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"QUALITY PREPRATORY WORK: THE BASIC NEED OF ANALYSIS".

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ABSTRACT
Quality is compliance to best known standards, processes and specifications. Although analysis of sample is frequently carried out by using sophisticated instruments, the importance of basic laboratory skills cannot be overlooked. The aim of this article is to provide basic techniques in key laboratory skills and to provide an introduction to important quality topics. It is important to have the knowledge about the safe handling of glassware's in order to reduce the injury and accidents. It is also essential to known the proper methods of glassware cleaning, to prevent the contamination of preparation need to meet quality band safety requirements every stage including weighing, transfeering and making solutions are important to improve efficiency and accuracy. This is especially important since inaccurate weighing can negatively impact the potency of the final product; through inconsistent ingredients. The present article summarizes the updated information on various aspects of laboratory skills involved in the quality preparatory work.

KEYWORDS: Quality, Analysis, laboratory skills, accuracy.

INTRODUCTION

The word 'QUALITY' has its origin in a Latin word- "Qualitas" means 'general exllence' or a distinctive feature'. Oxford Reference Dictionary defines quality as –"A standard of how good something is as measured against other similar things." If we try to analyze this definition come across some key words, they are:

1. Standard
2. Measurement
3. Goodness &
4. Comparison

Means you are trying to measure something (quality attributes), with a standard (may be reference standard or a set of specifications.) to know where the object in a question stands in the continuum of the expectation of its quality attributes being assured or measured. Sounds complicated, is it not? True, but that is it. Let us try to make out a simple understanding of the word Quality, about which we are going to talk more in this article. Quality's most simple definition is-"fitness for use." In this prime definition the customer or the user is at the focus. If the user of the product or service is happy and delighted when using the product or service then the product or service may be called as a quality product or service.

Our objective in this article is to define the word quality, relate this to pharmaceutical preparation, by identifying the various quality attributes which are required to be present in the pharmaceutical preparation, before it can be declared as a quality pharmaceutical preparation. And also try to provide guidelines for achieving it.

SAFE HANDLING OF LABORATORY GLASSWARE"

- **Glassware is a marvelous accomplishment. It is:**

Designed efficiently.
Shaped "by science, for science".

- **But glassware is fragile and can break or shatter under a number of conditions:**

If it is bumped.
If it is dropped.
If too much pressure is applied.
If temperatures change too drastically.

- **Any of these problems can cause accidents.**

Some accidents are minor.
Others can result in serious injuries.
Contamination can also be a problem.

- **How do we protect ourselves from glassware accidents?**

Learn about our equipment.

Inspect it before use.
Follow proper procedures.

- **Glassware is everywhere.**

Beakers.
Flasks.
Bottles and jars.
Tubing and more.

- **Each type of glassware is made for a specific purpose.**

They should be used only for that purpose.
"Makeshift" apparatus is unstable and can lead to accidents.

- **You should always determine the compatibility of glassware with the chemicals you are using.**

Especially acids and alkalies.
Many chemicals react with glass.

- **Only certain grades of glassware can stand up to lab environments.**
- **Labware can often be heated to extreme temperatures.**

Inferior/flawed material can shatter or crack.

- **Certain operations require specifically designed glassware**

Vacuum operations.
Gas-producing reactions.

- **Before working with glassware, always inspect it for flaws.**

Glass should be pulled from service if defects are present.
Discard or send defective glassware to a glass blower for repair.

- **Proper handling of glassware is also important.**

Never carry a flask by its neck.
Never carry a beaker by its side.
Always use two hands carrying any glassware (position one hand under the glass for support).

- **Gloves should be worn whenever glassware is handled.**

Cut-resistant gloves are best.
Wear lab gloves underneath to keep out liquids.
Use insulated gloves with extreme temperatures.
Compromises must sometimes be made when a fine sense of touch is required.

- **Never heat or cool glassware unless it is designed for those processes.**

Round-bottom flasks are best for boiling liquids.
Never set hot glass on a cold bench top.

- **Scratches in glass can grow to cracks later on.**

So don't use glass/metal stirring rods.

- **Avoid any physical stresses to glassware.**

Where necessary, stabilize it.
Use clamps and platforms to relieve pressure.

