Laboratory Manual: Analytical Chemistry
BT 514-P

Virtual University of Pakistan

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Practical No: 1
Introduction to Analytical Chemistry

Analytical chemistry is a science field evolving and adapting methods, devices and strategy for obtaining information about chemical composition, structure and energy state of substances. Goals of analytical chemistry: to detect chemical elements, which compose particular substance?

Qualitative analysis to determine the ratios of different elements in investigative substance

Quantitative analysis various substances differ from each other by composition, structure, physical and chemical properties. Most of properties can be used to learn about qualities, which distinguish substance from others.

These qualities are analytical signals

Methods of analysis are based on obtaining analysis signals and measurement of intensity of signals. According to action, which gives analytic signal, methods of analysis are divided into physical and chemical instrumental methods. They are employed in both in qualitative and quantitative analysis.

Chemical methods of analysis methods based on chemical interaction of atoms, molecules and ions. These methods are employed to detect characterize chemical properties of element or ion. Methods of chemical analysis can be divided according the type of chemical reaction, rate of chemical reaction and advisability (gravimetry, titrimetry, gas analysis, kinetic methods of analysis).

Physical methods are based on different parameters of substance radioactivity, electromagnetic properties, and radiation.

Also there are:

Biological methods based on use of biologically active substances and biological systems.

Biochemical method when substances of biological origin are investigated with chemical methods.

Analysis of chemical composition of substance proceeds by following steps:

1. Choosing a sample; preparation of sample for analysis
2. Extraction of component to investigate, purification; choosing method and scheme of analysis
3. Disruption or solving of sample
4. Separation and concentration
5. Measurement of physical properties of sample, chemical reagent or product of chemical reaction
6. Calculation of analysis data
7. Estimation of results reliability.

Terminologies:

The **sample** is a material that we wish to analyze and also has a statistical meaning. The **analyte** is the substance or element in the sample whose presence or concentration we wish to determine. The matrix is other components of the sample other than the analyte.

The Analytical Process

Every scientist requires an understanding of analytical science so that the data produced fit the purpose. Analytical science can be considered as the science of problem solving. As can be seen in Figure, analytical science is a process rather than an end in itself:

• The technical approach to solving the problem requires the analyst to consider the analytical information, required level of accuracy, the cost, timing, availability of instruments and facilities before selecting the method.

• Knowledge and practical skills start with sampling. Without a representative sample, the results from the time consuming analysis are of little use. This is followed by the sample treatment to produce the analyte into suitable form for detection/measurement by the selected analytical technique. This may involve dissolution, separation of the analyte from the sample matrix or converting the analyte to another form.

• Interpretative skills are essential to determine the significance of the analytical results and their relevance in solving the original problem.

Fig 1.1: Analytical process of sample

Table 1.1: A few commonly used terminologies are given in the Table

Validation	The process of verifying that a procedure yields acceptable results.
QA/QC	Those steps taken to ensure that the work conducted in an analytical Lab is capable of producing acceptable results.
Mean	The average of a set of data
Median	Middle value(s) when a set of data is arranged in ascending or descending order
Range	Difference between the largest and smallest values in a set of data
Std. dev (s)	A statistical measure of average deviation from the mean of a set of

	data
Error	A measure of bias in a result
Variance	Square of standard deviation (s)
Sampling error	Error occurred during sampling process
Method error	Error due to limitations of the analytical method
Personal error	Error due to analyst & his/her approach
Measurement error	Error due to limitations in the equipment / instrument used
Determinate error	Error that can be traced to the source – above FOUR
Outlier	A datum which is far larger / smaller than the remaining data
Repeatability	The precision for analysis in which the only source of variability is the analysis of replicate samples
Reproducibility	Precision of results of several samples, several analysts or several method
Indeterminate errors	Sources are not known but affects the scatter around the central value (mean)
Uncertainty	The range of possible values for a measurement
Confidence intervals	Range of results around a mean value that can be explained by random error
Histogram	Profile of frequency as a function of the range of measured values
Normal distribution	Normalized frequency (probability) of measured values
Degrees of freedom	Number of independent values on which the result is based
Significance test	A statistical test to determine whether the difference between two Values are significant or not.
Null hypothesis	A statement that the difference between two values can be explained by indeterminate error
SRM / CRM	Material certified with known concentrations of the analytes
Type 1 error	The risk of falsely rejecting null hypothesis
Type 2 error	The risk of falsely retaining the null hypothesis
t-test	For comparing two mean values
F -test	For comparing two variances

Practical No: 2
Work Safety Instructions for Persons Working in Chemical Laboratory

GENERAL PART

- Student must obey established order in the work place, take care of his or her health and of colleagues' health, perform requirements of this instruction.
- Students can't use devices, which have defects and must report lecturer about them.

- Don't use defect sockets, plugs, switches and other defect equipment. Electrical devices must be grounded, if it is required by use rules. Switch off electrical device if current flow outside circuit is noticed.
- Don't connect to one socket several high power devices, if their requirement of current may exceed permeability of installation cables. Remember, voltage up to 36 volts is not dangerous to human.
- Work carefully with laboratory equipment, glassware and devices and start work with them only after learned how to use them. If equipment is broken, report to laboratory worker immediately.
- If gas, water supply, canalization, electricity system defect is noticed, report to laboratory worker. If gas flow is noticed, close gas valve and don't switch on any devices, which can induce flame or sparks.
- When leaving laboratory, check if all electrical and gas devices are switched off and if no water or gas flow is present. Last leaving laboratory person is directly responsible for this requirement.
- Wear lab coat during the practical classes and wear closed toe shoes.
- Wash your hands thoroughly when exiting and entering the lab. Biochemical experiments are very sensitive to contamination & the chemicals can harm you without any symptoms being apparent.
- Keep your desk and apparatus neat and clean. At the end of the laboratory period, clean and put away all apparatus, and wash and dry the desktop.
- Leave any food, beverages outside the lab. Never drink water or eat anything in the laboratory.
- Properly label and store all chemicals.
- Do not smell or taste chemicals
- Do not keep a vessel with the base or acid near your eyes.
- Never use mouth suction for pipetting or starting a siphon
- Do not discard any chemicals down the sink unless instructed to do so.
- Never leave spilled chemicals or water on a desktop or floor. Clean up immediately.
- Read the label carefully before using any chemical.

