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Analytical Chemistry

- Analytical chemistry is the science of obtaining, processing, and communicating information about the **composition and structure of matter.**
- In other words, **it is the art and science** of determining what matter is and how much of it exists.

- Analytical chemists use their knowledge of chemistry, instrumentation, computers, and statistics to solve problems in almost all areas of chemistry and for all kinds of industries.
- For example, their measurements are used to assure the **safety and quality** of food, pharmaceuticals, and water; to assure compliance with environmental and other regulations; to support the legal process; to help physicians diagnose diseases.
- Analytical chemists often work in service-related jobs and are employed in industry, academia, and government.
- They conduct basic laboratory research; perform process and product development; design instruments used in analytical analysis; teach; and work in marketing and law.
- Analytical chemistry can be a challenging profession that makes significant contributions to many fields of science.

Different types of chemical analysis may be classified as:

(i) Proximate Analysis: the amount of each element in a sample is determined with no concern as to the actual components present.

(ii) Partial analysis: deals with the determination of selected constituents in the sample,

(iii) Trace constituent analysis: a specialized form of partial analysis in which determination of specified components present in very minute quantity,

(iv) Complete Analysis: when the proportion of each component of the sample is determined.

Role of pharmaceutical analysis in pharmaceutical industry

- Pharmaceutical analysis may be defined as the application of analytical procedures used to determine the **purity, safety and quality of drugs and chemicals.**
- Pharmaceutical analysis rely upon both **qualitative and quantitative chemical analysis** to ensure that the raw material used meet all the desired specifications, and also to check the quality of the final product.
- The examination of raw material is carried out to ensure that there is no unusual substance present which might deteriorate the manufacturing process or appear as a harmful impurity in the final product.
- The quantity of required ingredient in raw material is determined by a procedure known as **Assay.**

- In the modern practice of medicine, the analytical methods are used in the analysis of chemical constituents found in the human body whose altered concentrations during disease states as diagnostic aids and also used to analyse the medicinal agents and their metabolites found in biological system.
- The term “quality” as applied to a drug product has been defined as the sum of all factors which contribute directly or indirectly to the safety, effectiveness and reliability of the product.
- These properties are built into drug products through research and during the manufacturing process by procedures collectively referred to as **quality control.**
- Quality control guarantees within reasonable limits that a drug products

i. is free of impurities
ii. is physically and chemically stable
iii. contains the amount of active ingredients as stated on label and
iv. provides optimal release of active ingredients when the product is administered.

- The quality of pharmaceutical products depends on the correct performance of al manufacturing operations and must be built in from the beginning of the manufacturing process.
- The principles of quality control procedures that should be applied to drug manufacturing practices are designated **Good Manufacturing Practices (GMP)** in the manufacture and quality control of drugs.
- The quantitative analytical methods such as assays are very much essential to find out the % purity, the active content of the pharmaceutical product etc.
- The choice and selection of the assay methods will depend on the type of substance to be estimated to find out its purity of the pharmaceutical product.

Some specific use of analysis is under mentioned:

(i) Quantitative analysis of air, water and soil samples is carried out to determine the level of pollution.

(ii) Chemical analysis is widely used to assist in the diagnosis of illness and in monitoring the condition of patients.

(iii) In farming, nature of soil and level of fertilizer application is analyzed

(iv) In geology, composition of the rock and soil is carried out.

In general analysis is divided into two major part:

(a) Qualitative analysis (what substances are present in the given sample)

(b) Quantitative analysis (to determine the quantity of each component in the given sample)

Different Techniques of Analysis

The main techniques are based upon:

1. The quantitative performance of suitable chemical reactions
2. Appropriate electrical measurements
3. Measurement of certain optical properties and
4. Combination of optical and electrical measurement followed by quantitative chemical reaction. Eg. Amperometry

1. Methods Based on Chemical Analysis: These are based on traditional method of analysis and may be divided as:

(a) Titrimetry

(b) Gravimetry

(a) Titrimetric Analysis (also termed as volumetric analysis) in this technique the substance to be determined is allowed to react with an appropriate reagent added as a standard solution, and the volume of solution needed for completion on reaction is determined. Following are the types of titrimetric analysis:

- **(i)** Neutralization (acid-base) reactions
- **(ii)** Complexometric titrations
- **(iii)** Precipitation titrations
- **(iv)** Oxidation-reduction titrations

(b) Gravimetric Analysis in this technique substance under determination is converted into an insoluble precipitate which is collected and weighed.

