Technically, the *petroleum* term comprises crude oil, gas, and bitumen in liquid, gaseous, and solid states, respectively.

Natural gas is originated by means of thermogenic (slow decomposition of organic matter), biogenic (methane formation by methanogenic bacteria), or abiogenic processes (methane is formed by hydrothermal reduction of carbon dioxide), and it is a naturally gaseous hydrocarbon mixture (Faramawy et al. 2016). Table 8.1 depicts the chemical composition of natural gas.

Oil—or crude oil—is originated from organic matter transformation by means of physical and chemical natural processing. In a general way and according to Malyshev (2013), the organic material in source rocks is transformed into oil at temperatures between 65 °C and 150 °C, which are reached at depths between about 2000 and 5500 m. At these temperatures the oil is irreversibly converted into natural

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Table 8.1 The chemical composition of natural gas. Source: Adapted from Speight (2015). Reproduced with permission from Taylor & Francis

Constituents	Composition (vol%)	
	Wet	Dry
Hydrocarbons		
Methane	84.6	96.0
Ethane	6.4	2.00
Propane	5.3	0.60
Isobutane	1.2	0.18
n-Butane	1.4	0.12
Isopentane	0.4	0.14
n-Pentane	0.2	0.06
Hexanes	0.4	0.10
Heptanes	0.1	0.80
Non-hydrocarbons		
Carbon dioxide	≤5	
Helium	≤0.5	
Hydrogen sulfide	≤5	
Nitrogen	≤10	
Argon	≤0.05	
Radon, krypton, xenon	Traces	

gas and graphite. The range of depths between 2000 and 5500 m is called the oil window. Only natural gas can be found below this window. Other important conditions for oil generation are pressure and time. Chemical reactions run faster at higher temperatures; at lower temperatures, or smaller depths, the oil may take millions of years to form. If a source rock has not been buried deep enough for a long period of time, the conventional oil (crude oil) does not form—an example is the shale oil rocks.

Regarding the chemical composition, the oil is a mixture of hydrocarbons, and a typical elemental composition of oil is as follows (Speight 2007):

- Carbon: 83–87%
- Hydrogen: 10–14%
- Sulfur: 0.05–6%
- Nitrogen: 0.1–2%
- Oxygen: 0.05–1.5%

And a typical chemical compounds presented in the oil are as follows (Fingas 2014):

- Saturates: alkanes or paraffins (e.g., dodecane), cycloalkanes or naphtenes (e.g., decalin), and waxes (e.g., *n*-alkanes C_{18}–C_{80}).
- Aromatics: benzene, toluene, ethylbenzene, and xylenes (BETX), polyaromatic hydrocarbons (PAHs, e.g., anthracene), and naphtenoaromatics (e.g., tetralin).
- Resins: anomalous polar compounds, which can contain oxygen, nitrogen, sulfur, and metals (e.g., carbazole).

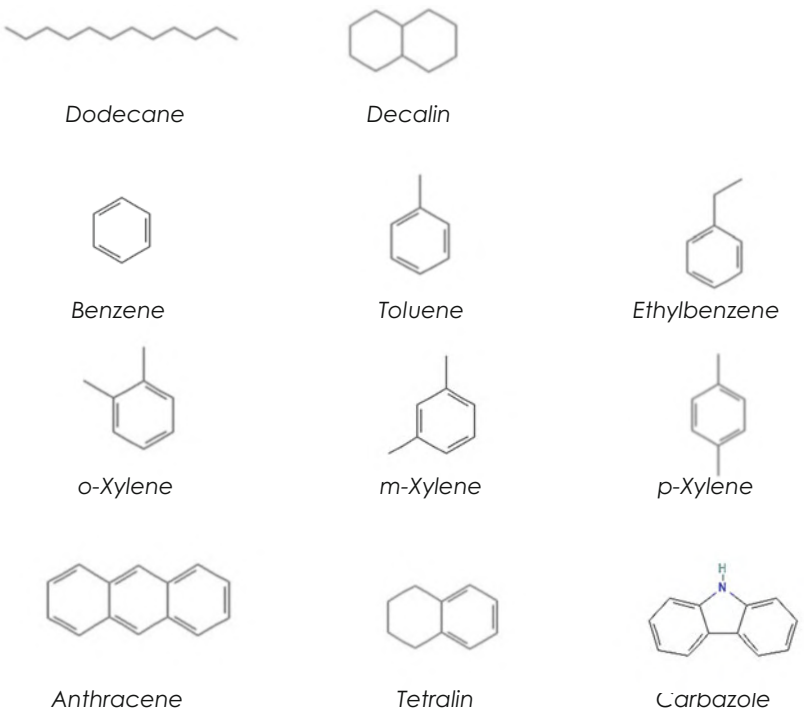

Fig. 8.1 Chemical structures of SARA compounds in oil

Table 8.2 Chemical composition of crude oil fractionated by the distillation based on the boiling point

Fraction	Boiling range (°C)
Light naphtha	−1 to 150
Gasoline	−1 to 180
Heavy naphtha	150 to 205
Kerosene	205 to 206
Light gas oil	260 to 315
Heavy gas oil	315 to 425
Lubricating oil	>400
Vacuum gas oil	425 to 600
Residue	>510

- Asphaltenes: large anomalous polar compounds, which can contain oxygen, nitrogen, sulfur, and metals with not well-defined structures.

Figure 8.1 depicts some compounds cited for SARA (saturates, aromatics, resins, and asphaltenes). And Table 8.2 depicts the fractions present in crude oil after the distillation.

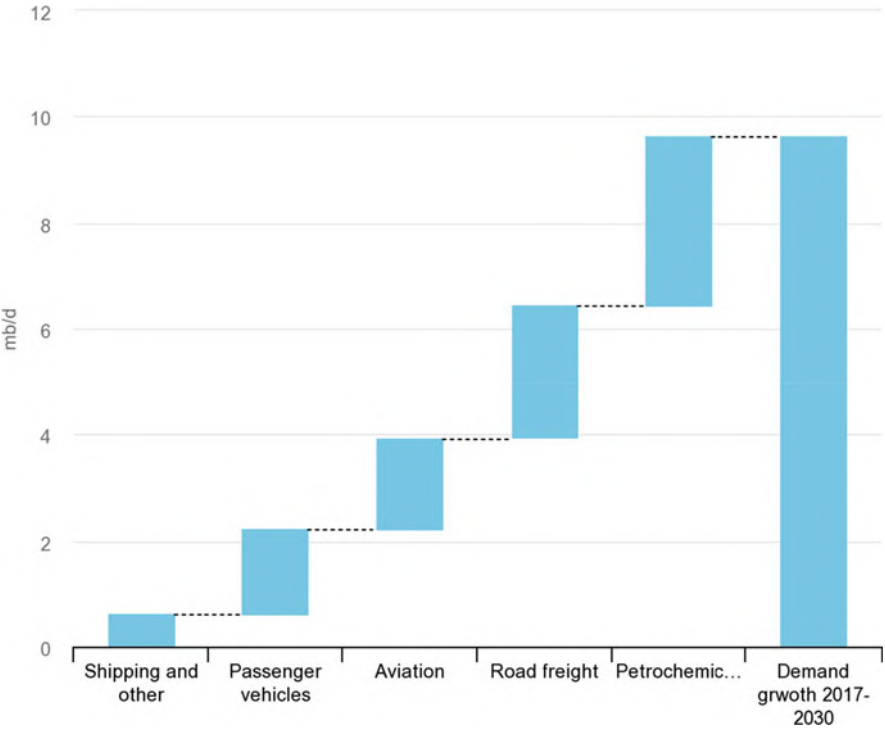

Fig. 8.2 Oil demand growth by sector, 2017–2030. Source: International Energy Agency (2022a). Reproduced with permission from International Energy Agency

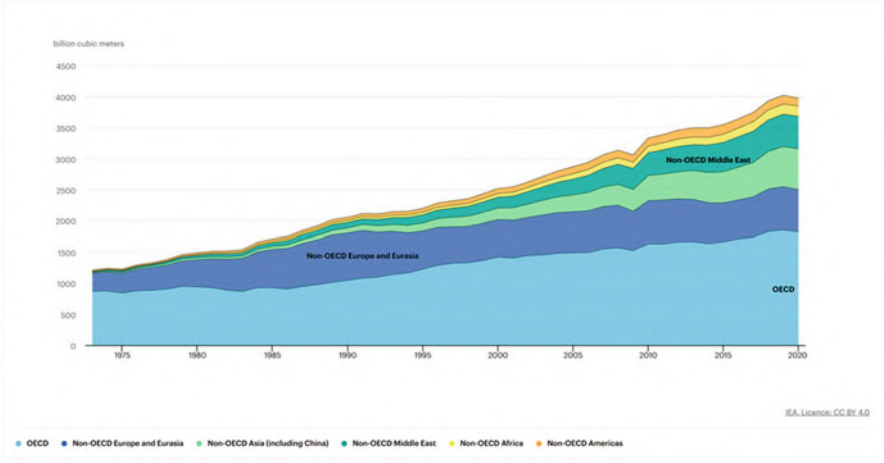

Fig. 8.3 World natural gas demand by region, 1973–2020. Source: International Energy Agency (2022b). Reproduced with permission from International Energy Agency

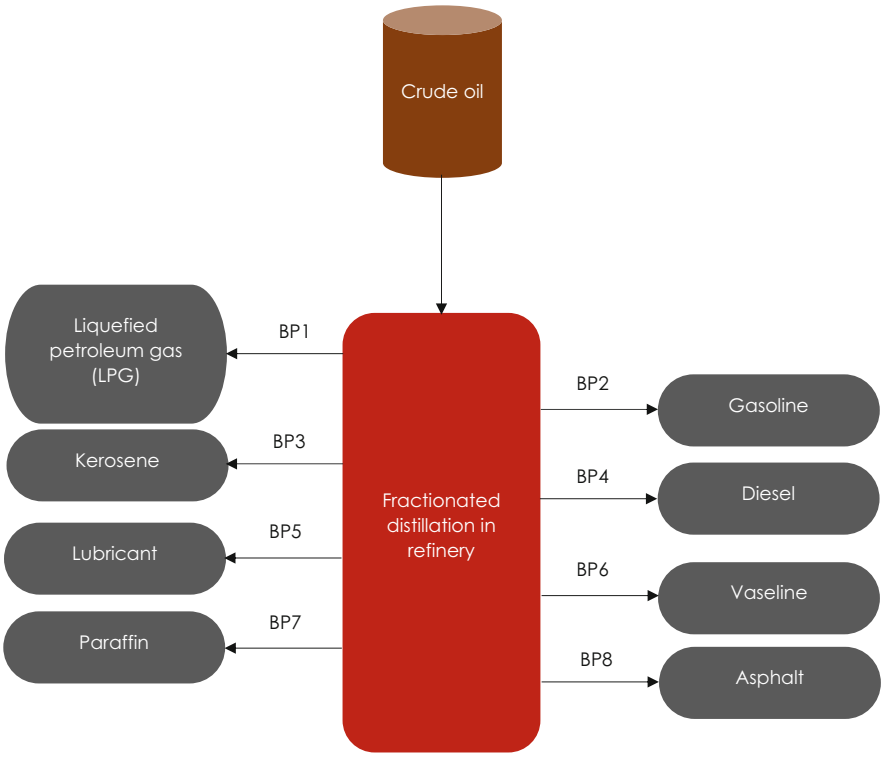

Fig. 8.4 Petrochemical value chains from the crude oil distillation according to the increasing boiling point (from LPG to asphalt). BPn: boiling point (in °C); lower BP, higher volatility and higher vapor pressure

Regarding the global demand for oil and gas, Figs. 8.2 and 8.3 describe it, while Figs. 8.4 and 8.5 depict the detailed petrochemical chains for the derived oil and gas products.

