Basic Concept in Laboratory Techniques

(PGS-504)

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2020

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Practical No 1

Introduction to 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 chemistry studies, uses instruments and methods to separate, identify, and quantify the matter. The analytical chemistry divided into two parts.

1) Qualitative Analysis: A qualitative analysis determines the presence or absence of a particular compound, but not the mass or concentration. By definition, qualitative analyses do not measure quantity.

2) Quantitative Analysis: Quantitative analysis is the measurement of the quantities of particular chemical constituents present in a substance. Quantitative analysis divided into several categories.

It is very necessary to know about the basic concept and principle of analytical chemistry. So some important definitions and formulas are described in this exercise as follows:

1) Atom and Atomic Weight:

Atom: Atom is the smallest particle of an element which retain its property and take part in chemical reaction. Every solid, liquid, gas, and plasma is composed of neutral or ionized atoms. Atoms are extremely small; typical sizes are around 100 picometers (a ten-billionth of a meter, in the short scale).

Atomic Weight: The average mass of an atom of an element, usually expressed relative to the atomic mass of carbon 12. The atomic weight expressed in gram. Example- 16 gm of oxygen means 1 gram atom of oxygen.

2) Molecule and Molecular Weight:

Molecule: A molecule is the smallest particle in a chemical element or compound that has the chemical properties of that element or compound and exit in free state. Molecules are made up of atoms that are held together by chemical bonds. These bonds form as a result of the sharing or exchange of electrons among atoms.

Molecular Weight: Molecular weight is defined as the weight of one molecule usually expressed as relative to the weight of carbon atom 12. It is calculated as the sum of the relative atomic masses of each constituent element multiplied by the number of atoms of that element in the molecular formula. The molecular weight expressed in gram. Example- 32 gm of oxygen means 1 gram molecule of oxygen.

Example 1 The molecular mass of methane, whose molecular formula is CH_4, is calculated as follows:

	Atomic mass	Number	Total mass
C	12.011	1	12.011
H	1.008	4	4.032
CH_4			16.043

3) Equivalent weight: Equivalent weight (also known as gram equivalent) is the mass of one equivalent, that is the mass of a given substance which will combine with or displace a fixed quantity of another substance. The equivalent weight of an element is the mass which combines with or displaces 1.008 gram of hydrogen or 8.0 grams of oxygen or 35.5 grams of chlorine. These values correspond to the atomic weight divided by the usual valence. The equivalent weight in gram is termed as gram equivalent weight.

Equivalent weight of salt = Molecular wt./ Valiancy

4) Standard Solution: The solution of accurately known strength (or concentration) is called a standard solution. It contains a definite number of gram equivalent or gram mole per liter of solution. If it contains 1 gram equivalent weight of a substance/compound, it is 1N solution. If it contains 2 gram equivalent weights of the compound, it is 2N. There are two types of standard solution-

1 Primary standard: Such substance, whose weight and purity is stable, is called as 'Primary Standard'.

Primary standard solution is one which can be prepared directly by weighing and with which other solutions of approximate strength can be titrated and standardized. Some Primary standards are given below:

Acids 1. Potassium hydrogen phthalate 2. Benjoic acid

Bases 1. Sodium carbonate 2. Borax

Oxidizing agents 1. Potassium dichromate 2. Potassium bromate

Reducing agents 1. Sodium oxalate 2. Potassium Ferro cyanide

Others 1. Sodium chloride 2. Potassium chloride

Secondary Standard Solutions are those which are prepared by dissolving a little more than the gram equivalent weight of the substance per liter of the solution and then their exact standardization is done with primary standard solution. Some Secondary standards are given below:

Acid 1. Sulphuric acid 2. Hydrochloric acid

Base 1. Sodium hydroxide.

Strength of a solution: Strength of a solution refers to the weight of a solute dissolved in a unit volume of the solution. It can be expressed in many ways as follows:

5) Normal Solution: A normal solution is one which contain 1 gram equivalent of the substances dissolved in one liter of the solution.

The normality of a solution is the number of gram equivalents of the solute per liter of the solution. It is usually designated by letter N. Semi-normal, penti-normal, desinormal, centi-normal and milli-normal solutions are often required, these are written (shortly) as 0.5N, 0.2N, 0.1N, 0.01N and 0.001N, respectively.

$$\textbf{Normality} = \frac{\text{Number of gram equivalent of solute}}{\text{Number of liters of solution}}$$

The equivalent weight of a substance is the weight in grams which in its reaction corresponds to a gram atom of hydrogen or of hydroxyl or half a gram atom of oxygen or a gram atom of univalent ion. When one equivalent weight of a substance is dissolved in one liter, it gives I N solution.

Normal solution of liquid reagents can be prepared using given formula:

$$\textbf{ml of liquid reagent required for preparation of N solution} = \frac{\text{Mol.wt. of liquid reagent x required normality x 100}}{\text{Purity \% x Specific gravity x Acidity/Basicity of reagent}}$$

Example 1 Prepare the 1 N H_2SO_4 solution by using following details- Purity of sulphuric acid-96%,specific gravity 1.84, equivalent weight 49 and basicity 2.

ml of H_2SO_4 required per liter for preparation of 1N H_2SO_4= $\frac{98x1x100}{96 x1.84 x 2}$

= 28ml/liter

Weight of substance= $\frac{\text{Eq. weight x desired normality x desired volume}}{1000}$

6) Molar solution: A molar solution is the one which contains a gram molecular weight of solute dissolved in liter of solution. It is denoted by M.

Molarity: It is the number of gram molecule of substance dissolved in one liter of solution.

Molarity = moles of solute/liters of solution

7) Molal solution(m): It is defined as the number of gram moles of solute per kilogram (1000 gms) of solvent.

molality = moles of solute/kilograms of solvent

8) Percentage by weight: The concentration is expressed in terms of grams of solute per 100 grams of solution.

The expression of weight percent is $\frac{\text{Mass of the solute}(g)}{\text{Volume of the solution}(mL)} \times 100$

Example: A 7% (w/v) NaCl solution means that a mass of 7 g of NaCl is dissolved in a solution containing volume 100 mL.

(9) Percentage by volume: Grams/ ml of solute dissolved per liter of solution.

For liquid solute, the concentration of the solution can be expressed in terms of volume percent.

The expression is $\frac{\text{volume of the solute}(mL)}{\text{volume of the solution}(mL)} \times 100$

Example: 1000 mL of a 7.3% by volume of a solution of ethanol in water. It means in 100 mL solution, ethanol is there 7.3 mL. Therefore, in a 1000 mL solution ethanol = 73 mL. Hence 73 mL ethanol and (1000-73) = 927 mL of water is needed.

10) Parts per million (ppm): The concentration is expressed in terms of grams of solute per milligram of solute per liter of solution.