In a special case of gravimetric analysis, electrolysis of the substance is carried out and the material deposited on one of the electrodes is weigh, this technique is called as **electrogravimetry.**

2. Electrical Methods of Analysis: These involve the measurement of current voltage or resistance in relation to the concentration of a certain species in a solution. These methods are of following types:

i. **Voltametry:** It is the measurement of current at a microelectrode at a specified voltage.

ii. **Coulometry:** It is the measurement of current and time needed to complete an electrochemical reaction or to generate sufficient material to react completely with a specified reagent.

iii. **Conductometry:** It is the measurement of electrical conductivity of a solution. The ionic reactions in which there is a sudden change in conductance after completion of reaction, can act as a basis of conductometric titration method.

iv. **Potentiometry:** It is the measurement of the potential of an electrode in equilibrium with an ion to be determined..

3. Optical Methods of Analysis: The optical methods of analysis depend upon:

i. Measurement of the amount of radiant energy of a particular wavelength absorbed by the sample.

ii. The emission of radiant energy and measurement of the amount of energy of a particular wavelength emitted.

- Several analytical techniques have been developed which involve the measurement of radiant energy. These are:
    - i. Emission spectrography
    - ii. Colorimetry
    - iii. Fluorimetry
    - iv. Turbidimetry & Nephelometry
    - v. Spectrophotometry
    - vi. Flame photometry
    - vii. Atomic absorption spectroscopy
    - viii. Polarimetry

The optical methods are basically of two types:

i. Absorption methods

ii. Emission methods.

Absorption methods are usually classified according to wavelength involved:

i. Visible spectrophotometry

ii. Ultraviolet spectrophotometry

iii. Infrared spectrophotometry

iv. Atomic absoption spectroscopy

- In **emission** method sample is subjected to heat or electrical treatment so that the atoms are raised to excited states causing them to emit-energy; and the intensity of this emitted energy is measured.
- The emission spectroscopy includes flame photometry and fluorimetry as common excitation techniques.

4. Other Methods: the others methods of analysis like

Chromatography,

Radioactivity,

Kinetic methods,

Mass spectrometry,

Thermal method of analysis,

NMR spectroscopy and

X-ray diffraction analysis etc.

Significant Figures

- A figure of digit denotes any one of the ten numerals (0,1,2,3,4,5,6,7,8,9). A digit alone or in combination serves to express a number.
- A significant figure is a digit having some practical meaning, *i.e.* it is a digit, which denotes the amount of the quantity in the place in which it stands.
- For example in 0.456, 4.56 and 456 there are three significant figures in each number.
- Zero may or may not be a significant figure. A zero is a significant figure except when it serves to locate the decimal point, while it is a significant figure when it indicates that the quantity in place in which *i.e.* in 1.3680 and 1.0082, zero is significant but in 0.0035, zeros are not the significant figures as they serve only to locate the decimal point. Thus, first two numbers contain five but the third one contains two significant figures.

Computation Rules

- **Rule 1 →** In expressing an experimental measurement, never retain more than one doubtful digit. Eliminate all the digits that are not significant.
- **Rule 2 →** Retain as many significant figures in a result or in any data as will give only one uncertain figure. *e.g. a* volume between 30.5 ml and 30.7 ml should be written as 30.6 ml. and not as 30.60 as it would be between 30.59 and 30.61.
- **Rule 3 →** Two rules are given for rejecting superfluous digits.
- 1. When the last digit dropped is greater than 5, the last digit retained is increased by one. e.g. in rejecting the last digit in 8.947, the new value will be 8.95 as 7 is greater than 5. But when 4.863 is rounded up to two digits, it gives 4.9 as the first digit discarded is 6 which is greater than 5. This is known as rounding up.