The natural gas—majority composed by methane—is used, mainly, for energy purposes (power generation). This gas is considered a clean option to substitute coal in industrial plants, especially in chemical and petrochemical industry. Additionally, natural gas can be used to produce ammonia—for fertilizers, using the Harber-Bosh process—and methanol by means of steam reforming of the natural gas components.

The demand for natural gas is divided into power (40%), building heating (21%), other industry (15%), others (12%), petrochemicals (8%), and transport (4%) (International Energy Agency 2018).

According the International Energy Agency (2018), already a major component of the global energy system, the importance of petrochemicals—obtained from oil and gas as feedstock—is continuing to grow. Demand for plastics—the most familiar group of petrochemical products—has outpaced that of all other bulk materials (such as steel, aluminum, or cement), and has nearly doubled since 2000.

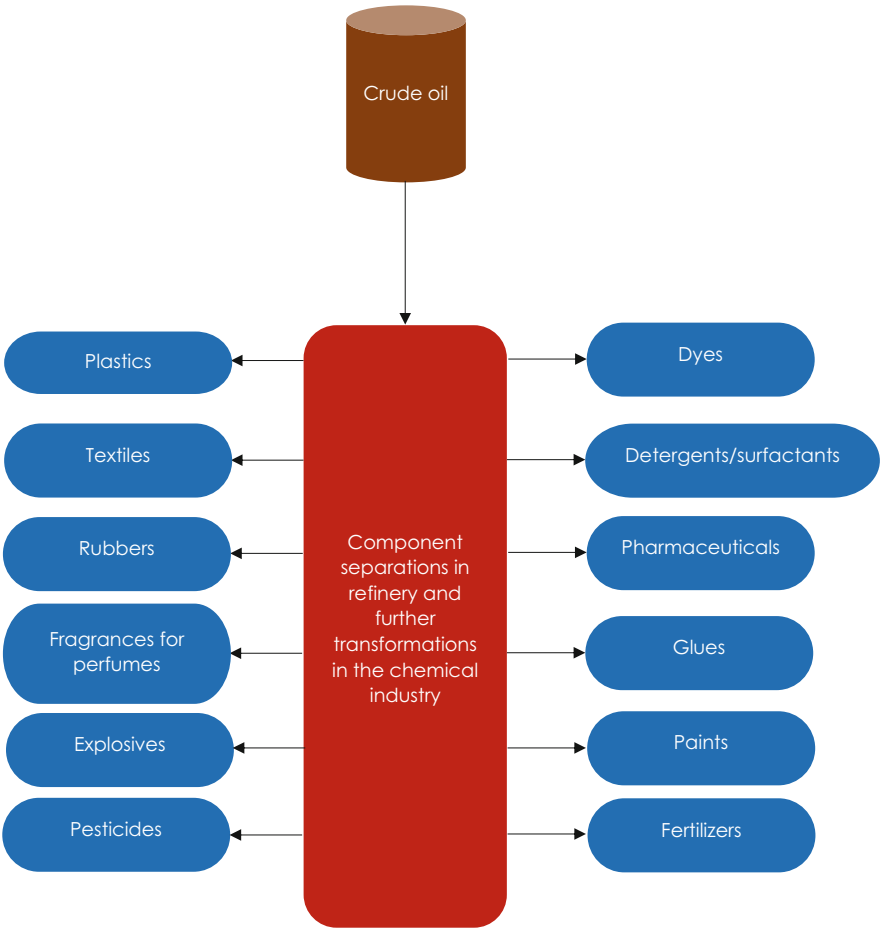

Fig. 8.5 Petrochemical value chain from the crude oil components transformation

Advanced economies, such as the United States and Europe, currently use up to 20 times as much plastic and up to 10 times as much fertilizer as developing economies such as India and Indonesia, on a per capita basis. This underscores huge potential for growth worldwide. More petrochemicals examples are as follows:

- Plastic packaging for food and other commercial products can be made from a range of petrochemical polymers, including polyethylene (PE) and polystyrene (PS).
- Globally, more than a half of ammonia is converted into urea, which is in turn to be used as a fertilizer to increase crop yields and boost food production.
- Synthetic rubber is a major component of tires for cars, trucks, and bicycles, and it is mainly derived from the petrochemical molecule butadiene.

- Many of the laundry detergents and items of clothing in our washing machines are derived from petrochemicals, such as surfactants and polyester fiber.

The growth in demand for petrochemical products means that petrochemicals are set to account for over a third of the growth in oil demand by 2030, and nearly half by 2050, ahead of trucks, aviation, and shipping. Petrochemicals are also poised to consume an additional 56 billion cubic meters of natural gas by 2030, equivalent to about half of Canada's total gas consumption today.

Despite their relevance for the global economy, petrochemicals from oil and gas are a potential source of pollution of ecosystems and contamination of people and animals due to oil exploration, processing, and end use of their derived products.

8.2 How This Industry Works

The oil and gas industry works, primarily, using extraction technologies and their processes to obtain the crude oil and the natural gas from geological reservoirs.

The main unitary operations for oil extraction and industrial usage are as follows:

- Extraction and recovery
- Evaluation, by means of physicochemical analyses, of the recovered material
- Distillation, the most fundamental step in the refining process to produce fractions
- Cracking, corking, hydrocracking, and reforming, to produce several derived molecules—from those previous fractions—using, generally, catalysts
- Treating for impurities removal

And the main unitary operations for natural gas are as follows:

- Inlet gas compression, when required pressure is higher than the pressure in the delivery pipeline
- Dehydration, involving vapor water extraction from the natural gas using dehydrating agents as diethylene glycol or thriethylene glycol
- Gas sweeting, involving the removal of CO_2 and H_2S using, for instance, primary, secondary, and tertiary amines
- Total refrigeration, involving the separation of natural gas liquids from the raw natural gas

Figure 8.6 depicts the flowchart for natural gas production and delivery. And Fig. 8.7 depicts a flowchart for oil exploration in a refinery.

8.3 Main Related Analytical Matrices

The analytical matrices for oil and gas involve gaseous (natural gas and oil fractions), liquid (crude oil and its fractions), and solid physical states (bitumen).

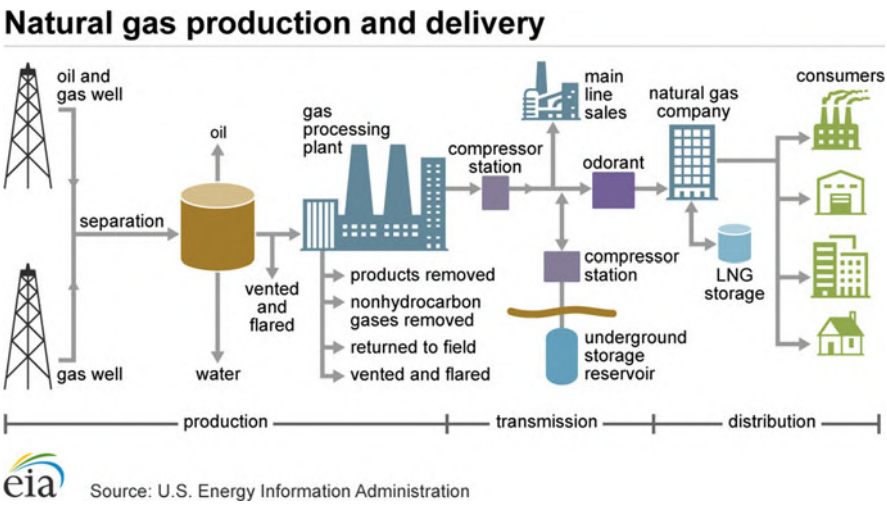

Fig. 8.6 The natural gas production and delivery; *LNG* liquified natural gas. Reproduced with permission from U.S. Energy Information Administration

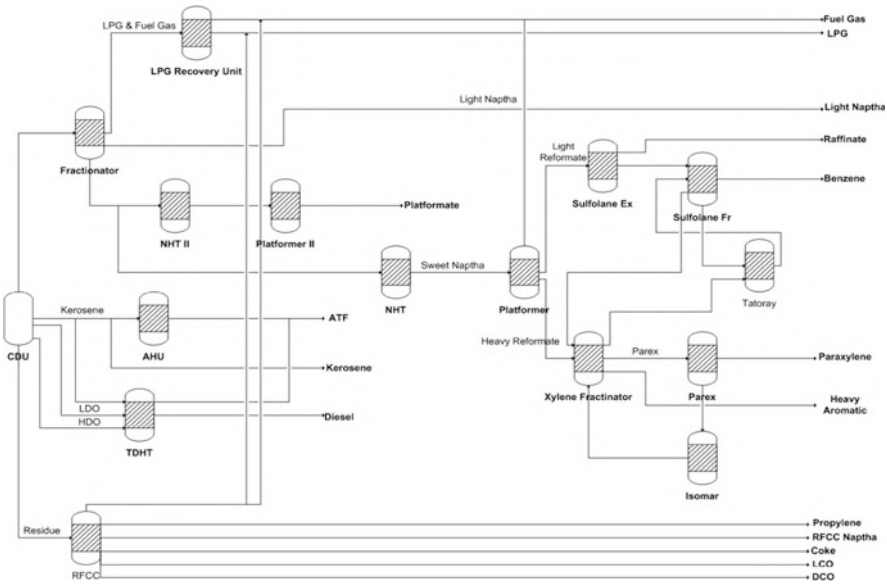

Fig. 8.7 An oil refinery and its processes. Source: Utomo et al. (2020). Reproduced with permission from Springer Nature

After the refining processing of the crude oil, we have majority gaseous and liquids substances. Regarding the natural gas, it can be maintained in the gaseous state or can be liquified for storage, transportation, or retail purposes.

