

Text book of PHARMACEUTICAL MODERN Analytical Techniques

About The Book

It Provides comprehensive yet concise analytical techniques as per syllabi of the B.PHARM, M.PHARM and PHARM.D course prescribed by P.C.I. The subject matter will be of interest to the students and



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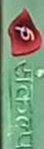
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Dr. Jitender K. Malik



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Modern Analytical Techniques

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CONTENTS

Unit- I	Atomic absorption Spectroscopy	1-8
Unit- II	Chromatography	9-47
Unit- III	UV Spectroscopy	48-69
Unit- IV	Potentiometry and Thermal Techniques	70-100
Unit- V	Electrophoresis	101-126
Unit- VI	IR- Infra-Rays Spectroscopy	127-150
Unit- VII	NMR Spectroscopy	151-163
Unit- VIII	Mass spectroscopy	164-183

UNIT-I

ATOMIC ABSORPTION SPECTROSCOPY

Atomic Absorption Spectroscopy

AAS is an analytical technique used to determine the concentration of metal atoms/ions in a sample. Metals make up around 75% of the earth's chemical elements. In some cases, metal content in a material is desirable, but metals can also be contaminants (poisons). Therefore, measuring metal content is critical in many different applications, which we will explore later in this article. Suffice to say for now that it finds a purpose in quality control, toxicology and environmental testing to name a few.

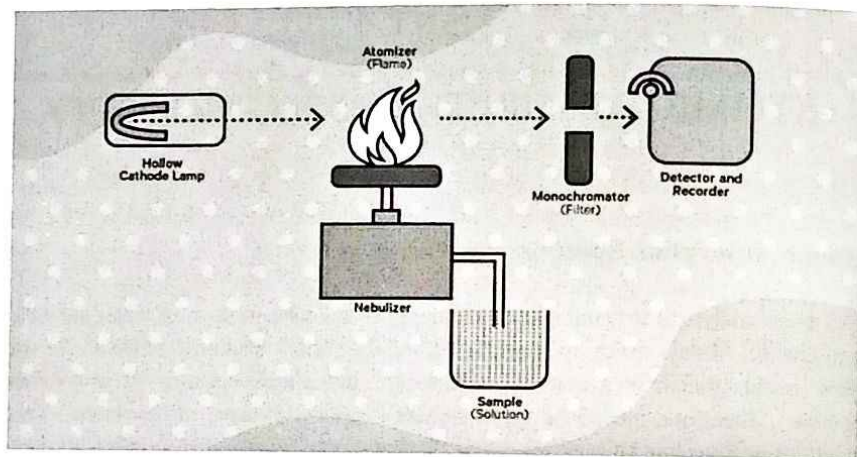
Principle of Atomic Absorption Spectroscopy

The basic principles of AAS can be expressed as follows. Firstly, all atoms or ions can absorb light at specific, unique wavelengths. When a sample containing copper (Cu) and nickel (Ni), for example, is exposed to light at the characteristic wavelength of Cu, then only the Cu atoms or ions will absorb this light. The amount of light absorbed at this wavelength is directly proportional to the concentration of the absorbing ions or atoms.

The electrons within an atom exist at various energy levels.

When the atom is exposed to its own unique wavelength, it can absorb the energy (photons) and electrons move from a ground state to excited states. The radiant energy absorbed by the electrons is directly related to the transition that occurs during this process. Furthermore, since the electronic structure of every element is unique, the radiation absorbed represents a unique property of each individual element and it can be measured.

An atomic absorption spectrometer uses these basic principles and applies them in practical quantitative analysis. A typical atomic absorption spectrometer consists of four main components: the light source, the atomization system, the monochromator and the detection system.