- 2. If the first digit discarded is less than 5, leave the last digit unchanged. It is known as rounding down. e.g. when the number 5.64987 is rounded to two digits, we get 5.6 as the first digit, discarded is 4, which is less than 5. Rounding never changes the power of 10. Thus, it is better to express numbers in exponential notation before rounding. e.g. in rounding 57832 to four figures, result $5.783 \text{ X } 10^4$

- **Rule 4.** In addition or subtraction, there should be in each number only as many significant figures as there are in the least accurately known number. e.g. sum of three values 35.6, 0.162 and 71.41 should be reported only to the first decimal place as the value 35.6 is known only to the first decimal place. Thus, the answer 107.172 is rounded to 107.2
- **Rule 5.** In multiplication or division, retain in each factor one more significant figure than is contained in the factor having the largest uncertainty. The percentage precision of a product or quotient cannot be greater than the percentage precision of the least precise factor entering into the calculation. e.g. the product of the three figures 0.0121, 25.64 and 1.05782 is 0.0121 X 25.6 X 1.06 = 0.328
- In a product or quotient of experimental numbers, the final result will have only as many significant figures as the factor with smallest number of significant figures.

e.g. in the calculation, $\frac{(0.0181057)\,(197.15)\,(0.218)}{0.4970}$,least number of significant figures

(3) is in 0.218. Thus, the answer should also be expressed in three significant figures.

- When a calculation involves both addition or subtraction and multiplication or division, addition is done first so as to determine the number of significant figures in the answer.
- **Rule 6** Computation involving a precision not greater than one fourth of 1 % should be made with a 10-inch slide rule. For greater precision, logarithm tables should be used.
- Slide rule is a good method for checking the calculations made by logarithms. Use of logarithms has been recommended where a large number of multiplications and divisions are to be made.

Errors

- In quantitative analysis, when numerical data and numerical results are measured with the greatest exactness.
- It has been observed that the results of successive determination differ among themselves to a greater or lesser extent.
- Evidently not all and perhaps none, of the values obtained are correct within the possible limits of measurements.
- The average value of a series of measurements is accepted as the most probable value. It should, however, be noted that the average value may not always be the true value. In some cases difference may be small and in others, it may be so large that the result is unacceptable.
- Thus, the reliability of the result depends upon the magnitude of the difference between the average value and the true value.

Classification of Errors: Errors in any set of measurements can be divided into the following categories:

1. Systematic, determinate or constant errors.
2. Random, accidental or indeterminate errors.

1. **Systematic, determinate or constant errors:** These errors can be avoided and their magnitude can be determined, thereby correcting the measurements.

- Determinate errors are characterized by the fact that it affects the results of a series of determination to the same degree. These errors occur with definite regularity owing to the faulty methods of technique or measuring instruments.

Types of determinate errors: Determinate errors may be of different types :

I. ***Personal errors:*** These errors are not connected with the method or procedure but the individual analyst is responsible for them. This type of errors may arise due to the inability of the individual making observations. Some important personal errors are:

A. Inability in judging colour change sharply in visual titrations.
B. Error in reading a burette.
C. Mechanical loss of material in various steps of an analysis.
D. Failure to wash and ignite a precipitate properly.
E. Insufficient cooling of crucible before weighing.
F. Using impure reagents.
G. Ignition of precipitate at incorrect temperatures.
H. Errors in calculations.

II. ***Operational errors:*** These errors are mostly physical in nature and occur when sound analytical technique is not followed.

III. ***Instruments and reagent errors:*** following factors are reasponsible for such errors:

A. Balance arms of unequal lengths.

B. Uncalibrated or improperly calibrated weights.

C. Incorrectly graduated burettes.

D. Attack of foreign materials upon glasswares.

E. Loss in weight of platinum crucibles on strong heating.

F. Impure reagents.

These errors can be avoided by using calibrated weights, glasswares and pure reagents.

IV. ***Methodic errors:*** These are the most serious types of errors encountered in chemical analysis. Some exmples involving methodic errors are:

A. Solubility of precipitate in medium and in wash liquid.

B. Decomposition or volatilization of weighing forms of precipitates on ignition of on heating.

C. Hygroscopicity of the weighing forms of the precipitates.

D. Co-precipitation.

E. Post-precipitation

F. Failure of a reaction to achieve completion.

G. Occurrence of side reactions.

These errors can be eliminated or reduced to a small magnitude by employing the proper technique.

V ***Additive and proportional errors:***

- Absolute error is independent of the amount of the constituent present in the determination

e.g., loss in weight of a crucible adds error to the weight of precipitate is ignited in it.

- On the other hand, the magnitude of proportional error depends upon the quantity of the constituent.
- e.g., impurity present in a standard substance gives a wrong value for the normality of a standard solution.

2. Random or Indeterminate Errors: These errors are accidental and analyst has no control over them. They may be of two types.

