chemical property of the analyte. This property must vary in a known and reproducible way with concentration cA of the analyte. The measurement of the property is directly proportional to the concentration.

cA = kX ... eq. 1.1

Where k is proportionality constant, the process of determining k is an important step in most analyses this step is called a calibration.

1.1.2.1.5. Calculate results

Computing analyte concentrations from experimental data is usually relatively easy, particularly with modern calculators or computers. These computations are based on the raw experimental data collected in the measurement step, characteristics of the measurement instruments and stoichiometry of the analytical reaction.

1.1.2.2.6. Estimate reliability of results

Analytical results are incomplete without an estimate of their reliability. The experimenter must provide some measure of the uncertainties associated with computed results if the data are to have any value.

1.2. VOLUMETRIC ANALYSIS OR TITRATION

Volumetric analysis or Titration involves the addition of a reactant to a solution being analyzed until some equivalence point is reached. Often the amount of material in the solution being analyzed may be determined. Most familiar to those who have taken college chemistry is the acid-base titration involving a color changing indicator. There are many other types of titrations and may use different types of indicators to reach some equivalence point. *Ex:* Potentiometric titrations.

Volumetric or titrimetric analyses are quantitative analytical techniques which employ a titration in comparing an unknown with a standard. In a titration, a volume of a standardized solution containing a known concentration of reactant A is added to a sample containing an unknown concentration of reactant B. The titration proceeds until reactant B is just consumed (stoichiometric completion); this is known as an equivalence point. At this point the number of equivalents of A added to the unknown equals the number of equivalents of B originally present in the unknown. Volumetric methods have the potential for a precision of up to 0.1%. In almost all cases, a burette is used to meter out the titrant. When a titrant reacts directly with an analyte or with a reaction the product of the analyte and some intermediate compound, the procedure is termed a direct titration. and to purify the sample and used in various industrial processes for isolating metals organic compounds etc. Separation techniques are chromatography, ion-exchange, liquid extraction etc.

1.5.7. Hyphenated techniques

A hyphenated separation technique refers to a combination of two or more techniques to detect and separate chemicals from solutions. Hyphenated techniques are widely used in chemistry and biochemistry. A slash is sometimes used instead of hyphen, especially if the name of one of the methods contains a hyphen itself. Several examples are in popular use today and new hybrid techniques are under development. Ex: Gas chromatographymass spectrometry, LC-MS, HPLC-MS, GC-IR, LC-NMR, CE-MS, CE-UV and GC-MS.

1.5.8. Microscopy

The visualization of single molecules, single cells, biological tissues and nano- and micro materials is very important and attractive approach in analytical science. Also, hybridization with other traditional analytical tools is revolutionizing analytical science. Microscopy can be categorized into three different fields: optical microscopy, electron microscopy, and scanning probe microscopy. Recently this field is rapidly progressing because of the rapid development of computer and camera industries.

1.6. ROLE OF ANALYTICAL CHEMISTRY

Analytical chemistry has played critical roles in the understanding of basic science to a variety of practical applications, such as biomedical applications, environmental monitoring, quality control of industrial manufacturing and forensic science. Many chemists', biochemists and medicinal chemists devote much time in the laboratory gathering quantitative information about systems that are important and interesting to them. Quantitative analytical measurements are also play a vital role in many research areas in chemistry, biochemistry, biology, geology, physics and other sciences. The central role of analytical chemistry in this enterprise and many other is illustrated in Fig. 1.4. Analytical chemistry has a similar function with respect to the many other scientific fields listed in the Fig. 1.4. The central location of analytical chemistry in Fig. 1.4 signifies its importance and the breadth of its interactions with many other disciplines. Chemistry is often called the central science; its top center position and the central position of analytical chemistry in this importance. The interdisciplinary

concentration. Measurements of substance C become less sensitive with increasing concentration. Sensitivity may also be expressed as the concentration of analyte required to cause a given instrument response. For example In atomic absorption spectroscopy, sensitivity is expressed as concentration in micrograms per milliliter of analyte that produces an absorbance of 0.0043 absorbance unit (1.0% absorption). When comparing different techniques or instrument, one should be alert to the procedures used by the practitioners to arrive at sensitivity values.

1.14. DETECTION LIMIT

Detection limit is commonly understood to be the smallest concentration we can measure with a particular technique. In fact it is the point at which we can make a decision whether the element or compound is present or not. To be able to measure it we need at least three times the detection limit. Three times the detection limit is often called the limit of determination. A different term used for detection limits is "Limits of Detection" with the abbreviation LoD.

The minimum single result with a stated probability can be distinguished from a suitable blank value. The limit defines the point at which the analysis becomes possible and this may be different from the lower limit of the determinable analytical range. In chemical analysis, the minimum amount of a particular component that can be determined by a single measurement with a stated confidence level. LOD or detection limit (DL), is the lowest amount of analyte in a sample that can be detected, but not necessarily quantities as an exact value. The LOD may be expressed as:

$$LOD = 3.3 * SD/S$$
 ... eq. 1.7

Where:

SD = the standard deviation of the response S = the slope of the calibration curve

As the concentration of the analyte approaches zero the signal disappears into the noise and the detection limit is exceeded. The detection limit is most generally defined as the concentration of analyte that give a signal, x, significantly different from the blank or background signal x_B . This definition leaves the analyst with considerable freedom to define the phrase significantly different. When working with analytes in trace amounts the analyst is confronted with two problems:

i. Reporting an analyte present when in fact it is absent and

Microscopes
of
types
five
of
Comparison
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Table

S. No.	Name of the Microscope	Maximum Magnification	Appearance of Specimen	Common Uses	Advantages	Disadvantages
	Bright-field Microscopy	1000X	Specimen stained or unstained; bacterium generally stained and appear colour of stain	Observing stained specimens; counting microscopes	Easy to use; readily available; relatively inexpensive; allows staining reactions to be interpreted	Lack of contrast; inability to resolve very thin bacteria and viruses; introduction of artifacts during
6	Dark-field Microscopy	1000X	Generally unstained; appears bright or lighted in an otherwise dark	Viewing unstained cells not observable with bright field microscope	Allow living microbes to be viewed Ex: Spirochetes	staining procedures Staining reactions cannot be used for examining stained specimens.
ë.	Fluorescence Microscopy	1000X	field. Bright and coloured; colour of the fluorescent dye	Detecting specific infectious agents in tissue, detecting immunological reactions	Rapid identification of infectious microorganisms	Specimens that naturally fluoresce or which are stained with a fluorescent dve are only
.4	Phase- Contrast Microscopy	1000X	Structures varying degrees of darkness	Observing living unstained cell; revcaling intracellular structures	Enhances subcellular anatomy; allows observation of motility,	observed. Inability to evaluate staining reactions
ý	Electron Microscopy	1,000.000X	Viewed on fluorescent screen	Examination of viruses and ultra structure of cells; diagnosis certain virus diseases and cancers; detecting certain giant molecules.	pnagocytes and biological activities	

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Instrumental Methods of Analysis