- Never return unused chemicals to the stock bottles. This may lead to contamination of the entire supply of reagent.
- Be careful not to mix stoppers or caps of reagent bottles.
- Never pour water into conc. acid. Pour the acid slowly into the water with stirring. (In a fume hood).
- Know the properties (i.e., flammability, toxicity, and reactivity) of the chemicals in use. All hazardous chemicals must be handled in the fume hood.
- Consult the teacher before using any unfamiliar equipment.
- Report any injury, no matter how slight, to your instructor

Personal care:

- Work only with clean laboratory robes.
- Wash hands before and after work with warm water and soap, use disinfection and neutralization measures.
- Don't keep food at the work place, eat only in special place.

HANDLING OF REAGENTS AND DEVICES

- All experiments with strong smelling, explosive, dangerous to health or volatile substances are performed in fume hood, with protecting glass lowered.
- When working with strong smelling, dusty, dangerous to health substances not in the fume hood, respiratory mask and safety glasses must be used.
- Flammable substances and heating devices must be handled extremely carefully. Don't heat ether (C2H5-O-C2H5), ethanol (C2H5-OH), petrol (Cs-C9) using opened flame or opened electrical heater. Heat them carefully, on closed electrical cooker or in water bath.
- Use for chemical experiment exact amount of substance, as indicated in laboratory work instruction.
- Avoid contact with chemicals which may cause external or internal injuries. External injuries are caused by skin exposure to caustic/corrosive chemicals (acid/base/reactive salts).
- Use pipettes with holders and avoid mouth pipetting.
- Store acids and bases separately, in well-ventilated areas and away from volatile organic and oxidisable substances.

Practical No: 3
Laboratory Technique, Materials and Fundamental Operations

Students, who work in a chemistry laboratory, must know the purpose, potential use and all features of the laboratory's equipment, devices and tools. The success of laboratory session is determined by accurately, thoroughly performed operations and actions, named in the description of an experiment, as well as acquirement.

While working in the analytical chemistry laboratory it is necessary to learn how to:

- Prepare laboratory glassware, Instruments and filters for work
- How to filter, to heat and to dry materials
- To measure liquid volume or weight with technical and analytical balance
- To assemble chemical equipment
- How to prepare solutions of required concentration
- To calculate the required amount of reagents, the yield of reaction products and the relative error
- To describe accomplished session
- To draw used devices and chart graphs.

Cleaning of laboratory glassware

Before use, all glassware should be thoroughly cleaned to prevent errors caused by contaminants. First of all the glassware is washed with tap water, using small amounts of soap or soda and a brush to scrub the glassware. If the glassware is not truly cleaned, the dirt is eliminated while washing with hydrochloric acid or "cleaning mixture": concentrated sulphuric acid (H_2SO_4) is poured in 200 ml of saturated potassium dichromic acid ($K_2Cr_2O_7$) solution mixture reaches 500 ml. The glassware surface is rinsed in such "cleaning mixture" for several minutes, but for ease of use the utensil can be filled with solution or dipped into it and left to stay for longer time.

Finally it should be washed with tap water and rinsed with deionized water, tipping and rolling the glassware. If rinsing a pipette, burette or other glassware with a tip, water needs to be discarded through the tip. The clean glassware should be inverted on a paper towel to dry.

Warming, drying, heating

Spirit lamps, electric and gas burners, water baths, drying and heating ovens, and thermostats are used for warming in the laboratory. Thermal resistant substances are dried in the electric drying ovens (100 - 125 ^{0}C), and thermal non-resistant substances – in the vacuum drier or desiccator, which is filled with moisture sorbent. The materials heated at 800 - 1000°C temperature lose all volatile impurities, and sometimes they decompose into the thermal resistant compounds. It is mainly heated in the electric muffle furnace and on the flame of gas burner at times. The heated material is placed in the porcelain, quartz or platinum crucible.

Filtration

The process usually employed to separate an insoluble solid from a liquid is called filtration. After the filtration process, the liquid that passes through the filter paper (filtrate), and/or the solid that remains on the filter paper (precipitate or residue), can both be used. It is used for filtration:

• **Filters of filter paper** are cut from simple filter paper and first folded in half.

Then paper is folded again in squeeze-box form, but not in perfect quarters: the two folded edges should not quite touch; the second edge should be about 3 mm from the first edge. The filter is cut to a diameter that fits snug to the funnel walls, not reaching edges. The liquid with residues is poured into the funnel through the glass stick insomuch that its height would not reach filter's border 10 mm. If only the filtrate is required for further work, it should be filtrated through the folded filter (speed of filtration is faster);

• **Ash less filters** are used to perform quantitative analysis. The density of these filters is indicated on a tag of a cover. We can decide about ash less filter's density from strip color of a cover: red - least density, white - medium density, blue - close;

• **Glass filters** - glass funnels with poured spongy glass plate. The density of filters is marked by four numbers: No. l - least density, No.4 - most density;

• **Vacuum filtration** - Buchner filter with holey porcelain bottom is used and moistened with water and paper filter is placed on it. Paper filter should freely go in a funnel; its edges shouldn't be turned back. Buchner filter that is prepared for filtration is inserted into Bunsen flask, which is connected with water or mechanical pump in order to make vacuum;

• **Warmed filters** are used to filter viscous liquid, saturated and oversaturated solutions. Collected residues are thoroughly washed several times with small amount of a solvent (water). Another portion of liquid is poured only then, when an initial is out flowed completely. If liquid over the residues is utterly clean, it can be decanted (poured without roiling liquid) before the filtration. It is possible to wash the residues, which remained after the decantation, on several occasions with a solvent (water) again in order to eliminate impurities.

