# PHOTOELECTRIC COLORIMETRY

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# Photoelectric colorimetry

Progree in the development of colorimetric method has resulted largly due to the application of photoeletric cell, which eliminates the difficultes of complicated visual comparition. In this method human eye is replaced by suitable photoelectric cell, to afford a direct measure of the light intensity. Instruments employing photoelectric cell measure the light absorption and not color of substance

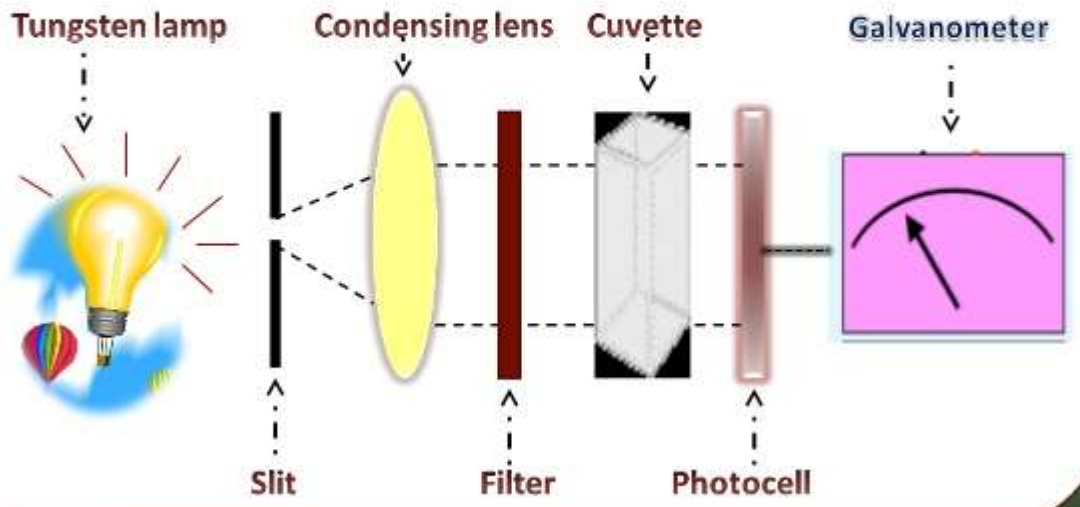
# Introduction

- **Photometry** is the most common analytical technique used in the biochemical laboratory. It is designed to measure the intensity of a beam of light.
- Photometric principles are applied to the several kinds of analytical techniques:

  (a) where absorbed or transmitted light is measured:
  - Colorimetry
  - Spectrophotometry
  - Atomic absorption, and
  - Turbidometry