(i) Variation within determinate errors: These cannot be prevented from variation e.g., in igniting a precipitate of $Al(OH)_3$ to constant weight of aluminum oxide, an analyst may obtain different values without regular variation. This is because igniting the precipitate at incorrect temperatures.

(ii) Erratic errors: Analyst has no control over such errors. Important examples of erratic errors are:

A. Vibration in balance while handling it.
B. Accidental loss of material during analysis.

The mathematic model that satisfies the magnitude of random error and the frequency of its occurrence is called normal distribution.

Minimization of Errors

The determinate error may be minimized by using following methods:

1. **Running a blank determination:** Errors arising from the introduction of impurities through the reagents and vessels are accomplished by running a blank. Such a procedure involves going through all the analysis, using the same solvent and reagent in the same quantities, but omitting the unknown component. Thus, in making a blank, sample is omitted; otherwise the details of the procedure are followed exactly as far as possible.

2. **Calibration of apparatus and application of corrections:** All instruments, such as burettes, pipettes, weights, measuring flasks, etc. must be properly calibrated and the appropriate corrections must be applied to the original measurements.

3. **Running a controlled determination:** It consists in carrying out a determination under identical experimental conditions as far as possible upon a quantity of a standard substance, which contains the same weight of the constituent as it contained in the unknown sample.

The weight of the constituent x in the unknown can then be calculated.

$$\frac{\text{Result found for standard}}{\text{Result found for unknown}} = \frac{\text{Wt. of constituent in standard}}{\text{x}}$$

4. **Running of parallel determination:** Parallel determinations serve as a check in the result of a single determination and indicate only the precision of the analysis. The values obtained in parallel determination should agree well among themselves. These values should not vary by more than three parts per thousand.

If larger variations are shown the determination must be repeated until satisfactory concordance is obtained. A good agreement between, duplicate and triplicate determinations does not justify the conclusion that the result is correct, but it merely show that the accidental errors or variations of the determinate errors are same in parallel determinations.

5.**Standard addition:**

- A known amount of the constituent being determined is added to the sample, which is then analysed for the total amount of constituent present.
- The difference between the analytical results for samples with and without the added constituent gives the recovery of the amount of added constituent.
- If the recovery is satisfactory, accuracy of the procedure is enhanced. This procedure is especially applied to physico-chemical processes, as polarography and spectrophotometry.

6.**Isotopic dilution:** It consists in adding a known amount of pure component containing a radioactive isotope to the unknown, now the element so isolated is obtained in pure form usually as a compound. Its activity is determined with the help of an electroscope. The activity is compared with the added element. The weight of element in the unknown sample can be calculated.

7.**Use of independent method of analysis:** Sometimes the complete analysis has to be carried out in an entirely different manner to get accuracy of results. e.g. strength of HCl may be determined by two methods :

i. Titrating it with a standard solution of a strong base.

ii. Precipitation with AgNO3 and weighing as AgCl.

If the results obtained by the two methods are in good agreement, it may be said that the values are correct within small limits of errors.

Calibration of Analytical Equipments

There are three general approaches to the calibration of volumetric glassware.

1. Direct, absolute calibration: In this, volume or water delivered by the burette or pipette or contained in volumetric flask is obtained directly from the weight of the water and its density.

2. Calibration by Comparison (Indirect): In this approach, the volumetric glassware is calibrated by using previously calibrated vessel. It is especially convenient if many pieces of glassware's are to be calibrated.

3. Relative calibration: Sometimes it is necessary to know only the relationship between two items of glassware's without knowing the absolute volume of either one.

Calibration of Burettes:

- A burette is a long calibrated glass tube with a fine end tip and a glass stopcock to allow controlled flow of volume.
- It affords greater precision, typically 0.1 to 0.2 %.
- Burette is principally used in titrations for the accurate delivery of a standard solution to the sample solution until the end point is reached.
- The conventional burettes for macro titrations are marked in 0.1 ml increments and are available in capacities of 10, 25, 50 and 100 ml.
- Micro-burettes are available in capacities of down to 2 ml where the volume is marked in 0.01 ml increments.