Measurement

The measurement of liquid volume can be performed using graduated cylinders, volumetric flasks and measuring vessels. Unlike counting, which can be exact, measurements are never exact but are always estimated quantities. Obviously, some instruments make better estimates than others, so more precise liquid volume is measured by calibrated measuring vessels:

• **Pipette** – vessel, used to suck, to drop and to measure liquid. Mohr pipette measures only one, definite and marked on it volume. Graduated pipettes allow measurement of any volume that would not exceed the volume of pipette's graduated section. Such pipettes commonly are graduated with 0.1 ml scale and allow to measure volume in 0.005 ml precision. Semi micropipettes and micropipettes can be graduated with 0.01 and 0.001 ml scale;

• **Automatic micropipette** – instrument, used to suck, to drop and to measure liquid.

• **Burette** - glass tube (generally with 0.1 ml scale), used to drop and to measure liquid volume. Semi micro burettes and micro burettes can be graduated with 0.01 or 0.001 ml scale;

• **Measuring flasks** – are used to measure various volumes and to prepare various concentration solutions.

Volume of liquid, which is colorless and moistens surfaces, is measured looking at the bottom of liquid's meniscus in the measuring vessels. Colorful liquid's volume, when we can't see the bottom of meniscus, is measured by deducting according to a top of meniscus. Meniscus should be in a level of a person who measures

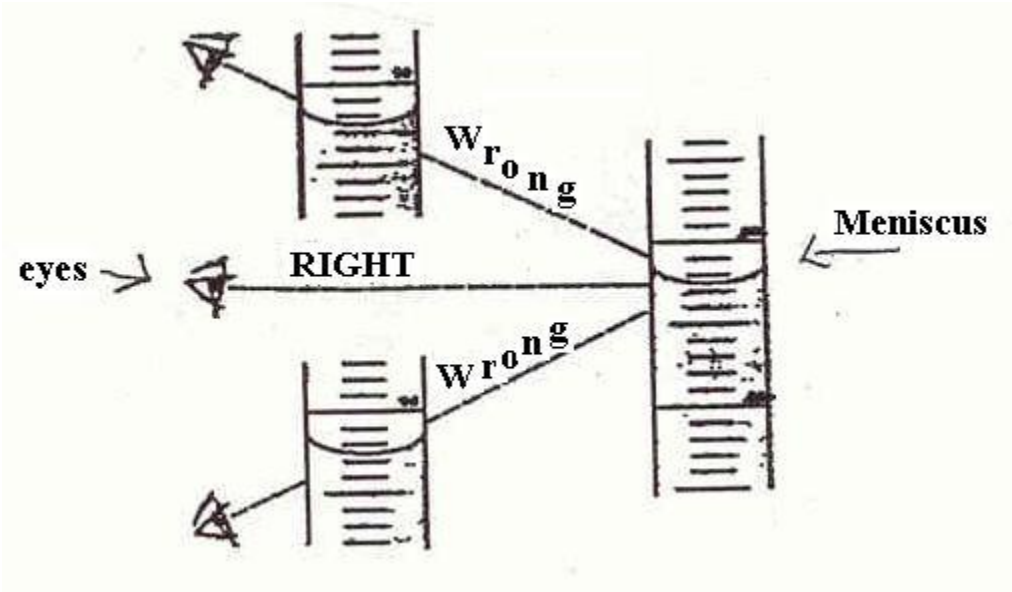

Fig 3.1: Measuring flask view

Practical No: 4
Sampling and Analysis Methods

Sampling and Sample Preparation

The term "sample" in analytical chemistry is applied to a portion of material selected in an appropriate manner, to represent a larger body of material. The result obtained from the samples is merely an estimate of the quantity or concentration of a constituent or property of the bulk material from which this sample is taken. The parent material may be homogeneous or heterogeneous. The use of a sample is always likely to introduce an uncertainty, arising from heterogeneity of the parent material and in extrapolating from the smaller portion to the larger portion called the "sampling error."

Sampling consists of two important steps- the sample collection step (sampling) and sample preparation step. The overall uncertainty is more often limited by problems with sampling or sample preparation than by the analysis of the prepared sample. If samples collected are the wrong size or type, even the best lab analysis of those samples may not be able to correctly represent the analysis of bulk material and thus may not serve the intended purpose.

Sampling Techniques:

Mixing

Mixing is combining of components, particles, or layers into a more homogeneous state.

The mixing may be achieved manually or mechanically by shifting the material with stirrers or pumps or by revolving or shaking the container. The process must not permit segregation of particles of different size or properties. Homogeneity may be considered to have been achieved in a practical sense when the sampling error of the processed portion is negligible compared to the total error of the measurement system.

Reduction of size

Decreasing the size of the laboratory sample or individual particles, or both is achieved by the division of the bulk material. Division of the size of the laboratory sample is generally accomplished manually by coning and quartering or by riffling or mechanically by rotary dividers. Reduction of particle size may be accomplished by milling or grinding. Simultaneous division and reduction may also be achieved with mills having stream diverters.

Coning and quartering

Coning and quartering is one of the methods for preparing representative sample from the powdered sample by forming a conical heap. The heap is spread out into a circular, flat cake. The cake is divided radially into quarters and two opposite quarters are combined. The other two quarters are discarded. The process is repeated as many times as necessary to obtain the quantity desired for the final use.

Riffling

The separation of a free-flowing sample into (usually) equal parts by means of a mechanical device composed of diverter chutes.

Milling/grinding

In milling and grinding mechanical reduction of the particle size of a sample is achieved by attrition (friction). The required particle size of a sample is related to the size of the test portion and the number of particles required to ensuring homogeneity among test portions. The reduction in particle size may sometimes result in particles of different hardness and density, which produces in-homogeneity during the preparation of the test sample or during the withdrawal of the test portion.

Sample Types

Random sample:

The sample **so** selected that any portion of the population has an equal chance of being chosen.

Representative sample

A sampling plan that adequately reflects the characteristics of the population. The degree of representativeness of the sample may be limited by cost or convenience.

Selective sample:

A sample that is deliberately chosen by using a sampling plan that screens out materials with certain characteristics and/or selects only material with other relevant characteristics.

Stratified sample:

A sample consisting of portions obtained from identified subparts (strata) of the parent population. Within each stratum, the samples are taken randomly. The objective of taking stratified samples is to obtain a more representative sample than that which might otherwise be obtained by random sampling.

Convenience sample:

A sample chosen on the basis of accessibility, expediency, cost, efficiency, or other reason not directly concerned with sampling parameters.

