Handbook for Analytical Chemist

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INTRODUCTION

Before we start to work in the laboratory, need to refresh some old lessons we have learnt and practised earlier.

There is no harm to forget things, but if we do not revise, then we are out of the race. There is something always which we have to imprint in our memory and no chances to erase it. It is same like our name, home address and our mobile number. We should not forget our name or mobile number; we should not forget the rules and safety process to walk on a street for our own benefit. Same, few things we should remember to work in a laboratory.

This chapter covers many of the basic skills and information that are necessary to be a good analytical chemist. Nothing can replace actual laboratory working experiences as a learning tool, but hope this chapter will help the beginner to learn proper laboratory techniques early rather than having to correct improper habits later.

All of this, which we are going to discuss now, detail attention is not always needed, but you must know what to ignore. The decision to use something other than the "best" technique must be conscious decision and based on knowledge of the analytical method being used along with how the resulting data will be used.

PRECISION AND ACCURACY

Precision refers to the reproducibility of replicate observations, typically measured as standard deviation (SD), standard error (SE), or coefficient of variation (CV). This we will discuss more elaborately in different chapter. The smaller the value, more precise the measurement is. Precision is determined not on reference standards, but by the use of actual samples, which cover a range of concentrations and a variety of interfering materials usually encountered by the analyst. Obviously, such data should not be collected until the analyst is familiar with the method and has obtained a reproducible standard curve (mathematical relationship between the analyte concentration and the analytical response). One of the methods among different available methods to determine precision as follows:

1. Three separate concentration levels should be studied, including a low concentration near the sensitivity level of the method, an intermediate concentration, and a concentration near the upper limit of application of the method.
2. Ten replicate determinations should be made at each of the concentrations tested.
3. To allow for changes in instrument conditions, the precision study should cover at least 2 hour of normal laboratory operation.
4. To permit the maximum interferences in sequential operation, it is suggested that the samples be run in the following order: high, low and intermediate. This series is then repeated ten times to obtain the desired replication.
5. The precision statement should include a range of standard deviations over the tested range of concentration. Thus, three

standards will be obtained over a range of three concentrations.

All laboratories have their own protocol to check and you need to follow. In most of cases, the protocol says to check reproducibility using standards and sequence of analysis is continuous of the same concentration. This cannot establish the precision of the method. We have to consider the real life. During the analysis, we do not know the concentration of target molecules in sample and get different concentration for different samples. So to establish the precision of the method, it is always good to shuffle the concentration sequence during analysis.

Accuracy refers to the degree (absolute or relative) of difference between observed value and "actual" values. The 'actual" value is often difficult to ascertain. IT may be the value obtained by a standard reference method (the accepted manner of performing a measurement). Another means of evaluating accuracy is by the addition of a known amount of the material being analyzed for the sample and then calculation of % recovery. This approach has following steps:

1. Known amount of the particular constituent are added to actual samples at concentrations for which the precision of the method is satisfactory. It is suggested that amounts be added to the low-concentration sample, sufficient to double that concentration, and that an amount be added to the intermediate concentration, sufficient to bring the final concentration in the sample to approximately 75% of the upper limit of application of the method.
2. Ten replicate determination at each concentration are made.
3. Accuracy is reported as the percent recovery at the final concentration of the spiked sample. Percent recovery at each concentration is the mean of the ten replicate results.

A fast, less rigorous means to evaluate precision and accuracy is to analyze a sample and replicate a spiked sample, and then calculate the recovery of the amount spiked. An example has shown in below table.

Measured calcium concentration (g/L) in milk and spiked milk:

Replicate	Milk	Milk + 0.75 g Ca/L
1	1.33	2.22
2	1.28	2.16
3	1.35	2.2
4	1.26	2.29
5	1.22	2.05
6	1.41	2.12
7	1.26	2.24
8	1.27	2.07
9	1.25	1.75
10	1.33	2.12
Mean	1.30	2.12
SD	0.06	0.15
%CV	4.43	7.12

%CV = (SD/Mean)*100

Note: Refer "CALCULATION" section to calculate standard deviation.

The accuracy can then be measured by calculating the % of the spike (0.75 g/L) detected by comparing the measured

values from the unspiked and spiked samples:

Accuracy ≈ % recovery =
{measured spiked sample / (measured sample + amount spiked)} x 100%
= { 2.12 g/L / (1.30 g/L + 0.75 g/L)} x 100%
= { 2.12 g/L / 2.05 g/L} x 100%
= 1.034 x 100%
= 103.4%

BALANCES

Two general types of balances are used in most of the laboratories – top loading balances and analytical balances. Top loading balances usually are sensitive to 0.1 – o.001 g, depending on the specific model in use (this means that they can measure differences in the masses of a sample to within 0.1 – 0.001 g). In general, as the capacity (largest mass that can be measured) increases, the sensitivity decreases. In other words, balances that can measure larger masses generally measure differences in those masses to fewer decimal places. Analytical balances are usually sensitive to 0.001 – 0.00001 g, depends on the specific model. It should be remembered, however, that the sensitivity (ability to detect small differences of mass) is not necessarily equal to accuracy (the degree to which the balances correctly report the actual mass). The fact that a balance can be read to 0.01 mg does not mean it is accurate to 0.01 mg. What does that mean is that the balance can distinguish between masses that differ by 0.01 mg, but may not accurately measure those masses to within 0.01 mg of the actual masses (because the last digit is often rounded). The accuracy of a balance is independent of its sensitivity.

Selection of types of balance depends on "how much accuracy" is required in a given measurement. One way to determine this is by calculating how much relative (%) error would be introduced by a given type of balance. As an example, suppose, if 0.1 g of a reagent was needed, weighing it on top loading balance accurate to within only ±0.02 g of the actual mass would introduce approximately 20% error:

% error in measured mass = (absolute error in measured mass / measured mass) x 100%
= (0.02 g/0.1 g) x 100%
= 0.02%

The decision on "how much accuracy" is needed can only be answered when one knows the function of the reagent in the analytical method.

This is one reason why it is necessary to understand the chemistry involved in an analytical method, and not to simply approach an analytical method in a cookbook fashion. Therefore, a general guideline regarding which balance to use is hard to define.

Another situation is which care must be exercised in determining what type of balance to use is when a difference in masses is to be calculated. For instance, a dried cruisible to be used in a total ash determination may weigh 20.05 g on a top loading balance, crucible plus sample = 25.05 g, and the ashed cruisible 20.25 g. It may appear that the use of the top loading balance with its accuracy of ±0.02 g would

introduce approximately 0.1% error, which would often be acceptable. Actually, since a difference in weight (0.2o g) is being determined, the error would be approximately 10% and thus unacceptable. In this case, an analytical balance is definitely required because sensitivity is required in addition to accuracy.

Use of Top Loading Balances

These instructions are generalized but apply to the use of most models of top loading balances:

1. Level the balance using the bubble level and the adjustable feet.
2. Either "zero" the balance or "tare" the balance with a container that will hold the sample on the weighing pan (so the balance reads "0").
3. Weigh the sample.

Use of Analytical Balances

Consult the specific instruction manual for an analytical balance before using it. Speed and accuracy are both dependent on one being familiar with the operation of ana analytical balance. If you have not used the a specific types of balance for a while, "practice" before actually weigh a sample. The following general rules apply to most analytical balances and should be followed for accurate result and to avoid and damage by improper use:

1. Treat the balance with extreme care as it is sensitive and expensive.
2. Make sure that the balance is on a sturdy bench, free of vibration and levelled.
3. Once above conditions are met, follow the same procedure as mentioned for the top loading balance.
4. Always leave the balance clean.

Additional information

Regarding the use of balances, following points also to be taken care:

1. Many analyses (moisture, ash, etc.) require weighing of the final dried or ashed sample with the vessel. The mass of the vessel must be known so that it can be subtracted from the final mass to get the mass of the dried sample or ash.
2. The accumulation of moisture from the air or fingerprints on the surface of a vessel will add a small mass to the sample. So, handle the weighing vessels with tongs or with gloved hands. Use desiccator.
3. Air currents or leaning on the bench can cause appreciable error in analytical balances. It is best to take the reading after closing the side or top doors of an analytical balance.
4. Most balances in modern laboratory are electric balances. Older level-type balances are no longer in wide use, but they are extremely reliable. So, do not upset if you see it in your laboratory.

PIPETTES

Pipettes may be glass volumetric pipettes or mechanical pipettes.

Mechanical pipettes

Mechanical pipettes, also known as automatic pipettors, are standard equipment now in most analytical laboratories, because of its convenience, precision and acceptable accuracy (when used properly and calibrated). Although it seems easier to use than glass volumetric pipettes, but cannot be obtained necessary accuracy and precision without attention and proper pipetting technique, and, will cause greater error than glass volumetric pipette.

Operation:

1. Set the desired volume on the digital micrometer / volumeter as per the instruction manual. For improved precision, always approach the desired volume by dialing downward from a larger volume setting. Make sure not to wind it up beyond its maximum capacity; this will break it beyond repair.
2. Attach a disposable tip to the shaft of the pipette and press on firmly with a slight twisting motion to ensure a positive. Airtight seal.
3. Depress the plunger to the first positive stop. This part of the stroke is the calibrated volume displayed. Going past the first positive stop will cause inaccurate measurement.
4. Holding the mechanical pipette vertically, immerse the disposable tip into sample liquid to a depth indicated in table, specific to the maximum volume of the pipette.
5. Allow plunger to slowly return to the “up” position. Never permit it to snap up (this will suck liquid up into the pipette mechanism and will cause inaccurate measurement and damaging the pipette).
6. Wait 1-2 s to ensure that full volume of sample is drawn into tip. If the solution is viscous such as glycol, you need to allow more time.
7. Withdraw tip from sample liquid. Should any liquid remain on the outside of the tip, wipe carefully with a lint-free cloth, taking care not to touch the tip opening (else, it will suck the sample from inside the tip).
8. To dispense sample, place tip end against side wall of vessel and depress plunger slowly past the first stop until the second stop (fully depressed position) is reached.
9. Wait.
10. With plunger fully depressed, withdraw mechanical pipette from the vessel carefully with tip sliding along wall vessel.
11. Allow plunger to return to top position.
12. Discard tip by depressing tip-ejector button smartly.
13. A fresh tip should be used for the next measurement if:
    a. A different solution or volume is to be pipetted.
    b. A significant residue exists in the tip (not to be confused with the visible “film” left by some viscous or organic solutions).

Table for appropriate pipette depth:

Pipette (for µL)	Depth (mm)
20, 100, 200	1-2
500, 1000	2-4
5000	3-6

Table for appropriate dispense wait time

Pipette (for µL)	Time (s)
20, 100, 200	1
500, 1000	1-2
5000	2-3

Pre-rinsing

Pipetting very viscous solutions or organic solvents will result in a significant film being retained on the inside wall of the tip. This will result in an error that will be larger than the tolerance specified if the tip is only filled once. Since this film remains relatively constant in successive pipetting with the same tip, accuracy may be improved by filling the tip, dispensing the volume into waste container, refilling the tip a second time, and using this quantity as the sample. This procedure is recommended in all pipetting operations when critical reproducibility is required, whether or not the tips are reused (same solution) or changed (different solutions/different volumes). Note that the "non-wettability" of the polypropylene tips is not absolute and that pre-rinsing will improve the precision and accuracy when pipetting any solution.

Pipetting Solutions of Varying Density or Viscosity

Compensation for solutions of varying viscosity or density is possible with any adjustable pipette by setting the digital micrometer slightly higher or lower than the requirement volume. The amount of compensation is determined empirically. Also, when dispensing viscous liquids, it will help to wait 1s longer at the first stop before depressing to the second stop.

Selecting the Correct Pipette

Although automatic pipettes can dispense a wide range of volumes, you may have to choose the "best" pipette with the best accuracy/precision from among several choices. For example, a 5000µL (5 mL) automatic pipettor can theoretically pipette anywhere between 0 and 5 mL. However, there are several limitations that dictate which pipettes to use. The first is a practical limitation: mechanical pipettes are limited by the graduations (the increments) of the pipette. The 5 mL and1 mL are typically adjustable in increment of 0.01 mL. Therefore, these pipettes cannot dispense volumes of <10µL. However, just because these pipettes can technically be adjusted to 10µL does not mean that they should be used to measure volumes anywhere near this small. Most pipettes are labelled with a working range that lists the minimum and maximum volume, but this is not the range for ideal performance. Mechanical pipettes should be operated from 100% down to 10-20% of their maximum capacity. Below 10-20% of their maximum capacity, performance suffers. A good way of thinking of this is to use the largest pipette capable of dispensing the volume in a single aliquot.

Mechanical pipettes are invaluable pieces of laboratory equipment and if properly treated and maintained, they can last for decades. Improper use can destroy them in seconds. Mechanical pipettes should be

calibrated, lubricated and maintained at least yearly through the manufacturer or by knowledgeable technician. Weighing dispensed water is often a good check to see if the pipette needs calibration.

Recommended volume ranges for mechanical pipettors

Pipette (maximum volume, µL)	Lowest recommended volume (µL)
5000	1000
1000	100-200
200	20-40
100	10-20
50	5-10
20	2-4
10	1-2

Glass Volumetric Pipettes

Pipettes, sometimes spelled pipet, commonly used to transport a measured volume of liquid. Pipettes come in several designs for various purposes with differing levels of accuracy.

According to the purpose of uses, there are three types of pipettes:

(a) Volumetric or transfer pipettes.
(b) Graduated or measuring pipettes (Mohr pipettes)
(c) Graduated or measuring pipettes (Serological pipettes)

Volumetric pipettes are designed to deliver accurately fixed volume of liquid. It is not graduated, more accurate, non-blown out. It consist of a cylindrical bulb joined at both ends to narrowed glass tubing.

Graduated pipettes are for measuring the desired volume. It is graduated, but less accurate compare to volumetric pipettes. Some are blown out.

Glass Serological pipettes

These are graduated all the way to the tip. For example, if you look at a 10 mL serological pipette, there will be a line at the top marked "0". When you fill the pipette to this line, it contains 10 mL. Below this line, there are ten smaller, unmarked graduations, representing 0.1 mL each, and then a line marked with 1mL. Below that are more 0.1 mL graduations and marked lines for 2 mL, 3 mL, etc. The last marked line will be 9 mL, but there will be 0.1 mL marks below that and down on the tip to indicate that to dispense the full 10 mL volume, you have to go all the way to the tip.

