People`s Democratic Republic of Algeria
University of Mohamed Khider Biskra
*Faculty of Exact sciences, Natural Sciences and Life Sciences*

Department of Material Sciences

1st year professional physics: MCPC

PPP2

Prof. Said BENRAMACHE

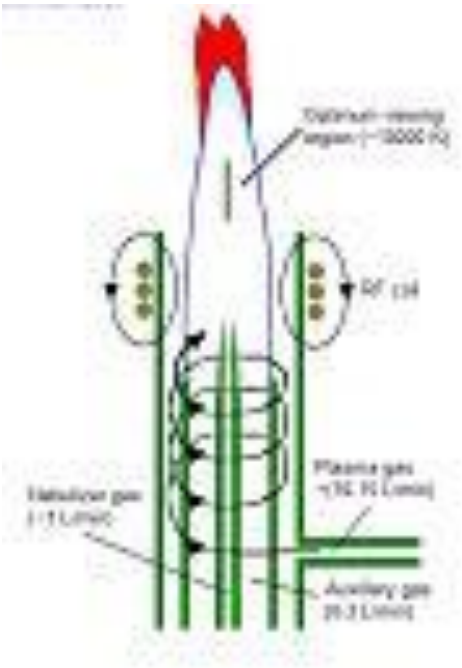
# Chapter one: Bibliographic research 2023/2024

## Instrumental Analysis Definition, Methods & Examples

Instrumental analysis investigates the use of scientific instruments to study systems. Typical topics that are included within this area are spectroscopy, nuclear spectroscopy, mass spectrometry, crystallography, electrochemical analysis, thermal analysis, separations, and Microscopy.

## [1] Atomic Emission Spectroscopy (AES)

This module provides an introduction to Atomic Emission Spectroscopy (AES). AES is a broad area that includes several analytical chemistry techniques focused on elemental analysis, the identification, quantification, and (sometimes) speciation of the elemental makeup of a sample. AES can be an extremely useful tool and is utilized in academic and industrial settings within biological and chemical sciences. It is mainly used in quantitative analysis, but can be used in qualitative work as well.



### History & Theory

Atomic spectroscopy began with the realization in the mid-19th century that salts in a flame could emit light of wavelength specific to metallic elements introduced to the flame as powders or solutions, and that light of the same wavelength might be missing from emission from stars (including the sun) because the elements absorbed light.

### Introduction of Analyte

The composition of the analyte and matrix along with the emission source play a role in creating excited atoms that can give off an emission. THE MAJOR GOAL IS TO GET AS MANY ATOMS IN THE EXCITED STATE AS POSSIBLE WHILE MINIMIZING ALL THE OTHER PROCESSES. In the figure below, one can see that there are several factors that control the ability of atoms to go from the ground state to an excited state.

# [2] Affinity Chromatography

This module provides an introduction to Affinity Chromatography separations. This is a technique commonly used to purify a biomolecule of interest based on the specific interaction of the biomolecule with a ligand immobilized on a stationary phase. Affinity chromatography is widely used in biotechnology research and in industry. Using this technique, molecules of interest can be purified in a one-step separation with high purity and high recovery. The purification of the molecule does not require harsh conditions and hence is preferred for biomolecule separation. Affinity chromatography has also found applications in studying drug/ligand binding to proteins of interest. This technique is useful in the purification of peptides, chiral molecules, antibodies, nucleic acids, and proteins.

The aim of this module is to present the basic theory and applications of affinity chromatography. This module is meant to be utilized in the classroom as a teaching resource or by anyone interested in learning about affinity chromatography. As a teaching resource, the module can be used directly, or the students can be given an in-class problem set, which will lead to further discussion on affinity chromatography.

## Objective

The students will be able to describe the principles of affinity chromatography. They will be able to apply and evaluate the technique of affinity chromatography to the purification of a biomolecule of interest.

### Goals:

Theory and applications of affinity chromatography **will be described** with emphasis on the following:

- Devising a method of purification for a biomolecule of interest
- Steps involved in affinity chromatography purification
- Methods for evaluating purity of the biomolecule of interest
- Broader applications of the technique

Links are provided for additional reading material in the field of affinity chromatography.

## History

Although the word affinity chromatography was coined in the 1960s, and has since been recognized as a powerful tool for bio-specific purification, the bio-specific binding was first used in 1910 by the German Pharmacologist Emil Starkenstein. He demonstrated specific binding of the enzyme alpha-amylase to starch as a means for purification of the enzyme. Subsequent studies reported the use of insoluble affinity material as both the stationary phase and the support material.