Duplicate (duplicate) sample:

Multiple (or two) samples taken under comparable conditions. A duplicate sample is a replicate sample consisting of two portions. The umpire sample is often used to settle a dispute and the replicate to estimate sample variability.

Umpire sample/referee sample/reserve sample:

A sample taken, prepared, and stored in an agreed manner for the purpose of settling a dispute, if arises.

Sequential sample:

Units, increments, or samples taken one at a time or in successive predetermined groups, until the cumulative result of their measurements (typically applied to attributes), as assessed against predetermined limits, permits a decision to accept or reject the population or to continue sampling.

Multistage sampling

Samples taken in a series of steps with the sampling portions constituting the sample (units or increments) at each step being selected from a larger or greater number of portions of the previous step, or from a primary or composite sample.

Sample Preparation, storage and handling:

In analytical chemistry, sample preparation refers to the ways in which a sample is treated prior to its analysis. Preparation is a crucial step in most of the analytical techniques, because the techniques are often not responsive to the analyte in its in-situ form, or the results are distorted by interfering species. Sample preparation may involve dissolution, reaction with some chemical species, pulverizing, treatment with a chelating agent (e.g. EDTA), masking, filtering, dilution, sub-sampling or many other techniques. Sample preservation, storage, and handling must be established in the work plan prior to sample collection.

Sample preparation, storage and handling should consider the following:

Sample volume:

Samples should be collected using equipment and procedures appropriate to the matrix, the parameters to be analyzed, and the sampling objective. The volume of the sample collected must be sufficient to perform the analysis requested, as well as the quality assurance/quality control requirements

Sample Container:

Containers must be compatible with the sample matrix, clean and labeled appropriately. The exterior of the sample containers must be wiped clean and dry prior to sample packaging. To prevent leakage of aqueous samples during shipping, sample containers should be no more than 90 percent full. If air space would affect sample integrity, fill the sample container completely and place the container in a second container to meet the 90 percent requirement.

Sample Preservation:

When a preservative other than cooling is used, the preservative is generally added after the sample is collected. If necessary, the pH must be adjusted to the appropriate level and checked with pH paper in a manner, which will not contaminate the sample. The laboratory performing the analysis should be contacted to confirm the requirements for sample volumes, container types, and preservation techniques.

Practical No: 5
Calculations Used In Analytical Chemistry

Measurements in Analytical Chemistry

Analytical chemistry is a quantitative science. Whether determining the concentration of a species, evaluating an equilibrium constant, measuring a reaction rate, or drawing a correlation between a compound's structure and its reactivity, analytical chemists engage in measuring important chemical things.

Units of Measurement

A measurement usually consists of a unit and a number expressing the quantity of that unit. We may express the same physical measurement with different units, which can create confusion. For example, the mass of a sample weighing 1.5 g also may be written as 0.0033 lb or 0.053 oz. To ensure consistency, and to avoid problems, scientists use a common set of fundamental units, several of which are listed in Table. These units are called SI units after the Système International d'Unités.

Table 5.1: SI base unites

Physical Quantity	Name of Unit	Abbreviation
Mass	Kilogram	kg
Length	Meter	m
Time	Second	s[a]
Temperature	Kelvin	K
Amount of substance	Mole	mol
Electric current	Ampere	A
Luminous intensity	Candela	cd

Table 5.2: Here is the table providing a list of some important derived SI units, as well as a few common non-SI units.

Quantity	Name	Symbol	Expression
Frequency	Hertz	Hz	1/s
Force	Newton	N	$kg \cdot m/s^2$
Pressure, stress	Pascal	Pa	$N/m^2 = kg/m \cdot s^2$
Energy, work	Joule	J	$N \cdot m = kg \cdot m^2/s^2$
Power, radiant flux	Watt	W	$J/s = kg \cdot m^2/s^3$
Electric charge	Coulomb	C	$A \cdot s$
Voltage, electric potential	Volt	V	$W/A = kg \cdot m^2/A \cdot s^3$
Capacitance	Farad	F	$C/V = s^4A^2/m^2kg$
Electric resistance	Ohm	Ω	$V/A = m^2kg/s^3A^2$
Conductance	Siemens or mho	S or Ω	$1/\Omega = s^3A^2/m^2kg$
Magnetic field	Tesla	T	$N/A \cdot m = kg/s^2A$
Magnetic flux	Weber	Wb	$T \cdot m^2 = m^2kg/s^2A$
Inductance	Henry	H	$V \cdot s/A = m^2kg/s^2A^2$

Chemists frequently work with measurements that are very large or very small. A mole contains 602 213 670 000 000 000 000 000 particles and some analytical techniques can detect as little as 0.000 000 000 000 001 g of a compound. For simplicity, we express these measurements using

scientific notation; thus, a mole contains 6.022 136 7 × 1023 particles, and the detected mass is 1 × 10–15 g. Sometimes it is preferable to express measurements without the exponential term, replacing it with a prefix (Table). A mass of 1×10–15 g, for example, is the same as 1 fg, or femtogram. Here is the table for some commonly used prefixes.

Table 5.2: View of common SI Prefixes

Power	Prefix	Abbreviation
10^{15}	peta	P
10^{12}	tera	T
10^{9}	giga	G
10^{6}	mega	M
10^{3}	kilo	k
10^{2}	hecto	h
10^{1}	deka	da
10^{-1}	deci	d
10^{-2}	centi	c
10^{-3}	milli	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p
10^{-15}	femto	f

Significant Figures

Significant figures are a reflection of a measurement's magnitude and uncertainty. The number of significant figures in a measurement is the number of digits known exactly plus one digit whose value is uncertain.

If the mass is 1.2673 g, for example, has five significant figures, four which we know exactly and one, the last, which is uncertain.

Suppose we weigh a second cylinder, using the same balance, obtaining a mass of 0.0990 g. Does this measurement have 3, 4, or 5 significant figures? The zero in the last decimal place is the one uncertain digit and is significant. The other two zero, however, serve to show us the decimal point's location. Writing the measurement in scientific notation (9.90 × 10–2) clarifies that there are but three significant figures in 0.0990.