1 ppm = 1mg/l = 1ug /ml = 1000ug/L , ppm = ug/g =ug/ml = ng/mg = pg/ug = 10^{-6}

ppm = mg/liters of water , 1 gram pure element dissolved in 1000ml = 1000 ppm

ppm= me/lit x eq. wt, ppm = % x 10,000, ppm x 2.24 = kg/ha

%= ppm/10000

11) Milli equivalent weight (mEq W)

Equivalent weight (Eq W) when expressed as milli-equivalent weight (mEq W), means the equivalent weight in grams divided by 1000. It is commonly expressed by "me". It is the most convenient value because it is the weight of a substance contained in or equivalent to one ml of I N solution.

Number of mEq = Volume x Normality

Strength of common acids used in laboratory

S.No.	Reagent	Eq. Wt	Normality	Molarity	Purity	Specific gravity	Ml required for 1N/liter solution
1	Nitric acid	65	16	16	70	1.42	63.7
2	Sulphuric acid	49	35	17.5	98	1.84	28
3	HCL	36.5	11.6	11.6	36.5	1.19	82.6
4	Phosphoric acid	32.7	45	15	85	1.71	22.7
5	Perchloric acid	100.5	10.5	10.5	65	1.60	108.7
6	Ammonium Hydroxide	35	15	15	28	0.90	67.6

12) Titration: It is a process of determining the volume of a substance required to just complete the reaction with a known amount of other substance. The solution of known strength used in the titration is called titrant. The substance to be determined in the solution is called titrate.

13) Indicator: A substance which indicates the end point on completion of the reaction is called as indicator. Most commonly used indicators in volumetric analysis are:

- **internal indicator**
- **external indicator**
- **self indicator**

i.) Internal indicator: The indicators which are added in the solution where reaction occurs, are called internal indicators. Example: methyl red, methyl orange, phenolphthalein and diphenylamine. On completion of the reaction of titrant on titrate, a colour change takes place due to the presence of indicator, which also helps in knowing that the titration is complete.

ii) External Indicator: Some indicators are used outside the titration mixture are called external indicator. Potassium ferricyanide is used as an external indicator in the titration of potassium dichromate and ferrous sulphate in acid medium. In this titration, few drops of indicator are placed on a white porcelain tile. A glass rod dipped in the solution being titrated is taken out and brought in contact with the drops of indicator placed on white tile. In the beginning deep blue colour is noticed which turns greenish on completion of titration.

iii) Self Indicator: When one of the reacting substances itself acts as an indicator by change in color is known as self indicator. In $KMnO_4$ titration with ferrous sulphate, the addition of $KMnO_4$ starts reacting with $FeSO_4$ which is colourless. On completion of titration, slight excess presence of $KMnO_4$ gives pink colour to the solution which acts as a self indicator and points to the completion of the titration.

pH range of various indicators

Name of indicator	pH range	Colour in acid medium	Colour in alkaline medium
Methyl orange	**3.1 to 4.5**	**Red**	**Yellow**
Methyl red	**4.2 to 6.3**	**Red**	**Yellow**
Bromothymol blue	**6 to 7.6**	**Yellow**	**Blue**
Phenol red	**6.4 to 8.2**	**Yellow**	**Red**
Phenolphthalein	**8 to 10**	**Colourless**	**Pink**
Thymolphthalein	**9.3 to 10.5**	**Colourless**	**Blue**

Practical No 2

Methods of soil, water and plant sampling and processing for analysis

Soil sampling and processing

Introduction:- The importance of having a true representative sample can be very well realized from the fact that only a minute fraction of huge soil mass of the field is actually used for the analysis in the laboratory to find out the quantity of essential nutrients available to plants and other relevant physical and chemical characteristics. Therefore, while collecting soil samples the following aspects should be carefully considered. The soil samples collected should be representative of the area. A field can be treated as single sampling unit if it is appreciably uniform in all respects. Variation in slope, colour, texture, crop growth and management practices should be taken in to account and separate set of composite soil samples should be collected from each unit of such area.

The main purpose for which samples collected are:

a. Soil fertility evaluation and fertilizer recommendation.

b. Reclamation of problematic soils.

c. Plantation of orchards.

Tools and materials required :-

1. Soil auger, tube auger, spade, pick-axe, khurpi.
2. Bucket or tray.
3. Paper tages (Labels).
4. Information sheet
5. Cloth bags (alternatively polythene bags).
6. Ball point pen or copying pencil

Sampling for fertility evaluation, reclamation and plantation of orchard

Normally the samples are taken from the plough layer i.e., 0-15 cm depth. This is applicable for the fields growing cereals and other crop. In case of deep-rooted crops and under dry farming conditions, it may be necessary to obtain samples from different depths (or layers) of soil. For collecting proper soil samples following steps should be kept in mind:

1. Divide the field into small areas so that each sample represents an area of approximately 1 hectare.

2. A sample should be collected separately from areas which differ in soil colour or past management, e.g., liming, manuring, fertilization, cropping pattern etc.

3. Scrap away the surface litter and insert soil auger or sampling root to a plough depth (about 15 cm). Take at least 15 samples randomly distributed over each area and place them in a clean bucket. A spade or khurpi can be very well used if auger is not available.

4. If a spade or khurpi is used for taking samples, then dig a V-shaped hole to a plough depth and cut 1.5 cm thick slice of soil from top to bottom of the exposed face of the V-shaped hole and collect soil in a clean bucket.

5. In orchard, Dig a pit 1.80 meter deep and make its one side vertical, put marks at 15, 30, 60, 90, 120, 150 and 180 cm depths from the surface.

6. Collect samples separately from 0-15, 15-30, 30-60, 60-90, 90-120, 120-150 and 150-180 cm depths in the same way that of saline alkali soils.

7. Thoroughly mix the soil samples collected from 15 or more spots in a bucket.

8. Collect only ½ to 1 kilogram soil and discard remaining soil samples by quartering.

9. Quartering is done by dividing the thoroughly mixed soil in to four equal parts and discarding two opposite quarters. Remix the remaining two quarters and again divide it into four parts and reject two of them, repeat this procedure until about one half kilogram of soil is left.

Precautions

1. Do not draw any sample from the extreme corners of the field, area recently manured or fertilized, old bounds and marshy spots.

2. Avoid sampling from furrows, acidic or alkaline pockets.

3. Keep the sample in a bag and tag it properly.

4. Do not take less than 0.5 kg of a composite sample.

5. Sampling should be done from a uniform piece of land.

6. If there is a hard pan in the pit, it should be sampled separately and also note down its depth and thickness.

Preparation of samples for analysis

Drying: Wet soil sample should not be stored as changes may occur in the chemical nature of certain ions and organic matter. Samples are generally air dried at temperature (25-35^{0}C) and relative humidity (20-60%) then after are stored. Fresh samples from the field without any drying are required. For certain determinations such as ammonium and nitrate N, exchangeable K, acid

extractable P and ferrous iron fresh sample from the field without any drying are required. Results of soil analysis are expressed on oven dry weight basis. This necessitates determination of moisture percentage by drying a small sample in an oven at 105^{0C} for 2 hours.