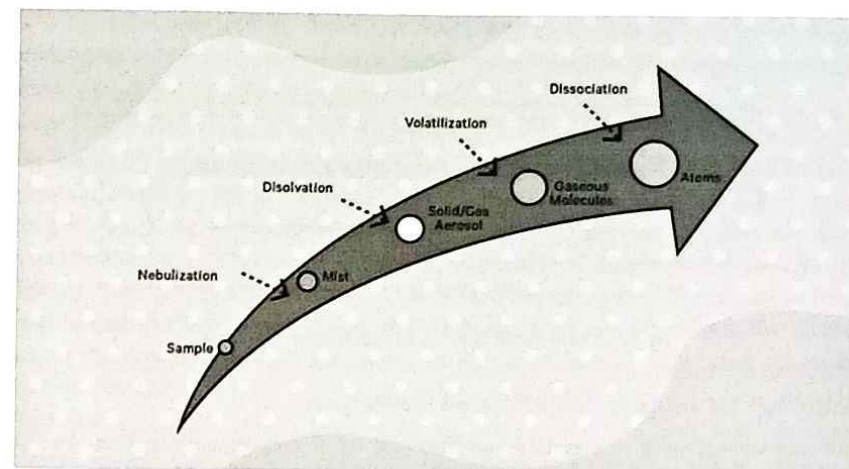
Atomic Absorption Spectrometer

In a typical experiment, the sample, either liquid or solid, is atomized in either a flame or a graphite furnace. The free atoms are then exposed to light, typically produced by a hollow-cathode lamp, and undergo electronic transitions from the ground state to excited electronic states. The light produced by the lamp is emitted from excited atoms of the same element that is to be determined, therefore the radiation energy corresponds directly to the wavelength absorbed by the atomized sample. A monochromator is placed between the sample and the detector to reduce background interference. From here, the detector measures the intensity of the beam of light and converts it to absorption data.

While solid samples can be used for AAS this analysis is usually restricted to the more expensive graphite furnaces where the sample can be heated by controlled electrical heating as opposed to a direct flame. Also, AAS is normally only used to analyse metal atoms. The main reason for this is that metals have narrow, bright and clear single emission and absorption lines.

Atomizing techniques - flame atomic absorption spectroscopy (FAAS)

FAAS is mainly used to determine the concentration of metals in solution in parts per million (ppm) or parts per billion (ppb) ranges. The metal ions are nebulized as a fine spray into a high-temperature flame where they are reduced to their atoms and subsequently absorb light from an element-specific hollow cathode lamp.



Atomization Process in FAAS.

While this method has proved to be a robust technique for routine metal determinations, it does have drawbacks. Firstly, it has limited sensitivity because of the spectral noise created by the flame. This aspect has, however, been improved as the technology has evolved. The main drawback is that one can only measure one metal at a time and as different lamps are required for each element, the lamp must be changed each time you want to analyse for something different. Also, a large part of the sample is lost in the flame (up to 90%) in FAAS, further influencing the sensitivity.

Atomizing techniques - graphite furnace atomic absorption spectroscopy (GFAAS)

In GFAAS, a type of electrothermal atomization, a sample is placed in a hollow graphite tube which is heated until the sample is completely vaporized. GFAAS is much more sensitive than FAAS and can detect very low concentrations of metals (less than 1 ppb) in smaller samples. Using electricity to heat the narrow graphite tube ensures that all of the sample is atomized in a period of a few milliseconds to seconds. The absorption of the atomic vapor is then measured in the region immediately above the heated surface. Naturally, the detection unit does not have to contend with spectral noise, leading to improved sensitivity.

In mining, a lot of focus is on the recovery of precious metals like gold from old mine heaps. With the help of AAS, the amount of gold can be quantified to determine whether it would be profitable to extract it. The analysis of drinking water is probably one of the most important applications of AAS, especially in places where the environment is not properly cared for.

UNIT- II

CHROMATOGRAPHY

Chroma- "colour" and graphein - "to write". Chromatography is a technique in which substance are separated, purified and identified from a mixture for qualitative and quantitative analysis. On the basis of hydrophobic interactions, Polarity, enzymes and net charges are separated by using chromatography. Chromatography is a physical method of separation of compound. TSWETT, referred as the father of chromatography.

This technique was first used by Tswett, to separate various plants pigments such as chlorophylls and xanthophylls by passing these pigments through glass column packed with finely divided calcium carbonate.

Purpose of chromatography

- **Analytical:** To determine chemical composition of a sample.
- **Preparative:** Used in purification of a substance.

Principles

The principle of chromatography is very simple. The sample to be examine or analysing is termed as solute or analyte is allowed to interact or react with two immiscible phases-

1. **Mobile phase**
2. **Stationary phase**

The stationary phase which may be a solid is or a liquid supported on a solid, which is immobile it means it does not move. The mobile phase migrates the sample through stationary phase. The mobile phase may be either liquid or gas. All chromatographic methods involve separation of mobile phase through a stationary phase. The rate of travel of individual solute molecules through a column or thin layer is not uniform. It is directly related to the partition of the molecules between the mobile phase and stationary phase.