Example

How many significant figures are in each of the following measurements, Convert each measurement to its equivalent scientific notation or decimal form.

- 0.0120 mol HCl
- 605.3 mg CaCO3
- 1.043 × 10–4 mol Ag+
- 9.3 × 104 mg NaOH

Solution

- Three significant figures; 1.20 × 10–2 mol HCl.
- Four significant figures; 6.053 × 102 mg CaCO3.
- Four significant figures; 0.000 104 3 mol Ag+.
- Two significant figures; 93 000 mg NaOH.

Significant Figures in Calculations

Significant figures are also important because they guide us when reporting the result of an analysis. In calculating a result, the answer can never be more certain than the least certain measurement in the analysis. Rounding answers to the correct number of significant figures is important.

For addition and subtraction round the answer to the last decimal place that is significant for each measurement in the calculation. The exact sum of 135.621, 97.33, and 21.2163 is 254.1673. Since the last digit that is significant for all three numbers is in the hundredth's place

135.6 1
97.3
+ 21.2 63

_____

157.1673

_____

To avoid "round-off" errors it is a good idea to retain at least one extra significant figure throughout any calculation. Better yet, invest in a good scientific calculator that allows you to perform lengthy calculations without recording intermediate values. When your calculation is complete, round the answer to the correct number of significant figures using the following simple rules.

1. Retain the least significant figure if it and the digits that follow are less than half way to the next higher digit. For example, rounding 12.442 to the nearest tenth gives 12.4 since 0.442 is less than half way between 0.400 and 0.500.
2. Increase the least significant figure by 1 if it and the digits that follow are more than half way to the next higher digit. For example, rounding 12.476 to the nearest tenth gives 12.5 since 0.476 is more than half way between 0.400 and 0.500.

3. If the least significant figure and the digits that follow are exactly halfway to the next higher digit, then round the least significant figure to the nearest even number. For example, rounding 12.450 to the nearest tenth gives 12.4, while rounding 12.550 to the nearest tenth gives 12.6. Rounding in this manner ensures that we round up as often as we round down.

Concentration and its units

Concentration means the relative amounts of the components of a solution. It tells the ratio of the quantity of one component to the quantity of the other or to the total quantity of solution. It has many units. Some common units are discussed below.

Mass Percentage

The ratio of the mass of the solute to the mass of the solution multiplied by 100 is called mass percentage.

Mass Percentage of a solute = Mass of the solute x 100

Mass of solution

For liquid-liquid solutions, it is sometimes more convenient to express the concentration in the units of percentage by volume:

Volume Percentage of a liquid = Volume of the liquid x 100

Total volume

Parts per Million (ppm)

This is used to express very dilute concentrations of a substance. One ppm is equal to 1 mg of solute dissolved per litre of solvent or 1 mg of solute dissolved per kg of solvent.

Molarity (M)

Molarity or the molar concentration is the number of moles of solute dissolved per dm3 of solution. (1 dm3 is equal to 1 Liter)

Molarity = Number of moles of solute

Volume of solution in liters

Molality (m)

Molality is the number of moles of solute dissolved per kilogram of solvent.

Molality = Number of moles of solute

Kg of solvent

Normality (N) Normality is the number of equivalents dissolved per liter of solution.

Normality = No. of equivalents

Volume of solution in liters

pH

The pH is defined as follows:

"The pH of a solution is the negative logarithm to the base 10 of the hydrogen ion (or hydronium ion) concentration".

$pH = -\log_{10} [H^+]$

$pH = -\log_{10} [H_3O^+]$

The pH of a neutral solution is 7, the pH of acids is less than 7 while that of bases is higher than 7.

Practical No: 6
Basic Tools of Analytical Chemistry

Equipment for Measuring Mass

An object's mass is measured using a digital electronic analytical balance. An electromagnet levitates the sample pan above a permanent cylindrical magnet. The amount of light reaching a photo detector indicates the sample pan's position. Without an object on the balance, the amount of light reaching the detector is the balance's null point. Placing an object on the balance displaces the sample pan downward by a force equal to the product of the sample's mass and its acceleration due to gravity.

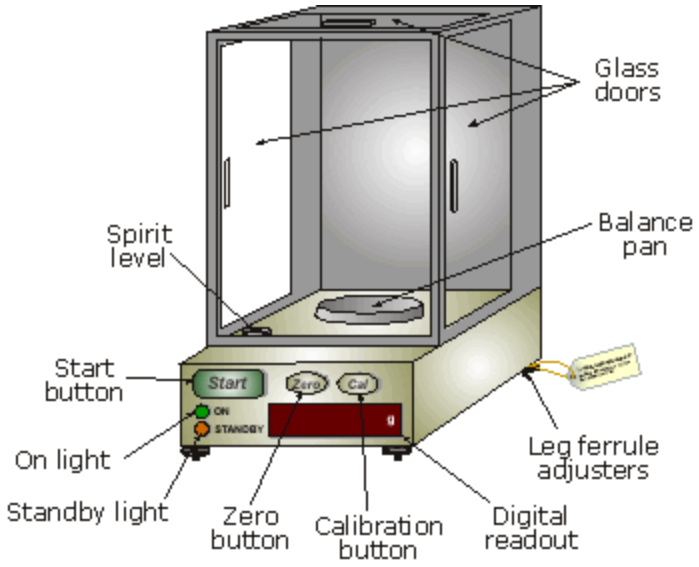

Fig 6.1: View of digital electronic analytical balance

If the sample is not moisture sensitive, a clean and dry container is placed on the balance. The container's mass is called the tare. Most balances allow you to set the container's tare to a mass of zero. The sample is transferred to the container, the new mass is measured and the sample's mass determined by subtracting the tare. Samples that absorb moisture from the air are treated differently. The sample is placed in a covered weighing bottle and their combined mass is determined. A portion of the sample is removed and the weighing bottle and remaining sample are reweighed. The difference between the two masses gives the sample's mass.

Equipment for Measuring Volume

Analytical chemists use a variety of glassware to measure a liquid's volume. The choice of what type of glassware to use depends on how accurately we need to know the liquid's volume and whether we are interested in containing or delivering the liquid.