Sieving: Field moist samples prior to drying can be made to pass through a 6 mm sieve (about 4 mesh per inch) by rubbing with fingers. The practice seems of much advantage in case of heavy soils. Soil in the right moisture condition can be passed through a 2 mm sieve (about 10 mesh per inch). The common practice of sieving a portion of the gross sample through a 2 mm sieve and discarding the rest is undesirable as it increase the concentration of most of the elements involved in soil fertility. When the gravels in the soil exceeds 2% limit over a 2 mm sieve their exact percentage should be recorded.

Grinding: A roller, rubber pestle in an agate mortar, or a motorized grinder is commonly used. Crushing of the gravel or primary sand particles should be avoided for heavy soils, it is better to pass these through a 2 mm sieve before allowing them to get completely air dried.

Mixing: Sample should be thoroughly mixed by rolling procedure. Place the dried ground and sieved sample on a piece of cloth. Hold all the four corners of the cloth and then up the one corner and down the other corner across the sample alternatively. Now repeat the process in the reverse direction to roll the soil from one corner to another. Continue this until thorough mixing is assured.

Storage: Store the soil in paper carton (soil sample box) using a polythene bag as in inner lining. Label the carton mentioning cultivators name, plot number, date of sampling and initials.

Water sampling and processing

Methods of Water Sample Collection

A representative sample (500 ml) is collected in glass or polyethylene bottle which should be properly washed/rinsed with the same water which is being sampled. The floating debris or any other contaminant should be avoided while collecting the samples. After proper labeling, such as source of water, date of collection and the type of analysis required, the sample should be sent to the laboratory without undue delay.

Some of the anions like SO_4 and NO_3 may be quite low in irrigation waters. Hence, large volume of sample has to be first concentrated by evaporating to about 100 ml to obtain their detectable amounts.

Plant sampling and processing

Proper sampling requires that a specific plant part be taken such as a particular leaf, group of leaves or portion of the plant. Instructions also include number of individual parts, as well as the number of plants to sample. This will ensure that a sufficient quantity of plant tissue is submitted for analysis and that the collected sample is statistically representative of the area under study.

• When sampling mixed stands, particularly forages and pastures, separate plant species. Similarly, the sample should be of only leaves or petioles or whole tops and not mixtures. • When no specific sampling instructions are given for a particular crop, the general rule of thumb is to sample the uppermost recently mature leaves.

• Young emerging leaves, older mature leaves and seed are not usually suitable plant tissues for analysis since they do not ordinarily reflect the general nutrient status of the whole plant.

• The recommended time to sample usually occurs just prior to the beginning of the reproductive stage for many plants. However, sampling earlier or even later than the specified time may be recommended for specific plants or circumstances.

• Sample plants that are showing a suspected nutrient deficiency symptom at the time or shortly after the visual symptoms appear. Do not sample or include in a sample plants under a nutrient stress for an extended period of time, dead plant tissue or plants or tissue mechanically injured, diseased, or insect-damaged.

Washing to Remove Contaminants

Avoid dusty or soil-covered leaves and plants whenever possible. When leaves are dusty, brush or wipe with a damp cloth to remove the contaminants. If this is not effective or when leaves are covered with spray materials, wash in a mild detergent solution (0.30 percent) and rinse in running water to remove attached substances. Do not prolong the washing procedures or allow the plant material to stand in either the washing or rinsing baths. Wash and rinse briskly. Wash leaves which have been sprayed with nutrient solutions while they are still fresh. If iron is of primary interest, wash leaves regardless of their outward appearance. Wash whole plants sampled shortly after emergence to remove soil particles that are frequently attached to the new tissue.

Packaging Plant Tissue

• Air-dry plant tissue samples before shipment to the laboratory. Package samples in clean paper bags or envelopes for mailing to the laboratory. Never place fresh samples in a plastic bag.

What Not to Sample

Do not include diseased or dead plant material in a sample. Do not sample or include plants or leaf tissue that have been damaged by insects or mechanically injured in a sample. When whole plants are sampled, remove the roots and wash the upper portion to remove soil particles. Do not sample plants, which have been stressed extensively by cold, heat, moisture deficiency, or by excess moisture. Examine both the below ground as well as the above ground portion of the plant. The presence of nematodes or roots damaged by other insects or diseases should preclude the need to sample.

Laboratory Processing

Sample preparation is critical in obtaining accurate analytical data and reliable interpretation of plant analysis results. Proven procedures must be followed during handling in the laboratory, decontamination, drying, grinding and mixing, and storage. Such preparatory procedures enhance the accuracy and reliability of the analytical results.

Handling in the laboratory

1. As soon as the plant samples are received at the plant preparation laboratory, they should be checked with the accompanying information list. Information regarding samples should be entered in a register and each sample be given a laboratory number.

2. Keep plant samples refrigerated until cleaning. Take care that fermentation does not occur.

Decontamination

Decontamination procedures involving washing and rinsing should only be used for fresh, fully-turgid plant samples. After decontamination, samples should be dried immediately to stabilize the tissue and stop enzymatic reactions.

A. Reagents and Apparatus

Deionized water , 0.1 to 0.3 % detergent solution (non-phosphate) , Medium-stiff nylon bristle brush , Plastic containers suitable for washing and rinsing tissue samples

B. Cleaning processing

- A preliminary dry-wiping can be done if the plant sample is very dirty.
- If the plant samples are too dirty and a dry-wiping is not possible, washing through the nylon bag can be done.

- The samples must be properly cleaned, but no part of it should be under water for more than a few seconds.
- Cleaning plant tissue to remove dust, pesticide and fertilizer residues, normally by washing the plants with DI water or with 0.1 – 0.3 % P-free detergent (like HCl 1%), followed by DI water.
- Rinse each portion of the plant sample into a bath of DI water, into which it is plunged, agitated, and immediately withdrawn. Change the water and repeat the rinsing. Dry by shaking vigorously by hand.
- Plant samples for soluble element determination may not be washed, particularly for long periods. However, **plant samples for total Fe analysis must be washed**.
- Excessive washing is worse than no decontamination since soluble elements, including B, K, and N, are likely to leach from the tissue.
- The wash and rinse periods should be as short as possible to avoid danger of N, B, K, and Cl leaching from the tissue.

Drying

Water is removed from plant tissue to stop enzymatic reactions and to stabilize the sample. Enzymes present in plant tissue become inactive at temperatures above 70 oC. As a result, air-drying may not stabilize samples and prevent enzymatic decomposition. Samples should, therefore, be properly dried as soon as possible after taking the sample. Some technical guidelines are as follows:

1. The plant sample material should be evenly and thinly spread in a container.

2. Place containers in well-ventilated drying oven.

3. If samples absorb significant amounts of moisture during grinding, additional drying may be required prior to weighing for analysis.