Considering the gas chromatography, the main analytical technique to be applied in the oil and gas industry for quantification, the samples (analytical matrix more analyte) can be easily handled because the analytes are, generally, volatile compounds at room temperature promoting the thermal desorption—for solid and liquid matrices—on their molecules inside the instrument.

Rocks, gaseous moisture, and water with the presence of hydrocarbons are the most common matrices to be analyzed.

8.3.1 Geochemistry and Their Matrices

According to Dembicki-Jr (2017), geochemistry can be defined as the study of the processes that control the abundance, composition, and distribution of chemical compounds and isotopes in geologic environments. It is paramount to evaluate and to explore the geological sources of oil and gas.

Sample matrix has a direct influence on the sample preparation, which is a critical step in the geochemical analysis, and complete sample dissolution is a pre-requisite for obtaining accurate and precise data for geological materials in most studies (Balaram and Subramanyam 2022), by means of elemental analysis and isotopic analysis (to be seen ahead).

For geochemistry rocks, minerals, ores, soils, sediments, fossil fuels (as crude oil and coal), and natural water are the common analytical matrices to be analyzed.

8.4 Main Related Analytical Techniques

The advanced analytical techniques described here can be applied to exploratory and extraction studies, quality control of products and processes, and research and development.

8.4.1 Gas Chromatography

The basis of chromatography is presented in detail in Chap. 4.

In GC the components of a sample are separated as a function of their partition between a gaseous mobile phase, usually the helium gas, and a liquid or solid phase contained within the column. One limitation of GC is when the analyte to be analyzed is not volatile (i.e., it is thermally stable); an alternative is the *derivatization*, when the formation is of another molecule from the analyte with lower boiling values. The elution of the components is done by an inert mobile phase (carrier gas) flow; that is, the mobile phase does not interact with the molecule of analyte.

Table 8.3 Most common GC detectors. Source: Adapted from Vaz Jr (2018). Reproduced with permission from Springer Nature

Detector	LOD
Flame ionization detector (FID)	0.2 pg
Thermal conductivity detector (TCD)	500 pg
Electron capture detector (ECD)	5 fg
Thermal-ionic detector (TID) or nitrogen-phosphorus detector (NPD)	0.1 pg
Mass spectrometer (MS)	<100 pg

1 pg = 10^{-12} g; 1 fg = 10^{-15} g; *LOD* limit of quantification

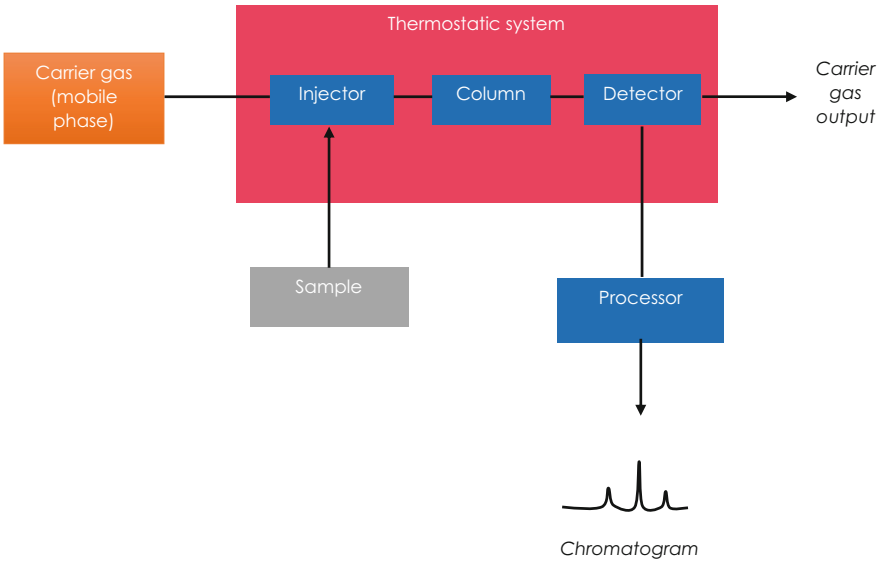

Fig. 8.8 Block diagram of a GC equipment

The modernization of the equipment, through the development of new stationary phases and data processing software, also led to an investment in systems that provide higher speed during the chromatographic analysis. The shortest analysis time has the direct consequence of reducing the cost of the analytical process and increasing the analytical capacity of the laboratory. The increase in the speed of the chromatographic analysis can be related to the reduction of the size of the column, and reduction of its internal diameter, which compensates the loss of resolution in the determinations.

Regarding the choice of the most suitable detector to be used, the nature of the sample (analytical matrix more analyte) should be considered. Several detectors are commercially available for use in GC, with thermal conductivity (TCD), flame ionization (FID), electron capture (ECD), and mass spectrometer (MS) detectors

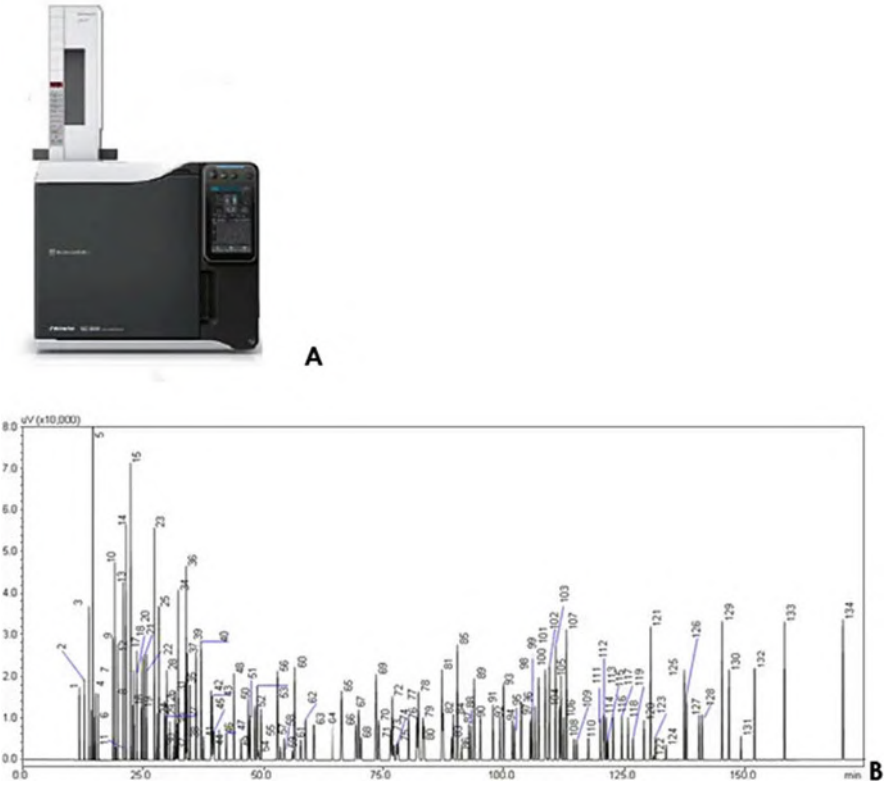

Fig. 8.9 A GC equipment (**a**), and an obtained DHA (detailed hydrocarbon analysis) chromatogram (**b**). Courtesy of Shimadzu

being most commonly used. An ideal detector should meet the following characteristics:

- Adequate sensitivity
- Good stability and reproducibility
- Linear response to analytes, extending to several orders of magnitude
- Temperature range from ambient to at least 400 °C
- Ease of use
- Similarity of response to all analytes in the sample

In practice, the detectors rarely meet all the features described above. Table 8.3 shows the most common detectors used in GC and their limit of detection (LOD).

Figure 8.8 describes a block diagram for a GC equipment. And Fig. 8.9 depicts the equipment.

8.4.2 Mass Spectrometry

MS is essentially a technique for detecting molecular components having the mass/charge ratio (m/z) as the unit of measurement, which are obtained by means of the original molecule fragmentation into derived chemical species (e.g., the molecular ion M^+). Depending on the ionization technique used, analytes may present with one or multiple charges. In single-charge components, the m/z ratio corresponds to the total mass of the ion in Daltons. In cases where ions with two or more charges are more frequent, the calculation of the original ion mass will depend on deconvolutions of the original signal.

Regarding the fragmentation, it is based on the removal of the electron from the molecule resulting in its ionization. Removal of electrons from either *sigma* bond, *pi* bond, or nonbonding orbitals causes the ionization. The fragmentation pattern promotes the distinction among the analytes.

The direct analysis of the sample in the mass spectrometer seldom generates results that can be considered quantitatively, even if the sample is pure. This is a consequence of the high sensitivity of the technique and the efficiency of the ionization process, besides the intrinsic characteristics of each sample that allow greater or less easiness of ionization.

MS is often associated with a separation technique, usually gas chromatography or liquid chromatography—or the *hyphenated techniques*—where a separation technique coupled to a detection and quantification technique is used. In this case, the mass spectrometer functions as a detector. Such hyphenated techniques make it possible to separate complex mixtures, identify the components, and quantify them in a single operation. Almost all measurements of MS are done under high vacuum, as this allows the conversion of most of the molecules into ions, with a lifetime enough to allow their measurement. The mass spectrometer consists essentially of three components: ionization source, mass analyzer, and ion detector.

There are several commercially available ionization systems: electron impact ionization (EI), chemical ionization (CI), fast atom bombardment (FAB), particle beam bombardment (PBB), matrix-assisted laser desorption ionization (MALDI), electrospray ionization (ESI), atmospheric pressure photoionization (API), and atmospheric pressure chemical ionization (APCI). For high molecular weight, non-volatile and heat-sensitive materials, MALDI, APCI, and FAB techniques are used. The most common analyzers are quadrupole, quadrupole ion trap, and time-of-flight tube. The detection is done by electron multiplier tube.

The high-resolution MS provides comprehensive accurate mass information in a single analysis by MS^n technology (tandem mode); detects more low-level components in complex samples; and is designed and well suited for large molecule analysis (Vaz Jr 2021). However, the main limitation is that a large volume of data to process requires an experienced analyst to operate.