- **Ground-glass joints are crafted for a perfect fit.**

Because of this they sometimes stick.
Never force a joint free (the glass can shatter).
Lubricate surfaces or use a teflon sleeve.
A heat gun can gently loosen the joints.

- **Cutting and bending tubing can also cause problems.**

Make sure you are wearing gloves and safety glasses.

- **Several specific steps should be followed to cut tubing**

Position a triangular file where the cut is to be.
Score the tube with your a single, light stroke.
Grip the tube with your fingers on either side of the score mark
Gently pull the ends of the tube toward you.
The glass should snap at the score mark.

- **Remember to fire-polish the tube's ends.**

Removes sharp edges.
Keeps cracks from appearing.

- **Bending tubing has its own procedures**

Heat it in a flame until the glass turns red.
Pull the ends toward you to form desired angle.

- **Setting up apparatus can involve pushing glass tubes through a cork or stopper.**

This should be approached with caution.
Determine that holes are the correct size for the tubing.
Lubricate the hole and tube (with water or glycerin).
Hold the tubing with a towel.
Position the tube close to the insertion point.
Gently twist the tube into the stopper.

- **Using proper techniques when stirring materials is also important.**

Make sure that electrodes, tubing, etc. are placed high enough to avoid the stir bar.
Avoid contact with any portion of the apparatus.

- **Some glassware can present unusual safety risks.**

Make sure you have had the necessary training before working with specialized equipment.

- **Vacuum operations can severely test glassware.**

Container walls must be able to withstand pressure differences.

Containers can implode if they are not strong enough.
Round-bottomed or thick-walled flasks should always be used.

- **Glassware that is showing repairs should be avoided.**

It is more apt to break through thermal shock.
Checking for flaws before use is very important.

- **Often, protective measures should also be taken.**

Place all vacuum apparatus behind a blast shield.
Always wear appropriate protective equipment (goggles, gloves and even a face shield).
Cover flasks, dewers and desiccators with tape or mesh, or use PVC coated containers.

- **Using containers made of other materials can also prevent accidents. Alternatives include**

Metal.
Plastic.
Teflon.

- **Make sure the containers you select are appropriate for the task.**
- **More glassware accidents occur during clean-up than any other activity.**

Keep glassware clear of the sides of sinks.
Never use worn out cleaning brushes (they can scratch the glass).
Avoid cleaning with "aqua-regia", "chromic acid" or other caustics.

- **Be careful when drying glassware.**

Place small articles on towels or in lined baskets.
Large containers should be hung on pegs.

- **It is also important to know how to store glassware properly.**

Keep it well away from shelf edges.
Don't let instruments roll around in drawers (use drawer pads).
Place glassware well back in hoods or on benches.

- **Know proper procedures in case of a mishap.**

If something is falling, let it drop.

- **Use common sense when doing cleanup.**

Wear leather or other cut-resistant gloves.
Never pick up fragments with your fingers… use a dustpan and broom instead.
Dispose of glass pieces in "glass-only" receptacles.

- **Also be aware of any spilled substances. Look for**

The substance itself. & Contaminated broken glassware.

- **Spilled materials may have to be disposed of as a hazardous/biological waste.**

The situation could conceivably require evacuation.

- **Know the location of eye washes and safety showers.**

Make sure you can use them effectively.

GLASSWARE CLEANING

Procedure

Preparation of cleaning solutions

For the cleaning of glassware those are used for chemical analysis special precautions will take place to avoid any chemical residue in glassware, which may later interfere with the results of chemical analysis. Following cleaning solutions were used for cleaning of glassware:
A] 0.5% Labolene
B] 2% Liquid soap solution

1) Measure 5 ml Labolene and add it to container containing 995 ml of purified water and stir it well. For better results use hot water
2) Use it for cleaning of laboratory glassware such as bottles, flasks, beakers and tubing.
3) Record the solution preparation details in glassware cleaning solution preparation record. 2% Liquid soap solution.
4) Measure 20 ml soap solution and add it to container containing 980 ml of purified water and stir it well.
5) Use it when 0.5% Labolene is not available then it can be used for glassware cleaning.
6) Record the solution preparation details in glassware cleaning solution preparation record.