4. **Drying time required will vary**. Dry to constant weight.

5. The original condition and sample size will affect drying time.

6. The drying temperature should not exceed 70^{oC}, because higher temperatures may cause volatilization loss.

7. Drying at temperatures less than 70^{oC} may not remove all combined water and may result in poor homogenization and incorrect analytical results.

8. Drying temperatures above 70^{oC} may result in thermal decomposition and reduce dry weight.

9. A drying time of 24 hours may be sufficient in normal conditions.

10. Drying times longer than 24 hours may be required depending on the type and number of plant samples in the dryer.

11. Quick drying of a limited number of samples can be done using a microwave oven and the drying process is closely monitored.

Grinding and Mixing

Plant tissue samples are reduced to 0.5 to 1.0 mm particle size to ensure homogeneity and to facilitate organic matter destruction.

A. Apparatus

1. Standard mills equipped with 20, 40, and 60-mesh screens and stainless steel contact points.

2. Tecator Cyclotec sample mill (standard equipped with a 1-mm sieve) or equivalent high-speed grinder.

3. Medium bristle brush.

4. Vacuum system.

B. Procedure

1. After drying, samples should be ground to pass a 1.0-mm screen (20 mesh) using the appropriate Wiley mill. A 20-mesh sieve is adequate if the sample aliquot to be assayed is >0.5 g. However, if the sample aliquot to be assayed is less than 0.5 g, a 40-mesh screen should be utilized.

2. After grinding, the sample should be thoroughly mixed and a 5 to 8 g aliquot withdrawn for analyses and storage.

Practical No. 3

Determination of pH of Soil.

Principle: Glass surface in contact with H^+ ions of the solution under test, acquires an electrical potential, which depends on the concentration of H^+ ions. This is measured potentiometrically against some reference electrode, which is usually a calomel electrode. The potential difference between glass electrode and calomel electrode is expressed in pH units.

Two electrodes are used in the determination of pH. One is reference electrode, which provides a standard voltage. The reference electrode is usually a saturated calomel electrode which has two layers

(1) Saturated solution of KCl and

(2) mixture of solid $HgCl_2$ and Hg.

Method of pH determination: The methods for the determination of pH of the soil solution are broadly classified into two group i.e. Potentiometric or electric pH meter method and Calorimetric or Kuhn's method.

Electric pH meter method: The instrument commonly used in this method is a glass electrode pH meter with calomel reference electrode introducing salt bridge. Most digital pH meter at present have single (combined) electrode assembly. The instrument being a potentiometric requires to be calibrated before use with buffer solutions of known pH values such as 4, 7 and 9.2.

Instruments & Reagents: Glass electrode pH meter, beaker (100 ml), glass rod, distilled water, filter paper with rubber and standard buffer solutions of pH 4, 7 and 9.2 in pure water.

Procedure:

1. Standardization of pH meter: Switch on the instrument and wait for 5 minutes. Insert the electrodes in a beaker containing standard solution of known pH buffer solutions of pH 4, 7 and 9.2. Calibrate the instrument at 4.0 and 9.2. This is done with the help of knobs provided for this purpose and pH is read on marking presented as pH scale of instrument. Adjust the temperature at 25^0C with the help of temperature knob.
2. For soil water suspension: Weigh 20g of prepared soil into a 100 ml beaker and add 40 ml of distilled water.
3. Shake the mixture with the glass rod for 30 minutes. Insert electrode in soil water suspension; record the reading as shown by pH scale.

4. Remove the suspension and wash the electrodes with distilled water.

Precautions & practical suggestions:

1. Allow the pH meter to warm for 10 minutes before recording the pH.
2. Adjust the pH meter on two pH values (4 and 9.2) of unknown buffer solution.
3. Never allow the lower portion of electrodes to touch bottom of the beaker.
4. Always put the switch to neutral, zero or release pH button whenever solution is charged.
5. Ensure that the reference electrode is clean and filled with saturated KCl solution.
6. The electrodes should be immersed in a beaker of distilled water to avoid drying of electrodes.
7. The electrodes should not be dipped in the suspension longer than necessary, essentially in solution of pH > 9.0.
8. The electrodes must be washed with a jet of distilled water, removing water from the surface with filter paper prior to use.

Soil reaction classes

Reaction rating	pH range	Inference
Acidic		
Extremely acidic	< 4.5	Requires liming
Strongly acidic	4.6 – 5.0	
Medium acidic	5.1 – 5.5	
Slightly acidic	5.6 – 6.0	
Normal		
Neutral	6.6 – 7.5	No treatment requires
Saline/Calcareous		
Mild alkaline	7.6 – 8.0	Requires leaching of soluble salts or consumption of salts by growing algae
Alkaline		
Strongly alkaline	8.1 – 9.0	Requires an amendments like gypsum application
Very strongly alkaline	> 9.1	

Result: pH of the soil is……. which comes in the category of ……..

Practical No. 4

Determination of Electrical Conductivity of Soil

Principle: A solution offers some resistance to the passage of electric current through it depending upon the concentration and type of ions present. Higher the salt content, lesser the resistance to the flow of current. The resistance (R) is defined by Ohm's law as the ratio of electrical potential in volts (E) and strength of current in ampere (I).

E/I = volts/current = R in ohm

Electrical conductivity or conductance is the reverse of resistance.

I/R = I/Ohm = mho (reverse of ohm)

At present the unit of EC mhos/cm is expressed in terms of dS/m.

Instruments & Reagents:

1. Conductivity meter: Meter and a conductivity cell with known cell constant.
2. Beaker (50 ml), Erlenmeyer flasks (250 ml), pipettes, glass rods, china dish, vaccum pump, spatula.
3. Filter paper
4. Saturated solution of Calcium sulphate.
5. 0.01 M KCL solution: Dissolve 0.7456g of AR grade KCl previously dried at 60^0C for 2 hours and dissolve in freshly prepared distilled water and make the volume to 1 litre. This gives 1.412 mS/cm at 25^0C.

Procedure:

1. The saturation extract of the soil or 1:2 soil water suspensions by taking 20g prepared soil into 100ml beaker, add 40ml of distill water, and shake for 30 minutes. Filter through Whatman No.1 filter paper. The filtrate is ready for measurement of conductivity or allows the soil suspension in the beaker to settle for 30 minutes. Dip the conductivity cell and note the reading of conductivity meter.
2. Switch on the conductivity meter and adjust the temperature at 25^0C.
3. Pour some 0.01M KCl solution into a 25ml beaker and dip the electrode in the solution. Adjust the conductivity meter to read 1.412 mS/cm, corrected to 25^0C. OR Adjust conductivity meter reading to 2.2 dS/m at 25^0C by dipping electrode in saturated calcium sulphate solution before proceeding for sample reading.