The method was further developed by Prof. Pedro Cuatrecasas from the University of California San Diego and Prof. Meir Wilchek from Weizmann institute of Science in Israel in 1968. Their method was developed to purify the common enzymes staphylococcal nuclease, a-chymotrypsin, and carboxypeptidase A using sepharose-enzyme inhibitor as the matrix. You can read more about their discovery in the following paper:

- Cuatrecasas P, Wilchek H, and Anfinsen CB (1968). "Selective enzyme purification by affinity chromatography". Proc. Nat. Acad. Sci. USA61: 636-43.

The scientists were awarded the Wolf Prize in Medicine in 1987 for their work in Affinity Chromatography.

## [3] Chromatography

Chromatography is a method by which a mixture is separated by distributing its components between two phases. The stationary phase remains fixed in place while the mobile phase carries the components of the mixture through the medium being used. The stationary phase acts as a constraint on many of the components in a mixture, slowing them down to move slower than the mobile phase. The movement of the components in the mobile phase is controlled by the significance of their interactions with the mobile and/or stationary phases. Because of the differences in factors such as the solubility of certain components in the mobile phase and the strength of their affinities for the stationary phase, some components will move faster than others, thus facilitating the separation of the components within that mixture.

- Chromatographic Columns
  Chromatography is an analytical technique that separates components in a mixture. Chromatographic columns are part of the instrumentation that is used in chromatography. Five chromatographic methods that use columns are gas chromatography (GC), liquid chromatography (LC), Ion exchange chromatography (IEC), size exclusion chromatography (SEC), and chiral chromatography. The basic principals of chromatography can be applied to all five methods.

- Chromatography
  Chromatography is a method by which a mixture is separated by distributing its components between two phases. The stationary phase remains fixed in place while the mobile phase carries the components of the mixture through the medium being used. The stationary phase acts as a constraint on many of the components in a mixture, slowing them down to move slower than the mobile phase.

- Gas Chromatography
  Gas chromatography is a term used to describe the group of analytical separation techniques used to analyze volatile substances in the gas phase. In gas chromatography, the components of a sample are dissolved in a solvent and vaporized in order to separate the analytes by distributing the sample between two phases: a stationary phase and a mobile phase. The mobile phase is a chemically inert gas that serves to carry the molecules of the analyte through the heated column.

Liquid Chromatography

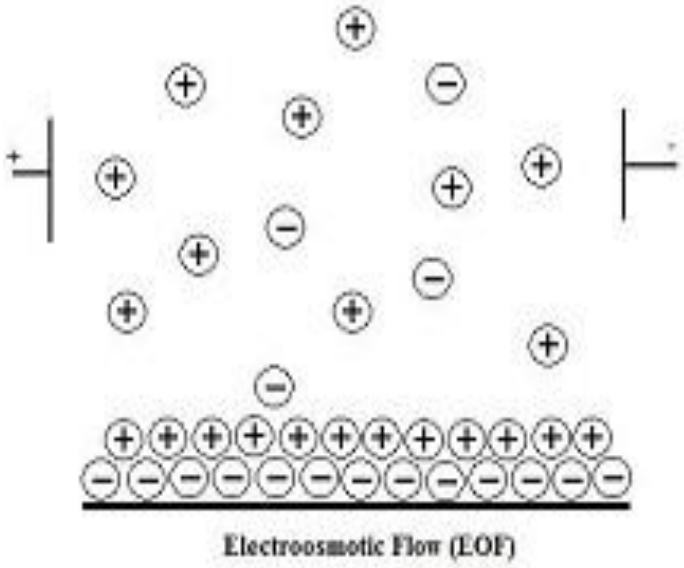
Liquid chromatography is a technique used to separate a sample into its individual parts. This separation occurs based on the interactions of the sample with the mobile and stationary phases. Because there are many stationary/mobile phase combinations that can be employed when separating a mixture, there are several different types of chromatography that are classified based on the physical

states of those phases. Liquid-solid column chromatography, the most popular chromatography technique.

- High Performance Liquid Chromatography
  High Performance Liquid Chromotagraphy (HPLC) is an analytical technique used for the separation of compounds soluble in a particular solvent.