General cleaning

1. Cleaning of glassware, which has contained hazardous materials, must be solely undertaken by experienced personnel.
2. Most new glassware is slightly alkaline in reaction. For precision chemical tests, new glassware should be soaked several hours in acid water (1% solution hydrochloric acid or nitric acid) before washing.
3. Wash glassware as quickly as possible after use if it is not possible then the articles should be allowed to soak in water.
4. For cleaning of glassware such as bottles, flasks, beakers, test tubes, etc use 0.5% Labolene. For better results use hot water
5. For general cleaning 2% liquid soap solution may also be used when Labolene are not available.
6. During the washing all parts of the article should be thoroughly scrubbed with a brush selected for the shape and size of the glassware. Brushes should always be in good condition to avoid any abrasion of the glassware.
7. Special types of precipitate material may require removal with nitric acid, aqua regia or fuming sulphuric acid. These are very corrosive substances and should be used only when required
8. Before cleaning of glassware remove the labeling of marker pen with the help of IPA or acetone. For plastic ware do not use acetone for removing marker pen labeling.
9. It is imperative that all soap detergents and other cleaning fluids be removed from glassware before use. This is especially important with detergents, slight traces of which will interfere with serological and culture reactions. After cleaning, thoroughly

rinse with tap water ensuring that containers are partly filled with water, shaken and emptied several times. Finally rinse with purified water.

10. After cleaning dry the glassware in Hot air oven at 60 °C temperature ± 5 °C. 5.4.11 Always protect clean glassware from dust by use of temporary closures or by placing in a dust free cabinet. Cleaning of specific types of glassware

GLASSWARE LABELING

Primary labeling

- Containers by the manufacturer
- Specific information is set by Federal Lab Safety Standard (FLSS)
- Secondary Labeling
- used to label smaller, secondary containers
- must be done by user to provide point-of-use information
- must contain

– Common or scientific name of chemical
– Name of person who is responsible for it
– Date at which container was filled
– Any specific precautions; minimum is NFPA 704M symbol

Secondary labeling methods in the lab

• Solvent wash bottles use the pre-labeled polypropylene squirt bottles and just refill then when needed.
• Glass beakers
- For long term use in the same process, use a sharpie felt marker on the white labeling area, or create a tape label and apply this to the beaker. Tape labels will resist most chemicals, solvents will dissolve the sharpie felt tip marks.
– For short term use, label a filter paper and place the beaker over top of it to identify it.
• Screw cap chemical bottles
– use a clean, new bottle and put a pressure sensitive label on it, or use tape labels.

As mentioned above, all samples should be clearly labelled with a unique identifier when received into the laboratory. Factors to consider in designing a suitable label include:
• The label should be securely attached to the body of the container, not the closure;
• The label must remain legible while the sample is being stored.

It may also be helpful to label the closure of the sample container in some way, to make sure that the original closure is used once the sample container has been opened .It is important that any subsamples or aliquots taken from the laboratory sample are also clearly labeled so that the sample can be tracked through the analytical process .Laboratories handling large numbers of samples frequently use laboratory information Management system for tracking samples. Such systems often make use of barcode labels to help identify and track samples.

ACCURATE WEIGHING

a) SOLID:-Using balances

The primary method that you will use to measure the amounts of chemicals is to weigh them--that is, to determine their mass. To do this you use a balance. You'll be learning to use two balances: a standard laboratory balance and an analytical balance.

GENERAL RULES FOR USING BALANCES

Do not place chemicals directly on the balance pan unless they are inert and at room temperature. Containers, glassware, or pieces of metal can generally be put directly on the balance pan, but for most other chemicals, you need to use a container for the chemicals. The reason for this rule is that many chemicals react with the balance pan. Some attract moisture and cause corrosion. Any residue that sticks to the pan can interfere with weighing. It could also contaminate any other chemicals weighed later in the same way. So, instead of weighing materials directly on the balance pan, always weigh the chemicals in or on something--a weighing dish, a beaker, or a piece of folded paper. When you do, remember to weigh the container first or adjust for its weight. Be careful not to spill any chemicals on or around the balance. If you do, clean up immediately! If for some reason your balance doesn't seem to be adjusted properly, let the instructor know. Don't just try to fix it yourself. Tell us, so we can deal with it and get it functioning properly. When you're finished with the balance, return the weights back to zero. Then it will be ready for the next person to use it.