A graduated cylinder is the simplest device for delivering a known volume of a liquid reagent. The graduated scale allows you to deliver any volume up to the cylinder's maximum. Typical accuracy is ±1% of the maximum volume. A 100-mL graduated cylinder, for example, is accurate to ±1 mL.

A volumetric pipet provides a more accurate method for delivering a known volume of solution. Several different styles of pipets are available.

Fig 6.2: Measuring Cylinders

Graduated Cylinders

Transfer pipets provide the most accurate means for delivering a known volume of solution. A transfer pipet delivering less than 100 mL generally is accurate to the hundredth of an mL. Larger transfer pipets are accurate to the tenth of a mL. For example, the 10-mL transfer pipet in Figure will deliver 10.00 mL with an accuracy of ±0.02 mL.

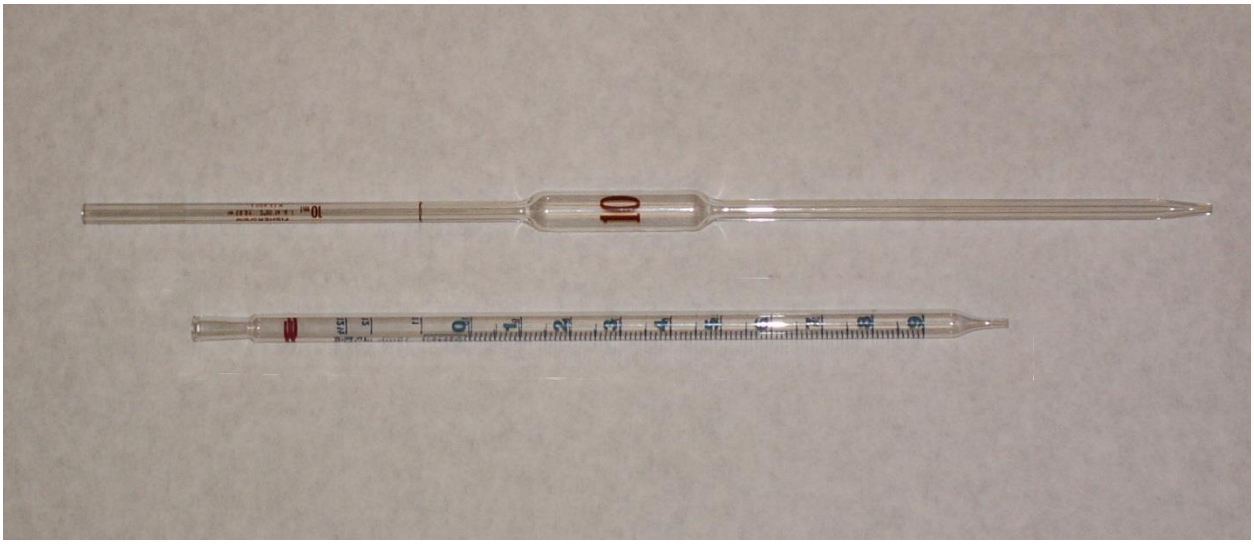

Fig 6.3: Transfer pipets

Delivering microliter volumes of liquids is not possible using transfer or measuring pipets. Digital micropipettes which come in a variety of volume ranges, provide for the routine measurement of microliter volumes.

Fig 6.4: View of pipets

A volumetric flask, on the other hand, contains a specific volume of solution.

Fig 6.5: A volumetric flask

Equipment for Drying Samples

Many materials need to be dried prior to analysis to remove residual moisture. Depending on the material, heating to a temperature between 110 ºC and 140 ºC is usually sufficient. Other materials need much higher temperatures to initiate thermal decomposition.

Conventional drying ovens provide maximum temperatures of 160 ºC to 325 ºC (depending on the model). Some ovens include the ability to circulate heated air, allowing for a more efficient removal of moisture and shorter drying times. Other ovens provide a tight seal for the door, allowing the oven to be evacuated. In some situations a microwave oven can replace a conventional laboratory oven. Higher temperatures, up to 1700 ºC, require a muffle furnace.

After drying or decomposing a sample, it should be cooled to room temperature in a desiccator to prevent the re-adsorption of moisture. A desiccator (Figure 2.11) is a closed container that isolates the sample from the atmosphere. A drying agent, called a desiccant, is placed in the bottom of the container. Typical desiccants include calcium chloride and silica gel. A perforated plate sits above the desiccant, providing a shelf for storing samples. Some desiccators include a stopcock that allows them to be evacuated.

Desiccator

Muffle Furnace

Fig 6.5: Equipment for Drying Samples

Practical No: 07
Separation of Biomolecules by Paper Chromatography

Paper chromatography of amino acids

Materials Required: Whatmann filter paper (12x22 cm), Butan-1-ol, Acetic acid, ninhydrin spray (2% solution of ninhydrin in ethanol), Capillary tube, beaker (500 ml), oven.

Chromatography

Chromatography is the separation of sample components based on differential affinity for a mobile versus a stationary phase. The mobile phase is a liquid or a gas that flows over or through the stationary phase, which consists of spherical particles packed into a column. When a mixture of proteins is introduced into the mobile phase and allowed to migrate through the column, separation occurs because proteins that have a greater attraction for the solid phase migrate more slowly than do proteins that are more attracted to the mobile phase.

Several different types of interactions between the stationary phase and the substances being separated are possible. If the retarding force is ionic in character, the separation technique is called ion exchange. Proteins of different ionic charges can be separated in this way. If substances absorb onto the stationary phase, this technique is called absorption chromatography. In gel filtration or molecular sieve chromatography, molecules are separated because of their differences in size and shape. Affinity chromatography exploits a protein's unique biochemical properties rather than the small differences in physicochemical properties between different proteins. It takes advantage of the ability of proteins to bind specific molecules tightly but non-covalently and depends on some knowledge of a particular protein's properties in the design of the affinity column.

Paper Chromatography

Chromatography is an analytical method used for separation of different biomolecule on the basis of their chemical properties. In paper chromatography, a biomolecule (or mixture of biomolecules) is spotted on a piece of filter paper and is placed in an organic solvent. The hydrophobic organic solvent moves up the paper by capillary action. As the solvent reaches the biomolecule, the biomolecule starts to move up the paper. The degree of movement of biomolecule up the paper is associated to its relative affinity for paper (hydrophilic in nature) and the solvent (hydrophobic in nature).