Glass Mohr pipettes

The other types of pipettes is a measuring pipette. This one isn't graduated all the way to the tip, and there's actually a line for 10 mL. That means that if the pipette is filled to the zero line, it actually contains more than 10 mL, and to dispense 10 mL, you don't empty the pipette completely but instead just let out fluid until it reached to 10 mL line.

Pipette classification

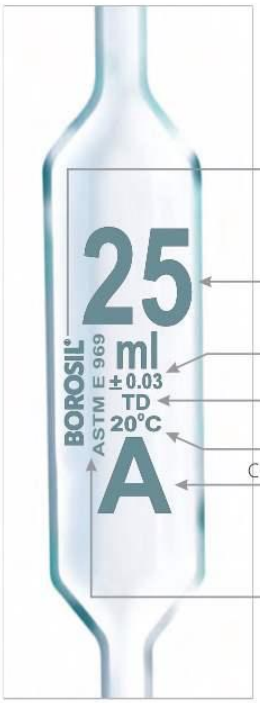
I. Design
   a. To contain (TC)
   b. To deliver (TD)
II. Drainage characteristics
   a. Blowout
   b. Self-draining
III. Type
   a. Measuring or graduated
      i. Serologic
      ii. Mohr
      iii. Bacteriologic

iv. Ball, Kolmer, or Kahn

b. Transfer
    i. Volumetric
    ii. Ostwald-Folin
    iii. Pasteur pipettes

The proper technique for using volumetric pipettes is as follows (this technique is for TD pipettes, which are much more common than TC pipettes):

1. Draw the liquid to be delivered into the pipette above the line on the pipette. Always use a pipette bulb or pipette aid to draw the liquid into the pipette. Never pipette by mouth.
2. Remove the bulb (when using the pipette aid, or bulbs with pressure release valves, you can deliver without having remove it) and replace it with your index finger.
3. Withdraw the pipette from the liquid and wipe off the tip with tissue paper. Touch the tip of the pipette against the wall of the container from which the liquid was withdrawn (or a spare beaker). Slowly release the pressure of your figure (or turn the scroll wheel to dispense) on the top of the pipette and allow the liquid level in the pipette to drop so that the bottom of the meniscus is even with the line on the pipette.
4. Move the pipette to the beaker or flask into which you wish to deliver the liquid. Do not wipe off the tip of the pipette at this time. Allow the pipette tip to touch the side of the bealer or flask. Holding the pipette in a vertical position, allow the liquid to drain from the pipette.
5. Allow the tip of the pipette to remain in contact with the side wall for several seconds. Remove the pipette. There will be a small amount of liquid remaining in the tip of the pipette. Do not blow out this liquid with the bulb, as TD pipettes are calibrated to account for this liquid that remains.



Manufacturer

Nominal Capacity

Tolerance

Calibration (TD= to deliver)

Ref Temperature

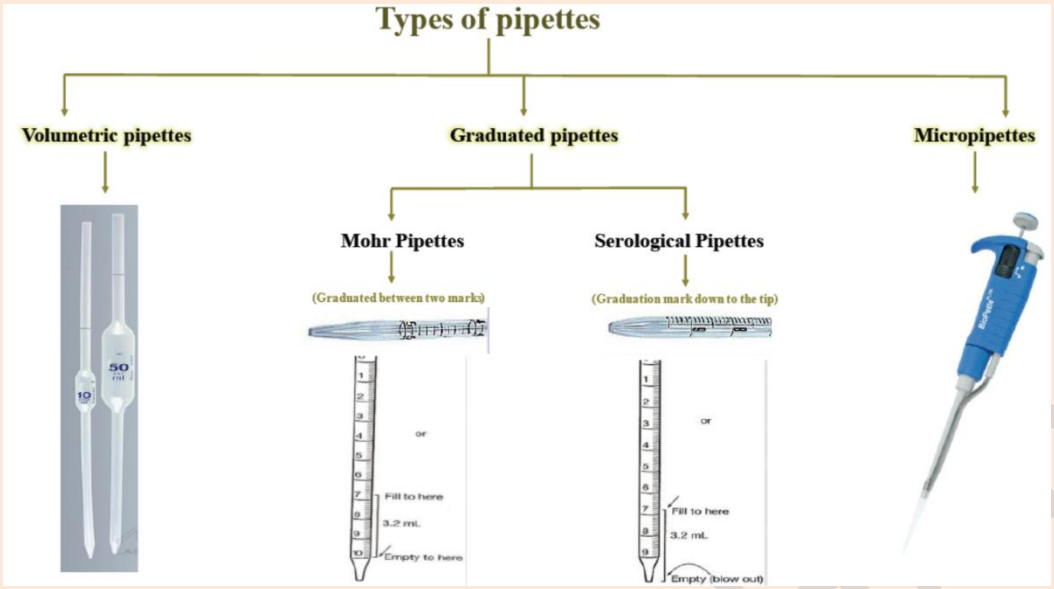
Class A Certified (Quality Grade)

Standards

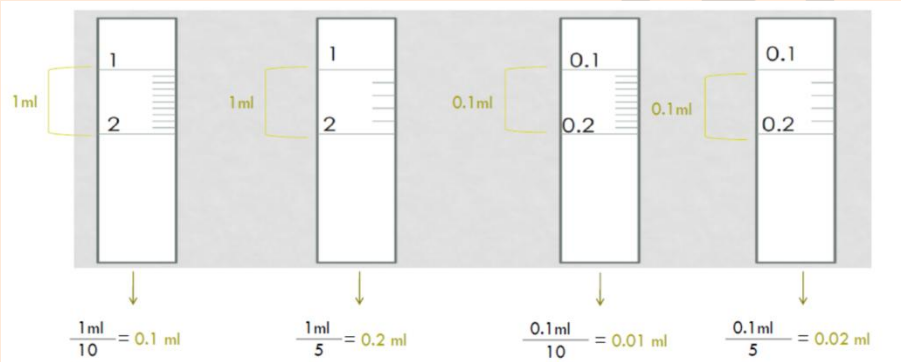
Tips are carefully tooled and ensure proper flow rates

Tip edges are fire polished

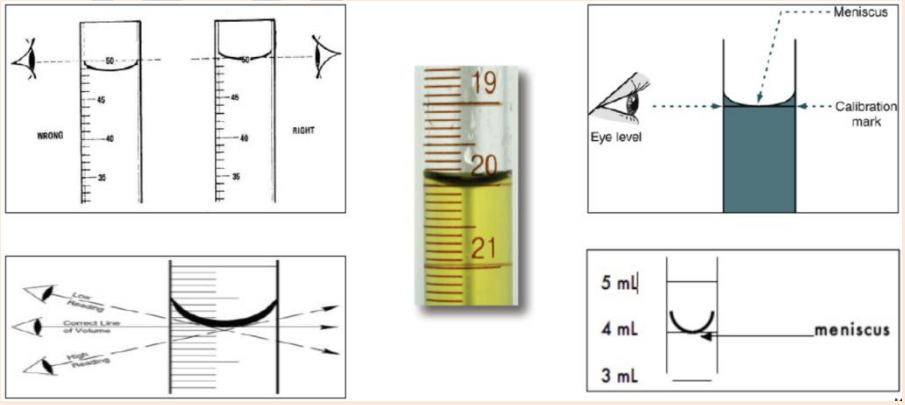
All pipettes are colour coded at the top to allow fast identification

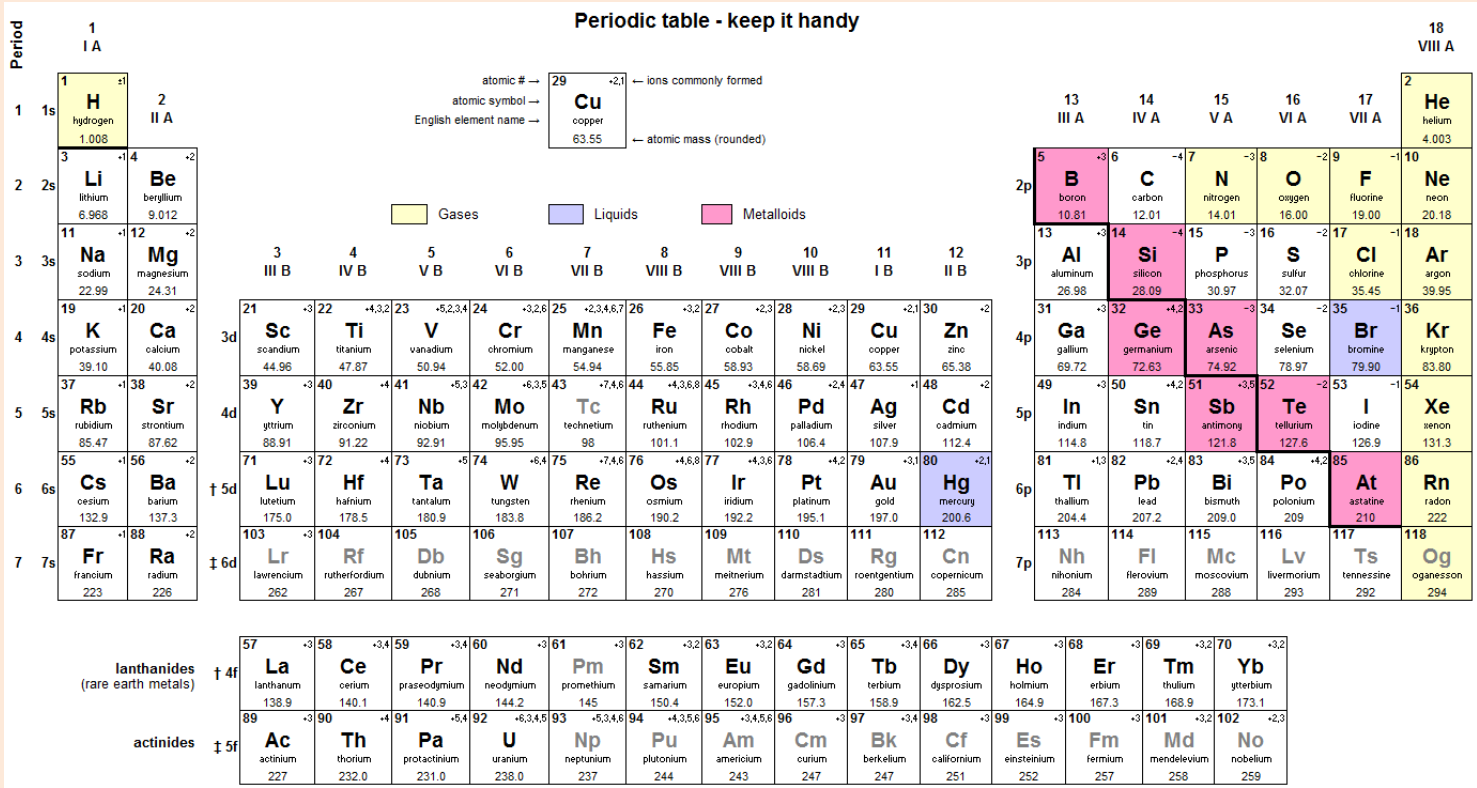


Smallest division of graduated pipette



Reading the meniscus





Common Polyatomic Ions

ammonium	NH_4^{+1}	perchlorate	ClO_4^{-1}	hydrogen sulfate	HSO_4^{-1}	sulfate	SO_4^{-2}	oxalate	$C_2O_4^{-2}$
hydronium	H_3O^{+1}	chlorate	ClO_3^{-1}			sulfite	SO_3^{-2}	silicate	SiO_3^{-2}
acetate	$C_2H_3O_2^{-1}$ CH_3COO^{-1}	chlorite	ClO_2^{-1}	permanganate	MnO_4^{-1}	phthalate	$C_8H_4O_4^{-2}$	peroxide	O_2^{-2}
		hypochlorite	ClO^{-1}	periodate	IO_4^{-1}	chromate	CrO_4^{-2}	tetraborate	$B_4O_7^{-2}$
hydroxide	OH^{-1}	nitrate	NO_3^{-1}	hydrogen carbonate	HCO_3^{-1}	dichromate	$Cr_2O_7^{-2}$	borate	BO_3^{-3}
cyanide	CN^{-1}	nitrite	NO_2^{-1}			carbonate	CO_3^{-2}	arsenate	AsO_4^{-3}
cyanate	OCN^{-1}	bromate	BrO_3^{-1}	dihydrogen phosphate	$H_2PO_4^{-1}$	hydrogen phosphate	HPO_4^{-2}	phosphate	PO_4^{-3}
thiocyanate	SCN^{-1}	iodate	IO_3^{-1}					orthosilicate	SiO_4^{-4}

GLASSWARE

Type of Glassware/Plasticware

Glass is the most widely used material for construction of laboratory vessels. There are many grades and types of glassware to choose from, ranging form student grade to others possessing specific properties such as resistance to thermal shock or alkali, low boron content, and super strength.

Choosing Glassware/Plasticware

Consider below minimum points to choose:

1. Generally special types of glass are not required to perform most analysis.
2. Reagents and standard solutions should be stored in borosilicate or polyethylene bottles.
3. Certain dilute metal solutions may plate out on glass container walls over long period of storage. So, dilute metal standard solutions should be prepared fresh at the time of analysis.
4. Strong mineral acids (such as sulphuric acid) and organic solvents will readily attack polyethylene. These are best to store in glass or a resistant plastic.
5. Borosilicate glassware is not completely inert, particularly to alkalis. So, standard solutions of silica, boron and the alkali metals (such as NaOH) are usually stored in polyethylene bottles.
6. Certain solvents dissolve some plastics, including plastic used for pipette tips. This is especially true for acetone and chloroform. When using solvents, check the compatibility with the plastics you are using. Plastic dissolved in solvents can cause various problems, inlcuding binding /precipitating the analyte of interest, interfering with the assay, clogging instruments, etc.
7. Ground-glass stoppers require care. Avoid using bases with any ground glass because the base can cause them to "freeze" (.e., get stuck). Glassware with ground-glass connections (burette, volumetric flask, separatory funnels, etc.) are very expensive and should be handled with extreme care.

For additional information, you should refer the catalogs of the various glass and plastic manufacturers. These contain very important information which you may not get in different books easily.

