S. S. College, Jehanabad

Department: Zoology

Class: M.Sc. Semester II

Subject: Zoology

Topic: Introduction to Principles and Uses of Analytical Instruments

Mode of teaching: Google classroom & WhatsApp

Date & Time: 08.08.2020 & 10:30

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INTRODUCTION TO PRINCIPLES AND USES OF ANALYTICAL INSTRUMENTS

Everywhere we need measurement, be it our kitchen, businesses, research stations, clinical practices, industries, and other places. It is an important aspect of life and therefore without the measurement life will become tough. We measure time, length, weight, temperature, air pressure, flow, level, density, chemical composition, etc. in day-to-day life using various relative measuring device. Therefore, in other words measurement eases of our way of life and give a new direction of life and our livelihood.

It always requires the use of specific device to measure these parameters, that device is termed as **measuring device** or **measuring instruments**, and it is the science of measurement and control, which is known as **instrumentation**. **Analytical instruments** are those instruments which deal with the measurements and control of various physical and chemical properties such as pH, conductivity, density, viscosity, Octane number, chemical composition, etc. The analytical instruments that are used in laboratory are generally called as Bench top Laboratory Analyzers. These analytical instruments are mostly complex and maintenance intensive. Analytical instruments enable us to determine the chemical compositions of matters viz. "what are the elements present? What are the compounds present? In what quantities are they present?" Thus, it helps in gathering both qualitative as well as quantitative information of matter of test. Qualitative analysis utilizes the physical properties of matter such as boiling points, melting points, solubility in solvents, refractive indices and colour reactions, while quantitative analysis utilizes gravimetry, volumetry and tetrimetry of a matter. Because, it is found that presence of even minor impurities may completely change the overall characteristic of the matter. Below is the table which shows the different physical and chemical properties and instrumental methods for their measuring:

Physical/Chemical Property of Matter	
1. Optical	
Principle	**Analytical techniques**
Absorption of radiation	Photometry
	Spectrophotometry
	Atomic Absorption
Emission of radiation	Flame photometry
	Emission spectroscopy
	Fluorescence
Scattering of radiation	Turbidimetry
	Nephalometry
Diffraction of radiation	X ray diffraction
	Electron diffraction
2. Electrochemical methods	
Measurement of potential of electrode or change in potential	Potentiometry
Measurement of mass of substance deposited on electrode	Electrogravimetry

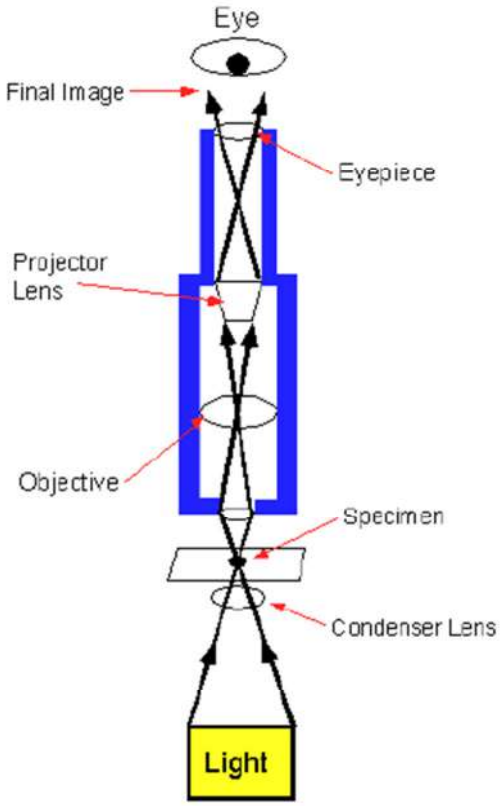
– Change of conductivity	
Measurement of conductivity or change in conductivity of a solution	Conductimetry
Change in diffusion current with respect to potential	Voltammetry/polarography
3. Mass to change determination	Mass spectrometry
4. Radioactivity Measurements	Radio analytical methods

However, the measurement of mass is fundamental in Analytical chemistry. Practically, all determinations depend directly or indirectly on the measurement of mass. This is the basis of gravimetry. Even for titrimetric measurements, the concentrations of standard solutions are expressed in terms of molarity, molality and normality, which are based on mass.