Paper chromatography is very valuable method for characterizing amino acids. Different amino acids migrate at different rates on the paper because of difference in their R groups. The rate of movement of a biomolecule during paper chromatography is known as its relative mobility (Rf). Rf is also known as retardation factor. Rf is simply "the distance the biomolecule moved through the filter paper divided by the distance the moved by solvent through the paper".

Rf = Distance travelled by sample

Distance travelled by solvent

Procedure

i. Place enough chromatography solvent [Butan-1-ol: Acetic acid : Water in 60 : 15 : 25 ratio] in tank or beaker to achieve a depth of about ½ inch. Cover tank and allow solvent to saturate the atmosphere in the tank for at least 30 minutes.

ii. Draw a line across the bottom of the chromatography sheet about 2.5 cm from the bottom edge of the chromatography paper with the aid of lead pencil.

iii. Take a small volume of amino acid into the capillary and deposit it onto the paper by touching the capillary to the line drawn. The spot should not be larger than ¼ inch in diameter and the two spots should be separated by 2 cm. Allow the spots to dry.

iv. After drying, roll the paper into a cylinder and staple so that the ends do not touch.

Development of the Chromatogram

i. Place the cylinder (chromatography paper) in a chromatography chamber/beaker (under the hood). Cover the chamber and allow the solvent to migrate up the paper for 60-90 minutes or till the solvent line is about an inch from the top of the sheet.

ii. Remove the chromatography paper from the tank and mark the solvent front line with pencil. Allow it to dry.

iii. Spray the paper with Ninhydrin solution (used to detect the location of amino acids).

iv. Dry the chromatography paper in a drying oven for about 100oC for 3-4 minutes to allow the color to develop. Amino acids give blue/purple color when they react with ninhydrin.

v. Measure the distance the solvent migrated and the distance each of the amino acids migrated. Calculate the relative mobility (Rf)/retardation factor for each amino acid.

Rf = distance the amino acid migrated

Distance the solvent migrated

Practical No: 08
Separation of Biomolecules by Thin Layer Chromatography

Principle

TLC involves the same principles of separation as column chromatography but the apparatus and technique for development is different. Instead of a column, the silica (or alumina) is adhered to a plate of plastic or glass. A capillary spotter is used to apply the dissolved sample onto the plate about 1 cm from the bottom (a line with pencil is drawn). Once the solvent has evaporated, only the sample remains. The plate is carefully placed into a closed developing chamber, which has a shallow layer of solvent that does not submerse the spot.

The chamber is lined with a folded piece of filter paper to ensure a uniform and saturated atmosphere of solvent vapor.

The plate is removed when the solvent front has reached about 0.5 cm from the top, and is quickly marked with pencil. The capillary action of the solvent causes the initial spot to be separated into individual components that may be visualized by color identification or with the following techniques for colorless compounds:

(i) Irradiation with ultraviolet light

(ii) Reversible staining with iodine vapor (formation of brown spots which fade)

(iii) Spraying with a reagent that irreversibly colors the spots, e.g. H_2SO_4, $KMnO_4$

Procedure

1. On a balance weigh 1.0 grams of fresh spinach and combine with 1.0 gram of anhydrous magnesium sulfate and 2.0 grams of sand. Transfer the materials to a mortar and using a pestle grind the mixture until a fine powder is obtained (if the leaves are wet to begin with, you may not get a dry powder).
2. Transfer the powder to a large test tube and combine with 2.0 ml of acetone. Stopper the test tube and shake vigorously for approximately one minute. You need to make sure that the solvent and solid are well mixed. Allow the mixture to stand for 10 minutes.
3. Use a pipette to carefully transfer the solvent above the solid (should be green) into a small test tube. Cover the tube to avoid evaporation.

4. Obtain a TLC chamber (a glass jar with a cover) and add developing solvent (a mixture of pet ether, acetone, cyclohexane, ethyl acetate and methanol). The solvent should completely cover the bottom of the chamber to a depth of approximately 0.5 cm.
5. Obtain a TLC plate (a silica gel coater plastic sheet) which has been precut and make a dot with a pencil on the coated side approximately 1.0 cm from the bottom of the strip.
6. Fill a capillary tube by placing it in the leaf extract. Keep your finger on the end of the tube. Apply the extract to the center of the dot on the TLC plate by quickly touching the end of the TLC applicator to the plate. Allow to dry. Repeat several times to make a concentrated dot of extract (your instructor will demonstrate this process). Be sure to let dry between applications.
7. Carefully place the TLC plate in the TLC chamber. The TLC plate should sit on the bottom of the chamber and be in contact with the solvent (solvent surface must be below the extract dot). Screw the lid on the TLC chamber.
8. Allow the TLC plate to develop (separation of pigments) for approximately 10 minutes (do not allow the solvent front to reach the top of the plate!). As the solvent moves up the TLC plate you should see the different colored pigments separating.
9. Remove the TLC plate from the chamber when the solvent is approximately 1.0 cm from the top of the TLC plate. With a pencil, mark the level of the solvent front (highest level the solvent moves up the TLC plate) as soon as you remove the strip from the chamber.
10. Calculate and record the Rf values (see below).

Rf = distance the pigment migrated

Distance the solvent migrated

Pigment	**Distance moved**	**Rf**

Practical No: 09
Analysis of electrolytes by UV/Visible spectrophotometer

Analysis of Potassium Permanganate

Outcomes

After completing this experiment, the student should be able to:

1. Determine wavelength of maximum absorption
2. Use Beer's Law to determine molar absorptivity.
3. Explain the fundamental principal behind spectrophotometric analysis

Spectrophotometry Principle:

Spectrophotometry is a technique that uses the absorbance of light by an analyte (the substance to be analyzed) at a certain wavelength to determine the analyte concentration. UV/VIS (ultra violet/visible) spectrophotometry uses light in UV and visible part of the electromagnetic spectrum. Light of this wavelength is able to effect the excitation of electrons in the atomic or molecular ground state to higher energy levels, giving rise to an absorbance at wavelengths specific to each molecule. The complex formed between ASA and Fe3+ is intensely violet coloured, and therefore can be determined by spectrophotometry using the visible part of the electromagnetic spectrum.