4. Now wash the electrode using distill water and wipe excess water from the tip of electrode with the help of ordinary filter paper then dip it in soil extract for reading.
5. Record the digital display corrected to 25^0C. The reading in mS/cm of electrical conductivity is a measure of the soluble salt content in the extract, and an indication of salinity status of the tested soil. The conductivity can also be expressed as mmhos/cm.

Observation and Calculation:

Cell constant = Actual conductivity of 0.01M KCl/Observed conductivity of 0.01M KCl

EC (dSm^{-1} at 25^0C) = (Dial reading x Knob reading x cell constant)/10^3

(The divisor 10^3 is used to convert micromhos into millimhos)

Precautions:

1. The conductivity should be taken at 25^0C.
2. For each conductance cell its cell constant should be denoted or calculated.
3. No air bubble should remain in the conductivity tube.
4. Adjust the conductivity meter using standard KCl or saturated solution of $CaSO_4$.

Interpretation of results for EC of 1:2 soil water suspension

EC of 1:2 soil water suspension (dSm^{-1} at 25^0C)	**Nature of soil**
< 0.8	Normal
0.8 – 1.6	Critical for salt sensitive crops
1.6 – 2.5	Salt tolerant crops may be grown
> 2.5	Injurious to all crops
> 16	Only few tolerant crops yield satisfactorily

Results: **The EC of given soil is ------- dSm^{-1} which comes in the category of** ----------
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Practical No 5

Determination of organic carbon in soil by Walkley and Black (1934) rapid titration method

Principle; In this method the soil is digested with chromic and sulphuric acids, making use of the heat of dilution of the sulphuric acid. The excess of chromic acid, not reduced by the, organic matter of the soil is then determined by titration with standard ferrous sulphate. Carbon in the sample is oxidized as follows:

$2K_2Cr_2O_7 + 6\ H_2SO_4 + 3\ C = 2\ K_2SO_4 + 3Cr_2\ (SO_4)_3 + 8H_2O + 3CO_2$

Thus $2K_2Cr_2O_7 = 3C$

1ml of 1N $K_2Cr_2O_7 = 0.003$ g of C

Reagents

1. 1 N $K_2Cr_2O_7$: Dissolve 49.04 g of A.R. grade $K_2Cr_2O_7$ in distilled water and make up the volume to 1 litre in volumetric flask.

2. 0.5 N Fe $(NH_4)_2\ (SO_4)_2.6H_2O$: Dissolve 392 g ferrous ammonium sulphate in distilled water and add 40 ml concentrate H_2SO_4 and make the volume to 2 litre. The FAS should be a fresh lot and light green in appearance. Yellowing of the salt indicates its oxidation.

3. Concentrate H_2SO_4. (Add 1.25% silver sulphate in salphuric acid in case of saline soil to check the chloride interferes in the estimation)

4. Orthophosphoric acid (85%) or sodium fluoride (NaF gives sharper end point)

5. Diphenylamine indicator: Dissolve 0.5 g diphenylamine in a mixture of 20 ml water + 100 ml conc. H_2SO_4.

Procedure:

1. Soil sample should be ground to pass a 0·5 mm. screen and transfer 1 g soil into 500ml conical flask.

2. Add 10 ml 1 N $K_2Cr_2O_7$ and 20 ml of conc. H_2SO_4 solution and shake to mix.

3. Allow the flask to stand for 30 minutes on an asbestos sheet for complete reaction.

4. Add 200 ml distilled water to the flask.

5. Add 10 ml 85% orthophosphoric acid and 1 pinch of NaF.

6. Pour 1 ml (6-10 drops approximately) of diphenylamine indicator.

7. Titrate the solution with 0.5 N ferrous ammonium sulphate till the colour changes from violet to bright green.

Observations

1. Weight of soil = ---------- W (g)

2. Volume of 0.5 N ferrous ammonium sulphate used in Blank titration (B) = ... ml.

3. Volume of 0.5 N ferrous ammonium sulphate used in Soil sample titration (S) = ... ml.

Calculation:

$$\% \text{ organic carbon in soil} = \frac{10\,(B - S) \times 0.003 \times 100}{B \times \text{Weight of soil}}$$

% Organic matter = % organic carbon x 1.724 (Since organic matter contains 58% Carbon, Van Bemmelen factor)

Interpretation of results for organic carbon:

(i) Below 0.50 % - Low

(ii) 0.50 to 0.75% - Medium

(iii) More than 0.75% - High

Result: The percent organic carbon in the soil sample is---------, therefore soil is ---------- in organic carbon. The organic matter in the soil sample is -------

Practical No-6

Principle of Important Instruments

Principle of Spectrophotometer

It is based on the determination of the concentration of a substances by measuring the relative absorption or transmission of light with respect to a known concentration. Colorimetric analysis is based on the measurement of intensity of radiant energy after it passes through a sample solution. A monochromatic light beam of known intensity is passed through the test solution and the intensity of the transmitted beam is determined with the help of a photo-electric cell. Thus, colorimetry can be considered as the absorption spectrophotometry in the visible range. It is based on Bear's law which states that the intensity of a monochromatic light beam decreases exponentially as the concentration of a absorbing substances increases aritmatically, as expressed below

Log I_0/I_t =kC

Where I_0 and I_t are the intensities of the incident radiation and transmitted radiation, respectively, C denotes the concentration in solution and k is a constant.

Flame photometry (flame atomic emission spectrometry)

Flame photometry is suitable for qualitative and quantitative determination of several cations, especially for metals that are easily excited to higher energy levels at a relatively low flame temperature (mainly Na, K, Rb, Cs, Ca, Ba, Cu). It is a two step process-

- In this procedure atoms under investigation are excited by light. Absorption techniques measure the absorbance of light due to the electrons going to a higher energy level.
- Emission techniques measure the intensity of light that is emitted as electrons return to the lower energy levels.

Principle of Flamephotometer

Flame photometry relies upon the fact that the compounds of the alkali and alkaline earth metals can be thermally dissociated in a flame and that some of the atoms produced will be further excited to a higher energy level. When these atoms return to the ground state they emit radiation,

which lies mainly in the visible region of the spectrum. Each element will emit radiation at a wavelength specific for that element. The table below gives details of the measurable atomic flame emissions of the alkali and alkaline earth metals in terms of the emission wavelength and the colour produced. Element Emission Wavelength (nm) & Flame Colour

Element	Emission wave length nm	Flame colour
Sodium	589	Yellow
Potassium	766	Violet
Barium	554	Lime green
Calcium	622*	Orange
Lithium	670	Red

*Note: Calcium is measured by using the calcium hydroxide band emission at 622nm as the Calcium main atomic emission occurs at 423nm.