  (b) where emitted light is measured:
  - Flame emission photometry

# Components of the colorimeter

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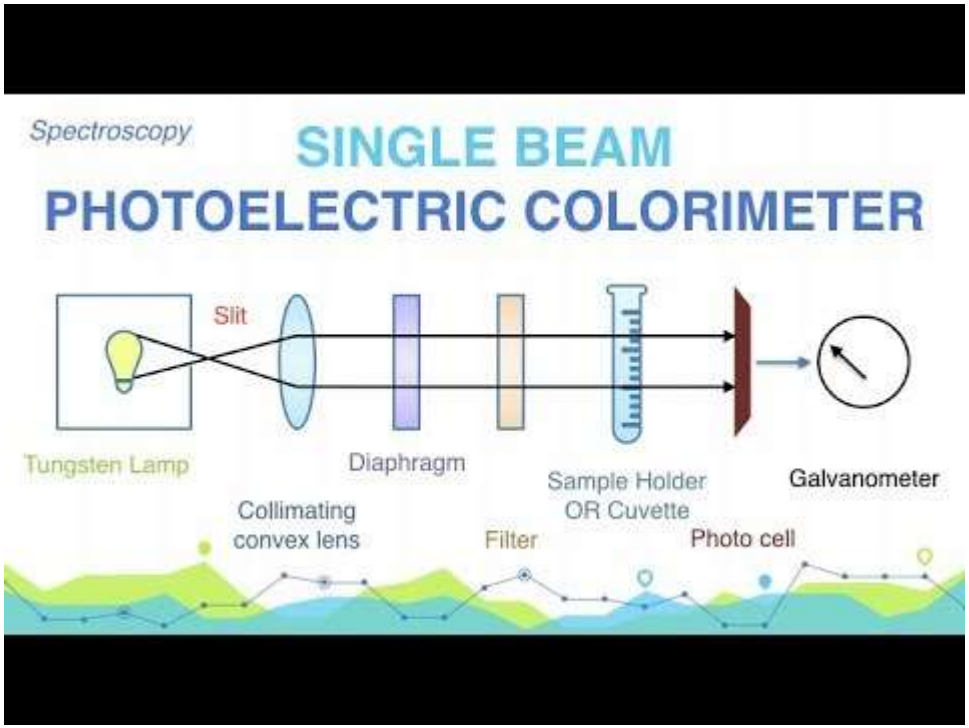
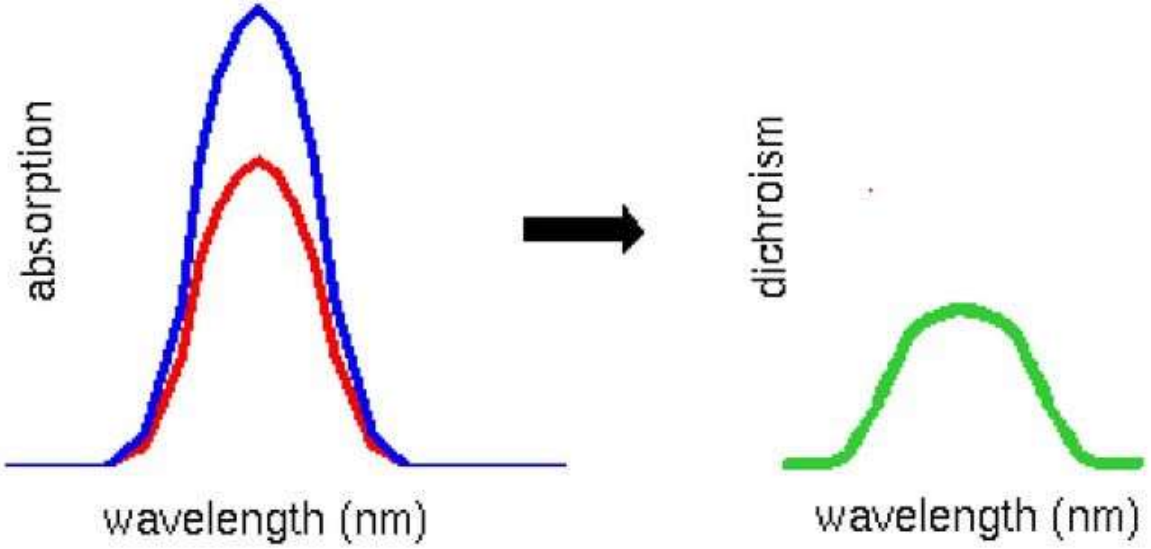

Spectroscopy
SINGLE BEAM
PHOTOELECTRIC COLORIMETER
Slit
Tungsten Lamp
Diaphragm
Sample Holder
OR Cuvette
Galvanometer
Collimating
convex lens
Filter
Photo cell

# Difference between colorimeter and spectrophotometer

| Colorimeter | Spectrophotometer |
| --- | --- |
| Colorimeter is the **general type** | Spectrophotometer is the specific type. |
| Both of them measure color and intensity of color through light. | |
| Basic method of operation is similar for all instruments. | |
| colorimeter utilizes a **three color source** (Red, green, and blue) generated by either a **color wheel** with **colored filters** or, sets of **specially designed LEDs**. | Spectrophotometer utilizes either a **diffraction grating** or **prism** in the sensor |
| Colorimeter is limited to the **visible light** only with WL 400-700 nm | spectrophotometer can be extended to x-ray, UV light, infrared and radiofrequencies |