Volumetric glassware

Glassware which are accurately calibrated for accurate and precise measurements of volume is known as volumetric glassware. This group includes volumetric flasks, volumetric pipettes, and accurate calibrated burets. Less accurate types of glassware, including graduated cylinders, serological pipettes and measuring pipettes, also have specific use in laboratory when exact volumes are unnecessary. Volumetric flasks are to be used in preparing standard solutions, but not for strong reagents. The precision of an

analytical method depends in part upon the accuracy with which volumes of solutions can be measured, due to the inherent parameters of the measurement instrument. For example, a 10ml volumetric flask will typically be more precise (i.e., have smaller variations between repeated measurements) than a 1000mL volumetric flask, because the neck on which the "fill to" line is located is narrower, and therefore smaller errors in liquid height above or below the neck result in smaller volume differences compared to the same errors in liquid height for the larger flask. There are certain sources of error, which must be carefully considered. The volumetric apparatus must be read correctly; the bottom of the meniscus should be tangent to the calibration mark. There are other sources of error, however, such as changes in temperature, which results in changes in the actual capacity of glass apparatus and in the volume of the solutions. The volume capacity of an ordinary 100 mL glass flask increases by 0.025 mL for each 1^0 rise in temperature, but if made of borosilicate glass, the increase is much less. One thousand mL of water (and of most solutions that are ≤0.1N) increase in volume by approximately 0.20 mL per 1^0C increase at room temperature. Thus, solutions must be measured at the temperature at which the apparatus was calibrated. This temperature (usually 20^0C) will be indicated on all volumetric ware.

A volumetric apparatus is calibrated "to contain" or "to deliver" a definite volume of liquid. This will be indicated on the apparatus with the letters "TC" (to contain) or "TD" (to deliver). Volumetric flasks are calibrated to contain a given volume, means that the flask contains the specified volume ± a defined tolerance (error). The certified TC volume only applies to the volume contained by the flask and it does not take into account the volume of solution that will stick to the wall of the flask if the liquid is poured out. Graduated cylinder on the other hand, can be either TC or TD. For accurate work the difference may be important.

Some of the volumetric pipettes have calibration markings for both TC and TD measurements. Make sure to be aware which marking refers to which measurement. The TC marking will be closer to the dispensing end of the pipette.

Some pipettes are designed to have the small amount of liquid remaining in the tip blown out and added to the receiving container; such pipettes have a frosted band near the top. If there is no frosted band near the top of the pipettes, do not blow out any remaining liquids.

Note: Factor to be considered is that liquid volumes are often not strictly additive. For example, exactly 500 ml 95% v/v ethanol aq. Added to 500ml distilled water will not equal to 1000ml; in fact, the new volume will be closer to 970 ml. Where did the missing 30ml go? Polar molecules such as water undergo different three-dimentional intermolecular bonding in a pure solution versus in a mixture with other solute or chemicals such as ethanol. The difference in bonding causes an apparent contraction in this case. As well, addition of solute to an exact volume of water will change the volume after dissolved. To account for this effect, volumetric glassware I sused to

bring mixed solutions up to a final volume after initial mixing. When two liquids are mixed, the first liquid is volumetrically transferred into a volumetric flask, and then the second liquid is added to volume, with intermittent swirling of vortexing to mix the liquids as they are being combined. For mixing solids into solvents, the chemicals are first placed in a volumetric flask, dissolved in a partial volume, and then brought to exact volume with additional solvent.

Buretes

Burettes are used to deliver definite volumes. The more common types are of 25 or 50 ml capacity, graduated to tenths of a milliliter, and are provided with stopcocks. For precise analytical methods in microchemistry, microburets are also used. Microburets generally are of 5 or 10 ml capacity, graduated in hundredths of a millilitre division. General rules in regard to the manipulation of a buret are as follows:

1. Do not attempt to dry a buret that has been cleaned for use, but rather rinse it two or three times with a small volume of the solution with which it is to be filled.
2. Do not allow alkaline solutions to stand in a buret, because the glass will be attacked, and the stopcock, unless made of taflon, will tend to freeze.
3. A 50 ml buret should not be emptied faster than 0.7 ml per second; otherwise too much liquid will adhere to the walls; as the solution drains down, the meniscus will gradually rise, giving a high false reading.

Cleaning of glass and porcelain

In the case of all apparatus for delivering liquids, the glass must be absolutely clean so that the film of liquid never breaks at any point. Careful attention must be paid to this fact or the required amount of solution will not be delivered. The method of cleaning should be adapted to both the substances that are to be removed and the determination to be performed. Water-soluble substances are simply washed out with hot or cold water and the vessel is finally rinsed with successive small amounts of distilled water. Other substances more difficult to remove, such as lipid residues or burned material, may require the use of a detergent, organic solvent, nitric acid, or aqua regia (25% v/v conc. HNO_3 in conc. HCl). In all cases it is good practice to rinse a vessel with tap water as soon as possible after use. Material allowed to dry on glassware is much more difficult to remove.

The “gold standard” providing maximal accuracy and precision is a Class A glassware. During manufacture, glassware to be certified as Class A is calibrated and tested to comply with tolerance specifications established by the ASTM (American Society for Testing and Materials). These specifications are the standard for laboratory glassware. Class A glassware has the tightest tolerance and therefore the best accuracy and precision.

REAGENTS

Chemical reagents, solvents and gases are available in a variety of grades of purity and their selection and utilization depends of the types of use and technology. These includes technical grade, analytical reagent grade and various "ultrapure" grades. The parameter being measured and the sensitivity and specificity of the detection system are important factors in determining the purity required. In methods for which the purity of reagents is not specified, it is intended that analytical reagent grade be used. Reagents of lesser purity than that specified by the method should not be used. Technical grade is useful for making cleaning solutions, such as alcoholic potassium hydroxide solutions.

Acids

The concentration of common commercially available acids mentioned below:

Acids	Molecular Weight (g/mol)	Conc. (M)	Specific Gravity
Acetic acid, glacial	60.05	17.4	1.05
Formic acid	46.02	23.4	1.20
Hydrochloric acid	127.9	7.57	1.70
Hydrofluoric acid	20.01	32.1	1.167
Hypophosphorous acid	66.0	9.47	1.25
Lactic acid	90.1	11.3	1.2
Nitric acid	63.02	15.99	1.42
Perchloric acid	100.5	11.65	1.67
Phosphoric acid	98.0	14.7	1.70
Sulfuric acid	98.0	18.0	1.84

Distilled water

Distilled or demineralised water is used in the laboratory for dilution, preparation of reagent solutions, and final rinsing of washed glassware.

There are a variety of methods for purifying (removing anion, cation, VOCs, etc.) water, such as distillation, filtration, and ion exchange.

Water purity has been defined in many different ways, but one generally accepted definition states that high purity water is water that has been distilled and/or deionized so that it will have a specific resistance of 500,000 Ω (2.0 μΩ/cm conductivity) or greater. This is satisfactory as a base to work from, but for more critical requirements, shown below:

Degree of purity	Maximum Conductivity (μΩ/cm)	Approx. conc. Of electrolytes (mg/L)
Pure	10	2-5
Very pure	1	0.2-0.5
Ultrapure	0.1	0.01-0.02
Theoretically pure	0.055	0.00

Carbon Dioxide-Free water

Carbon dioxide (CO_2) dissolved in water can interfere with many chemical measurements. So, CO_2-free water may need to be produced. It can be done by boiling distilled water for 15 min and cooling to room temperature. Or distilled water may be vigorously aerated with a stream of inert gas (e.g., N_2 or He_2) for a period sufficient to achieve CO_2 removal. The final pH of the water should lie between 6.2 to 7.2. It is not advisable to store CO_2-free water for extended periods. To ensure that this remains that way, an ascarite trap should be fitted to the container such that air entering the

container is CO_2-free. Ascarite is silica coated with NaOH, and it removes CO_2 by the following reaction:

$2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$

Gases

The gases are one of the most important parameters in analytical chemistry and use for multiple purposes. According to its use it depends that what purity needed. Suppose you are using FID (flame ionization detector) in GC, then Nitrogen, as a carrier gas, to be 99.99% pure. But at the same time if it needs to be used for ECD (electron capture detector) on the same GC, that purity is not enough and you need 99.999% pure.

DATA HANDLING AND REPORTING

Significant figures

The term significant figure is used rather loosely to describe some judgement of the number of reportable digits in a result. Often the judgement is not soundly based and meaningful digits are lost or meaningless digits are accepted. Proper use of significant figures gives an indication of the reliability of the analytical method used. Thus, reported values should contain only significant figures. A value is made up of significant figures when it contains all digits known to be true and one last digit in doubt. For example, if a value is reported at 21.2 mg/l, the "21" must be a firm value, while "0.2" is somewhat uncertain and may be between "0.1" or "0.3". The number zero may or may not be a significant figure:

1. Final zeros after a decimal point are always significant figures. For example, 7.8g to the nearest mg is reported as 7.800g.
2. Zeros before a decimal point other preceding digits are significant. With no preceding digit, a zero before the decimal point is not significant.
3. If there are no digits preceding a decimal point, the zeros after the decimal point but preceding other digits are not significant. These zeros only indicate the position of the decimal point.
4. Final zeros in a whole number may or may not be significant. In a conductivity measurement of 1000μΩ/cm, there is no implication that the conductivity is 1000±1 μΩ/cm. Rather, the zeros only indicate the magnitude of the number.

Once the number of significant figures is established for a type of analysis, data resulting from such analyses are reduced according to the set rules for rounding off.

Rounding off Numbers

Rounding off numbers is a necessary operation in all analytical areas. However, it is often applied in chemical calculations incorrectly by blind rule or prematurely and, in these instances, can seriously affect the hinal results. Rounding off should normally be applied only as follows:

1. If the figure following those to be retained in less than 5, the figure is dropped, and the retained figures

are kept unchanged. As an example 32.553 is round off to 33.55.

2. If the figure following those to be retained is greater than 5, the figure is dropped, and the last retained figure is raised by 1. As an example, 33.556 is round off to 33.56.
3. When the figure following those to be retained is 5 and there are no figures other than zeros beyond the 5, the figure is dropped, and the last place figure retained is increased by 1 if it is an odd number, or it is kept unchanged if an even number. As an example, 11.435 is rounded off to 11.44, while 11.425 is rounded off to 11.42.

Rounding off single arithmetic operations

Addition: When adding a series of numbers, the sum should be rounded off to the same numbers of decimal places as the addend with the smallest number of places. However, the operation is completed with all decimal places intact and rounding off is done afterward. As an example:

11.1 + 11.12 + 11.13 = 33.35

The sum is rounded off to 33.4

Multiplication: When two numbers of unequal digits to be multiplied, all digits are carried through the operation, and then the product is rounded off to the number of significant digits of the less accurate number.

Division: When to numbers of unequal digits are to be divided, the division is carried out on the two numbers using all digits. Then the quotient is rounded off to the lower number of significant digits between the two values.

Powers and roots: When a number contains n significant digits, its root can relied on for the n digits, but its power can rarely be relied on for n digits.

Special Notes:

Analytical Chemistry

Don't confuse yourself by thinking that analysis by high tech instruments are analytical chemistry.

Analytical chemistry is the science inventing and applying the concepts, principles, and strategies for measuring the characteristics of chemical systems. So, performing a routine samples using an instruments is known as analysis only.

Qualitative analysis

Identify what is present in the sample is known as qualitative analysis. Here the scope is only to find out the composition or the presence of absence of a target molecule. It does not deal with quantity.

Quantitative analysis

Measure the quantity of the molecules present in the sample. This is the most common and industrial requirement and knows as quantification.

Characterization analysis

Another important area of analytical chemistry, is the development of new methods for characterizing physical and chemical properties. Determination of chemical structure, equilibrium constant, particle size, and surface structure are examples of a characterization analysis.

Fundamental analysis

The purpose of qualitative, quantitative or characterization analysis is to solve a problem associated with a particular sample and is known as fundamental analysis. Extending and improving the theory on which an analytical method is based, studying and analytical method's limitation, and designing and modifying existing analytical method are examples of fundamental studies in analytical chemistry.

The day you will be selected first time by an organization as chemist, it will be your one of the best day of your life. You will share your excitement and dream with your parents, relatives and friends. So, prepare yourself to make your first day memorable, you need to be ready for the job.

Basic work in a laboratory is performing the analysis, as per the SOP (Standard Operating Procedure). So, when you will get an assignment, you need to refer the SOP. This SOP will cover everything from sample preparation, instrument operation and reporting. But you need to understand what you are doing, as doing work by understands it will make the job simple. Don't be a push button chemist.

Following are the common steps which need to follow to maintain GLP (Good Laboratory Practices), fulfil the requirement of different accreditations. This not only help the organization to grow but will help you to grow systematically, with clear knowledge about your work and domain.

To perform these steps, now we need to prepare ourselves by recollecting some basic knowledge, which will be required multiple times during the process.

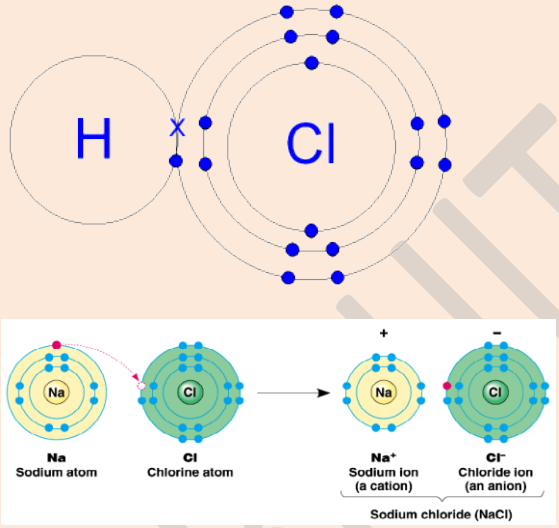
Atomic structure

An atom is the smallest constituent unit of ordinary matter that has the properties of a chemical element. It consists of three basic sub-atomic particles – electron, proton and neutron. You can imagine the solar system.

The neutron (mass ~1 atomic mass unit, elementary charge = 0) and proton (mass ~1 atomic mass unit, elementary charge = +1) are in the center forming the nucleus and electrons (mass ~0 atomic mass unit, elementary charge = -1) are moving around it at different orbital. Each orbit has different energy level and when the electron gets excited through external energy, the absorbed the energy and shift from ground level to higher energy level. By losing the absorbed energy, they return to ground state. Using this character, the spectroscopy instrument works.