Atomic absorption spectrophotometer

Principle

In the analysis employing Atomic Absorption Spectrophotometer (AAS) the sample in the form of a homogeneous liquid is aspirated into a flame where "free" atoms of the element to be analysed are created. A light source (hollow cathode lamp) is used to excite the free atoms formed in the flame by the absorption of the electromagnetic radiation. The decrease in energy (absorption) is then measured which follows the Lambert-Beer law, i.e. the absorbance is proportional to the number of free atoms in the ground state (Baker and Suhr, 1982).

Practical No 7

Simple Staining Technique

Bacterial morphology may be examined in two ways (1) By observing the living, unstained organisms, as is done in demonstrating bacterial motility. (2) By observing dead cells stained with dyes. Bacteria differ chemically. It is this chemical difference that enables to distinguish bacteria by staining, the stain or dye generally reacting with the bacterial cell but not with the background.

Stains or dyes are generally salts in which one of the ion is colored, and colored ion referred to as chromophore and the other ion is called as auxochrome. Dyes are divided into two groups as basic and acidic. If the colour is in the positive ion of the dye, it is a basic stain eg. Crystal violet and carbol fuchsion. And if the color is in the negatively charged ion then it is acidic stain e.g. Eosin Y.

MATERIALS REQUIRED:

Slides

Methylene blue

Crystal violet

Bacterial cultures

Microscope

Procedure

1. Place a drop of distilled water on a clean glass slide and take a loopful of the pure culture and make thin smear on the slide.

2. Fix the smear by passing it over the flame or air dry.

3. Flood the fixed smears with 3-5 drops of methylene blue or Crystal violet and allow to act for 30 seconds.

4. Wash the stained preparation with water and blot dry.

5. Examine under low power and afterwards oil immersion objective and make sketches.

Observations:

Observe the difference in cell size, shape and arrangements. Make sketches of variety

of cells as seen under the microscope.

Practical No 8

Sterilization Techniques

Sterilization is a method of freeing up of an article from all living organisms. The common methods of sterilization are as below.

A) Dry heat: Sterilization by dry heat is carried out in many ways.

1. Red heat: Inoculation loop: forceps and spatula are sterilized by heating them in flame up to red-hot.

2. Flaming: Stoppers, culture tubes, flasks etc., are sterilized by passing them through the flame

3. Hot air oven: It is electrically heated and fitted with thermostatic arrangement with blower for ensuring rapid and controlled heating of the materials, equipments such as Petri plates, pipettes, test tubes, conical flasks are sterilized at a temperature of 160 ^{0}C for one hour. The time and temperature for sterilization of glass ware are as follows**:**

120 ^{0}C – for 8 hours.

140 ^{0}C – for or 2 ½ hrs.

160 ^{0}C for 2 hr. is most commonly used.

180 ^{0}C – for 20 minutes.

B. Moist heat: For most types of media, cloth, rubber and other materials that would be destroyed by dry heat is sterilized by moist heat in an autoclave at 121^{0}C for 20 minutes using steam under 15 pounds pressure.

Water boils when vapour pressure equals the surrounding atmospheric pressure. This occurs at 100^{0}C, when water is boiled in a closed vessel at increased pressure. Thus the temperature at which is boils and that of the steam will also rise. The temperature boiling time and pressure used are as follows.

Steam Pressure	Temp.	Time in min
10 – Ibs	115^{0}C	45 min
15 – Ibs	121^{0}C	20 min
30 – Ibs	134^{0}C	3 min

At 15 lbs pressure most of the organisms including spores of *Bacillus* and *Clostridium* are readily killed hence the sterilization in the autoclave is carried out under 15 lbs. pressure.

I. Chemical methods: Disinfectants like alcohols, phenol, mercuric chloride etc., are used to surface sterilize any materials so that bacteria present on the outer surface are killed.

II. Filtration: Many materials for example sugars, blood sera, which are destroyed by heating at temperatures, which are normally used for sterilization. To sterilize such heat labile materials filters can be used. The filters remove bacteria by the sieve-like action of the pores in the filter and also by adsorption of microbes to the filters. The types of filters used are membrane filter, sintered glass filter, Seitz asbestos pad filter, the chamber land and selas candle type filter etc., All these filters are used by being attached to a suction flask to draw the liquid materials through them.

III. Gaseous sterilization: The use of ethylene oxide vapours under pressure in special equipment is a common method of cold sterilization: ethylene oxide is highly toxic for viral particles, bacterial and fungal cells and the heat-resistant endosperms.

Exercise No. 9

Preparation of Culture Media (Nutrient Agar)

It is not always possible to identify organisms by morphology alone. One of the most important methods for studying and identifying the organisms is by growing them on simple artificial food material called 'culture media'. The microbial growth on solid culture media itself is called 'culture'.

Some naturally occurring substances are used for the cultivation of bacteria. Notable among these is milk, usually skimmed rather than whole. Such natural materials are merely dispensed into tubes or flasks an sterilized before use. Media of the nutrient broth or nutrient agar type are prepared by compounding the required individual ingredients or, more conveniently, by adding water to a dehydrated product which contains all the ingredients. Practically all media are available commercially in powdered form.

Some of the common ingredients of culture media are :

1) Carbohydrates: These are the energy sources in the form of maltose, glucose etc., which serves as a carbon and energy source for microbial growth.

2) Proteins: In the form of peptone, tripeptone etc., which serves as nitrogen requirement of microbial cell.

3) Beef extract and yeast extract: They are in the form of reduced organic bases, which helps to supply nitrogen, vitamins, minerals etc.,

4) Amino acids: They are in the form of reduced organic bases, which helps to supply nitrogen, vitamins etc.,

5) Growth factors: In the form of vitamins like riboflavin, niacin etc.,

6) Solidifying agents: In the form of Agar, silica gel and Gelatin. The most common in use is agar, which has certain unique properties. It is obtained from a sea –weed called, Geladium cornea,. Its melting point is 100^0C and solidifies at a temperature of 42^0C- 45^0C.

7) Mineral salts: In the form of chloride, sulphate, nitrite and nitrate etc.,

Types of culture media

A) Media can be classified on the basis of physical state, as

1) Liquid Media 2) Solid Media3) Semi solid media

B) Another way of classifying the media is on the basis of their components.

1) Synthetic Media (Reproducible media). The synthetic media are made with pure chemical substances and the exact composition of the medium is known. It can be reproduced any number of items. Eg. Norris nitrogen free medium.

2) Non-synthetic Media: non-synthetic media are represented by an almost infinite variety of naturally occurring substances for growing microorganism Eg. Milk, Blood serum, Coconut milk, Eggs, Potatoes, etc.,

C) Special Media: Special media are further classified into:

1) Enriched media: In these media substances such as blood serum, and egg are added to basal medium (NA). These media are used to grow bacteria, which are more exacting in their nutritional needs. e. g. Blood agar.