# Circular Dichroism

The difference between the absorption of left and right handed circularly-polarised light and is measured as a function of wavelength. CD is measured as a quantity called **mean residue ellipticity**, whose units are *degrees-cm$^2$/dmol.*



What is Circular Dichroism? • Circular Dichroism (CD) is a type of absorption spectroscopy that can provide information on the structures of many types of biological macromolecules • It measures the difference between the absorption of left and right handed circularly-polarized light by proteins. CD is used for; • Protein structure determination. • Induced structural changes, i.e. pH, heat & solvent. • Protein folding/unfolding. • Ligand binding • Structural aspects of nucleic acids, polysaccharides, peptides, hormones & other small molecules.

## Application of CD

A primary use is in analysing the secondary structure or conformation of macromolecules, particularly proteins as secondary structure is sensitive to its environment, temperature or pH, circular dichroism can be used to observe how secondary structure changes with environmental conditions or on interaction with other molecules. Structural, kinetic and thermodynamic information about macromolecules can be derived from circular dichroism spectroscopy.

# OPTICAL ROTATRY DISPERSION:–

- The specific rotation [α] changes with wavelength is called **optical rotatory dispersion (ORD) .**

OR

- The rate of change of specific rotation with wavelength is called ***Optical rotatory dispersion (ORD) .***

# Optical rotatory dispersion & Circular Dichroism

- **Optical rotatory dispersion** is the variation in the optical rotation of a substance with a change in the wavelength of light.

- For wavelengths that are absorbed by the optically active sample, the two circularly polarized components will be absorbed to differing extents. This unequal absorption is known as **circular dichroism**.