Normally, each atom has equal number or Electron, Proton and neutron (exception Isotope and Isobar) and for the stable condition, the outer

shale of the atom need to have 2 or 8 electrons. As the electron (negative charge) and proton (positive charge) are same, so the atom is neutral. But to be stable they also need to maintain the electron number (2 or 8). That's why two atoms of an element always stay together and known as molecule. All the chemical reactions are based on this electron transfer. A simple example is HCl. Hydrogen have one electron, which need another one to make it 2 in outer shale, chlorine have 7 electrons and he needs 1 more to make it 8. So, each will share one electron and will be used by both of them. And in case of NaCl, Na has one electron in outer shale 1 electron, and 2nd last orbit has 8 electrons. So for him, it is easy to leave one electron and for chlorine it is easy to take 1 electron.



Atomic mass

The mass number of an atom is the total number of protons and neutrons in its nucleus. As the mass number of an electron is very, very small compared to the mass of either a proton or a neutron, counting the number of protons and neutrons tells about the total mass of an atom.

An atom's mass number is very easy to calculate provided you know the number of protons and neutrons in an atom.

Example:
What is the mass number of an atom of Helium that contains 2 neutrons?

Solution:
Number of Proton = 2
Number of Electron = 2
Mass number = Number of Proton + Number of Neutron
Mass number = 2 + 2 = 4

*But there are some atoms of the same elements, where the number of neutrons can be different, even in atoms of the same element. These are known as **Isotope**. We can then calculate the atomic mass (or average mass) for them if we know the relative abundance (the fraction of an element that is a given isotope) the element's naturally occurring isotopes, and the masses of those different isotopes. We can calculate this by the following equation:*

$$\textbf{Atomic mass} = (\%_1)(Mass_1) + (\%_2)(Mass_2) + \ldots..$$

Example:
Boron has two naturally occurring isotopes. In a sample of Boron, 20% of the atom are B-10, which is an isotope of Boron with 5 neutrons and a mass of 10 amu (atomic mass unit). The other 80% of the atoms are B-11, which is an isotope of Boron with 6 neutrons and a mass of 11 amu. What is the atomic mass of Boron?

Solution:
Boron has two isotopes. We will use the equation:
$\textbf{Atomic mass} = (\%_1)(Mass_1) + (\%_2)(Mass_2) + \ldots..$
Isotope 1: %1 = 0.2, mass1 = 10
Isotope 2: %2 = 0.8, mass2 = 11

Substitute these into the equation, and we get:
Atomic mass = (0.2)(10) + (0.8)(11) = 10.8 amu

The mass of an average Boron atom, and thus Boron's atomic mass, is 10.8 amu.

*In this context, I also want to tell about **Isobar**.*

Isobars are the atoms of different elements, who have same atomic mass but different atomic numbers. As they have <u>different numbers of Protons</u>, so they have different atomic numbers, but as they have enough neutrons (different

numbers)to make the total of nucleons, so they have same atomic mass.

$^{32}Ge_{76}$ and $^{34}Se_{76}$ are isobars. The Ge has 32 protons and 44 neutrons, whereas Se has 34 protons and 43 neutrons. Each nucleus has 76 nucleons.

Whereas, if the atom have same neutron but different proton number, they are called ***Isotones****.*

Boron-12 and Caarbon-13 nuclei, both contains 7 neutrons.

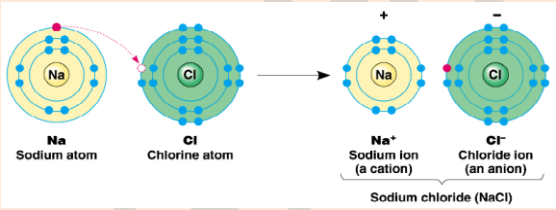
Atomic number

Atomic number is equal to the number of protons in the nuclei of any atoms. The atomic number is whole number usually written above the chemical symbol of each element in periodic table. For Hydrogen it is 1 as it has one Proton, for Helium it is 2 as it has 2 Protons.

Of course, since neutral atoms have to have one electron for every proton, an element's atomic number also tells how many electrons are in a neutral atom of that element.

Valancy

IT is the combining capacity of an element.



In the above picture, we can see that Sodium (Na), who had one electron in outer shale, has donated one electron to Chlorine (Cl) so that his current outer shale will have 8 electron. So, the valency of NA is 1. Chlorine who had 7 electron in his outer shale, taken 1 electron from Na, and made 8 electron in the present outer shale. So, his valency also 1.

Comparison chart of Atom and Molecules

Basis for comparison	***Atom***	***Molecule***
Meaning	*The tiny particle of a chemical element, which may or may not exist independently, is called an atom.*	*A molecule refers to the set of atoms held together by bond, indicating the smallest unit of a compound.*
Existence	*May or may not exist in free state.*	*Exist in free state.*
Comprise of	*Nucleus and electrons.*	*Two or more identical or different atoms, bonded chemically.*
Division	*Indivisible.*	*Divisible into individual atoms.*
Shape	*Spherical.*	*Linear, angular and triangular.*
Visibility	*Neither visible through naked eyes, nor magnifying microscope.*	*Not visible through naked eyes, but can be seen with the help of magnifying microscope.*
Reactivity	*Highly reactive, subject to certain exceptions.*	*Comparatively less reactive.*
Bond	*Nuclear bond.*	*Covalent bond.*

Mole

Moles are a SI unit of measurement for chemicals, just as meters are measurement units for length and grams are measurement units for mass. A chemical can be atoms of a single element or atoms of many elements combined into molecules, so a single molecule of a chemical can weigh three or four times what a single molecule of another chemical weighs. For this reason, chemists can't just measure the weight of different chemicals to have the right proportions of reactants. When chemists want to have a chemical reaction

come out they need to know how many molecules of each kind of chemical they have, so they measure the chemicals in moles. A mole is the atomic weight of a molecule of the chemical in grams. So a mole of a molecule like hydrogen (H) with an atomic weight of 1 is one gram. Meanwhile, a complex molecule like glucose ($C_6H_{12}O_6$) has an atomic weight of 180, so one mole is 180 grams. But even though the weight is different, the two moles contain the exact same number of molecules, 6.02 x 10^{23}.

The mole (symbol: mol) is the unit of measurement for amount of substance in the International System of Units (SI). A mole of a substance or a mole of particles is defined as exactly 6.02214076×10^{23} particles, which may be atoms, molecules, ions, or electrons. A mole is the quantity of anything that has the same number of particles found in 12.000 grams of carbon-12. That number of particles is Avogadro's Number, which is roughly 6.02x10^{23}. 1 A mole of carbon atoms is 6.02x10^{23} carbon atoms. A mole of chemistry teachers is 6.02x10^{23} chemistry teachers.

MOLE DAY: A DAY AFTER A CHEMIST'S HEART

You probably won't get the day off work or find your local store flush with cards celebrating the occasion, but Mole Day is celebrated every year by chemists throughout the world. Since Avogadro's number is 6.022 × 10^{23}, it only makes sense that the holiday starts at 6:02 a.m. every Oct. 23. Revelers tell chemistry jokes, blow bubbles of natural gas that they set ablaze, toast with drinks chilled by dry ice and even recite the mole pledge of allegiance.

Special thanks to Meisa Salaita, all-around chemistry whiz and the Director for Education & Outreach, NSF Center for Chemical Evolution, for her assistance with this article.

PREPARATION OF SOLUTIONS

Molar weight (MW)
= mass of one mole of a substance in grams (g/mol).
The MW of a molecule is a sum of relative atomic weights A_r (expressed in grams per mole) of all elements building the molecule. The values of A_r are found in the Periodic table, e.g. A_r of H = 1, N = 14, O = 16 → molar weight of HNO_3 = 1x1 + 1x14 + 3x16 = 63 g/mol.

Molar Solution

A 1 molar solution is a solution in which 1 mole of a compound is dissolved in a total volume of 1 litre.

For example:
The molecular weight of sodium chloride (NaCl) is 58.44, so one gram molecular weight (= 1 mole) is 58.44g. If you dissolve 58.44g of NaCl in a final volume of 1 litre, you have made a 1M NaCl solution.
To make a 0.1M NaCl solution, you could weigh 5.844g of NaCl and dissolve it in 1 litre of water; OR 0.5844g of NaCl in 100mL of water (see animation below); OR make a 1:10 dilution of a 1M sample.

Density of a solution (r)
= mass of a specified volume of the solution (g/cm^3 = g/mL = kg/dm^3 = kg/L); it is often labeled on a bottle
containing the solution (e.g. r = 1.8 g/mL means that 1 mL of the solution weights 1.8 g).

Concentration
= quantity of a substance found in a specified volume (or mass) of a solution.

a) molarity (molar concentration) = number of moles of a substance per litre of a solution (mol/L) – it can be used if the molar weight (MW) of the substance is known.

b) osmolarity = number of moles of all particles (including ions to which a molecule dissociates) found in one litre of a solution (osmol/L); the osmotic active particles show an osmotic pressure of the solution.

c) mass concentration = mass of a substance per specified volume of a solution (g/L, mg/dL,...).

d) percent concentration (it is a special type of the mass concentration) = parts (g or mL) of a solute per 100 parts (g or mL) of total solution.

Molarity is another standard expression of solution concentration. Molar solutions use the gram molecular weight of a solute in calculating molar concentration in a liter (L) of solution.

The gram molecular weight (GMW) of a substance (sometimes called the "formula weight") is the sum of the combined atomic weights of all atoms in the molecule expressed in grams. For example, the GMW of NaCl is equal to the atomic weight (these atomic weights may be found on the periodic table given at the beginning of this book or as a formula weight on the bottle of substance) of Na (22.99) and the atomic weight of Cl (35.45) for a total of 58.44 g.

Molarity (mol x L^{-1} = mol x dm^{-3} = mol/L = mol/dm = M).
The molarity can be calculated either using the formula c = n/V (c = molarity, n = substance amount in moles, V = final volume of the solution in L) or directly from the definition* of the molar concentration. A direct proportionality between the concentration (c) and a related substance amount (n) is used.

(*) 1 M solution (read: one molar solution) means that 1 L of the solution contains 1 mol of a substance.

(*) 0.5 M solution (read: half molar solution) means that 1 L of the solution contains 0.5 mol of a substance.

Example 1:
1M (Mol) of NaOH = 1 mol of NaOH in 1 L of the solution = 40g of NaOH in 1 L of the solution. (40 is the molecular weight of NaOH).
0.1M (Mol) of NaOH = 0.1 mol of NaOH in 1 L of the solution = 4g of NaOH in 1 L of the solution. (40 x 0.1 = 4).
Problem: 300 ml of a solution contain 17.4 g of NaCl (MW = 58 g/mol). What is the concentration of the solution?
Ans.: For NaCl, 1mol = 58g, so 17.4g = 17.4/58 mol = 0.3 mol.
Means, 0.3 mol NaCl in 300 ml solution = (0.3/300) x 1000 mol in 1 L = 1M.
So, the concentration of the solution is 1M.

Example 2:

The GMW of HCl would be the atomic weight of H added to the atomic weight of Cl: H = 1 + Cl = 35.45 = 36.45 g. A liter of 1M solution of HCl would contain 36.45 g.
Monosodium dihydrogen phosphate (NaH_2PO_4) buffer is used in enzyme histochemistry. A liter of 1M solution of NaH_2PO_4 would contain Na (22.99) + 2 H (1x2=2) + P (30.97) + 4 O (16x4=64) for a total GMW of 119.98 g.

To simply calculate the amount or weight of a substance needed for a desired molar solution, the following formula may be used:

Weight in grams = desired molarity x volume needed in liters x GMW

-- OR --

(W = M x V x GMW)

Example:
500 mL of a 0.1M solution of NaOH is needed for a procedure. Calculate the amount of solute (NaOH) needed to prepare the solution. (atomic weights: Na = 22.99; O = 16; H = 1)
W= M x V x GMW
W = X (?), M = 0.1M, V = 500 mL = 0.5 L, GMW = 39.99
X g = 0.1M x 500 mL x 39.99
X = 0.1 x 0.5 x 39.99
X = 1.9995 or 2

2.0 g of NaOH must be diluted to 500 mL to prepare a 0.1M solution.

Normal solution and normality

1g equivalent amount dissolved in 1 L of solution is 1N (normal) solution.

1g equivalent weight = MW/replaceable H^+ ion.

Normality (N) is another way to quantify solution concentration. It is similar to molarity but uses the gram-equivalent weight of a solute in its expression of solute amount in a liter (L) of solution, rather than the gram molecular weight (GMW) expressed in molarity. A 1N solution contains 1 gram-equivalent weight of solute per liter of solution.

Expressing gram-equivalent weight includes the consideration of the solute's valence. The valence is a reflection of the combining power of an element often as measured by the number of hydrogen atoms it can displace or combine with. A 1.0 gram-equivalent weight is the amount of a substance that will combine with or displace 1 atom of hydrogen.

To determine gram-equivalent weight of a substance:

Divide the GMW (formula weight) of a solute by the valence (number of hydrogen ions that can be displaced).

Example 1:

HCl have GMW 36.45 g. So, 1M (molar) solution of HCl means, a 1 L solution contains 36.45 g HCl. HCl have 1 replaceable H ion. So, its valancy is 1. So, its GEW (gram equivalent weight) is 36.45/1 = 36.45 So, 1N (normal) solution of HCl means, 1 1 L solution contains 36.45 g HCl. Some compounds, however, will not have the same normality as molarity. Now, consider H2SO4 (atomic weight of H = 1; atomic weight of S = 32.06; atomic weight of O = 16) GMW of H2SO4 = 1(2) + 32.06 + 16(4) = 98 g H2SO4 have 2 replaceable H ion, so its valancy is 2. So, its GEW is 98/2 g = 49 g So, 1M solution of H2SO4 means, a 1 L solution contains 98 g H2SO4, and, 1N solution of H2SO4 means, 1 1 L solution contains 49 g H2SO4 The molarity of this 1N solution of H2SO4 would be 0.5 (M = g /GMW per liter or 49g/98g = 0.5) To simply calculate the amount or weight of a substance needed for a desired normal solution, the following formula may be used: Weight in grams = desired normality x volume needed in liters x GMW/valence (W = N x V x GMW/valence)

Example 2:

The normality of a 1.0 liter NaCl solution that contains 1.0 gram-equivalent weight will be the GMW of NaCl divided by the valence of NaCl: (atomic weight of Na = 22.99; atomic weight of Cl = 35.45) GMW of NaCl = 22.99 + 35.45 = 58.44 g N = GMW/valence (the valence for NaCl is 1.0)

58.44 g/1.0 = 58.44 g = 1.0 gram-equivalent weight of NaCl = 1N solution of NaCl In this situation, because NaCl has a valence of one, the molarity and normality of the solution are the same.