2) Selective media: The incorporation of certain special chemicals to nutrient agar will prevent growth of one group of bacteria without inhibiting others. Eg: Crystal violet at a specific concentration in Macconkeys agar will prevent the growth of gm+ve bacteria without affecting the growth of gm-ve bacteria eg. Macconkey's Agar.

3) Differential media: It is used to bring out differentiating characters of bacteria and thus helping them to distinguish. Eg: Eosine methylene blue agar (EMB agar), Yeast extract mannitol agar with congored (YEMA).

Preparation of Nutrient Agar

Materials required:

Peptone Yeast extract

Distilled water Autoclave

Conical flask with cotton plug

Procedure:

1) Each ingredient, or the complete dehydrated medium, is dissolved in the appropriate volume of distilled water.

2) The pH of the fluid medium is determined with a pH meter and adjusted if necessary.

3) If a solid medium is desired, agar is added and the medium is boiled to dissolve the agar.

4) The medium is dispensed into conical flasks.

5) The medium is sterilized, generally by autoclaving. Some media that are heat labile are sterilized by filtration.

Exercise No 10

Laboratory glassware and their uses

Laboratory glassware refers to a variety of equipment used in scientific work, and traditionally made of glass. Glass can be blown, bent, cut, molded, and formed into many sizes and shapes, and is therefore common in chemistry, biology, and analytical laboratories. Many laboratories have training programs to demonstrate how glassware is used and to alert first–time users to the safety hazards involved with using glassware.

Laboratory glassware selection

Laboratory glassware is typically selected by a person in charge of a particular laboratory analysis to match the needs of a given task. The task may require a piece of glassware made with a specific type of glass. The task may be readily performed using low cost, mass-produced glassware, or it may require a specialized piece created by a glass blower. The task may require controlling the flow of fluid. The task may have distinctive quality assurance requirements.

Type of glass

Laboratory glassware may be made from several types of glass, each with different capabilities and used for different purposes. Borosilicate glass is transparent and can withstand thermal stress. Quartz glass can withstand very high temperatures and is transparent in certain parts of the electromagnetic spectrum. Darkened brown or amber (actinic) glass can block ultraviolet and infrared radiation. Heavy-wall glass can withstand pressurized applications. Fritted glass is finely porous glass through which gas or liquid may pass. Coated glassware is specially treated to reduce the occurrence of breakage or failure. Silanized (siliconized) glassware is specially treated to prevent organic samples from sticking to the glass.

Scientific glass blowing

Scientific glass blowing, which is practiced in some larger laboratories, is a specialized field of glassblowing. Scientific glassblowing involves precisely controlling the shape and dimension of glass, repairing expensive or difficult-to-replace glassware, and fusing together various glass parts. Many parts are available fused to a length of glass tubing to create highly specialized piece of laboratory glassware.

Controlling fluid flow

When using glassware it is often necessary to control the flow of fluid. It is commonly stopped with a stopper. Fluid may be transported between connected pieces of glassware. Types of interconnecting components include glass tubing, T-connectors, Y-connectors, and glass adapters. For a leak-tight connection a ground glass joints is used (possibly reinforced using a clamping method such as a Keck clips). Another way to connect glassware is with a hose barb

and flexible tubing. Fluid flow can be switched selectively using a valve, of which a stopcock is a common type fused to the glassware. Valves made entirely of glass may be used to restrict fluid flows. Fluid, or any material which flows, can be directed into a narrow opening using a funnel.

Quality assurance

Metrology

Laboratory glassware can be used for high precision volumetric measurements. With high precision measurements, such as those made in a testing laboratory, the metrological grade of the glassware becomes important. The metrological grade the can be determined by both the confidence interval around the nominal value of measurement marks and the traceability of the calibration to an NIST standard. Periodically it may be necessary to check the calibration of the laboratory glassware.

Dissolved silica

Laboratory glassware is composed of silica. Silica is considered insoluble in most substances with a few exceptions such as hydrofluoric acid. Though insoluble a minute quantity of silica will dissolve which may affect high precision, low threshold measurements of silica in water.

Cleaning

Cleaning laboratory glassware is sometimes necessary and may be done using multiple methods. Glassware can be soaked in a detergent solution to remove grease and loosen most contaminations. These contaminations are then scrubbed with a brush or scouring pad to remove particles which cannot be rinsed. Sturdy glassware may be able to withstand sonication as an alternative to scrubbing. For certain sensitive experiments glassware may be soaked in solvents, such as aqua regia or mild acids, to dissolve a trace quantities of specific contaminations known to interfere with an experiment. When cleaning is finished it is common practice to triple rinse glassware before suspending it upside down on drying racks.

There are many different kinds of laboratory glassware items:

Examples of glassware containers include:

Beakers are simple cylindrical shaped containers used to hold reagents or samples.

Flasks are narrow-necked glass containers, typically conical or spherical, used in a laboratory to hold reagents or samples. Examples flasks include the Erlenmeyer flask, Florence flask, and Schlenk flask.

Bottles are containers with narrow openings generally used to store reagents or samples. Small bottles are called vials.

Jars are cylindrical containers with wide openings that may be sealed. Bell jars are used to contain vacuums.

Test tubes are used by chemists to hold, mix, or heat small quantities of solid or liquid chemicals, especially for qualitative experiments and assays.

Desiccators of glass construction are used to dry materials or keep material dry.

Glass evaporating dishes, such as watch glasses, are primarily used as an evaporating surface (though they may be used to cover a beaker.)

Glass Petri dishes are used to culture living cells.

Microscope slides are thin strips used to hold items under a microscope.

Examples of glassware used for measurements include:

Graduated cylinders are cylindrical containers used for volumetric measurements.

Volumetric flasks are for measuring a specific volume of fluid.

Burettes are used to disperse precise amounts of liquid reagents.

Glass pipettes are used to transfer precise quantities of fluids.

Glass Ebulliometers are used to accurately measure the boiling point of liquids.

Other examples of glassware includes:

Stirring rods are used to mix chemicals.

Condensers are used to cool hot liquids or vapors.

Glass retorts are used for distillation.

Drying pistols are used to free samples from traces of water, or other impurities.

Exercise No 11

General Guidelines for Safe Handling of Chemicals In Laboratory

Laboratory hazards can be minimized by following these safety measures in handling.