Choosing the proper instrument for weighing

All weighing in the chemistry laboratory is done with a balance. However, depending on the requirements of the experiment at hand, different balances may be used. Preparative work, such as measuring out a rough amount of an excess reagent, does not require a high level of accuracy. For example, if an experimental procedure calls for 1.0 grams of a reagent known to be in excess in the reaction, amounts of 0.9 or 1.1 grams are perfectly acceptable. For these purposes, a standard laboratory balance is used to obtain an initial, approximate mass that is within the range specified by the procedure. The sample's mass is then determined more accurately before being presented in a laboratory report. A top-loading standard laboratory balance is a relatively simple electronic device that measures the mass of an object to

an uncertainty of ±0.02 grams, thus being suitable for preparative applications in the chemistry laboratory.

In analytical work, such as precisely determining the mass of a lead salt precipitate meticulously isolated from an aqueous solution, an analytical balance should be used to.

Minimize uncertainty. An analytical balance is a highly sensitive instrument that is much more prone to errors from environmental conditions and requires much more careful operation than a top-loading balance. This balance measures an object's mass to an uncertainty of ±0.00002 grams and is used any time a very accurate mass determination is needed.

Technique Notes for More Accurate Weighing
Presented below are some general pointers to increase the accuracy of your mass measurements, no matter which balance you use.

Weigh objects at room temperature	A warm or hot object will create convection current in the air around the balance pan. This fluctuating force reduces the air pressure on the balance pan and can make it difficult to obtain a stable reading
Weigh only dry objects.	Moisture can corrode the balance pan. Moisture evaporating from your sample can lead to unstable mass readings.
Use approximate amounts and then measure them accurately.	Approximate amounts should be weighed out on a top-loading balance and then weighed accurately on an analytical balance.
Use the same balance.	It is critical to always use the same balance especially if calculations require more than one mass measurement, as each device might be calibrated slightly differently.
Uses weigh boats.	Weigh boats are containers used to prevent reagents from contacting the balance pan. They are made of polypropylene, a plastic that does not adsorb water. They are inexpensive and do not need to be handled with care. If one is torn or too dirty to be wiped clean, simply discard it.

Standard laboratory balance

A standard laboratory balance is used for preparative work where an error of ±0.02 g is acceptable. It is also often used for pre-weighing samples to determine the mass approximately. The exact mass is then determined using an analytical balance.

1. Place a weigh boat on the balance

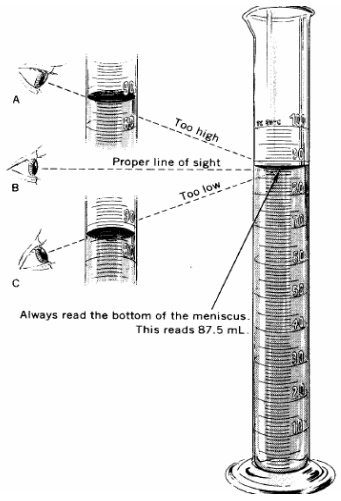
2. Press the TARE or ZERO button to get a reading of 0.00 g.

3. out the sample

4. Record the value. If asked to weigh, say, 0.8 g, a range of 0.7 – 0.9 g is OK.

B) LIQUIDS

Volumetric measurements of liquids are made with measuring cylinders, burettes, or pipettes. The measuring cylinder is usually used to measure approximate volumes of liquids. Aqueous solutions wet the glass walls, forming a concave meniscus, the bottom of the meniscus are used to indicate the volume of the liquid. To avoid parallax error (caused by change of observational position), your eye should always be level with the meniscus when you are making a reading. The volume is estimated to one tenth of the smallest division. Measuring cylinders may be calibrated to contain (TC) or to deliver (TD). If it is calibrated to deliver, the cylinder actually contains slightly more than the volume read, thus compensating for the thin film of liquid left on the walls when the contents are poured out. Calibrated for inflow means the same as calibrated to contain, calibrated for outflow is the same is calibrated to deliver.



The proper method of reading a meniscus to avoid parallax error.