## [4] Capillary Electrophoresis

Capillary electrophoresis is an analytical technique that separates ions based on their electrophoretic mobility with the use of an applied voltage. The electrophoretic mobility is dependent upon the charge of the molecule, the viscosity, and the atom's radius. The rate at which the particle moves is directly proportional to the applied electric field--the greater the field strength, the faster the mobility. Neutral species are not affected, only ions move with the electric field. If two ions are the same size, the one with greater charge will move the fastest. For ions of the same charge, the smaller particle has less friction and overall faster migration rate. Capillary electrophoresis is used most predominately because it gives faster results and provides high resolution separation. It is a useful technique because there is a large range of detection methods available




# Instrumental Setup

A typical capillary electrophoresis system consists of a high-voltage power supply, a sample introduction system, a capillary tube, a detector and an output device. Some instruments include a temperature control device to ensure reproducible results. This is because the separation of the sample depends on the electrophoretic mobility and the viscosity of the solutions decreases as the column temperature rises.[3] Each side of the high voltage power supply is connected to an electrode. These electrodes help to induce an electric field to initiate the migration of the sample from the anode to the cathode through the capillary tube. The capillary is made of fused silica and is sometimes coated with polyimide.[3] Each side of the capillary tube is dipped in a vial containing the electrode and an electrolytic solution, or aqueous buffer. Before the sample is introduced to the column, the capillary must be flushed with the desired buffer solution. There is usually a small window near the cathodic end of the capillary which allows UV-VIS light to pass through the analyte and measure the absorbance. A photomultiplier tube is also connected at the cathodic end of the capillary, which enables the construction of a mass spectrum, providing information about the mass to charge ratio of the ionic species.

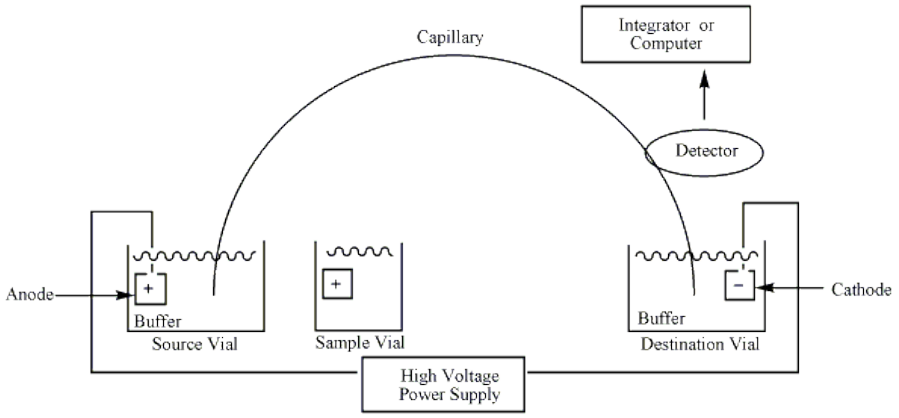
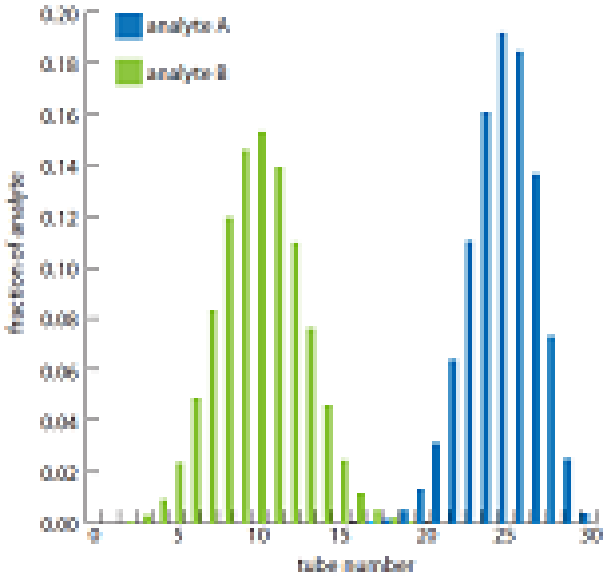



Figure 1: Instrumental Setup. from Wikipedia

## [5] Countercurrent Separations




In 1949, Lyman Craig introduced an improved method for separating analytes with similar distribution ratios.1 The technique, which is known as a countercurrent liquid–liquid extraction, is outlined in Figure A16.1 and discussed in detail below. In contrast to a sequential liquid–liquid extraction, in which we repeatedly extract the sample containing the analyte, a countercurrent extraction uses a serial extraction of both the sample and the extracting phases. Although countercurrent separations are no longer common—chromatographic separations are far more efficient in terms of resolution, time, and ease of use—the theory behind a countercurrent extraction remains useful as an introduction to the theory of chromatographic separations.