Example 3:

500 mL of a 0.1N solution of NaOH is needed for a procedure. Calculate the amount of solute (NaOH) needed to prepare the solution. (atomic weights: Na = 22.99; O = 16; H = 1) Valence = 1 X g= 0.1N x 500 mL (0.5 L) x GMW 39.99 / 1.0 X = 0.1 x 0.5 x 39.99/1.0 X = 1.99 1.99 g of NaOH must be diluted to 500 mL to prepare a 0.1N solution.

Normal solution to Molar solution

Multiply molarity by the number of hydrogen 9or hydroxide) ions in the acid (or base).

For example, a 2M H2SO4 solution = 4N H2SO4

(H2SO4 have 2 hydrogen ions, molarity of the solution is 2, so N = 2 x 2 = 4)

The process will reverse to convert from molar solution to normal solution.

Molal solution and molality

A molal solution is a solution that contains 1 molecular weight of solute in a kilogram of solvent. It is the strength or concentration of a solution, especially the amount of dissolved substance in a given volume of solvent. It is a concentration of a solution expressed in moles or molality (m).

A solution obtained by dissolving one gram of the solute in 1000 grams of solvent is known as a 1 molal solution. For example, when 60 g of NaOH are dissolved in 1000 g of solvent, the solution contains 1.5 moles of solute in 1 kg of solvent. Therefore, the molality is 1.5.

The factors needed to calculate molality are moles of solute and the mass of solvent in kilograms. The SI unit for molality is mol/kg. A solution with a molality of 3 mol/kg is often described as "3 molal" or "3 m."

Because volume is not part of the molality equation, molality is independent of temperature. Using molalities rather than molarities for lab experiments keeps the results within a closer range.

The primary advantage of using molality to specify concentration is that unlike its volume, the mass of the solvent does not change with changes of temperature or pressure; molality remains constant under changing environment conditions. Molality, like mole fraction, is used in applications dealing with certain physical properties of solutions.

As the molality changes, it affects the boiling point and freezing point (also known as the melting point) of the solution. A higher molality increases the boiling point and decreases the freezing point of the solution. As molality is a more accurate measure of solutes in solution in dynamic conditions, it is often used in comparing and determining colligative properties of solution.

Molality is a property of solutions. If the solvent is reactive, and one needs to know the stoichiometry between the solvent and the solute, knowing the molality can be very important. The mass-based nature of molality implies that it can be readily converted into a mass ratio.

Example 1:

Suppose we had 1.00 mole of sucrose (it's about 342.3 grams) and proceeded to mix it into exactly 1.00 liter water. It would dissolve and make sugar water. We keep adding water, dissolving and stirring until all the solid was gone. We then made sure everything was well-mixed. What would be the molality of this solution? Notice that my one liter of water weighs 1000 grams (density of water = 1.00 g / mL and 1000 mL of water in a liter). 1000 g is 1.00 kg, so:

$$Molality = \frac{1.00\ mol}{1.00\ kg}$$

The answer is 1.00 mol/kg. Notice that both the units of mol and kg remain. Neither cancels. A symbol for mol/kg is often used. It is a lower-case m and is often in italics, m. Some textbooks also put in a dash, like this: 1.00-m. However, if you write 1.00 m for the answer, without the italics, then that usually is correct because the context calls for a molality. Having said that, however, be aware that often m is used for mass, so be careful. (A lower-case m is also used for meter, but the context should be clear that m means molality.) Maybe including the dash would be wise if there might be a potential misunderstanding When you say it out loud, say this: "one point oh oh molal." You don't have to say the dash. And never forget this: replace the m with mol/kg when you do calculations. The m is a symbol that stands for mol/kg. It is not the actual unit.

Example 2:

Suppose you had 2.00 moles of solute dissolved into 1.00 L of solvent. What's the molality?

$$Molality = \frac{2.00\ mol}{1.00\ kg}$$

The answer is 2.00 m.

Notice that no mention of a specific substance is mentioned at all. The molarity would be the same. It doesn't matter if it is sucrose, sodium chloride or any other substance. One mole of anything contains 6.022×10^{23} units.

Example 3: What is the molality when 0.75 mol is dissolved in 2.50 L of water?

$$Molality = \frac{0.75\ mol}{2.50\ kg}$$

The answer is 0.300 m.

Now, let's change from using moles to grams. This is much more common. After all, chemists use balances to weigh things and balances give grams, NOT moles.

Example 4:

Suppose you had 58.44 grams of NaCl and you dissolved it in exactly 2.00 kg of pure water (the solvent). What would be the molality of the solution?

The solution to this problem involves two steps. Step One: convert grams to moles. Step Two: divide moles by kg of solvent to get molality.

In the above problem, 58.44 grams/mol is the molar mass of NaCl.

Step One: 58.44 g / 58.44 g/mol = 1.00 mol.

Step Two: 1.00 mol / 2.00 kg = 0.500 mol/kg (or 0.500 m).

Sometimes, a book will write out the word "molal," as in 0.500-molal.

Example 5:

Calculate the molality of 25.0 grams of KBr dissolved in 750.0 mL pure water.

$$\frac{25.0\ g}{119.0\ g/mol} = 0.210\ mol$$

$$\frac{0.210\ mol}{0.750\ kg} = 0.280\ m$$

Example 6:

80.0 grams of glucose (C6H12O6, mol. wt = 180. g/mol) is dissolved in1.00 kg of water. Calculate the molality.

$$\frac{80.0\ g}{180.0\ g/mol} = 0.444\ mol$$

$$\frac{0.444\ mol}{1.00\ kg} = 0.444\ m$$

Example 7:

Calcuate the molality when 75.0 grams of MgCl2 is dissolved in 500.0 g of solvent.

$$\frac{75.0\ g}{95.2\ g/mol} = 0.788\ mol$$

$$\frac{0.788\ mol}{0.500\ kg} = 1.58\ m$$

Example 8:

100.0 grams of sucrose ($C_{12}H_{22}O_{11}$, mol. wt. = 342.3 g/mol) is dissolved in 1.50 L of water. What is the molality?

$$\frac{100.0\ g}{342.3\ g/mol} = 0.292\ mol$$

$$\frac{0.292\ mol}{1.50\ kg} = 0.195\ m$$

Example 9:

49.8 grams of KI dissolved in 1.00 kg of solvent. What is the molality?

$$\frac{49.8\ g}{166.0\ g/mol} = 0.300\ mol$$

$$\frac{0.300\ mol}{1.00\ kg} = 0.300\ m$$

pH

pH is a measure of the hydrogen ion activity, typically in aqueous solution.

In pH, the p stands for -log10 and the H stands for hydrogen ion activity:

pH = -log10aH^+

Why is pH=7 Neutral in Aqueous Solutions?

To see why this should be so, consider the following:

Water exists as an equilibrium between molecules and ions:

$H_2O \rightleftharpoons H^+ + OH^-$

The multiplication product of the concentrations [H^+] and [OH^-] is a constant; it is water's dissociation constant, which has a known value of 10^{-14} M2.

$K_w = [H^+][OH^-] = 10^{-14}$ M2

For neutrality, [H^+] must be equal to

[OH^-]. This means these quantities must both equal 10^{-7} M.

If we put $[H^+] = 10^{-7}$ M into the equation for pH:

$pH = -\log10[H^+]$

we get pH = 7 for neutrality.

Dissociation Constant And Ionic Product Of Water

Pure water is poor conductor of electricity. Water is a weak electrolyte i.e. it is ionized to a very small extent as:

$H_2O = H^+ + OH^-$

$H_2O + H_2O = H_3O^+ + OH^-$

This ionization is called self ionization of water.

$$Keq = \frac{[H+][OH-]}{[H2O]}$$

Where K_{eq} is the dissociation constant of water.

$K_c = [H^+][OH^-]/[H_2O]$

As water is only slightly ionized [H2O] is much bigger than $[H^+]$ or $[OH^-]$

So, we can regard $[H_2O]$ as constant

So, $K_c[H_2O] = [H^+][OH^-]$

As K_c is a contant and $[H_2O]$ is a constant we can replace both with a new constant called Kw the ionic product of water

So, $K_w = [H^+][OH^-]$

K_w is the ionic product of water

Ionic product of water

K_w is the ionic product of water.

Ionic product of water may be defined as the product of the molar concentration of H^+ ions and OH^- ions.

As H^+ ions in water exist as H_3O^+ ions, therefore, ionic product may also be defined as the product of molar concentration of H_3O^+ ions and OH^- ions, i.e.

$K_w = [H^+][OH^-]$

Or

$K_w = [H_3O^+][OH^-]$

Ionic product of water is constant only at constant temperature.

K_w at 298 K

$K_w = 1.00 \times 10^{-14}$

Dissociation or ionization of constant of water is different from ionic product of water.

$K_{eq} = K_w / 55.55$

$K_w = K_{eq} \times 55.55$

$K_{eq} = 10^{-14} /55.55 = 1.8 \times 10^{-16}$

In pure water

$[H^+] = [OH^-] = 1 \times 10^{-7}$ mol/L

$K_w = [H^+][OH^-]$

$= (1 \times 10^{-7})(1 \times 10^{-7})$

$= 1 \times 10^{-14}$

Effect of temperature on K_w

The ionic product of water increases with increase of temperature.

With increase of temperature, the degree of ionization of water increases. More of water molecule dissociate into H^+ ions and OH^- ions. The concentration of H^+ and OH^- ions increases and hence the ionic product also increases.

H_3O^+ ion and OH^- ion concentration in aqueous solution of acid and bases

$[H^+] = [OH^-] = 1 \times 10^{-7}$ mol/L

If some acid is added to pure water, then $(H_3O^+) > 10^{-7}$M

$[OH^-] = K_w / [H^+]$

If some base is added to pure water, then $[OH^-] = 10^{-7}$ M

$[H_3O^+] = K_w / [OH^-]$

The increase or decrease of the H_3O^+ ion concentration in an aqueous solution of an acid or a base may be explained qualitatively on the basis of Le Chatelier's principle.

$2H_2O = H_3O^+ + OH^-$

If some acid is added to pure water, H_3O^+ ion concentration increases, therefore the equilibrium shifts in the backward direction. Thus OH^- ion concentration decreases.

If a base added, OH^- concentration increases. Again the equilibrium shifts in the backward direction and hence the H_3O^+ ion concentration decreases.

DOCUMENTATION

If you have not documented it, you have not done it

Why need proper documentation

Proper documentation process and practice helps us by different ways. It's not only important in work place but also for our daily life. It helps a lot in time management also. Most of the time, we spend lots of time to find a record or reference document when needed, and we end up with frustration. Then following words comes in mind…"if, would, supposed to….". A proper documentation helps us to manage the time efficiently, keep us cool to do problem solving, helps to think and evaluate the work and to plan next action in research, justify the correctness of our work in work place, and so on. Very simple example is bank pass book, if it is not updated, we do not know the balance available, major payment made recently, find out the expenses which can nut or reduce for better financial stability.

So, it is very important to document each action, activity, process and plan from the beginning systematically.

Advantages

Documentation is a valuable recourse, as explained above and can be divided into two:

- Documents – procedure or instruction
- Records

Both documents and records are an invaluable communication tool for any activities in personal and professional life.

Benefit	Description	Form of documentation
Provide back ground history	Information storage point for anyone to access. Information can be added at any time, providing a full history.	Record
Preserves learning and knowledge	Reference for future use or a means to communicate information to others. Practically important to ensure that knowledge is not lost when an employee leaves.	Document
Protects intellectual property	Evidence of an idea or a finding including the date, record and the responsible person.	Record
Provides legally valid evidence	Documents, events, processes, ideas, communications, etc., which can show that something did or did not happen.	Record
Ensure the quality and consistency of process / activities / manufacturing	Provides the same critical information and training to all relevant persons, to ensure the quality and safety of the end products.	Documents

Different types of documents in a testing laboratory

There are different types of documents needed to maintain in a testing laboratory and clearly identified in ISO 15189 (for clinical lab) and ISO 17025 (for non-clinical lab), which includes all the sectors/departments of an organization. We will only discuss which are related to laboratory activities. Some of this may/may not linked with other sectors/departments.

Following are the minimum important documents needed (but are not limited to):

- Attendance record
- Holiday list
- List of members of the department

- Laboratory lay out with storage plan and lay out with identification
- Work process flow chart
- List of test to be conducted
- Supplier record
- Training plan and training record
- Instrument performance history
- Calibration plan and record
- Chemical/reagent/standard record
- Consumables record
- Spares record
- Accessories record
- Reference book, journals, methods
- Method validation report
- Work procedure / instruction
- Daily work check list
- Data storage record

General requirements to apply documentation

The following requirements should be applied to all documentation:

Clearly written	All documents must be accurate and written in a manner that prevents errors and ensures consistency. If documents are to be used together, then each should reference the other, e.g., in a SOP the form should be mentioned which to be documented, and that for should have reference number to that SOP. Ensure there is a traceability between two or more documents/records using for mal document numbers or record identification.
Using indelible ink	All records must be filled out in indelible ink for long term legibility. Do not use pencil or ink that can be erased. Colour should be specified by the procedure; often this is limited to blue or black because historically copy/scanning technology was limited to reproduction quality. However, this is less of a factor with the advent of high resolution scanners and colour copiers.
Legible handwritten entries	A document is unusable if it cannot be read, so care must be taken to ensure that handwriting is legible. All entries must be made at the time of tasks are performed and should be legibly signed and dated. Many times, people prefer to put the time also.
Reviewing and approving	Documents and records should be reviewed by someone who did not perform the task to ensure that the information is correct and accurate. A signature and date by the reviewer / approver confirms that a review has taken place.
Signatures	Hand written signatures must be unique to the individual and listed within the signature register to ensure that the signature is traceable to a member of the team.
Signed delegation of responsibility	In the event that a critical member is absent for a time, they must delegate responsibility to another qualified person. The delegate must be either: ✓Proceduralised in a document (SOP, WI, etc.), or ✓Documented with names of all people involved and signed by the person that is delegating their responsibility. The delegation should be approved with the signature of a more senior member.

Record must be permanent

Traceability of records is of critical importance so that:

- The work / process can prove that it is complying the process and procedures.
- The root cause of a non-compliance or a justified customer complaint can be identified.
- Corrective or preventive actions can be implemented and their effectiveness checked over time.