The burette is used for more precise volumetric work and for titrations. If it is clean, the solution will leave an unbroken film when it drains; if drops of solution adhere to the inside of the burette, it should be cleaned with a special cleaning chemical (potassium dichromate dissolved in concentrated sulfuric acid) until it drains properly. Absolute cleanliness is important because the volume of a 25- or 50-mL burette can ordinarily be estimated to the nearest 0.01 mL and the error caused by a single drop adhering to the inside of a burette causes an error of about 0.05 mL. The presence of several drops would obviously result in poor measurement of the volume delivered.

When filling a freshly cleaned burette with solution, add a 5- to 10-mL portion of the solution, being sure the stopcock is turned off, and tip and rotate the burette so that the solution rinses the walls of the burette completely. Repeat the procedure with at least two more fresh portions of solution; then fill the burette above the zero mark and clamp in a burette holder. Then open the stopcock wide to flush out any air bubbles between the stopcock and the tip of the burette. Next drain the burette below the zero mark and take an initial reading, being careful to avoid parallax error. The smallest division on a 25- or 50-mL burette is ordinarily 0.1 mL; estimate the volume to the nearest tenth of the smallest division, usually 0.01 mL. Burettes may have stopcocks made of glass, which must be periodically cleaned and lubricated. Teflon stopcocks ordinarily require no lubrication, but they may have to be cleaned if they are plugged, or adjusted if the tension nut is too tight or too lose.

ACCURATE TRANSFER

A) Solid

Solids to be weighed generally fall into one of three categories. The easiest to work with are small, free flowing granules that slide from spatula to weigh paper to vessel without leaving significant traces behind (potassium carbonate is a typical example). Crystalline materials are a little more challenging, as stray static charge usually holds them to both spatula and weigh paper, necessitating a quick rinse to "help" the residue into the reaction vessel. Gummy solids and hygroscopic materials are the worst, as both are difficult to scrape from their original container, and tend to hold tight to the weigh paper and spatula. With a gummy solid the usual solution is simply to dissolve the compound in a suitable solvent and transfer it via syringe or pipette.

Solids that weigh 15-150 milligrams

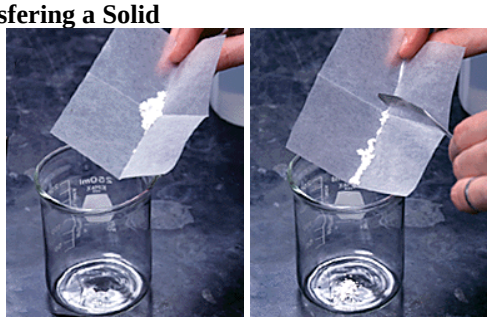
A good analytical balance (ideally with an error of ±0.1 mg) is required to accurately measure solids in this range. Luckily, these balances are not expensive, and most labs will have at least one. Weighing directly into your reaction vessel is viable for granular solids, but I generally use a 6 cm x 6 cm piece of weigh paper, torn into quarters and diagonally creased. The crease guides the solid into the reaction vessel, while the smaller size reduces overall surface area.

Solids that weigh less than 15 milligrams

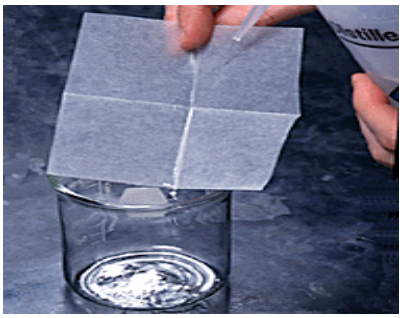
With an analytical balance that measures to ±0.01 mg well-behaved solids less than 15 milligrams can be accurately weighed, though not without some difficulty. Slight breezes are enough to disturb the balance and prevent precise measurements, while a weak shake in the hand can spill the precious cargo en route to the reaction vessel. Work cautiously with plenty of room around you, and double check the number of grains before and after transfer to the reaction vessel.

Solids that are at all difficult to handle or precious should be dissolved in a carrier solvent at a known concentration, then transferred as a liquid (diethyl ether and dichloromethane good solvents, as they are relatively easy to remove under reduced pressure/nitrogen/argon [1]). If reactions are being set up in parallel the liquid transfer route is also significantly faster for more than ~2 samples.

Transfering a Solid



This can be done be carefully tipping the creased weighing paper to pour the solid into the beaker. Tapping the paper with a spatula will knock particles into the beaker.