Materials and Equipment

UV/VIS spectrophotometer and polystyrene cuvettes, 2 100 mL volumetric flask, 1000 µL micropipette, 100 µL pipet, Hot plate, solid KMnO4, 1 10 mL graduated cylinders, 6 10 mL volumetric flasks, 2 125 mL Erlenmeyer flasks or 150 mL beakers

Procedure

Preparing the stock solution and four standard solutions

1. Preparation of 100 mL of a stock standard solution of 0.008M KMnO4: Accurately weigh 126 mg solid KMnO4. Transfer quantitatively to a 100 mL volumetric flask and fill to the mark with water. This is the stock solution.

2. Prepare four standards in 10.o mL volumetric flask with concentrations of 0.00008 M (solution #1), 0.00016 M (solution #2), 0.0004 M (solution #3) and 0.0008 M (solution #4) by diluting the stock solution prepared in Step 1 as following.

For the 0.00008, 0.00016 and 0.0004 M standards use the 100 μL micropipets (100 μL = 0.1 mL; calculate how many 100 μL samples stock solution are needed in each case) to make 10 mL standard solution.For the 0.0008 M standard use the 1 mL (1000 μL) micropipet, Mark the four 10 mL flasks with standard solutions #1-4 (lowest concentration #1 = 0.00008 M, highest concentration #4 = 0.0008 M).

Measuring the absorption spectrum and determining λmax

This part of the experiment may be done by all students together, with each pair of students determining the absorbance at one wavelength. Each pair of students should record all absorbances at each wavelength and draw the absorption spectrum.

3. Rinse one of the cuvettes with distilled water and fill it with water. Put the cuvette in the sample compartment. This is the reference solution. Set the wavelength to 400 nm, then set the Absorbance to zero.

4. Rinse a second cuvette once with distilled water and once with standard solution #1, then fill it with standard solution #1 (0.0008 M KMnO4). Place the cell in the sample compartment, measure the Absorbance at 400 nm and record in your notebook.

5. Repeat this procedure (steps 3 and 4 above) for the two cuvettes at wavelengths 420, 440, 460, 600 nm, first setting A = 0 for the cuvette with water, then measuring A for the cuvette with 0.0008 M KMnO4, recording the absorbance at each wavelength. Prepare a graph of absorbance A vs. wavelength λ and determine λmax (maximum wavelength). Attach this graph to the lab report.

The calibration curve

This part of the experiment must be done by each pair of students separately.

7. Set the wavelength at 525 nm (λmax). Place the cuvette with distilled water in the cell compartment and again set the Absorbance to zero.

8. Measure and record the Absorbance of each of the four standard solutions, starting with the most dilute standard. After each measurement, rinse the cuvette with the next standard, not with distilled water!

9. Draw a plot having X-axis as concentration (mole/L) and Y-axis as Absorbance at λmax (525 nm). 10. Use Beer's law to calculate ε for KMnO4, given the cell width (path length l) to be 1 cm.

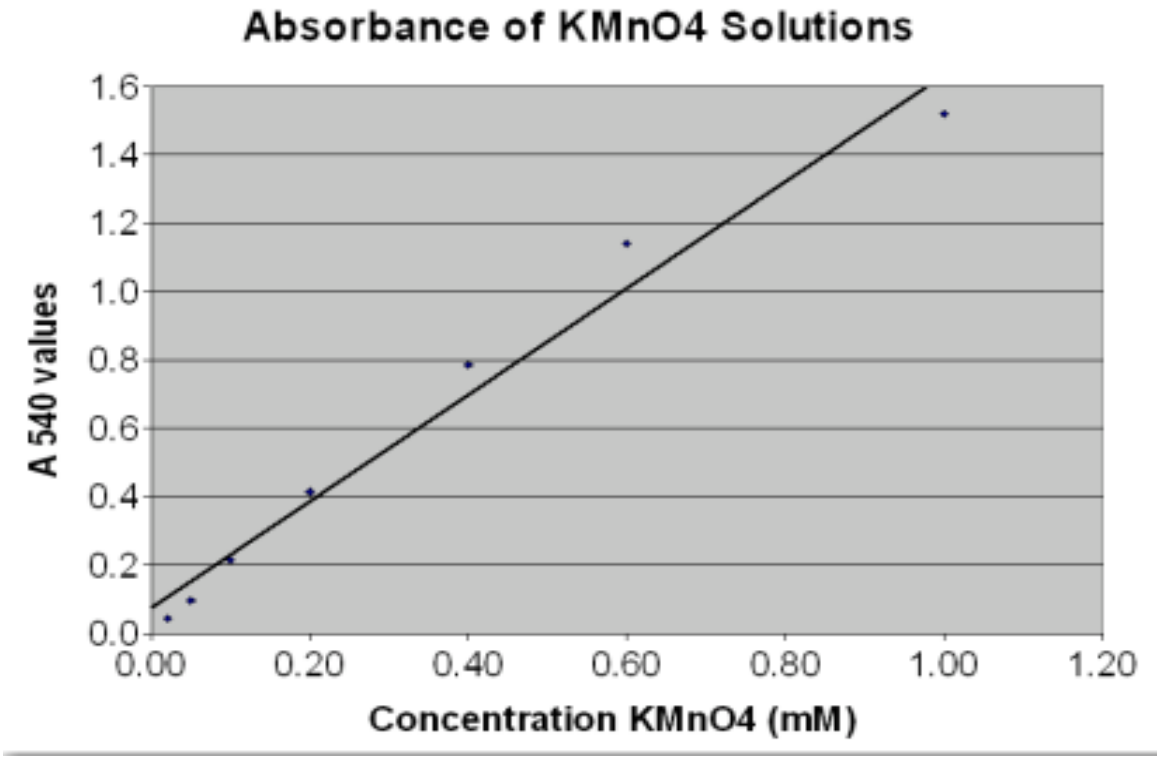

Fig 13.1: Absorbance and concentration of KMnO4