UNIT- II

UV SPECTROSCOPY

UV-VISIBLE SPECTROSCOPY

- Introduction to EMR & spectroscopy
- Theory of spectroscopy {EMR, wavelength, wave no., frequency, energy, absorption of light (I_a), transmission of light (I_t)}
- Types of spectroscopy (molecular absorption spectroscopy, molecular emission spectroscopy)
- Principles of UV-visible spectroscopy
- Region of UV-visible light (UV: 200nm-400nm, Visible: 400nm-800nm)
- Principle in colorimetry
- λ_{max} , calibration curve,
- Laws involved in principle (Beer and Lambert's law, derivation)
- Absorbance, Transmittance, Absorptivity, molecular extinction co-efficient
- Electronic states of molecule (ground state, excited state)
- Various types of electronic transitions states of molecules ($\sigma\text{-}\sigma^*$, $n\text{-}\sigma^*$, $\pi\text{-}\pi^*$, $n\text{-}\pi^*$)

Instrumentation

- **Visible or colorimeter**
 - ❖ Source of light (Tungsten lamp, carbon arc)
 - ❖ Filters (Absorption, Interference)
 - ❖ Sample cells (Glass/Plastic: - Rectangular/cylindrical)
 - ❖ Detectors
 - ❖ Single/double beam colorimeter

UV Spectroscopy

- ❖ Source of light (Hydrogen lamp, Deuterium lamp, Xenon discharge lamp, mercury arclamp)
- ❖ Monochromators {Prism (Refractive and Reflective), Grating (Diffraction, Transmission)}
- ❖ Sample cells (Quartz: - Rectangular/cylindrical)
- ❖ Detectors (Photovoltaic cells, Phototubes, Photomultiplier tubes)
- ❖ Readout device
- ❖ Single/double beam UV spectrophotometer.
- ❖ Deviation of Beer's law, Factors involved for deviation (Instrumental, Physiochemical changes).
- ❖ Solvent used and effect of solvent on λ_{max} .
- ❖ Some specific effects: Chromophore/ chromogenic agent, Auxochrome, Isosbestic point, Bathochromiceffect, Hypsochromic effect,

Hyperchromic effect, Hypochromic effect.

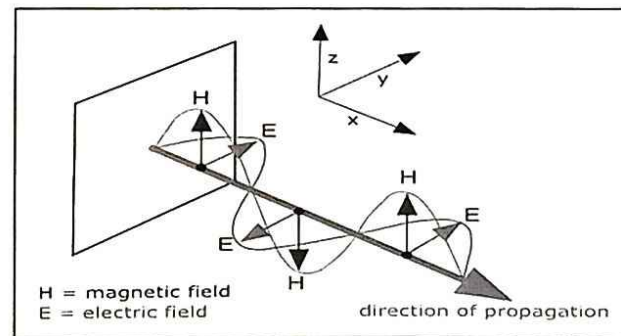
Applications in Pharmacy

- Qualitative analysis
- Quantitative analysis
- Structure elucidation of organic compounds
- Impurity studies

Introduction to Electro Magnetic Radiation (EMR)

- Light travels in a straight line, but phenomenon like interference, refraction, diffraction, etc. could not explain this. To explain these phenomena, light is supposed to travel in waves.
- Light or EMR is a form of energy that is transmitted through space at a constant velocity of 3×10^8 m/s. these radiations are said to have dual nature exhibiting both:
 - Wave character
 - Particle character or corpuscular theory

According to wave theory, light travel in the form of waves. This wave motion consists of oscillating *electric* (E) & *magnetic* (H) fields (vectors) directed perpendicular to each & perpendicular to the direction of the propagation of wave as shown in the figure.



- These are characterized by their wavelengths or frequencies or wave numbers.
- The energy carried by an EMR is directly proportional to the frequency.
- The emission or absorption of radiation is quantized & each quantum of radiation is called *Photon*.
- All types of radiations travel with the same velocity & no medium is required for their propagation. They can travel through vacuum also.

		Cell	(TC)	
Thermo mechanical Analysis	TMA	Dilatometer	Volume or Length	Volume or Length vs. T
Electrical Conductivity	EC	Electrometer	Current (I) or Resistance (R)	I or R vs. T
Emanation Thermal Analysis	ETA	ETA apparatus	Radioactivity (E)	E vs. T

UNIT- V

ELECTROPHORESIS

INTRODUCTION

This method is mainly based on the movement of the charged particles towards the opposite charge by the influence of the direct current which is obtained by the electric field. Electrophoresis is first proposed by Arne Tiselius for the separation of proteins. In this method, the charged particles move towards the opposite charge. The mobility of the ions in the electric field is first observed by Reuss in 1807. After that many scientists proposed different methods based on the principle of electrophoresis.

In 1939, zone electrophoresis is proposed. In 1950, gel electrophoresis with agar is proposed. In 1955, gel electrophoresis with starch is proposed. In 1983, capillary electrophoresis is proposed.