The concept of *petroleomics* contemplates the ultimate characterization of all the chemical constituents of petroleum (crude oil and natural gas), along with their interactions and reactivity (Marshall and Rodgers 2004). The application of this

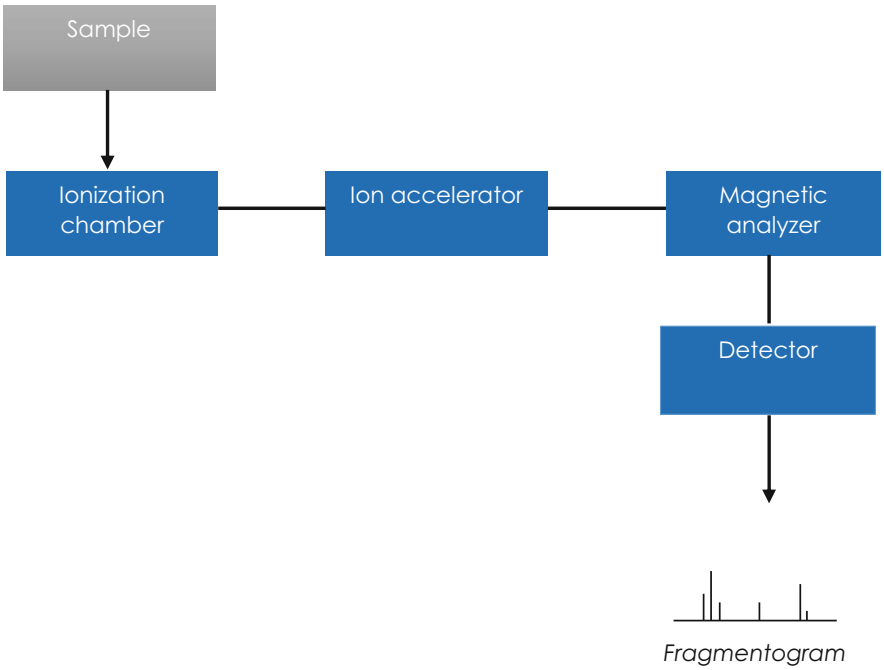

Fig. 8.10 Block diagram of a mass spectrometer

concept as an analytical approach demands the extensive use of MS techniques—e.g., ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry and high-field mega orbitrap Fourier transform mass spectrometry (Schmidt et al. 2018)—in order to determine the variability of the elemental composition of samples of crude oil.

Figure 8.10 depicts a block diagram of a MS instrument, and Fig. 8.11, the instrument.

8.4.3 Atomic Emission Spectrometry or Optical Emission Spectrometry

Atomic emission spectrometry (AES), or optical emission spectrometry (OES), is based on the measurement of the emission of the electromagnetic radiation in the ultraviolet-visible (UV-Vis) region by neutral and ionized atoms, not in excited state, being widely used in elemental analysis. The most common OES system uses an argon plasma torch that can reach up to 9000 K (as the hyphenated inductively coupled plasma, ICP) for the electrons excitation in gaseous state. ICP can also be

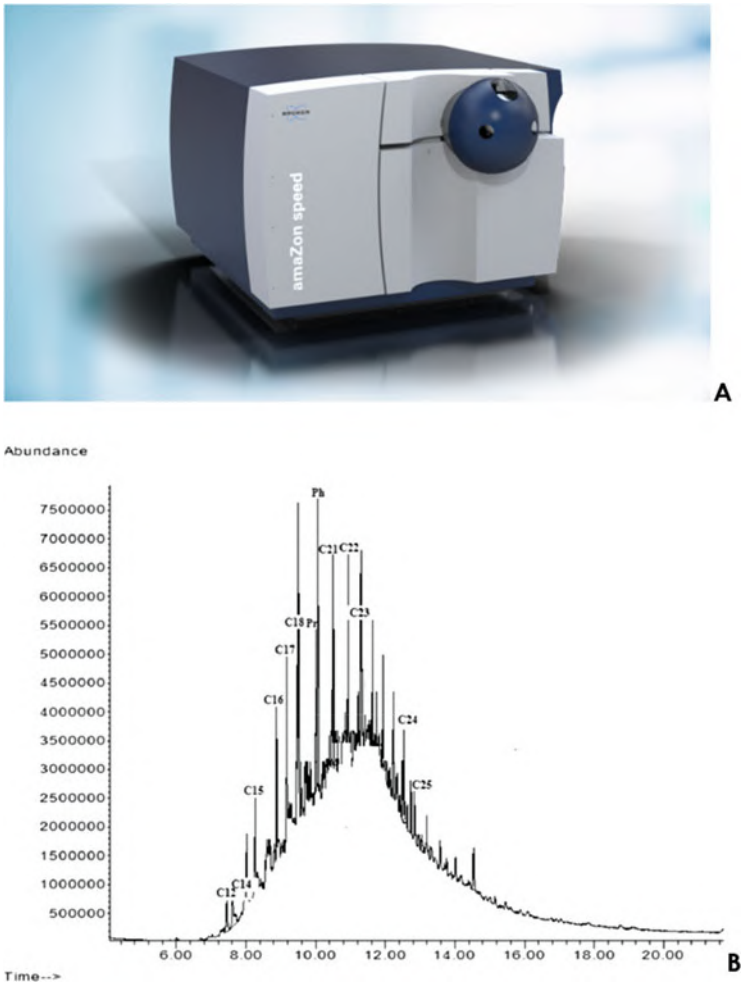

Fig. 8.11 An ion trap mass spectrometer (**a**), and the obtained fragmentogram (**b**). Courtesy of Brüker

coupled to a quadrupole mass analyzer (ICP-OES-MS); it offers extremely high sensitivity to a wide range of elements.

The technique has high stability, sensitivity, low noise—S/N or signal/noise ratio—and low background emission intensity. However, it involves relatively expensive methods that require extensive operator training. All metals or non-metals of interest for oil analysis can be determined by OES—favoring, for some elements, the achievement of lower values of limit of detection (LOD) and limit of quantification (LOQ).

For AES/OES we measure the excited-state population by means of the Maxwell-Boltzman expression (Eq. 8.1):

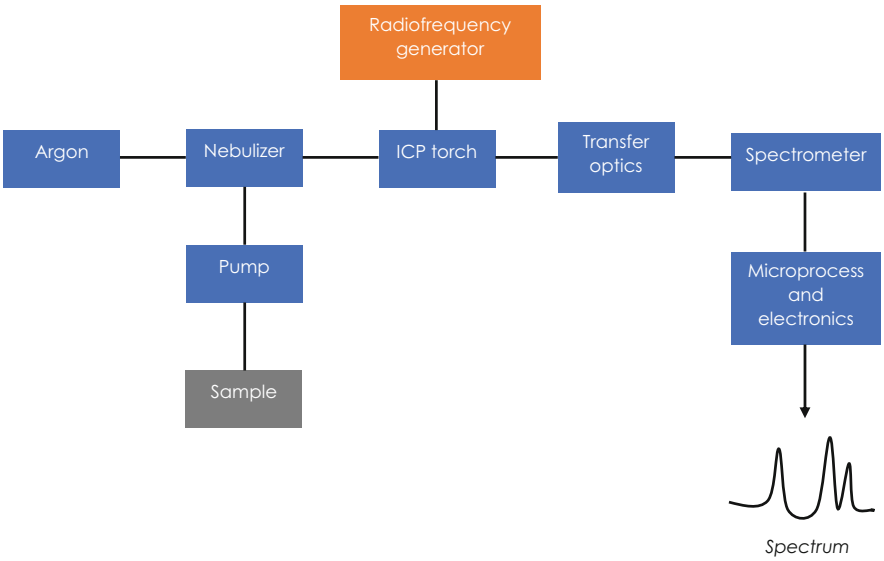

Fig. 8.12 Block diagram for an ICP-OES (or ICP-AES) instrument

$$N_e/N_0 = (g_e/g_0)e^{-(E_e - E_0)Kt} \tag{8.1}$$

Where N_e is the relative population of the excited state, N_0 the relative population in the ground state (measured in atomic absorption spectrometry), g_e and g_0 are the statistical weights of the excited and ground states, respectively; E_0 is the energy in the ground state; E_e is the energy in the excited state k is the Boltzmann constant; and t the absolute temperature.

According the EAG Laboratories (2023), advantages of OES are as follows: bulk chemical analysis technique that can determine simultaneously up to 70 elements in a single sample analysis; the linear dynamic range is over several orders of magnitude; instrumentation is suitable to automation, thus enhancing accuracy, precision, and throughput. Limitations are as follows: the emission spectra are complex and inter-element interferences are possible if the wavelength of the element of interest is very close to that of another element; in MS mode, determination and quantification of certain elements can be affected by interference from polyatomic species, matrix elements, and atmospheric elements; the sample to be analyzed must be completely digested, or dissolved prior to analysis in order to determine the element(s) of interest.

The use of microwave plasma (MP) coupled to AES/OES is very useful for the multi-elemental determination of metallic contaminants directly in the oil sample. It permits several advantages such as smaller footprint, multi-element capability, relatively inexpensive, low maintenance cost, good detection power, and speed for routine analysis (Poirier et al. 2017).

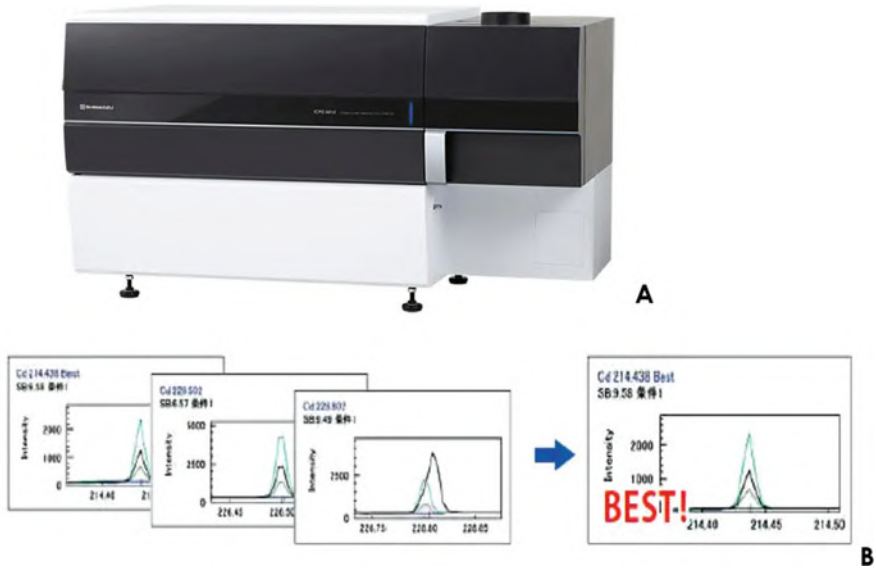

Fig. 8.13 An ICP-OES (or ICP-AES) instrument (**a**), and the obtained spectra (**b**). Courtesy of Shimadzu

Figure 8.12 depicts a block diagram for an ICP-OES instrument, and Fig. 8.13, the instrument.

8.4.4 X-ray Atomic Emission or Fluorescence Spectrometry

This technique allows a rapid and non-destructive multi-element analysis for solid and liquid samples (identification and quantification). When an atom is excited by the removal of an electron from its inner layer, it emits X-rays (from 10^{-12} to 10^{-8} m) when returning to its ground state; such radiation has a typical signal intensity for each element, which is used in the analysis. Here, we can use the Lambert-Beer law (Eq. 8.2) to operationalize the physical phenomenon.

$$A = \varepsilon bc \tag{8.2}$$

Where A is the absorption in arbitrary unities, ε is the molar absorptivity (cm^{-1} L mol^{-1}), b is the optical way or cell wavelength (cm), an c the molar concentration (mol L^{-1}).