## [6] Cyclic Voltammetry

Cyclic Voltammetry (CV) is an electrochemical technique which measures the current that develops in an electrochemical cell under conditions where voltage is in excess of that predicted by the Nernst equation. CV is performed by cycling the potential of a working electrode, and measuring the resulting current.

# Introduction

The potential of the working electrode is measured against a reference electrode which maintains a constant potential, and the resulting applied potential produces an excitation signal such as that of figure 1.[2] In the forward scan of figure 1, the potential first scans negatively, starting from a greater potential (a) and ending at a lower potential (d). The potential extrema (d) is call the switching potential, and is the point where the

voltage is sufficient enough to have caused an oxidation or reduction of an analyte. The reverse scan occurs from (d) to (g), and is where the potential scans positively. Figure 1 shows a typical reduction occurring from (a) to (d) and an oxidation occurring from (d) to (g). It is important to note that some analytes undergo oxidation first, in which case the potential would first scan positively. This cycle can be repeated, and the scan rate can be varied. The slope of the excitation signal gives the scan rate used.

## [7] Mass Spectrometry


Mass spectrometry is an analytic method that employs ionization and mass analysis of compounds in order to determine the mass, formula and structure of the compound being analyzed. A mass analyzer is the component of the mass spectrometer that takes ionized masses and separates them based on charge to mass ratios and outputs them to the detector where they are detected and later converted to a digital output.

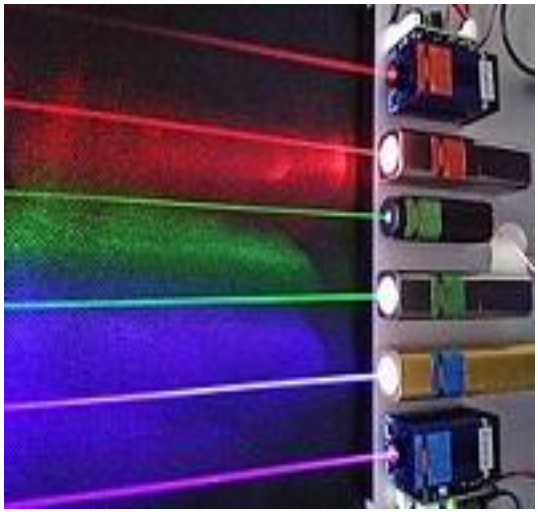
## [8] Lasers

LASER is an acronym for Light Amplification by Stimulated Emission of Radiation. Laser is a type of light source which has the unique characteristics of directionality, brightness, and monochromaticity. The goal of this module is to explain how a laser operates (stimulated or spontaneous emission), describe important components, and give some examples of types of lasers and their applications.

- Gas Lasers
  Gas lasers have lasing media that are made-up of one or a mixture of gases or vapors. Gas lasers can be classified in terms of the type of transitions that lead to their operation: atomic or molecular. The most common of all gas lasers is the helium-neon (He-Ne) laser.

- Laser Theory
  There are four laser demands: population inversion, laser threshold, energy source and active medium.

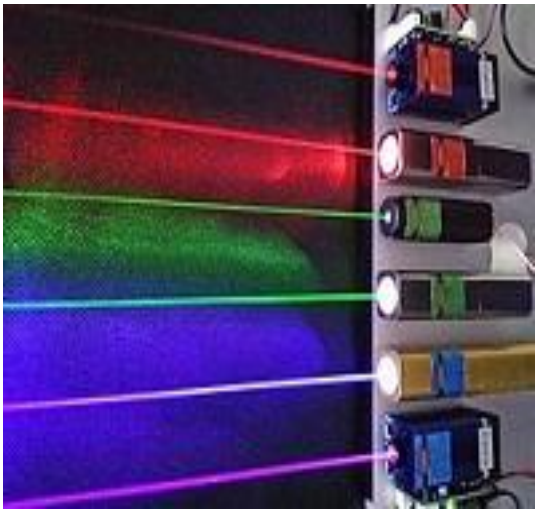
- Overview of Lasers
  LASER is an acronym for Light Amplification by Stimulated Emission of Radiation. Laser is a type of light source which



has the unique characteristics of directionality, brightness, and monochromaticity. The goal of this module is to explain how a laser operates (stimulated or spontaneous emission), describe important components, and give some examples of types of lasers and their applications.

- Semiconductor and Solid-state lasers
  In both solid-state and semiconductor lasers the lasing medium is a solid. Aside from this similarity, however, these two laser types are very different from each other. In the case of the solid-state lasers the lasing species is typically an impurity that resides in a solid host, a crystal of some sort. The crystal modifies some of the quantized energy levels of the impurity, but still the lasing is almost atomic - similar to gas lasers.