PRINCIPLE

The main principle involved in the separation of the compounds by electrophoresis is the following:

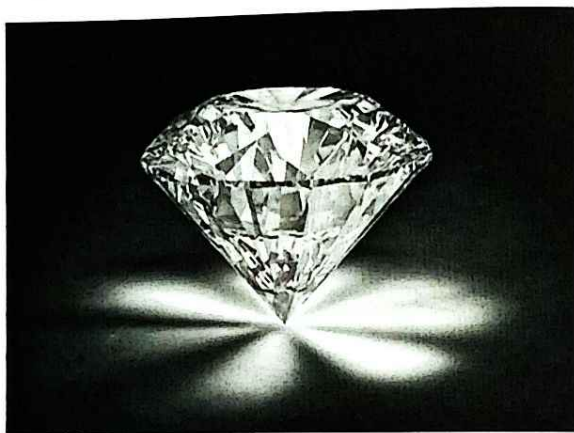
- According to the size.
- According to the charge: When charged molecules are placed in the electric field, they move towards to the anode or cathode based on their charge.

When the sample is placed in the constant electric field, based on the size, the particles are separated that is high molecular weight compounds are eluted first and low molecular weight compounds are eluted later.

Based on the charge, the positively charged molecules move towards the cathode which is negatively charged and the negatively charged particles move to anode which is positively charged.

Covalent Crystals:

Covalent Crystals are those crystals that are bonded together with Covalent bonds. These bonds are extremely strong and brittle since the atoms share electrons to create the bond. Since these atoms are packed together and covalently bonded, these materials are extremely hard to break like diamond and quartz. Diamonds are even a 10 out of 10 on the Mohs Hardness Scale, and quartz is a 7.



Ionic Crystals:

Since covalent crystals use covalent bonds, it only makes sense that Ionic Crystals are created of ionic bonds. Ionic Crystals are solid crystals with a high melting point, held together by the attraction of the ions (i.e. positive to negative). The most well-known ionic crystal is found in your salt shaker.



Metallic Crystals:

Metal isn't the first thing to pop into your head when you think of crystals, but that is exactly what Metallic Crystals are composed of. Metallic crystals are made of metals and held together using metallic bonds. These crystals have a shiny appearance and include copper, gold, aluminium, and iron, to name a few. To see an example of metallic crystals, just look at a wedding band or car.



Molecular Crystals:

The weakest of all the crystals are the Molecular Crystals. Held together by weak hydrogen bonds, molecular crystals have low boiling points and break apart relatively easily. Some you could even break apart with your hands. Dry ice is a great example of molecular crystals. You might even find molecular crystals in your pantry in the form of rock candy. Who knew crystals could be yummy?



droplets in to sequentially arranged containers. This technique is used in the counting the cells, separation of cells, purification of cells from a mixture.

8. Microspectrofluorimetry

A spectrofluorimetre is equipped with microscope which enables to observe the binding of antibody to a single bacterial cell or subcellular organelle and also helps in the identification of cancerous cells from normal cell as they express different set of protein for which fluoresce labelled specific antibody can be applied. Since some malignant cells have more nucleic acid content than do normal cell have, therefore these malignant cells take up more acridine orange dye which binds to the DNA and can be differentiated from the normal cells.

UNIT- VII

NMR SPECTROSCOPY

NUCLEAR MAGNETIC RESONANCE (NMR)SPECTROSCOPY

The objective of this chapter is to make the student able to identify magnetic and non-magnetic nuclei, determine nuclear spin angular momentum and corresponding magnetic moment and determine the interaction energy of nuclear spin states under the influence of an external magnetic field. Combined with ^{13}C NMR, ^1H NMR (PMR) will make the student able to reveal most of the structural and stereochemical features of an organic compound.

INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is the study of molecules by recording the interaction of radiofrequency (RF) electromagnetic radiations with the nuclei of molecules placed in a strong magnetic field. The number of types of hydrogen atoms in a molecule.

- (i) The chemical environment of hydrogen atoms.
- (ii) The number of hydrogen atoms in a given chemical environment.
- (iii) The molecular structure and its stereochemical features.

It is a research technique that exploits the magnetic properties of certain atomic nuclei. The NMR spectroscopy determines the physical and chemical properties of atoms or molecules.

It relies on the phenomenon of nuclear magnetic resonance and provides detailed information about the structure, dynamics, reaction state, and chemical environment of molecules.

Basis of NMR Spectroscopy

Nuclear Magnetic Resonance (NMR) was first detected experimentally at the end of 1945, nearly concurrently with the work groups Felix Bloch, Stanford University and Edward Purcell, Harvard University. The first NMR spectrum was first published in the same issue of the Physical Review in January 1946. Bloch and Purcell were jointly awarded the 1952 Nobel Prize in Physics for their research of Nuclear Magnetic Resonance Spectroscopy.