General requirements

No	*Requirements*
1	*Do not discard a record just because you might have made a mistake, it is still needed for traceability. Moreover, it will help you to understand where the mistake made and can take preventive action to avoid the same mistake in future.*
2	*Avoid writing anything in loose paper. It can be lost any time.*
3	*Do not use note books/writing pads with easily removed pages, scrap papers or post-it-notes to record important data.*

Basic tools

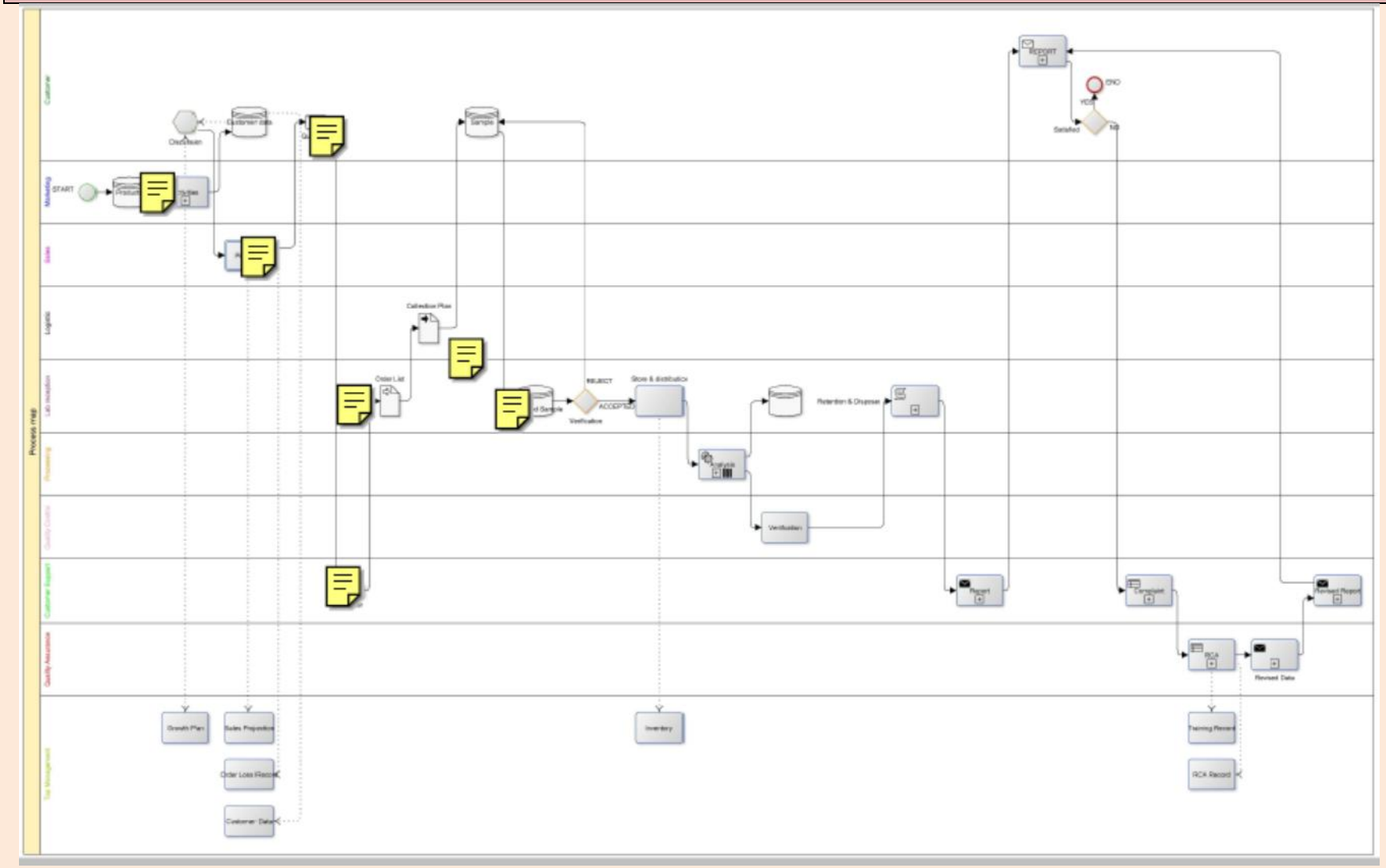
1. Work/process flow chart maker
2. Project planner for work activities
3. Planner for work plan for yearly, half-yearly, quarterly, monthly, weekly and daily
4. To-do-list software
5. SIPOC
6. FMEA

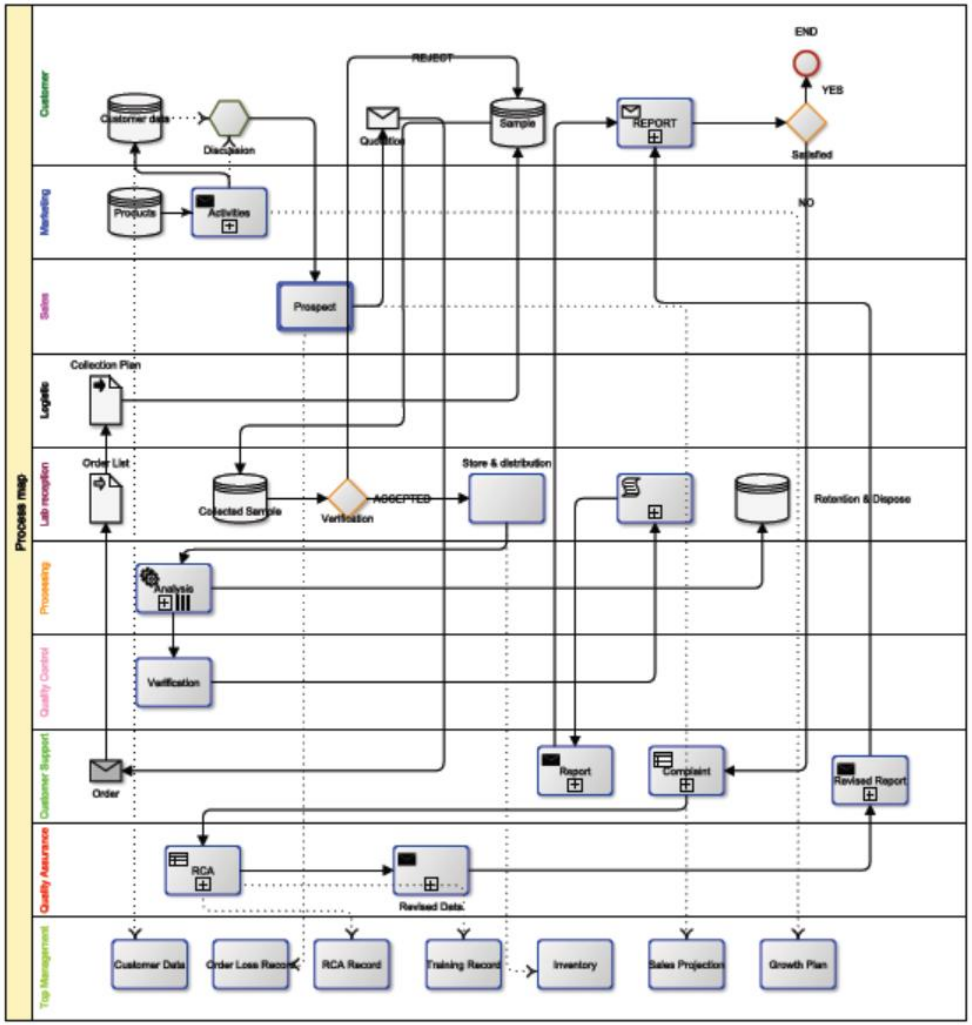
How to start

1. Prepare work flow chart
2. Prepare SIPOC using the flow chart
3. Make a work plan using planner/project charter or "To-Do-List" software (many professional softwares are available now).
4. Prepare SIPOC (Supplier, Input, Process, Output, Customer).
5. Perform the activities and evaluate where you failed, what is the reason and how to overcome the failure. This can be done using PDCA cycle. The parameters to check can be find out using FMEA (Failure Mode Effect Analysis).
6. Identify each document to be referred, records to be generated.
7. Identify the location for each document and record to be kept.
8. Be honest yourself. Remember, what you are doing, end of the day you are going to be benefited.
9. Stick to your plan; give extra time if needed to complete the task. This will help to improve your planning and time management.
10. Set a target and give yourself a treat or gift once the target achieved.

Typical process flow of laboratory work has given in next page (High Level Process Map).

HIGH LEVEL PROCESS MAP




Process map
Customer
Marketing
Sales
Logistic
Lab reception
Processing
Quality Control
Customer Support
Quality Assurance
Top Management
END
YES
NO
REJECT
Customer data
Discussion
Quotation
Sample
REPORT
Satisfied
Products
Activities
Prospect
Collection Plan
Order List
Collected Sample
Verification
ACCEPTED
Store & distribution
Retention & Dispose
Analysis
Verification
Order
Report
Complaint
Revised Report
RCA
Revised Data
Customer Data
Order Loss Record
RCA Record
Training Record
Inventory
Sales Projection
Growth Plan

Notes:

SAMPLE REGISTER

Register means a document where the details will be registered, it may be a physical book or it may be electronic copy. Advanced laboratory using special software, e.g., LIMS – Laboratory Information Management System), where everything records in the software, from material in to material out. Much software includes the data of the employee movement also inside the premises.

A typical register should have minimum following details. More information you get, easier to perform the job and more reliable the analysis will be:

Sr. No., sample receives date, customer details, sample source, types of samples (e.g., food, water, soil, air, etc.), test request, expected detection range, analysis started, analysis ended on, report release date, signature of the report receiver, remarks.

Many more information will be added in each steps of processing, multiple documents will be generated. Benefit of using the software is that you can avoid multiple entries for the same parameters and each individual department / person can get the required information only. It will help to avoid wrong entry, save time and avoid errors. Many people use excel file, but you can understand how difficult it will be to scroll and find out the required sample details. Moreover, using same file by multiple department or person, can do mistake in data feeding or probability is there of data alteration by mistake or intentionally.

Let's understand the importance of each record in sample register.

Sr. No. – Indicates how many samples entered into the laboratory.

Sample receives date – Confirm if the sample has been analyzed within specified time gap after sample collection / receiving date. Different types of sample have different sample retention time.

Customer details – Need to generate the technical and commercial reports / bills / invoice / etc.

Sample source – This is one of the most important data for the analyst. On the basis of this the analysts check if the sample collection has done properly to issue a test report.

Types of samples – Need to plan the sample preparation work.

Test request – Need to select methodology and to do work plan.

Expected detection range – Need to set the calibration standard and technology to be adopted.

Analysis started – The date confirms that analysis started within stipulated time of sample stability and also use to evaluate the performance of the process day to day.

Analysis ended – The day confirms that the analysis has completed as per plan and if not, then the necessary RCA (Route Cause Analysis) needs to be done.

Report release date – Ensure customer has got the report on time.

Signature of the report receiver – Ensure that the report has reached to the destination – the customer may be internal (e.g., report preparation department) or external (sender of sample).

Work plan

This is the most critical part in processing. Lots of practice in planning and time

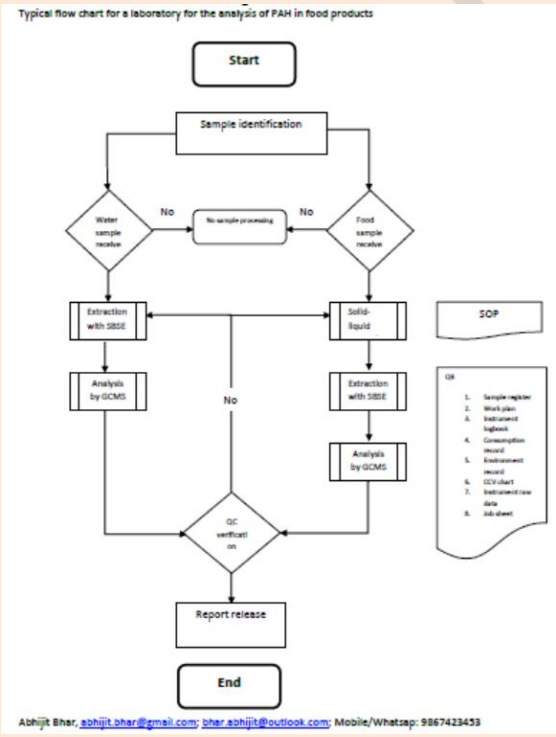
management needed for this step for continual improvement.

Before planning you need the following minimum information in hand (there are many more test and matrix other than which mentioned in below examples):

Information needed	Example
1. How many samples	25 nos
2. Types of samples	Water – 10 Food – 6 Air – 5 Soil – 4
3. Test requested	Water: Elements – 10 Alkalinity – 6 Cyanide – 10 PAH – 8 Pesticides – 4 VOCs – 2 Food: Fat – 6 Cholesterol – 6 Vitamin C – 4 Vitamin A – 3 Pesticide – 6 Solvents – 6 Elements – 6 Air: Elements – 3 VOCs – 5 Soil: Pesticide – 4 Elements – 4
4. Sample preparation needed	Water: PAH – 8 Pesticide – 4 VOCs – 2 Food: Fat – 6 Cholesterol – 6 Vitamin C – 4 Vitamin A – 3 Pesticide – 6 Solvents – 6 Elements – 6 Air: Elements – 3 Soil: Pesticides – 4 Elements – 4
5. Technology needed	Elements – ICP-OES. Total 23 samples. If the calibration ranges for all matrices are same, all 23 can be run simultaneously after digestion of the samples other than water. Else, different set of analysis to be done. PAH, pesticide – GCMS. Extraction needed. VOCs – GCFID with Headspace for water samples and GCFID-TDU for air samples. No sample preparation needed. Fat, Cholesterol – GC-FID after sample preparation. Solvents in food – GCFID with headspace, no sample preparation needed. Vitamin A – HPLC Vitamin C – HPLC
6. Work plan a) First start the analysis for which sample preparation not needed. b) Once started start sample preparation steps.	a) Direct analysis Elements on ICP, VOC in water, solvent in food b) Sample preparation • Digestion of samples for elements • Extraction of Pesticides and PAH • Fat extraction and esterification • Cholesterol extraction • Vitamin A extraction • Vitamin C extraction
7. According to the sample preparation time, plan for the analysis sequence.	Analysis sequence: • PAH in day time on GCMS • Vitamin C on HPLC • Fat on GCFID • Vitamin A will be done tomorrow
8. Identify the test for which sample will be prepared today and analysis will be performed tomorrow.	Vitamin A will be analyzed tomorrow
9. Plan for overnight analysis using autosampler.	Overnight analysis plan: • Pesticide by GCMS • Cholesterol by GCMS
10. Check list for each technology	a. Instrument check b. Calibration done,

	acceptable c. Analysis started d. CCV checked, acceptable e. Report updated
11. Check list for documentation	a. Instrument logbook updated b. Consumption record updated c. Reagent/standard preparation record updated d. Sample register updated with reporting date e. Overnight sample processing note kept on each instrument table f. Planned for next day

All the above steps and examples taken from one of my working day and this totally depend on the technology available, man power and methodology. Considering these three points, the plan may vary.