- Always label all containers with chemicals.
- Use protective equipments for eye protection and make sure to wear a laboratory coat.
- Avoid intentional smelling, inhaling and tasting of chemicals.
- Always avoid direct contact with chemicals, far from your hands face, clothes and shoes.
- Hazardous chemical should be used only as directed.
- Use separate cabinets for acid solutions with concentration more than 6 M.
- Mark the date on all containers upon receipt and again when reopened.
- Attach chemical labels with all necessary information to all containers.
- Immediately read the warning labels when opening newly received reagent chemicals. This will help to be aware of any special storage precautions such as refrigeration or inert atmosphere storage.
- A periodic check on chemical containers for rust, corrosion, and leakage is a must.
- Store bottles in chemical safe bags especially those hazardous and moisture-absorbing chemicals.
- Avoid use of mouth suction to fill a pipette. Use a pipette bulb or other filling devices.
- Smoking, drinking, eating and the application of cosmetics is forbidden in areas where hazardous chemicals are used or stored.
- Always use chemicals with adequate ventilation. Check with the MSDS and also the Standard Operating Procedure to work out what type of ventilation is required.
- Whenever you leave the lab after handling any chemicals wash thoroughly with soap and water. Keep your hands and face clean free from any trace of chemicals.

Exercise No-11

Lab Safety Rules and Guidelines

General lab safety rules

The following are rules that relate to almost every laboratory and should be included in most safety policies. They cover what you should know in the event of an emergency, proper signage, safety equipment, safely using laboratory equipment, and basic common-sense rules.

1. Be sure to read all fire alarm and safety signs and follow the instructions in the event of an accident or emergency.

2. Ensure you are fully aware of your facility's/building's evacuation procedures.

3. Make sure you know where your lab's safety equipment—including first aid kit(s), fire extinguishers, eye wash stations, and safety showers—is located and how to properly use it.

4. Know emergency phone numbers to use to call for help in case of an emergency.

5. Lab areas containing carcinogens, radioisotopes, biohazards, and lasers should be properly marked with the appropriate warning signs.

6. Open flames should never be used in the laboratory unless you have permission from a qualified supervisor.

7. Make sure you are aware of where your lab's exits and fire alarms are located.

8. An area of 36" diameter must be kept clear at all times around all fire sprinkler heads.

9. If there is a fire drill, be sure to turn off all electrical equipment and close all containers.

10. Always work in properly-ventilated areas.

11. Do not chew gum, drink, or eat while working in the lab.

12. Laboratory glassware should never be utilized as food or beverage containers.

13. Each time you use glassware, be sure to check it for chips and cracks. Notify your lab supervisor of any damaged glassware so it can be properly disposed of.

14. Never use lab equipment that you are not approved or trained by your supervisor to operate.

15. If an instrument or piece of equipment fails during use, or isn't operating properly, report the issue to a technician right away. Never try to repair an equipment problem on your own.

16. If you are the last person to leave the lab, make sure to lock all the doors and turn off all ignition sources.

17. Do not work alone in the lab.

18. Never leave an ongoing experiment unattended.

19. Never lift any glassware, solutions, or other types of apparatus above eye level.

20. Never smell or taste chemicals.

21. Do not pipette by mouth.

22. Make sure you always follow the proper procedures for disposing lab waste.

23. Report all injuries, accidents, and broken equipment or glass right away, even if the incident seems small or unimportant.

24. If you have been injured, yell out immediately and as loud as you can to ensure you get help.

25. In the event of a chemical splashing into your eye(s) or on your skin, immediately flush the affected area(s) with running water for at least 20 minutes.

26. If you notice any unsafe conditions in the lab, let your supervisor know as soon as possible.

Housekeeping safety rules

Laboratory housekeeping rules also apply to most facilities and deal with the basic upkeep, tidiness, and maintenance of a safe laboratory.

1. Always keep your work area(s) tidy and clean.

2. Make sure that all eye wash stations, emergency showers, fire extinguishers, and exits are always unobstructed and accessible.

3. Only materials you require for your work should be kept in your work area. Everything else should be stored safely out of the way.

4. Only lightweight items should be stored on top of cabinets; heavier items should always be kept at the bottom.

5. Solids should always be kept out of the laboratory sink.

6. Any equipment that requires air flow or ventilation to prevent overheating should always be kept clear.

Dress code safety rules

1. Always tie back hair that is chin-length or longer.

2. Make sure that loose clothing or dangling jewelry is secured, or avoid wearing it in the first place.

3. Never wear sandals or other open-toed shoes in the lab. Footwear should always cover the foot completely.

4. Never wear shorts or skirts in the lab.

5. When working with Bunsen burners, lighted splints, matches, etc., acrylic nails are not allowed.

Personal protection safety rules

Basic hygiene rules to follow to avoid any sort of contamination.

1. When working with equipment, hazardous materials, glassware, heat, and/or chemicals, always wear face shields or safety glasses.

2. When handling any toxic or hazardous agent, always wear the appropriate gloves.

3. When performing laboratory experiments, you should always wear a smock or lab coat.

4. Before leaving the lab or eating, always wash your hands.

5. After performing an experiment, you should always wash your hands with soap and water.

6. When using lab equipment and chemicals, be sure to keep your hands away from your body, mouth, eyes, and face.

Chemical safety rules

1. Every chemical should be treated as though it were dangerous.

2. Do not allow any solvent to come into contact with your skin.

3. All chemicals should always be clearly labeled with the name of the substance, its concentration, the date it was received, and the name of the person responsible for it.

4. Before removing any of the contents from a chemical bottle, read the label twice.

5. Never take more chemicals from a bottle than you need for your work.

6. Do not put unused chemicals back into their original container.

7. Chemicals or other materials should never be taken out of the laboratory.

8. Chemicals should never be mixed in sink drains.

9. Flammable and volatile chemicals should only be used in a fume hood.

10. If a chemical spill occurs, clean it up right away.

11. Ensure that all chemical waste is disposed of properly.

Chemistry lab safety rules

As chemistry labs are one of the most common types, these basic chemistry lab safety rules are relevant to many scientists, dealing with the safe performance of common activities and tasks in the average chemistry lab:

1. Before you start an experiment, make sure you are fully aware of the hazards of the materials you'll be using.

2. When refluxing, distilling, or transferring volatile liquids, always exercise extreme caution.

3. Always pour chemicals from large containers to smaller ones.

4. Never pour chemicals that have been used back into the stock container.

5. Never tap flasks that are under vacuum.

6. Chemicals should never be mixed, measured, or heated in front of your face.

7. Water should not be poured into concentrated acid. Instead, pour acid slowly into water while stirring constantly. In many cases, mixing acid with water is exothermic.

Electrical safety rules

Like almost every other workplace, laboratories contain electronic equipment. Electrical safety rules help prevent the misuse of electronic instruments, electric shocks and other injuries, and

ensure that any damaged equipment, cords, or plugs are reported to the appropriate authorities so they can be repaired or replaced.

1. Before using any high voltage equipment (voltages above 50Vrms ac and 50V dc), make sure you get permission from your lab supervisor.

2. High voltage equipment should never be changed or modified in any way.

3. Always turn off a high voltage power supply when you are attaching it.

4. Use only one hand if you need to adjust any high voltage equipment. It's safest to place your other hand either behind your back or in a pocket.

5. Make sure all electrical panels are unobstructed and easily accessible.

6. Whenever you can, avoid using extension cords.