UNIT- VIII

MASS SPECTROSCOPY

Introduction and History

The earliest forms of mass spectrometry go back to the observation of canal rays by Goldstein in 1886 and again by Wien in 1899. Thompson's later discovery of the electron also used one of the simplest mass spectrometers to bend the path of the cathode rays (electrons) and determine their charge to mass ratio. Later, in 1928, the first isotopic measurements were made by Aston.

These basic experiments and instruments were presented to most readers in first-year general chemistry. More modern aspects of mass spectrometry are attributed to Arthur Jeffrey Dempster and F.W. Aston in 1918 and 1919. Since this time there has been a flurry of activity [not only concerning minor advances in components of mass spectrometers such as different types of instrument interfaces (direct injection, GC, and HPLC)] to different ionization sources (electron and chemical ionization) but also new types of ion separators. For example, double focusing magnetic sector mass filters were developed by Mattauch and Herzog in 1934 (and recently revised into a new type of mass filter), time of flight MS by Stephens in 1946, ion cyclotron resonance MS by Hipple and Thomas in 1949, quadrupole MS by Steinwedel in 1953, and ion trap MS by Paul and Dehmelt in the 1960s.

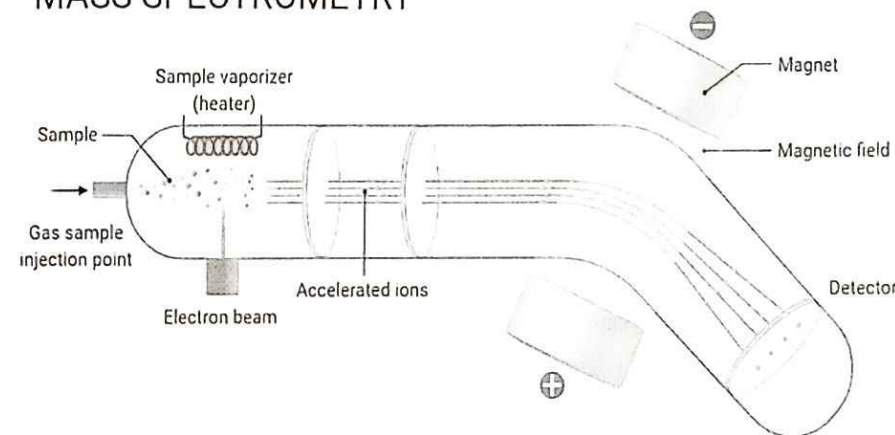
Mass spectrometry was first coupled with GC as a means of sample introduction in 1956 by Golhke et al. and with HPLC via electro-spray ionization in the mid-1980s (Blakely and Vestal, 1983; Yamashita and Fenn, 1984). New methods of mass spectrometry are constantly under development and even as recent as 1985, Hillenkamp and Michael Karas developed the MALDI technique (a laser-based sample introduction device) that radically advanced the analysis of protein structures and more types of mass analyzers will certainly be developed.

mass spectrometry, also called **mass spectroscopy**, analytic technique by which chemical substances are identified by the sorting of gaseous ions in electric and magnetic fields according to their mass-to-charge ratios. The instruments used in such studies are called mass spectrometers and mass spectrographs, and they operate on the principle that moving ions may be deflected by electric and magnetic fields. The two instruments differ only in the way in which the sorted charged particles are detected. In the mass spectrometer they are detected electrically, in the mass spectrograph by photographic or other nonelectrical means; the term mass spectroscopy is used to include

both kinds of devices. Since electrical detectors are now most commonly used, the field is typically referred to as mass spectrometry.

Principle of Mass Spectrometry (MS)

MASS SPECTROMETRY



Working of Mass Spectrometry (MS)

- In a typical procedure, a sample, which may be solid, liquid, or gas, is ionized, for example by bombarding it with electrons.
- This may cause some of the sample's molecules to break into charged fragments. These ions are then separated according to their mass-to-charge ratio, typically by accelerating them and subjecting them to an electric or magnetic field:
- Ions of the same mass-to-charge ratio will undergo the same amount of deflection.
- The ions are detected by a mechanism capable of detecting charged particles, such as an electron multiplier. Results are displayed as spectra of the relative abundance of detected ions as a function of the mass-to-charge ratio.
- The atoms or molecules in the sample can be identified by correlating known masses (e.g. an entire molecule) to the identified masses or through a characteristic fragmentation pattern.