STANDARD PREPARATION

If the weighing balance is not correct, you may shown over weight….

In recent analytical chemistry, most of the analysis are done using high-tech analytical instruments where the measurement done comparing with a standard. Lots of CRMs (Certified Reference Material) are available using which the tuning of the instruments done, known as calibration. But every time, the required concentrations are not available and it is costly affair. Many times, we will need to prepare the standards from a salt or from a concentrated CRM. This is needed as practically the shelf life of CRM will be less when the concentrations are at ppb level. So, we need to learn how to prepare standards more accurately.

You may have 99.9% pure CRM compound available, and you need to prepare 1 ppm of that for calibration. Immediately your management will not provide it. So, you have to prepare it yourself. Also, it is important to know for your own benefit as during a research work of method validation, you cannot depend on the third person to prepare standard for you. You may not able to find out the cause of error, if the result obtained is not as per your expectation.

Before we proceed further we need to remember some basic rule as listed below:

1. Use calibrated balance, micro pipette.
2. Use class ‘A’ glass apparatus.
3. Use high pure solvent.
4. Use valid CRM.
5. Use ultra pure water (whenever needed).
6. Use clean area.
7. Maintain temperature between 18 to 22^0C (cool temperature needed for compounds as well as your comfort).
8. Keep your mobile away.
9. Keep your writing pad handy.
10. Keep marker handy.
11. Use properly cleaned sample vials (better do cleaning at yourself).
12. Let other know that you are preparing standard so that no one will disturb you.
13. Do not take standard or reagent directly from bottle, take an aliquot in a beaker or volumetric flask and take from it (in case of liquid). Do not pore it again in the bottle, instead, label it for future use.
14. Do not reuse micro tip by any means. You will waste costly CRM by trying to save a micro expenses.

Now we are ready to proceed further. This is the second step. You have to be master in pipetting. It is not that you don’t know how to pipette; you have done multiple times in your school and college laboratory. But now you are in a different world. You cannot take chance to do silly mistakes.

1. Pre-Wet the Pipette Tip

Aspirate and expel any sample liquid at least three times before aspirating a sample for delivery. Evaporation within the tip can cause significant sample loss before delivery. Pre-wetting increases humidity within the tip, thus reducing any variation in sample evaporation. Using the same tip to deliver multiple samples without pre-wetting can result in a lower volume in the first few samples. The need to pre-wet increases when working with volatile samples (i.e. organic solvents).

2. Immerse the Tip to the Proper Depth During Aspiration

Before aspirating, immerse the tip adequately below the meniscus. Large volume pipettes (1-5 mL) should be immersed to 5-6 mm, while smaller volume pipettes should be immersed to 2-3 mm. Too little immersion, particularly with large volume pipettes, can lead to air aspiration. Too much immersion can cause samples to cling to the outside of the tip. Touching the container bottom with the tip may restrict aspiration.

3. Pause Consistently After Aspiration

Leave the tip still in the liquid for about one second after aspirating the sample. It takes a moment for the liquid in the tip to finish moving after the plunger stops, so failure to do so will cause the volume to be too low. Make this pauses as consistent as possible.

4. Use Consistent Plunger Pressure and Speed

Press down and release the plunger smoothly and consistently. Try to apply the same pressure and speed when aspirating and dispensing each sample. Repeatable actions produce repeatable results.

5. Pull the Pipette Straight Out

During sample aspiration always hold the pipette vertically and avoid touching the sides of the container. After sample aspiration pull the pipette straight out of the liquid from the centre of the container. This technique is especially important when pipetting small volumes (<50 µL). Holding the pipette at an angle as it is removed from the sample alters the volume aspirated. Touching the sides of the container can cause wicking and a loss of volume due to the effects of surface tension.

6. Examine the Tip BEFORE Dispensing a Sample

Before dispensing, carefully remove droplets on the outside of the tip with a lint-free cloth, being sure to stay clear of the tip opening. Absorbent material rapidly sucks the sample from the tip if it comes into contact at the opening, and unnecessary wiping of the tip increases the possibility of sample loss so use caution.

7. Examine the Tip AFTER Dispensing a Sample

While dispensing a sample, position the tip so that it touches the side of the container to deliver any residual sample remaining in the tip. Keep your thumb pressed on the second stop of the plunger and remove the tip to avoid sample re-aspiration into the pipette tip. Make sure that you see the sample leaving the tip.

8. Use Standard Mode Pipetting

Choose “standard (or forward) mode” pipetting rather than “reverse mode” for all aqueous samples, but you might consider it for particularly viscous or volatile samples. If the reverse mode is used with normal aqueous samples, the pipette tends to deliver more than the calibrated volume. On the other hand, using the standard mode with viscous or volatile samples may result in under-delivery.

9. Use the Appropriate Pipette

It is important to use a pipette with a volume range closest to the volume you plan to aspirate and dispense. The accuracy of your test will improve if there is a small difference between a pipette’s minimum volume and the volume being tested. For example, if you need to dispense 15 µL, a 1 mL pipette would be

the wrong choice, whereas a 20 µL pipette would be ideal.

10. Use the Correct Pipette Tip

Use high quality tips intended for use with specific pipettes. In most cases, manufacturer tips perform well. Alternative brands are also acceptable if their performance has been proven with a specific pipette model. Mismatched tips and pipettes can result in inaccuracy, imprecision, or both. Quality tips provide an airtight seal without the need for excessive force, are made of superior materials and are free of molding defects, thus ensuring reliable liquid delivery.

11. Work at Ambient Temperature Equilibrium

Allow liquids and equipment to reach an equilibrium at an ambient temperature before you begin pipetting. The volume of a sample delivered by air displacement pipettes varies with air pressure, relative humidity and the liquid's vapor pressure, all of which are temperature dependent properties. Working at a single, constant temperature minimizes this variation and improves overall precision.

12. Minimize Pipette Handling

Hold the pipette loosely, return it to the pipette stand or set it down when you are not pipetting. Always wear gloves to reduce body heat transfer to the pipette and avoid handling pipette tips or containers of samples yet to be pipetted. Body heat transferred during handling disturbs the temperature equilibrium, which can lead to variations in delivered volumes.

Now, do practice:

1. Take 10 µl of water by pipette and take the weight.
2. Do the same 10 times and note down the weight.
3. Make a bar graph of weight for all 10 readings. See the trend line.
4. Calculate the SD (standard deviation) of all 10 readings.
5. Do this every day at least for 1 time.
6. You can do the same for any other volume, which is critical for you.
7. Do the same with glass pipette also. For glass pipette you need more skill, so more you practice and check the error faster will be your improvement.

Now you are ready for pipetting. We have to select now the proper glass parts. Most of the time we do mistake here. To save the money or to reduce the work, we select the wrong items. Like an example is that I have two glass pipette – 1ml and 5 ml. Now I can take 0.5 ml using both the pipette. But I should use the 1 ml pipette. As the 1 ml pipette need to clean, so I have used 5 ml pipette, as it was clean. Same happens in selecting volumetric flask. I should not use 50 ml volumetric flask to prepare 25ml volume or 25 ml volumetric flask to prepare 20 ml stock.

Preparation of linear standard

To prepare linear standard, there are two ways – serial dilution and individual spiking. In serial dilution, we need to prepare a stock solution form, from which we can do serial dilution to prepare all the calibration point. In individual spiking, we prepare each standard individually by adding known concentration of stock standard to the diluents. Both have

advantages and disadvantages. It is always better to prepare each standard separately provided you have proper accessories (e.g. proper calibrated micro pipette).

To prepare calibration standard, use the following formula:

C1V1 = C2V2

Where, C1 is the concentration of stock standard, V1 = Volume of stock standard to be taken, C2 = Desired final concentration, V2 = Final volume of the standard

Example: You have CRM of 1001 ppm of Pb. You have to make 5 standards of 1ppb, 5 ppb, 10 ppb, 15ppb, 20ppb and 30 ppb.

First case – Serial dilution:

If we want to make 30ppb directly from 1001 ppm, the calculation will be:

C1V1 = C2V2

C1 = 1001 ppm, V1 = ?, C2 = 30 ppb = 30/1000 ppm (1ppm = 1000 ppb), V2 = 50ml (50 ml as we have to prepare the next 20ppb from it).

So, 1001 * V1 = 30/1000 X 50

V1 = (30 * 50) / (1000 * 1001) = 0.001498ml

So, we have to take 0.001498ml of 1001 ppm and dilute upto 50 ml to get 30 ppb.

Do you think you can take 0.001498 ml? So, first you have to make a stock solution near to 100 ppm or preferably 10 ppm from which we can make 30 ppb. Remember, lesser we will take the volume more the chances of error. So, first remove the thought to save money by using less volume of CRM.

So, lets prepare 10 ppm first.

Again, C1V1 = C2V2

C1 = 1001 ppm, V1 = ?, C2 = 10ppm, V2 = 50ppm

So, 1001 * V1 = 10 * 50

V1 = 500/1001 = 0.499 ml

As I can take 0.5 ml, so I have to recalculate the actual concentration now.

C1V1 = C2V2

C1 = 1001 ppm, V1 = 0.5ml, C2 = ? V2 = 50ml

So, 1001 * 0.5 = C2 * 50

C2 = (1001 * 0.5)/50 = 10.01 ppm

Now you can see the difference. It is not 10 ppm, it is 10.01 ppm.

0.01 ppm = 10ppb.

If you do not consider this 0.01 ppm in calculation, means you are adding 10ppb error in standard.

Now we will prepare all standards from this 10.01 ppm stock.

Lets prepare 30ppb.

C1 = 10.01 ppm, V1 = ?, C2 = 30ppb = 30/1000 ppm, C2 = 50ml

So, 10.01 * V1 = 30/1000 * 50 V1 = (30 * 50)/ (10.01 *1000) = 0.149ml.

Again, I can not take 0.149ml, instead I shall take 0.15ml

So, 10.01 * 0.15 = C2 * 50 C2 = (10.01 * 0.15)/50 = 0.03003 ppm = 30.03 ppb

So, from 30.03 ppb, now I shall make 20ppb, from 20ppb to 15ppb, from 15ppb to 10ppb, from 10ppb to 5 ppb and from 5 ppb to 1 ppb using same C1V1 = C2V2 formula. But we have to write actual value.

So, 2nd standard is 19.81ppb (taken 33ml of 30.03ppb diluted upto 50ml).

Second case – Individual spiking:

Prepare a final stock standard of 1ppm step by step (1001ppm to 100ppm, then 100ppm to 10ppm and finally 10 ppm to

1ppm). Then from 1ppm we can make each standard separately using same formula. Here the steps are more, but chances of error are less.

SAMPLE PREPARATION

If preparation is not correct, the taste of food with costly recipe also bitter……

Maximum time we need to spend on sample preparation. We need to extract the target molecules from complex matrix with maximum recovery and at the same time avoid the interferences. It is the most challenging job and needs lots of practice, skill, observation and thought process.

As we are going to deal with organic and inorganic compounds, we have to understand different process, like, extraction, digestion, esterification, etc. for different requirements using modern equipments and methodology. Ultimately our goal is to get maximum recovery, minimum interferences and lowest time and cost.

In this chapter we will discuss on extraction only.

Erase the line from your mind "if end is good, all is good"; not applicable in analytical instrumentation. Here end is good, if everything is good, starting from sample collection to reporting. In this chapter we will discuss on sample preparation only.

There is a growing need for applications in food control safety analysis for a large number of analytes in very complex matrices. No analytical technique can give the proper result unless the sample preparation is not correct. Sample preparation means to ready the sample for analysis; means extract the target molecules from matrices before introducing it to the instrument. A simple example is analysis of pesticides in soil. You can prepare the pesticide standard in solvents from CRM (Certified Reference Material) and then can inject to GCMS or GCMS/MS which will nicely be detected and will give a clear spectral matching. But how you will inject the soil sample? You can add water and the pesticide from soil can dissolve in water. That water you can inject in GCMS. But how you can be sure that all pesticides from soil have come to water (recovery %)? Or how much the dilution has happened (related to detectivity)? Same is for elemental analysis in soil.

So, now you can understand how important it is in analytical instrumentation.

There are two types of target molecules – organic and inorganic. Matrices can be solid, liquid or gas. Depending on the target molecule and matrices, the sample preparation to be decided. Sample preparation can be basically divided as extraction, esterification and digestion. Extraction normally used for organic molecules, digestion for elemental analysis and esterification for high boiling compounds where we convert them in different molecules by chemical reaction (should be proportional). Some times, we also do the chemical reaction to analyze anions and cations by colour formation.

Extraction:

SPE (Solid Phase Extraction) – off line and on line

SPE has been considered as one of the most popular analytical extraction technique, due to its simplicity, quickness and low solvent consumption. It can be applied on a several complex liquid matrices (i.e., milk, drinking water, wine, aquous beverages, oils, etc.) and solid matrices (i.e., plant tissues, fruits, vegetables, grains, meat, fish and animal tissues) for many purposes, such as purification, trace enrichment, desalting, and class fractionation.

The SPE technique involves partitioning between a liquid (sample matrix or solvent with analytes) and a solid sorbent phase. The main objectives of SPE are removal of interfering matrix components and selective concentration and isolation of the analytes. In particular, the success of SPE depends on the knowledge about the properties of target molecules and the kind of samples.