We discuss only few analytical instruments such as Microscope, Centrifuge, Spectrophotometer and Spectroscopy (Atomic Absorption Spectroscopy) as given in syllabus. An introduction of these analytical instruments and its principles is given below;

Microscopy

A microscope is a device which is used to visualize the minute object through magnification by the use of magnifying glass. It was first discovered by Antoni van Leeuwenhoek (1632 – 1723). Working principle of a microscope is the same basic principle as Rheinberg illumination, achieving different results by using different optical components. The basic idea involves splitting the light beam into two pathways that illuminate the specimen. Light waves that pass through dense structures within the specimen slow down compared to those that pass through less-dense structures. As all of the light waves are collected and transmitted to the eyepiece, they are recombined, so that they interfere with each other. As depicted in the figure, it utilizes two magnifying glasses; one is called as objective which is used to focus the specimen and can be magnified up to 100x depending upon the type of objective lenses, whereas the lens which is immediate to eye, is called as eye piece that can magnify image up to 20x. Magnification of a image can be measured by following formula;



A Light Microscope

$$\boldsymbol{Magnification = Value\ of\ eye\ piece \times Value\ of\ objective}$$

If, eyepiece is 10x and objective lens is 20x then the magnification;

$$\boldsymbol{Magnification = 10 \times 20 = 200\times}$$

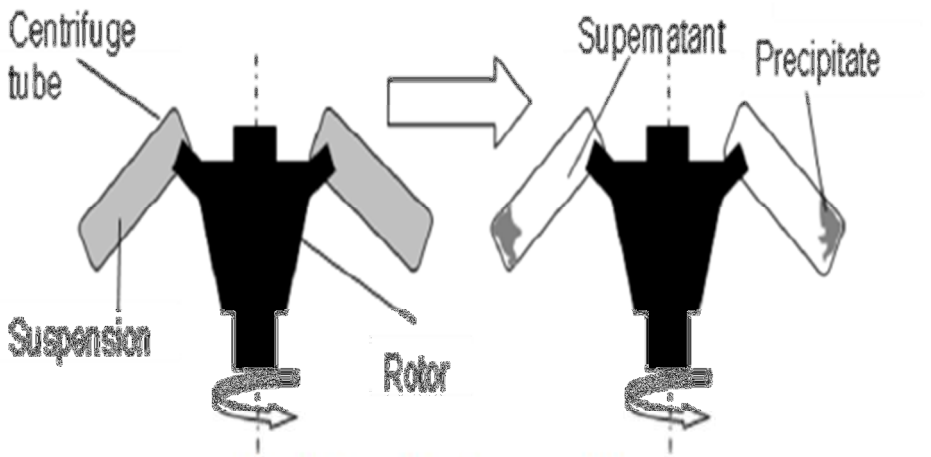
Similar to light microscope, electronic grids are used as lenses in place of glass lenses, and likewise the electron microscope also consists of eye piece and an objective lens but made of electronic grid.

Instruction of uses of microscope

- Do not touch the lenses. If they are dirty, use a lens paper. A soft cloth dipped in a small amount of isopropyl alcohol can also be used to clean the lenses.
- If using a microscope with a mirror, do not use direct sunlight as the light source. Eye damage can result. If using a microscope with a light, turn off light when not in use.
- Place a slide on the stage, and focus it as best as you can with the low power lens.
- Be cautious when handling glass slides and cover slips.
- Always clean slides and microscope when finished. Turn off light and cover microscope to avoid soiling.

Centrifuge

Centrifuge is a machine with a rapidly rotating container that applies centrifugal force to its contents to separate solutes of different densities in a solution, and the process is called as **centrifugation**. Best example of centrifugation is separation of cream from milk by churning. It was discovered by Gustaf de Laval in 1879.



Centrifugation in a centrifuge

The working principle of the centrifuge solely depends on the physical behavior of solids with different densities in a liquid. Centrifugal force (A type of work produced by a rotational movement that moves away from the centre of rotation) which builds up in the centrifuge, exceeds the gravitation force hundredfold is ideally adjusted to the input material for sorting medium and secures a quick and clean sorting of the material at a stable operating process. The rotors are placed at precise angles to overcome the centripetal force that settle some of the sample to the side of the container. Denser and heavier components of the sample end up on the bottom of the container, liquid materials float to the top while solid materials settle to the bottom. Spinning can be measured as the revolutions per minute (rpm) and as gravitational force (g-

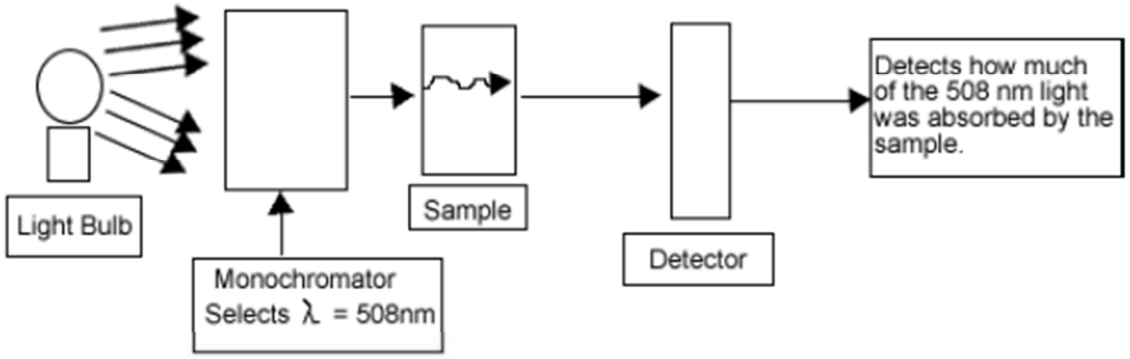
force), the unit of force equal to the force exerted by gravity. When a suspension is rotated at a certain speed or revolutions per minute (RPM), centrifugal force causes the particles to move radially away from the axis of rotation. The force on the particles (compared to gravity) is called Relative Centrifugal Force (RCF). For example, an RCF of 500 x g indicates that the centrifugal force applied is 500 times greater than Earth's gravitational force.