There are two X-ray fluorescence (XRF) systems available: the wavelength dispersive spectrometer (WD-XRF) and the energy dispersive spectrometer (ED-XRF)—the latter has higher signal throughput, which enables small area analysis or mapping.

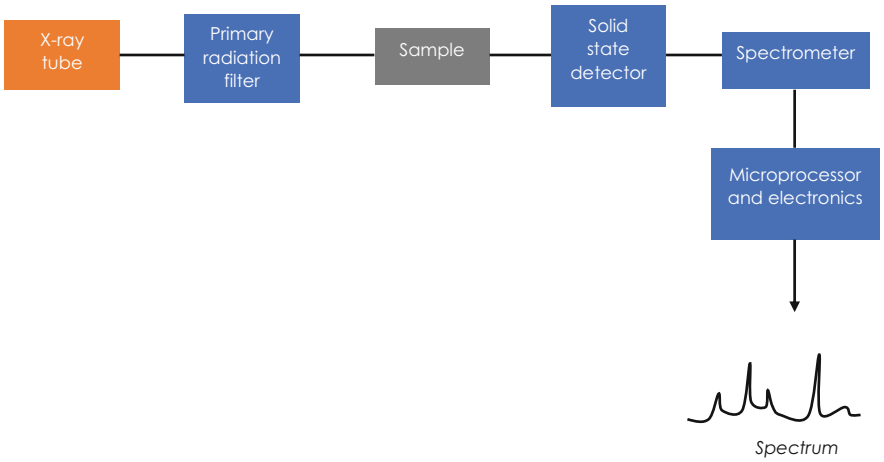

Fig. 8.14 Block diagram of an XRF instrument

According to the EAG Laboratories (2023), advantages of XRF are as follows: non-destructive technique; can analyze areas as small as ~150 μm; can analyze any solid material; and sampling depth ranging from a few micrometers to several millimeters depending on the material. Limitations are as follows: cannot detect elements lighter than aluminum using small spot ED-XRF; and highest accuracy measurements require reference standards similar in composition and/or thickness to the test sample.

The Fig. 8.14 presents a block diagram for an XRF instrument, and Fig. 8.15, the instrument.

8.4.5 Isotopic Analysis

Isotopes are nuclides which have the same atomic number but different mass numbers. Gaseous stable isotopes are used to help understand the formation mechanisms and compositional evolution of gas accumulations. This provides necessary information for petroleum system modeling and reservoir management enabling accurate exploration and production development planning (Airliquid 2023). For instance, for gas components such as H_2S, N_2, N_2O, CO, H_2, and CO_2 we can have $\delta^{34}S$, $\delta^{15}N$, $\delta^{13}C$, $\delta^{18}O$, and $\delta^{2}H$ isotope ratios (e.g., $\delta\ ^{13}C/\delta^{12}C$).

In order to standardize the isotope species for a certain chemical element, the Union of Pure and Applied Chemistry (2023) published a periodic table with the isotopic abundances for several atoms. It is especially useful in the observation of the variation in atomic weight with isotopic composition. For instance, for carbon atom in crude oil, the mole fraction of ^{13}C is between approximately 0.0105 and

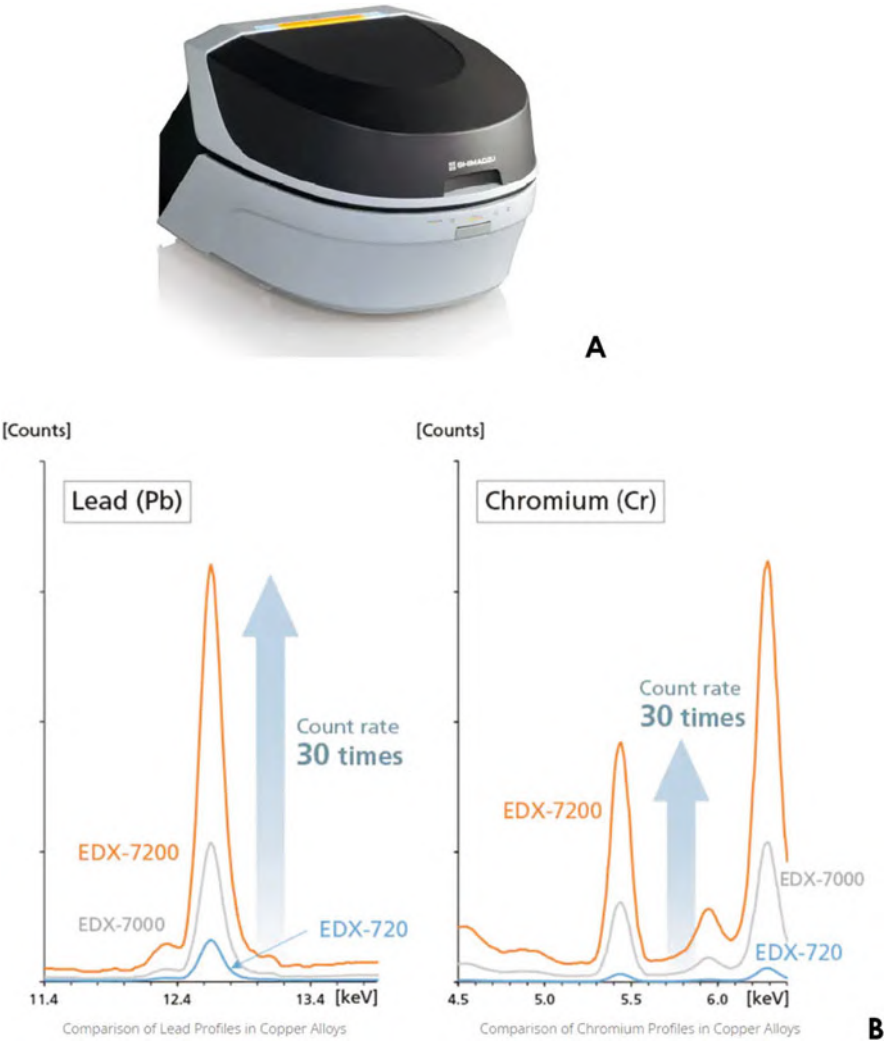

Fig. 8.15 An ED-XRF instrument (**a**), and the obtained spectra (**b**). Courtesy of Shimadzu

0.0110; and the atomic weight of the atom is between approximately 12.0105 and 12.0110.

Regarding the analytical instrumentation, the utilization of isotopes in petroleum exploration has more recently evolved due to the development of the gas chromatograph-isotopic ratios mass spectrometer (GCIRMS) system (EPCM 2023).

Carbon and hydrogen atoms are of special interest. The sample is injected into the GC, where compounds are separated on a capillary column and then converted to CO_2 in the reactor, using a source of O_2 and a catalyst. The CO_2 gas is then transferred into the mass spectrometer, where it is ionized. In carbon isotopes, the

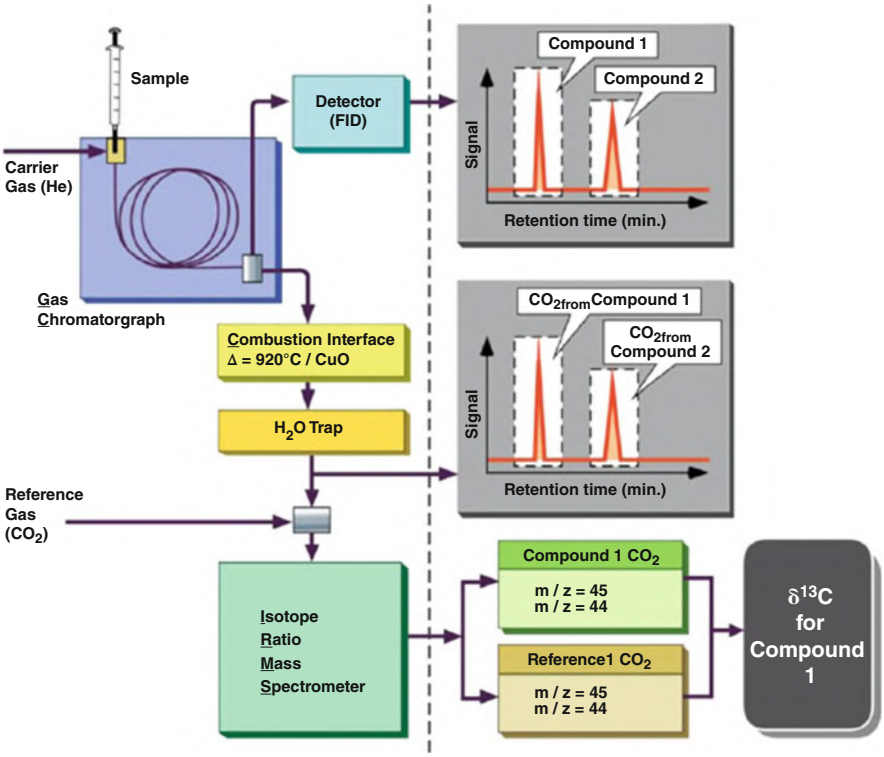

Fig. 8.16 A flowchart representation of a GCIRMS system for continuous flow determination of isotopic compositions of individual compounds. Source: Philp and Monaco (2012). Reproduced with permission from Springer Nature

$\delta^{13}C$ values of individual compounds are calculated relative to either a reference gas or a co-injected compound with a known isotopic $\delta^{13}C$ value. $\delta^{2}H$ measurements require a pyrolysis reactor to generate H_2 gas; organic compounds are carried from the GC column through a high-temperature conversion reactor; reduced H_2 gas is then transferred to the mass spectrometer.

Figure 8.16 depicts a block diagram of a GCIRMS equipment, while Fig. 8.17 depicts this instrument.

8.5 Commented Examples of Applications

Table 8.4 depicts examples of application of advanced analytical techniques for exploration, quality control, and research and development for oil and gas industry.

The detailed methodology for each example can be accessed directly in the reference citation.