Key properties of the sample matrix, analyte and eluent:

Sample matrix	Analyte solubility	Eluent recommended	Analyte polarity	Phase recommendation
Aqueous	Water soluble	Aquous	Non polar	Reversed
			Moderately polar	Reversed
			Polar	Ion exchange
			Cationic	Ion exchange
			Non polar and anionic	Reversed and ion exchange
			Non polar and ionic	
Aqueous	Organic soluble	Organic (water miscible)	Non polar	Reversed
Organic	Organic soluble	Organic	Polar	Normal
			Moderately polar	Normal
			Cationic	Ion exchange
			Anionic	Ion exchange
		Organic (water miscible)	Non polar and cationic On polar and anionic	Reversed and ion exchange

Applications of traditional SPE phase in food analysis

Analyte	Food matrix	SPE mechanism	SPE phase
Afflatoxins	Cereals, nuts, peanut butte	Normal phase	Silica (SiOH)
	Corn flour, liver	Reversed phase	C18
Antibiotics	Fish	Normal phase	Silica (SiOH)
	Honey	Reversed phase	C18
Aromatic hydrocarbons	Water	Reversed phase	C18
Attrazine	Vegetable oil	Normal phase	Silica-Diol
Biphenol (A)	Water	Reversed phase	PSDVB
Chromium (VI)	UHT milk and water	Normal phase	Silica-NH_2
Fungicides	Citrus fruit	Normal phase	Silica-Diol
	Fish	Normal phase	Silica (SiOH)
	Water	Reversed phase	C18
Herbicides	Water	Normal phase	Silica-CN
		Reversed phase	C18
		Ion exchange	SCX
Insecticides	Water	Reversed phase	C18
Metals	Water	Normal phase	Silica-NH_2
		Reversed phase	C18
Mycotoxins	Cereal, nuts, peanut butter	Normal phase	Silica (SiOH)
	Corn flour, maize, milk	Reversed phase	C18
Orchatoxin A	Cereal, foodstuff	Normal phase	Silica (SiOH)
Organochlorine pesticide	Homogenized milk, plants and meats, water	Reversed phase	C18 or C8
PAH	Oil	Normal phase	Silica (SiOH)
	Vegetables	Normal phase	Silica (SiOH)

PCB + pesticide	Adipose tissue	Ion exchange	SCX
	Animal fat	Reversed phase	C18
Phthalates	Water	Reversed phase	C18
Sulfonamides	Meat	Ion Exchange	SCX
Triazines	Homogenized milk, maize, water	Reversed phase	C18
	Soy beans	Ion exchange	SCX
Zeralenone	Cereals, foodstuffs	Normal phase	Silica (SiOH)
Zinc	Water	Reversed phase	C18

Note: above details are some of the targets and matrices. You need to refer application notes of manufacturers.

Sample pre-treatment before using SPE:

In addition to ensuring proper pH of the sample (see The Role of pH in SPE on page 5), you should consider other sample pretreatment needs. The following section describes how some difficult sample matrices should be pretreated before being applied to the SPE device:

Milk

Milk generally is processed under reversed phase or ion exchange SPE conditions. The sample may be diluted with water, or with mixtures of water and a polar solvent such as methanol (up to 50%). Some procedures may require precipitation of proteins by treatment with acid (typically HCl, H2SO4, or trichloroacetic acid). After precipitation, the sample is centrifuged and the supernatant is used for SPE.

Water samples

Drinking water, groundwater, and wastewater samples may be extracted directly by SPE, as long as they are not heavily loaded with solid particles. Groundwater and wastewater samples might need to be filtered prior to the SPE procedure. Filtering may reduce recoveries if compounds of interest are bound to the removed particles. If possible, do not filter the sample. Pass the unfiltered sample directly through the SPE device and, during elution, allow the solvent to pass through the particles on the adsorbent bed. This will improve recoveries, since particle-bound compounds of interest will be recovered using this process. In most cases, water samples are used with reversed phase or ion exchange SPE procedures.

Wine, Beer, and Aqueous Beverages

Aqueous and alcoholic beverages may be processed for SPE without pretreatment under reversed phase or ion exchange conditions. For reversed phase procedures, if alcohol content is high, dilution with water or buffer to <10% alcohol may be required. If necessary, solids in the sample can be removed by centrifugation or filtration prior to SPE.

Fruit Juices

Fruit juices typically are processed without pretreatment or are centrifuged for reversed phase or ion exchange SPE. If centrifuged, the resulting supernatant is used for the SPE procedure. Viscous juices may need to be diluted with water or buffer at the proper pH.

Oils

Hydrocarbon or fatty oils are commonly processed under normal phase conditions, because they cannot be diluted with water. The diluent is usually a mid-polar to nonpolar solvent such as hexane or a chlorinated solvent. The diluted sample is passed through a normal phase bonded silica or adsorption medium, and the

sample is collected as it passes through. The compound of interest should pass through unretained, while impurities remain in the adsorbent. If the compound of interest is retained on the packing, successive washes of the SPE packing with increasingly polar solvents, or with mixtures of the diluent with a polar solvent, are performed until the analyte is recovered in one of the fractions. For collecting oil in water samples, reversed phase SPE is used.

Soil and Sediment

Soil and sediment samples typically are extracted with mid-polar to nonpolar solvents via Soxhlet extraction or sonication. The resulting extracts are then processed by normal phase SPE to remove interferences. The cleaned extracts then can be evaporated and reconstituted with another solvent for additional SPE (reversed phase, ion exchange, or normal phase) if necessary. If extraction efficiency of the compound of interest is pH-dependent, soil and sediment samples may need to be homogenized with water at the appropriate pH prior to extraction and SPE cleanup. In some cases, small amounts of soil or sediment can be homogenized with an appropriate solvent and then passed through the SPE device without pretreatment, as long as the particles do not clog the device. The analyte is then eluted with the appropriate solvent by passing it directly through any particles that rest on the SPE tube packing or disk.

Plant Tissues, Fruits, Vegetables, and Grains

Plant tissues, fruits, vegetables, and commodities such as animal feeds and grains are homogenized either in water, in a polar organic solvent (e.g. methanol or acetonitrile), or in mixtures of water with these solvents, for reversed phase or ion exchange cleanup procedures. After centrifugation or filtration to remove the precipitated proteins and solids, the pH of the sample may need to be adjusted. The analyte may adsorb onto the SPE packing or may simply pass through, free from interferences. The sample also may be homogenized with a mid-polar to nonpolar solvent for normal phase SPE procedures. Again, the sample may need to be centrifuged or filtered prior to SPE.

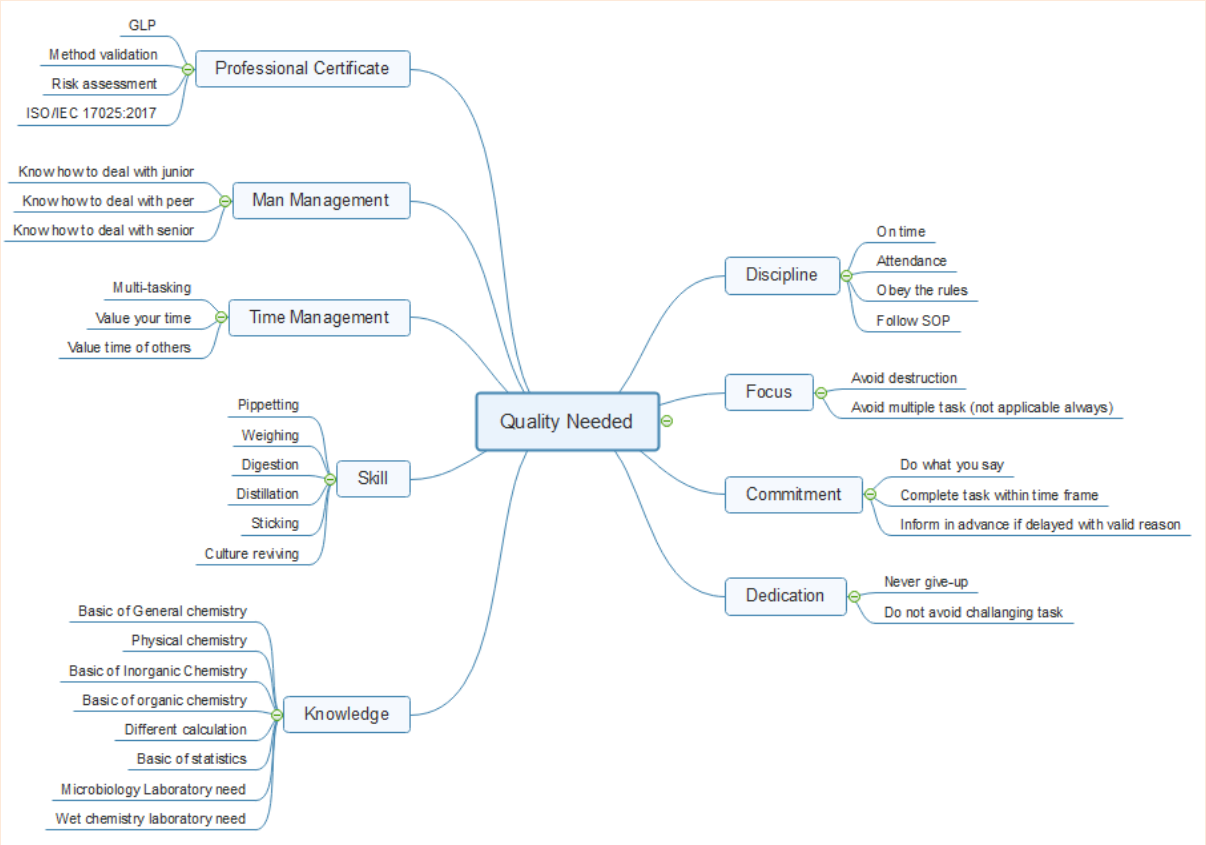
Meat, Fish, and Animal Tissues

Meat, fish, and other tissues can be processed in the same manner as described above for solid fruits and vegetables. In addition to homogenization with water, sample preparation for reversed phase and ion exchange SPE procedures may also involve hydrolysis or digestion of the meat or tissue with acid (typically HCl or trichloroacetic acid) or saponification with base (e.g. NaOH). Enzymatic hydrolysis also may be used. The sample can then be centrifuged and the supernatant used for the SPE procedure. Tissue extracts obtained with mid-polar to nonpolar solvents can be processed using normal phase procedures.

Hay….

Now you know what you are supposed to know. But do you know what quality you need to have?

Your senior expects….


Quality Needed
Professional Certificate
GLP
Method validation
Risk assessment
ISO/IEC 17025:2017
Man Management
Know how to deal with junior
Know how to deal with peer
Know how to deal with senior
Time Management
Multi-tasking
Value your time
Value time of others
Skill
Pippetting
Weighing
Digestion
Distillation
Sticking
Culture reviving
Knowledge
Basic of General chemistry
Physical chemistry
Basic of Inorganic Chemistry
Basic of organic chemistry
Different calculation
Basic of statistics
Microbiology Laboratory need
Wet chemistry laboratory need
Discipline
On time
Attendance
Obey the rules
Follow SOP
Focus
Avoid destruction
Avoid multiple task (not applicable always)
Commitment
Do what you say
Complete task within time frame
Inform in advance if delayed with valid reason
Dedication
Never give-up
Do not avoid challanging task

Your check-list:

Referring above mind map, prepare your own check list, and ensure that you gained those knowledge and skill. Below is one example:

Sr.No.	Description	Check (√)
1.	Knowledge	
1.1	Basic chemistry	√
1.2	GLP	
1.3	Laboratory safety	√
1.4	Method validation	
2.	Skill	
2.1	Pippetting	√
2.2	Standard preparation	
2.3	Software operation	

Make your learning plan:

Every day you have to learn new things and have to grow your knowledge and skill. In todays world in laboratory verticals, you need to know GC, HPLC, AAS, ICP-OES, UV-VIS Spectrophotometer, Spectrofluorometer, FTIR, GCMS, LCMS, ICPMS, Autochemistry.

It is not possible to write all about those instruments in details here. You can mail me on abhijit.bhar@outlook.com to more about each instruments.

Knowing instruments not means the operation. A good and smart chemist knows about the instrument operation, the working principle, minimum trouble shooting and applicability of the instrument. The person good in operation, knowing nothing else, is a god skilled worker/operator and not a chemist.

Maintain a note book

Maintain a laboratory practical book. If your organization does not allow bringing your personal note book, maintain it in home. Once you go back to home end of the day, write in details step by step what you have done. Write the details at right side (ruled side), and draw the flow chart and drawing to left side (white side). This will be your asset in long run, if you make it properly.

For a wet test, instead of writing a story, write the steps and draw the flow chart. Understand the chemistry of each step; it will help to avoid any wrong reporting of values.

I believe, being a fresher, you are now ready to work in the laboratory. Now once you are on-board, on the first day there will be induction training and then departmental training. On the first day ask for the below documents to refer:

1. HR policy manual
2. Laboratory Quality Manual and related procedures
3. Laboratory safety manual
4. Departmental manual (the department you are going to work)
5. Understand how your department is dependent on other departmental activities (we call it as "**S**upplier")
6. Understand what your department is getting ("**I**nput")
7. Understand what you are going to do with the "input" (your "**P**rocess")
8. Understand what you are getting from process ("**O**utput")
9. Understand whom you are going to deliver your analysis report ("**C**ustomer")

This flow of work is known as SIPOC.

Future reading

1. Time management
2. Documentation
3. Method validation
4. Estimation of Uncertainty of Measurement
5. Analytical instrumentation
6. Basic Gas Chromatography
7. Basic High Performance Liquid Chromatography
8. Basic of Molecular Spectroscopy
9. Basic of Atomic Spectroscopy
10. Overview of Statistical Quality Control
11. Guide to use Lean Six-Sigma for QA & QC
12. Seven Quality Tools and its applicability
13. ISO/IEC 17025:2017 implementation
14. How to improve the quality of work

Reference books:

More than 1000 reference books are available on request for instruments, laboratory manuals, Quality management systems, analytical instrumentation, Biochemistry, Antibiotics, Preservatives, Microbiology, Inorganic chemistry, Organic chemistry, Life science, Food analysis methods, Environmental analysis, Food safety, Goal setting, Human Resources, Human Physiology, Management, Marketing, Method validation, UoM, Organization behaviour, Quality Control, Risk management, Sample preparation, Shelf life and Six Sigma.

Last minute suggestion:

1. Read a lot, book is the best friend, knowledge is the ultimate assets.
2. Listen to your senior and do whatever they asked to do. Do not differentiate the work and don't think that the work is not for you.
3. Set a goal and focus on it.
4. Give value of your commitment.
5. Always be in touch with analysis on floor, else will lose your skill.
6. Speak less, listen more. You speak what you know and if you don't listen, you will lose the opportunity to learn what you don't know.
7. Never be satisfied with your knowledge and goal. There are more than what we have, what we do and what we know.
8. Always ask questions yourself.