# [9] Introduction to Lasers



The basic theory of lasers will be presented with emphasis on:

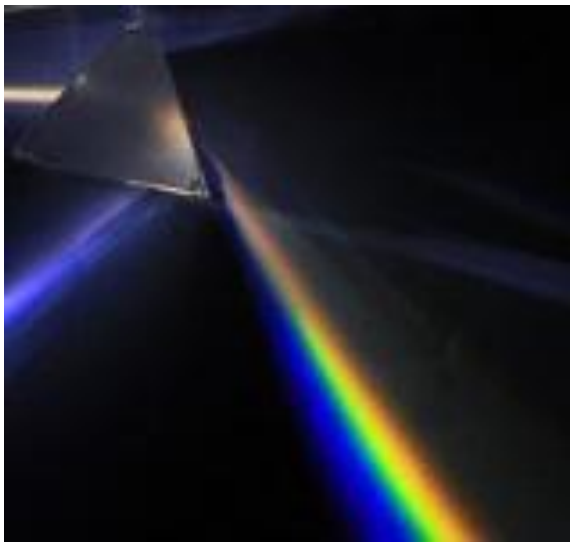
- laser radiation properties
- laser components and design
- laser light generation
- common laser types

This module discusses basic concepts related to Lasers. Lasers are light sources that produce electromagnetic radiation through the process of stimulated emission. Laser light has properties different from more common light sources, such as incandescent bulbs and fluorescent lamps. Typically, laser radiation spans a small range of wavelengths and is emitted in a beam that is spatially narrow. The word laser is an acronym for Light Amplification by Stimulated Emission of Radiation. Lasers are ubiquitous in our lives and are broadly applied in areas that include scientific research, medicine, engineering, telecommunications, industry and business (see the Applications page for examples). This module is aimed at presenting the most basic principles of lasers and discussing aspects of common types. Properties of laser radiation and laser optical components are introduced.

# [10] Spectrometer

Strictly speaking, a spectrometer is any instrument used to view and analyze a range (or a *spectrum*) of a given characteristic for a substance (for example, a range of mass-to-charge values as in mass spectrometry), or a range of wavelengths as in absorption spectrometry like nuclear magnetic radiation spectroscopy or infrared spectroscopy). A spectro*photo*meter is a spectrometer that only measures the intensity of electromagnetic radiation (light) and is distinct from other spectrometers such as mass spectrometers.

A spectrometer is typically used to measure wavelengths of electromagnetic radiation (light) that has interacted with a sample. Incident light can be *reflected* off, *absorbed* by, or *transmitted* through a sample; the way the incident light changes during the interaction with the sample is characteristic of the sample. A spectrometer measures this change over a range of incident wavelengths (or at a specific wavelength).



There are three main components in all spectrometers; these components can vary widely between instruments for specific applications and levels of resolution. Very generally, these components produce the electromagnetic radiation, somehow narrows the electromagnetic radiation to a specified range, and then detect the resulting electromagnetic radiation after is has interacted with the sample.

## [11] Diffraction Scattering Techniques

When an X-ray is shined on a crystal, it diffracts in a pattern characteristic of the structure.

- Bragg's Law
  The structures of crystals and molecules are often being identified using x-ray diffraction studies, which are explained by Bragg's Law. The law explains the relationship between an x-ray light shooting into and its reflection off from crystal surface.

- Powder X-ray Diffraction
  When an X-ray is shined on a crystal, it diffracts in a pattern characteristic of the structure. In powder X-ray diffraction, the diffraction pattern is obtained from a powder of the material, rather than an individual crystal. Powder diffraction is often easier and more convenient than single crystal diffraction since it does not require individual crystals be made. Powder X-ray diffraction (XRD) also obtains a diffraction pattern for the bulk material of a crystalline solid, rather than of a s

- X-ray Crystallography
  X-ray Crystallography is a scientific method used to determine the arrangement of atoms of a crystalline solid in three dimensional space. This technique takes advantage of the interatomic spacing of most crystalline solids by employing them as a diffraction gradient for x-ray light, which has wavelengths on the order of 1 angstrom (10-8 cm).

- X-ray Diffraction
  The construction of a simple powder diffractometer was first described by Hull in 1917 (1) which was shortly after the discovery of X-rays by Wilhelm Conrad Röntgen in 1895 (2). Diffractometer measures the angles at which X-rays get reflected and thus get the structural information they contains. Nowadays resolution of this technique get significant improvement and it is widely used as a tool to analyze the phase information and solve crystal structures of solid-state materials.