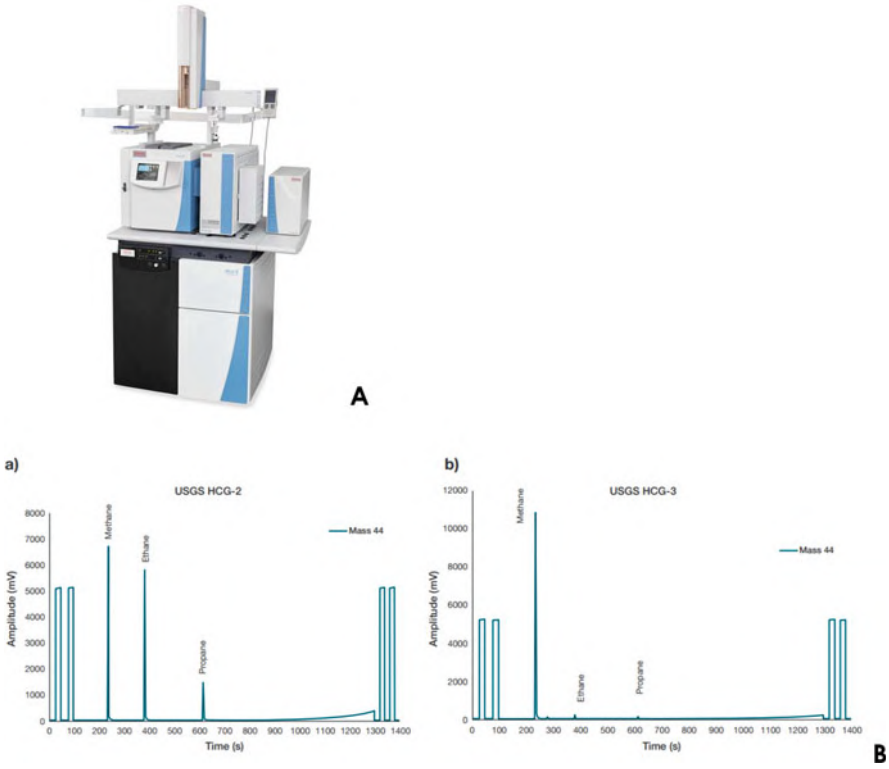

Fig. 8.17 A GCIRMS equipment (**a**), and the obtained chromatograms for natural gas samples (**b**). Courtesy of Thermo Fischer Scientific

Additionally, the ASTM International (2023) has a set of standards dedicated to petroleum and derived products, as lubricants, greases, and fuels. These standard methods are most frequently applied to quality control.

8.6 Conclusions

Oil and gas industry is a versatile branch of global economy, involving crude oil and natural gas extraction, processing, and usage.

In order to conduct the quality control and the research and development experiments, the use of advanced analytical techniques is paramount. And for both purposes, cited analytical techniques as GC, MS, AEOS/OES, XRF, and isotopic analysis can be explored for several analytical matrices in solid, liquid, and gaseous physical states.

These techniques can be applied from the quantification and characterization of the hydrocarbons to the determination of the presence of contaminants, as metals.

Table 8.4 Application of advanced analytical techniques for oil and gas industry

Analytical technique	Application	Brief method description	Reference
GC	Analysis of light olefins and aromatics after catalytic pyrolysis of heavy crude oil	Py-GC/MS analyses carried out using a pyroprobe pyrolyzer with a direct connection to a gas chromatograph/mass spectrometer	Niwamanya et al. (2022)
MS	Petroleomics (characterization of non-volatile polar compounds from Brazilian oils)	An FT-ICR mass spectrometer with an ICR cell. Prior to the acquisition of ESI(±)-FT-ICR mass spectra, experimental evaluations were performed of acquisition parameters such as concentration, TOF, ion accumulation time, skimmer, and collision voltage	Vanini et al. (2020)
AES/OES	Determination of metals (V, Ni, Fe, Na, K, Ca, Mo) in petroleum fractions	Direct injection of *o*-xylene solutions in an MP-AES with a nitrogen generator. On the MP-AES, a nebulizer and glass double-pass cyclonic spray chamber were used for the sample introduction system	Poirier et al. (2017)
XRF	Determination of chloride content in crude oil	Standard test method for chloride in aromatics, according to ASTM D7536, using a monochromatic wavelength dispersive X-ray fluorescence spectrometry	Katona et al. (2021)
Isotopic analysis	Geochemical characterization of presalt natural gas	A mass spectrometer coupled to a GC system. The temperature of the column oven was initially set at 30 °C, then heating to 80 °C at a rate of 8 °C min^{-1}, and eventually to 250 °C at a rate of 5 °C min^{-1}, which was held for 10 min. The results were measured relative to the Vienna Pee Dee Belemnite standard ($\delta^{13}C_{VPDB} = 0\%$)	Yang et al. (2022)

GC gas chromatography, *Py-GC/MS* pyrolysis-gas chromatography/mass spectrometry, *MS* mass spectrometry, *FT-ICR* Fourier transform-ion cyclotron resonance, *ICR* ion cyclotron resonance, *FT* Fourier transform, *TOF* time-of-flight, *MP-AES* microwave plasma-atomic emission spectrometry, *ESI* electrospray ionization

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Chapter 9
Environmental Issues of Industry

Abstract Environmental issues are a hotspot for the modern industry dedicated to pursuing more sustainable products and processes. They are imposed by environmental law (e.g., analytical parameters to control effluent emissions from processing) and by the market as a differential for investments and to gain market competition (e.g., net-zero carbon emission). Thus, environmental analyses are the ways to control and to monitor the emission of pollutants in the environment by the industrial sectors. Advanced analytical techniques as LC, GC, AAS, and AES/OES to supply quantitative information about the pollutant in aqueous and gaseous effluents are discussed in this chapter. Besides, size distribution analysis to determine the size of the particulate matter and LIBS, to supply quantitative information for carbon footprint, is discussed also. Issues related to analytical matrices and sample preparation are also addressed.

Keywords Liquid effluents · Gaseous effluents · Carbon footprint · Sustainability · Advanced analytical techniques · Standard methods

9.1 Introduction

Environmental issues are a hotspot for the modern industry dedicated to pursue more sustainable products and processes. They are imposed by environmental law (e.g., analytical parameters to control effluent emissions from processing) and by the market as a differential for investments and to gain market competition (e.g., net-zero carbon emission).

From those industrial sectors considered in this book, i.e., agrochemicals and pharmaceuticals, ores and mining, polymers, biotechnology, and oil and gas, all of them have processes that are industrial effluent emitters, considering the industrial effluent as a residue of the processing steps. It is worth to mention that these effluents are made up of several forms/species of chemical and biological constituents that could be detrimental, not only to the environment but to biodiversity resources (Izah et al. 2022).

Table 9.1 describes examples of industrial effluents according to the industrial sector previously treated in the book.

S. Vaz Jr, *Applications of Analytical Chemistry in Industry*,
https://doi.org/10.1007/978-3-031-38952-8_9

Table 9.1 Industrial effluents generated by several industrial sectors

Industrial sector	Example of industrial effluent	Physical medium
Agrochemicals and pharmaceuticals	Wastewater containing several AIs and APIs	Liquid (aqueous)
Ores and mining	Wastewater containing metals, AMD	Liquid (aqueous)
Polymers	Wastewater containing several additives	Liquid (aqueous)
Biotechnology	Wastewater containing microorganisms, enzymes, and/or chemicals	Liquid (aqueous)
Oil & gas	Wastewater containing hydrocarbons (e.g., BTEX, TPHs, and PAHs)	Liquid (aqueous)
Oil & gas	Hydrocarbon vapors and particulate matter	Gaseous

AMD acid mine drainage, *AIs* active ingredients, *APIs* active pharmaceutical ingredients, *BTEX* benzene, toluene, ethylbenzene, xylenes, *PAHs* polyaromatic hydrocarbons, *TPHs* total petroleum hydrocarbons

From Table 9.1 content we can observe that the liquid or aqueous effluents are the most predominant for the industrial sectors discussed in the book. However, gaseous effluents are observed, as expected, for oil and gas industry. Furthermore, the majority of the residues present in the aqueous effluents can be considered *emerging pollutants*, that are synthetic or naturally occurring chemical or microorganism that is not commonly monitored or regulated in the environment potentially known or suspected to cause adverse ecological and human health effects (Vaz Jr 2018).

Besides the control and monitoring of industrial effluents, the carbon footprint is a very important parameter to be evaluated, by means of a holistic view, to determine the sustainability of industrial processes and products. The quantity of emitted and captured greenhouse gases (GHG), i.e., carbon dioxide and methane, is a factor to be considered both for products and processes as a balance of mass to estimate the *sustainability*—composed of environmental, economic, and social impacts.

According to Fullana et al. (2009), carbon footprint (CF) is a method used to quantify the amount of GHG emissions associated with a company (corporate carbon footprint, CCF) or with the life cycle of an activity or a product/service (product carbon footprint, PCF) in order to determine its contribution to climate change. It is a characteristic explored to reach high percentages of market share. CF is part of the methodology to estimate the life cycle assessment of products and processes (Vaz Jr 2022).

Figure 9.1 depicts the general methodology for the CF calculation based on the ISO Norm 14040: 2006 (International Organization for Standardization 2006).

Regarding the carbon neutrality—or net-zero carbon emissions—obtained by means the CF, some authors suggest a certain prudence to consider it for processing and final products. For instance, for plastics Zheng and Suh (2019) calculated that the global life-cycle GHG emissions of conventional plastics were 1.7 Gt of

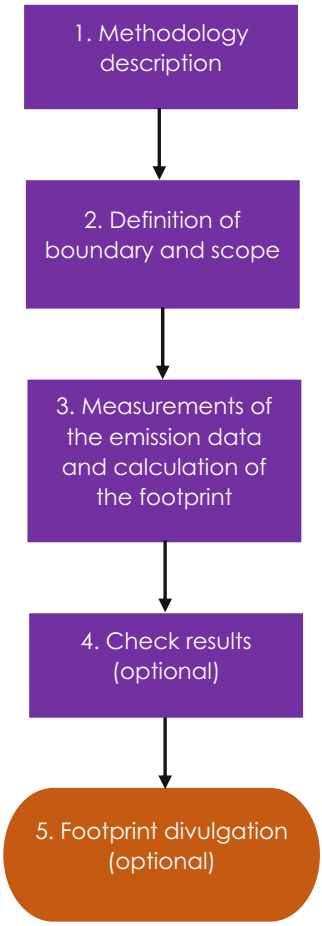

Fig. 9.1 Sequential steps involved in the carbon footprint calculation according to the ISO Norm 14040: 2006. Source: Adapted from Vaz Jr (2022). Reproduced with permission from Elsevier

CO_2-equivalent (CO_2eq) in 2015, which would grow to 6.5 $GtCO_2$eq by 2050 under the current trajectory of their production and use.

Regarding the official regulation, the main agencies to be highlighted worldwide are the U.S. Environmental Protection Agency[1] and the European Environment Agency.[2] Both agencies regulate the environmental aspects of several industrial sectors according to the environmental law for each country/region, establishing analytical parameters and their values to be analyzed and obeyed, respectively.

[1] https://www.epa.gov/

[2] https://www.eea.europa.eu/

9.2 Main Related Analytical Matrices

The analytical matrices of interest for environmental purposes can be considered according to the family of analytes, as described below.