Burettes can be calibrated by the following method

- Clean the burette thoroughly and lubricate the stopcock properly.
- Fill with water and test for leakage and wait for at least 5 minutes.
- During waiting period weigh a suitable receiver (generally an Erlenmeyer flask) to the nearest milligram. Record this weight.
- Fill the burette with distilled water. Measure and record the temperature. Sweep any air bubble from the tip of burette. Now, withdraw water more slowly until meniscus is at or slightly below the zero mark on burette. After drainage is complete read the burette to the nearest 0.01 ml. Record the initial reading. Remove the hanging drop.
- Now run about 10 ml of water from burette into a previously weighed flask. Quickly stopper the flask and weigh it to the nearest milligram. Record this weight.
- Read the burette reading after allowing the time for drainage and record the final reading. Calculate the correction as –

Intial B.R.	Final B.R.	App. Vol^{m}	Intial wt.	Final wt.	Act. Vol.	Temp.	Correction

- Now, refill the burette and obtain another initial reading. Run about 20 ml of water into the flask. Obtain the final burette reading and reweigh the flask. Note that we are calibrating the burette in 10 ml intervals but starting each time from the initial reading as titration starts generally from zero.
- This process is repeated for 30-40-50 ml volumes.
- The difference between actual volume and apparent volume is used as correction.
- Calibration of burette is repeated, as a check on work and the duplicate results should agree within 0.04 ml.

- For intermediate value, calibration is better by graphical methods. Plot correction against interval of 10,20 ml …Connect the points to get a straight line so as to obtain linear interpolations by simple inspection of graph.

The tolerances on capacity for burettes as per Indian pharmacopoeia are given below:

	BURETTES		
Normal capacity ml.	**10**	**25**	**50**
Subdivision ml.	**0.05**	**0.05**	**0.1**
Tolerance ± ml.	**0.01**	**0.03**	**0.05**

Calibration of Pipette:

- The pipette is used to transfer quantitatively a known volume of solution from one container to another. There are two common types of pipettes, the volumetric (transfer) pipette and measuring pipette.
- Volumetric pipette are used for high accuracy analytical work. They are calibrated to deliver a specified volume at a given temperature and are available in sizes from 0.5 to 200 ml.
- Measuring pipette are straight bore pipette that are marked at 6 different volume intervals. They are convenient for delivering various volumes with reasonable 0.5 to 0.01 ml accuracy. They are not as accurate because of non-uniformity of the internal diameter. Pipette must be calibrated if higher accuracy is required.

Pipette can be calibrated as follows:

- Clean and rinse the pipette thoroughly and try it.
- Weigh to nearest milligram a receiver generally Erlenmeyer flask.
- Fill the pipette to a level above the itched line using distilled water at the laboratory temperature. Remove any liquid on outside and release the pressure to allow the liquid to fall to itched line.
- Discharge the contents of pipette to a previously weighed receiver. Allow the pipette to drain completely for 20 to 30 seconds.
- Stopper the container and reweigh it. Calculate the volume of water delivered by the pipette from the weight and see the apparent volume from table.
- Calibration of pipette is repeated, as a check on work and duplicate results should not differ by more than 0.02mL.

The tolerances on capacity to pipettes as per Indian pharmacopoeia are given below:

			One mark Pipette
Normal capacity ml.	**10**	**25**	**50**
Tolerance ± ml.	**0.02**	**0.03**	**0.05**
			Graduated Pipette
Normal capacity ml.	**1**	**5**	**10**
Subdivision ml.	**0.01**	**0.05**	**0.10**
Tolerance ± ml.	**0.006**	**0.03**	**0.05**

Calibration of volumetric flasks:

- Volumetric flasks are used in the preparation of standard solution and are available with capacities from 5 to 1000 ml.
- They are normally calibrated to contain a specified volume at 27^0C when filled to the line etched on the neck.
- Calibration of a volumetric flask is necessary only for work of the highest accuracy and following ways can do it:
- Volumetric flask is cleaned, rinsed and then clamped in an inverted position to dry it.
- Stopper the flask and weigh to nearest milligram and record this weight.

- Fill the flask with distilled water at room temperature. Adjust the lower meniscus of water to the etched level mark by means of pipette or dropper.
- Stopper the flask and reweigh to the nearest milligram. Difference in weight gives the apparent volume of water contained and from the weight of water. Calculate the actual volume.
- Calibration should, be checked by repeating the procedure. Duplicate results should agree within 0.3 ml for the flask.

The tolerances on capacity for volumetric flask as per Indian Pharmacopoeia are given below:

Normal capacity ml.	10	25	50	100
Tolerance ± ml.	0.02	0.03	0.04	0.06