Instructions of uses of a centrifuge

- Autoclave centrifuge tubes only if absolutely necessary and only at 121°C for 15 min.
- Avoid cleaning plastic tubes in automated dishwashers or glassware washers, which may produce excessively hot temperatures.
- Clean tubes with a mild laboratory detergent in warm water, rinse, and thin air dry.
- Tube must be carefully matched with rotor type to prevent sample loss.

Spectrophotometer

It is an instrument that measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution and this process is known as **spectrophotometry**. It was invented by DeWitt B Brace in 1859.



Spectrophotometer

The spectrophotometer is working behind the law of Beer-Lambert Law[1]. When light beam passes through a slit, it is allowing for a specific wavelength to be selected. Only monochromatic light (single wavelength) successfully passes through a slit. The beams are allowed to pass through a sample cuvette and the reference cuvette. Some light of particular wavelength absorbed and rest of the light will be reflected. The intensities of the reflected light beams are then measured at the end. According to **Beer-Lambert Law**, absorption of light is directly proportional to the concentration of the molecules in the sample.

Instructions of uses of spectrophotometer

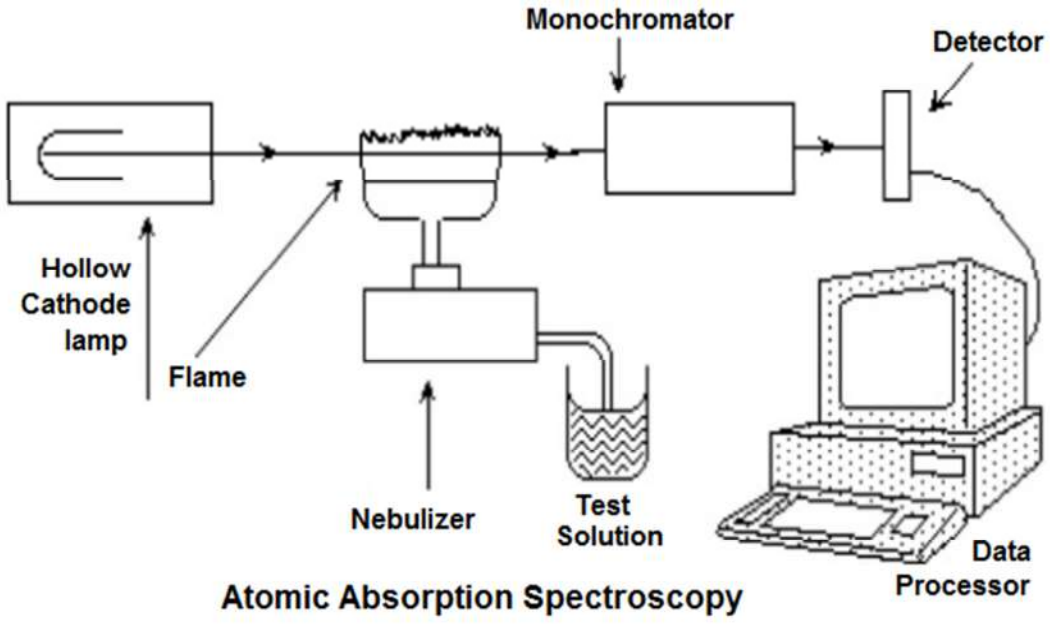
- Carefully handle the cuvettes.

[1] Beer-Lambert Law, also known as Beer's Law, states that there is a linear relationship between the absorbance and the concentration of sample.

- Wash the cuvettes with distilled water only.
- Ensure draining after washing out of the liquid in the cuvettes.
- Be care full on colour range and dilute the sample for higher concentration.

Atomic Absorption of Spectroscopy

Atomic absorption spectroscopy (AAS) is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. It was first used as an analytical technique and the underlying principles were established by Robert Wilhelm Bunsen and Gustav Robert Kirchhoff, both are professors at the University of Heidelberg, Germany in the second half of the 19th century on their study on sodium spectrum.



Atomic Absorption Spectroscopy

Atomized elements each absorb energy of a wavelength that is peculiar to that element. The atomic absorption method uses as its light source a hollow cathode lamp which emits light of a wavelength that is peculiar to each element. Elements within a solution are heated in a flame or electrically (2000K or 1727°C to 3000K or 2727°C) and subsequently determined using the fact that the degree of absorption varies with its concentration. It is widely used for the determination of metals at trace levels in solution and routine determination of alkali and alkaline earth metals.