# INTRODUCTION:-

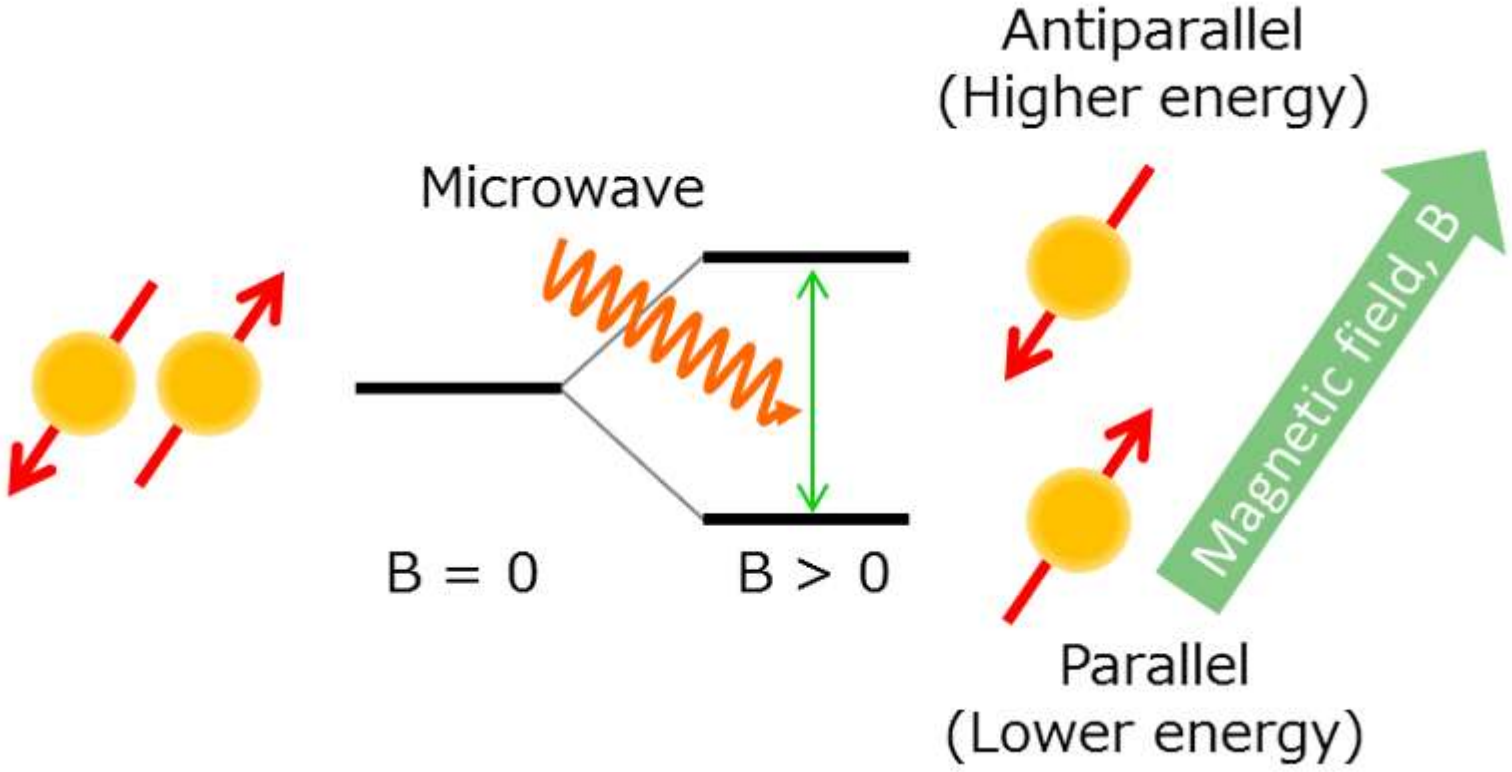
- ORD refers to the change in optical rotation with the change in wavelength of light source. i.e. applied only in optically active compounds.
- Optical rotation caused by compound changed with wavelength of light was first noted by ***Biot*** in **1817.**
- ORD curves in recent years are made use in structural determination by comparing the curve obtain from compound believed to have related structures particularly applied to carbonyl compounds.

# DIFFERENCE B/W ORD & CD

| ORD | CD |
| --- | --- |
| PLANE POLARIZED LIGHT | CIRCULARLY POLARIZED LUGHT |
| DISPERSIVE PHENOMENA | ABSORPTIVE PHENOMENA |
| PLANE POLARIZED IS USED & IS NOT CONVERTED TO ELLIPTICAL LUGHT | CIRCULAR POLARIZED LIGHT IS USED & IS CONVERTED TO ELLIPICITY |
| GRAPHS ARE OBTAINED BY SPECIFIC ROTATION V/S WAVELENGTH | GRAPHS ARE OBTAINED MOLAR ELLIPICITY V/S WAVE LENGTH |

# ESR Spectroscopy

- Electron Spin Resonance Spectroscopy
- Also called EPR Spectroscopy
  - Electron Paramagnetic Resonance Spectroscopy
- Non-destructive technique
- Applications
  - Extensively used in transition metal complexes
  - Deviated geometries in crystals


Antiparallel
(Higher energy)
Microwave
Magnetic field, B
B = 0
B > 0
Parallel
(Lower energy)

# Clinical application of ESR

- Nonspecific test.
- Prognostic not diagnostic.
- Monitor disease activity and response to therapy.
- ESR is a nonspecific marker of inflammation and is affected by other factors, ESR results must be used along with other clinical findings.

## APPLICATION

- ESR spectroscopy is one of the main methods used to study metalloproteins, particularly those containing molybdenum, copper, iron, etc.
- Both copper and non-haem iron do not absorb radiation in visible and ultra violet range, posses ESR absorbance peak in one of their oxidation state .
- Hence their appearance and disappearance of their ESR signal are used to monitor their activity in multi enzyme system