- For liquid effluents: liquid water is the main matrix with, in some cases, the observation of a physical medium of organic solvent.
- For gaseous effluents: generally, a gaseous mixture is the matrix, with the presence of organic vapors, water vapor, and inorganic gases; for particulate matter—which is a sum of solid and liquid particles suspended in the air—it can comprise organic and inorganic particles, such as dust, pollen, soot, smoke, and liquid droplets (U.S. Environmental Protection Agency 2023a).
- For carbon footprint: it can comprise air of the atmosphere, water, and soil.

The sample preparation for liquid and gaseous effluents must follow the same orientation as those presented for the respective industrial sector in the previous chapters.

9.3 Main Related Analytical Techniques

The advanced analytical techniques to be used for the chemical analysis of industrial effluent analysis generally is the same applied to the quality control and research and development due to the expected same physicochemical properties and physical characteristics of the analytes, with an especial interest on quantification to satisfy regulatory aspects. Thereby, in this chapter we will not scrutinize the analytical techniques for industrial effluents because they were discussed in the chapter dedicated to analytics for each industrial sector, except for the carbon footprint.

Table 9.2 describes the advanced analytical techniques according to the industrial sector, effluent, and chemical residue. In order to obtain detailed information about each technique the reader should consult the respective chapter dedicated to the respective industrial sector.

For the carbon footprint we can use LIBS (LASER[3]-induced breakdown spectroscopy) technology, the same technique that the North American Space Agency (NASA) used in robots to evaluate the soil of Planet Mars. It can be certified and used in global carbon market programs.

LIBS is a fast, reproducible and clean spectroscopic analytical technique for elemental analysis. It uses high-energy LASER pulses to create a plasm on the surface of the sample, and thus determine its chemical composition. As it is a non-destructive analytical technique, it can be applied to a wide variety of samples in different physical states of matter, such as solid, liquid, and gases.

[3] *LASER* light amplification by stimulated emission of radiation.

Table 9.2 Advanced analytical techniques applied to industrial effluents generated by several industrial sectors

Industrial sector	Example of industrial effluent	Examples of chemical residues present in the effluent	Analytical techniques to be applied
Agrochemicals and pharmaceuticals	Wastewater containing several AIs and APIs	Pesticides (e.g., herbicides, fungicides, insecticides, etc.),	Hyphenated LC (majority) and GC
Ores and mining	Wastewater containing metals, AMD	Almost all metals of the Periodic Table	AAS, AES/OES
Polymers	Wastewater containing several additives	Organic (e.g., starch, organic acids, organophosphates, phenols, etc.) and inorganic (e.g., carbonates, metal oxides, silica, etc.)	Hyphenated LC and GC, AAS, AES/OES
Biotechnology	Wastewater containing microorganisms, enzymes, and/or chemicals	Organic acids, alcohols, esters, several enzymes, bacteria, fungi, and yeast	Hyphenated LC and GC, biological analysis*
Oil & gas	Wastewater containing hydrocarbons (e.g., BTEX, TPHs, and PAHs)	Benzene and its homologues, naphthalene, and its homologues	Hyphenated GC
Oil & gas	Hydrocarbon vapors, and particulate matter	Benzene and its homologues, naphthalene, and its homologues, several inorganic or organic particles	Hyphenated GC, particle size distribution

AMD acid mine drainage, *AIs* active ingredients, *APIs* active pharmaceutical ingredients, *BTEX* benzene, toluene, ethylbenzene, xylenes, *PAHs* polyaromatic hydrocarbons, *TPHs* total petroleum hydrocarbons, *LC* liquid chromatography, *GC* gas chromatography, *AAS* atomic absorption spectrometry, *AES/OES* atomic emission spectrometry/optical emission spectrometry. *As this book is not dedicated to the biological and microbiological analysis, it is worth to indicate as reference book on these themes the Standard Methods for the Examination of Water and Wastewater (2022)

According to the equipment composition, LIBS requires a laser pulse to be focused on the sample of interest to ablate a small amount of material (usually a fraction of a microgram or less) and to create a plasma from the vaporized mass. This LASER-induced plasma has a typical lifetime of a few microseconds and a temperature in the range of 10,000 K, allowing the excitation of the vast majority of elements, as well as some radicals formed by recombination during the plasma cooling (Gardette et al. 2023).

Furthermore, near- and mid-infrared absorption spectroscopies (NIR and MIR) can be used associated to LIBS to increase the analytical applicability—these techniques were discussed in detail in previous chapters, as Chap. 4.

Table 9.3 Application of advanced analytical techniques for chemical analysis of industrial effluents

Analytical technique	Application	Brief method description	Reference
LC	Antibiotic pollutants (i.e., sulfadiazine, sulfamethazine, trimethoprim, and azithromycin) in aqueous effluent from a pharmaceutical plant	Use of PLE for sample pre-treatment associated to LC-MS/MS	Milaković et al. (2020)
GC	Hydrocarbon pollutants in aqueous effluent from an oil & gas refinery	Liquid-liquid extraction associated to GC-FID and GC-MS	Eldos et al. (2022)
AAS	Metallic pollutants in aqueous effluent from gold mining activities	Samples of refractory gold ore were digested in aqua regia and HNO_3 to determine their elemental composition by AAS	Espitia and Lapidus (2021)
AES/OES	Metallic pollutants in aqueous effluent from mining activities (abandoned Zn and Pb mine)	Samples were collected and filtered through a 1.2 μm pore diameter glass microfiber filter; after that, they were analyzed by MP-AES	Vendrell-Puigmitja et al. (2020)
Particle size distribution	Particulate matter type $PM_{2.5}$ (2.5 μm of diameter) from natural gas use in turbines	Microfiltration/cyclone combination associated to TEM for analysis	Brewer et al. (2016)

LC liquid chromatography, *PLE* pressurized solvent extraction, *LC-MS/MS* liquid chromatography-mass spectrometry/mass spectrometry, *GC-FID* gas chromatography-mass spectrometry, *GC-MS* gas chromatography-mass spectrometry, *AAS* atomic absorption spectrometry, *AES/OES* atomic emission spectrometry/optical emission spectrometry, *MP-AES* microwave plasma-atomic emission spectrometry, *TEM* transmission electron microscopy

9.4 Commented Examples of Applications

Table 9.3 describes examples of application of advanced analytical techniques for several industrial effluents to comply with environmental regulations. Generally, these techniques are used to monitor the effluent emission and the effluent treatment.

The detailed method for those examples presented in Table 9.3 can be accessed in the cited reference.

The U.S. Environmental Protection Agency is a very useful source of internationally recognized standard methodology for environmental pollutants. The SW-846 method compendium (U.S. Environmental Protection Agency 2023b) is one of the most used in the world for environmental analysis.

For carbon footprint analysis, we can determine, for instance, the carbon retention in soil for agricultural production chains and systems related to agrochemicals, as fertilizers. Stenio et al. (2022) applied mathematical model to quantify the total carbon in soil samples having different textures bypassing spectral interferences. A LIBS-specific method for removing outliers has been developed with 6% spectrum removal. Three repetitions were used to test the robustness of the methods and

presented an R^2 of 0.95 and 0.93, a mean error of about 20.38% and 24.12% for lines 193.03 and 247.85 nm, respectively, and a root mean square error of prediction lower than 0.40% for both lines.

9.5 Conclusions

Environmental analyses are paramount to control and to monitor the emission of pollutants in the environment by the industrial sectors.

Advanced analytical techniques as LC, GC, AAS, and AES/OES can supply mainly quantitative information about the pollutant in aqueous and gaseous effluent; while size distribution analysis can determine the size of the particulate matter. Furthermore, LIBS can supply quantitative information about carbon species for carbon footprint for studies related to sustainability aspects for products and processes.

These techniques can be better explored considering issues related to matrix and sample preparation according to standard methods.

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Chapter 10
Book Remarks and Conclusions

Abstract Remarks and conclusions are approaches used to facilitate the book's understanding and its applicability. Remarks cover the most representative statements, while conclusions summarize the performed discussions. Regarding the remarks, we saw from Chap. 1 to Chap. 3 the basis and terminological aspects of analytical chemistry, especially for industry; from Chap. 4 to Chap. 9 we saw the scientific and technical aspects of analytical techniques for different industrial sectors i.e., pharmaceuticals and agrochemicals, ores and mining, polymers, biotechnology, oil and gas, and environmental issues. Regarding the conclusions, we concluded from Chap. 1 to Chap. 3 that the relevance of analytical chemistry to the modern society is recognized, because it can provide information to the industry related to characterization, identification, and determination, by the use of figures of merit as paramount mathematical tools; and we concluded from Chap. 4 to Chap. 9 that the application of analytical chemistry and their technologies to guarantee the quality of industrial products and processes allied to the research and development activities is relevant.

Keywords Advanced analytical techniques · Industrial sectors · Sustainability · Industrial analytical chemistry · Instrumental analysis

10.1 Remarks

We saw in Chap. 1 that analytical chemistry is a strategic science by that supplies strategic knowledge (e.g., composition and concentration) for all substances and materials produced by man or found in nature. Analytical chemistry, as a scientific branch of chemistry, is a generator of knowledge related to the characterization, identification, and determination of several materials from several origins in all states of matter. Considering a broad context, analytical sciences are the experimental basis to understand the matter composition in completely different areas and economic sectors, in order to guarantee, for instance, the best uses of chemicals and related materials in the modern society. Concepts of green analytical chemistry and circular economy were explored for industrial purposes in order to attribute a sustainable characteristic to the chemical analysis. Trends (e.g., automation, miniaturization,

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chemometrics, machine learning) and challenges (e.g., computational tools, rapid and automated technologies) were considered in order to pave the road for this branch of the chemical science and to reach the whole potential to solve real problems.

We saw in Chap. 2 that chemical analysis—the practical branch of analytical chemistry—can be applied in three different or complementary situations: characterization, identification, and determination of organic and inorganic analytes. These sets of information are involved in the economic and social aspects of relevant industrial and technological activities in the modern society (e.g., agriculture, biotechnology, chemicals, oil and gas, materials, and pharmaceuticals). We observed a considerable variation related to the families of analytical techniques available for each economic sector, which comes from the complexity of the analytical parameters involved, as concentration of a certain compound and their physicochemical properties; for instance, in the case of chemicals and pharmaceuticals they require both qualitative (e.g., structural resolution obtained by spectroscopic techniques) and quantitative information (e.g., concentration of products and by-products by means chromatographic techniques)—this approach can be applied to all economic sectors considered here. Finally, the description of classical and instrumental techniques and examples ensure what can be obtained by industry from analytical chemistry.

We saw in Chap. 3 that in order to understand the application of analytical techniques for the analysis of several analytes of industrial and technological interest, it is paramount to introduce fundamental terms of analytical chemistry. Figures of merit is the first set because they are parameters of control used in analytical chemistry for the application of a certain analytical method—the most representative figures are accuracy, linearity, limit of detection, limit of quantification, precision, selectivity, sensitivity, and robustness. The processes of development and validation make up the modus operandi of any analytical method in its best performance condition. As an example of the importance of these both processes, we mentioned the constant and necessary evaluation of those figures of merit for the correct obtaining of an analytical result, a major procedure in an accreditation of the analytical laboratory. Then, these terms and applications were explored in this chapter in order to supply the necessary know-how to put into practice the chemical analysis.

We saw in Chap. 4 that pharmaceuticals and agrochemicals, the most representative family of biologically active molecules obtained by means industrial processing, embrace a huge diversity of compounds—mainly organic compounds—used as drugs for diseases, and pesticides for pest control in modern agriculture. As relevant industrial sectors for the modern society, both pharmaceuticals and agrochemicals demand advanced analytical techniques and methods for monitoring and quality control of processes, raw material, inputs, and products. However, these sectors generate a diversity of analytical matrices with their own physical characteristics. In this chapter several classes of analytical techniques, as chromatographic techniques, spectroscopic and spectrometric techniques, thermal techniques, among others were presented and discussed in order to be applied in the fine chemical industry and their analytes; furthermore, relevant auxiliary tools as artificial

intelligence, quality by design, and chemometrics were introduced also to obtain all potential and reliability from the analytical data.

We saw in Chap. 5 that mining corresponds to an economic and industrial activity that consists of research, exploration, extraction, and processing of ores present in the subsoil. And it is possible to say that mining is indispensable to socioeconomic development. This chapter considered the global outlook of mineral exploration and how this industry works regarding their most common processes. Aspects of physical properties of analytical matrices (e.g., powders, solids, semi-solids) and their preparation, and the most common analytical techniques (e.g., atomic emission and absorption spectrometries, X-ray fluorescence and diffractometry) were considered in order to describe relevant applications of those techniques. Furthermore, the process analytical technology approach was also considered.

We saw in Chap. 6 that polymers are one of the most relevant materials for the modern society to maintain its lifestyle and its life quality. Plastics, fibers, etc., are present in our day-to-day life from the morning (e.g., toothbrush) up to the night (e.g., lampshade). For the quality control and for the research and development of the polymeric materials we have several advanced analytical techniques, as microscopic (SEM, TEM, AFM), spectroscopic (EDS, NMR, SAXS), chromatographic (GPC), thermal (TGA, DTA, DSC), and sorption/desorption (BET) techniques. These techniques and their analytical methods were treated in this chapter. Furthermore, physical-chemical properties of natural and synthetic polymers, and aspects of market were also presented.

We saw in Chap. 7 that industrial biotechnology is a multidisciplinary branch which comprises aspects of organic chemistry, analytical chemistry, biochemistry, chemical engineering, and microbiology in order to explore the biochemical routes of conversion for several classes of organic compounds. This industry comprises bioproducts (e.g., alcohols, organic acids, vitamins, antibiotics, vaccines, etc.) obtained by means of bioprocesses using microbes and enzymes. In order to guarantee the quality of these bioproducts and their bioprocess and to support the research and development in this field, this chapter discussed chromatographies (gaseous and liquid phases), spectroscopies (absorption in the UV-Vis and infrared regions, Raman, and nuclear magnetic resonance), OMICS, and process analytical chemistry/process analytical technology. These analytical techniques promote a high-quality level of the biotechnological industry.

We saw in Chap. 8 that oil and gas are fossil mixtures with a large usage by the modern society to provide energy, chemicals, and materials. And this industry is a versatile branch of global economy, involving crude oil and natural gas extraction, processing, and usage. In order to conduct the quality control and the research and development experiments, the use of advanced analytical techniques is paramount. Analytical techniques as GC, MS, AEOS/OES, XRF, and isotopic analysis were explored in this chapter for their application from the quantification and characterization of the hydrocarbons to the determination of the presence of contaminants, as metals. Furthermore, issues related to market, industrial processing technologies, and natural resources were addressed too.

Finally, we saw in Chap. 9 that environmental issues are a hotspot for the modern industry dedicated to pursue more sustainable products and processes. They are imposed by environmental law (e.g., analytical parameters to control effluent emissions from processing) and by the market as a differential for investments and to gain market competition (e.g., net-zero carbon emission). Thus, environmental analyses are the ways to control and to monitor the emission of pollutants in the environment by the industrial sectors. Advanced analytical techniques as LC, GC, AAS, and AES/OES to supply quantitative information about the pollutant in aqueous and gaseous effluents were discussed in this chapter. Besides, size distribution analysis to determine the size of the particulate matter and LIBS, to supply quantitative information for carbon footprint, were also discussed. Issues related to analytical matrices and sample preparation were also addressed.

These remarks demonstrate the versatility of analytical chemistry and their advanced analytical techniques to be applied in the quality control and research and development of several matrices, analytes, and industrial sectors. Indeed, we can observe the industrial analytical chemistry as a natural and focused advancement of analytical chemistry based on the instrumental analysis.

10.2 Conclusions

We concluded in Chap. 1 that the relevance of analytical chemistry in the modern society, especially for the transformation of industries, as those related, for instance, to agroindustry and industrial chemistry, which are a few examples of application is recognized. Aspects of green chemistry and circular economy can promote a more sustainable approach for the chemical analyses allied to up-to-date technologies based-on trends as automation, miniaturization, chemometrics, and machine learning. However, we observed several challenges to overcome in the future of analytical chemistry, as the development of wearable sensors, computational tools, and rapid and automated new technologies. But they can turn into opportunities to be explored.

We concluded in Chap. 2 that analytical chemistry can provide to industry information related to characterization, identification, and determination of organic and inorganic analytes originated from several products and processes. The considered economic and technological sectors in this chapter (i.e., agriculture, biotechnology, chemicals, oil and gas, materials, and pharmaceuticals) have different analytical necessities based on their raw materials, processes, and products; with analytical chemistry—by means chemical analysis—having a high impact on the activity performance of products. Then, the use of classical and instrumental techniques contributes directly to the best usages of industrial products and to the maintenance of their whole production chain.

We concluded in Chap. 3 that figures of merit are paramount mathematical tools to guarantee the reliability of analytical method and, consequently, the reliability of any analytical result obtained from an analytical process. Method development and validation are relevant processes for the correct analytical method application and,

again, to ensure the reliability and, also, the uncertainty from a measurement. Here, the figures of merit are expanded and used coupled to other concepts (e.g., repeatability and reproducibility). Finally, quality control and quality assurance are useful approaches to maintain and to assess the quality of the measurements in order to achieve the accreditation for the analytical laboratory.

We concluded in Chap. 4 that chemical analysis applied to pharmaceuticals and agrochemicals is a fascinating branch of the analytical chemistry involving a large family of analytical techniques for several analytical matrices and analytical methods. Analytical techniques as GC, HPLC, NMR, DSC, AAS, ICP-OES, etc. give us the deep chemical knowledge of raw materials, inputs, and end products supplying constitution and properties in order to guarantee the quality and safety to those who make use of drugs and pesticides, highlighting the health and environmental aspects. Besides, PAC/PAT is a useful approach to monitor and control the processing steps in industries. Allied to the analytical techniques and their methods we have relevant tools as AI and chemometrics to automate the laboratory and to extract all data potential. Furthermore, and especially for pharmaceutical industry, QbD can promote a reliable and robust quality system for pharmaceuticals. Thus, these two classes of biologically active molecules can be well-monitored and controlled by the analytical instrumentation in order to offer reliable industrial end products to the modern society.

We concluded in Chap. 5 that analytics for ores and mining is an exciting branch of analytical chemistry dedicated to inorganic species with the highlighted use of spectroscopy-based analytical techniques, e.g., AAS, ICP-OES/ICP-AES, XRF, XRD, and particle size distribution. Furthermore, process analytical technology—based on spectroscopy also—supplies a strategic knowledge of material composition for quality control of products and their processes for mineral processing. The heterogeneous compositional characteristic of ores requires special attention with the sample preparation in order to avoid the effect of interferents on the chemical analysis. Additionally, the use of reference materials (or references standards) is essential to guarantee uncertainty and traceability of the analytical result.

We concluded in Chap. 6 that polymers are one of the most important family of industrial products for the modern society with the production of millions of tons each year. Certainly the modern way of life would not be possible without the polymeric materials—of course this huge production can promote negative impacts on the environment and health. The chemical and physical analyses of synthetic and natural polymers have a considerable arsenal of advanced analytical techniques available, comprising microscopic (SEM, TEM, AFM), spectroscopic (EDS, NMR, SAXS), chromatographic (GPC), thermal (TGA, DTA, DSC), and sorption/desorption (BET) techniques. And these large number of analytical technologies can be applied to quality control and to research and development activities.

We concluded in Chap. 7 that the biotechnological industry comprises bioproducts and bioprocess. The first one comprises ethanol and other industrial alcohols, organic acids, vitamins, antibiotics, vaccines, industrial enzymes, and proteins and amino acids; and the last one, the use of microbes (e.g., bacteria, fungi) and enzymes (free or immobilized). These products are used from

pharmaceutical purposes to food and feed purposes. Regarding the available analytical technologies for quality control and research and development of bioproducts and bioprocesses, we have chromatographies (gaseous and liquid phases), spectroscopies (absorption in the UV-Vis and infrared regions, Raman, and nuclear magnetic resonance), OMICS, and PAC/PAT for industrial application. These analytical techniques promote a high-quality level of the biotechnological industry.

We concluded in Chap. 8 that oil and gas industry is a versatile branch of global economy, involving crude oil and natural gas extraction, processing and using. In order to conduct the quality control and the research and development experiments, the use of advanced analytical techniques is paramount. And for both purposes, cited analytical techniques as GC, MS, AEOS/OES, XRF, and isotopic analysis can be explored for several analytical matrices in solid, liquid, and gaseous physical states. These techniques can be applied from the quantification and characterization of the hydrocarbons to the determination of the presence of contaminants, as metals.

Finally, we concluded in Chap. 9 that environmental analyses are paramount to control and to monitor the emission of pollutants in the environment by the industrial sectors. Advanced analytical techniques as LC, GC, AAS, and AES/OES can supply mainly quantitative information about the pollutant in aqueous and gaseous effluent; while size distribution analysis can determine the size of the particulate matter. Furthermore, LIBS can supply quantitative information about carbon species for carbon footprint for studies related to sustainability aspects for products and processes. These techniques can be better explored considering issues related to matrix and sample preparation according to standard methods.

These conclusions reinforce the relevance of the application of analytical chemistry and their technologies to guarantee the quality of industrial products and processes allied to the research and development activities to promote sustainable scientific and technological advances for the modern society.