Finally, the paper should be rinsed into the beaker, to remove all traces of the solid.

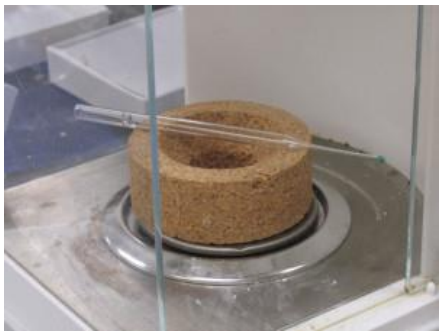
Transferring a solution or mixture

If you are transferring a solution or heterogeneous mixture to another vessel, rinse the container with solvent to be sure the transfer is quantitative. The rinsing should be transferred to the second vessel along with the rest of the mixture or solution.

B) LIQUID

Liquids of unknown density that weigh 50-200 milligrams

A 1 mL syringe with thin gauge needle (anywhere in the 19-22 range) is perfect for moving small quantities of poorly-characterized liquids. Weighing by difference, first measure the empty weight of the syringe, then draw up the liquid. Reweigh and repeat until the desired mass is achieved, then transfer the liquid to the reaction vessel. It's necessary to rinse the syringe at least three times with a neutral carrier liquid to remove any residue, and thick oils may need to be adulterated with solvent prior to transfer.



Weighing via pipette: Measuring marker ink in acetone, for visibility.

Liquids of unknown density that weigh 10-50 milligrams

The standard Pasteur pipette is your friend here. As above, tare an analytical balance with the empty pipette, then dab the pipette into your liquid/oil. Capillary action will draw up the liquid, which is then measured on the balance. A thumb to the top of the pipette will eject the liquid, and solvent applied to the top (via a second pipette, now with bulb) will rinse any traces into the reaction vessel .Liquids less than 10 milligrams and liquids of known density or concentration As with solids in this range, dissolve the liquid to a known concentration in a neutral carrier solvent (ex. 1 mg/mL), and transfer with a 1 mL graduated pipette or Hamilton micro syringe. The vials mentioned in this previous post will hold accurate concentrations for several months, provided the septa are not pierced.

Anhydrous conditions and the micro scale

Maintaining an air free environment is much more challenging on the micro scale. Working with reactions that are at worst stoichiometrically sensitive to water, my practice has been to transfer solids to the reaction vessel under argon, then flush for ~10 minutes with argon before adding in solvent. Carrier liquids should be removed during the argon flush, rather than attempting to keep them dry outside of the fume hood/glove box and all glassware should be flame/oven dried. If any readers have expertise here, please speak up.

MAKING SOLUTIONS

How to Make Accurate Stock Solutions

1. Make up solutions and standards using volumetric flasks.

For accurate and reproducible stock solutions your tool of choice should be a volumetric flask. Volumetric flasks are far more accurate than measuring cylinders and pipettes, especially if you use class a flasks, which are manufactured to extremely stringent standards. Volumetric flasks are available in various sizes from 1 mL upwards and you can find a guide to using them here.

2. Use the correct balance, correctly.
Obviously there's no point in using a highly accurate volumetric flask to measure the solvent volume, only to weigh out the solid using a balance that is too coarse for your needs. Make sure you use a balance that is made for weighing a mass in the range you require and that it is calibrated and on a level surface. For small masses, it is often quite difficult to weigh out exactly the amount you require. But normally, solutions don't have to be made up to an exact concentration – you just need to know their concentration exactly .A good approach is to weigh out the reagent as close to the target weight as possible then, note down the weighed mass and make up the solution in the volumetric flask. The actual concentration of the solution you made up can then be calculated exactly.

3. Make reagents in large batches where possible
Another way to ensure that stock solutions are reproducible from experiment to experiment is to make them up in large batches where possible. This not only means that you are working with the same solution in each experiment, but also that you are using a larger volume when making up the solution, which should help with the accuracy.

4. Take into account the strength of reagents you are using.
When making up stock solutions, the strength of the reagent is often overlooked. If the reagent is less than 100% strength i.e. less than 100% of the mass is the actual reagent (the remainder being impurities), it will normally be stated by the manufacturer on the container and you should take this into account when calculating how much of it to use.

B) SUBSEQUENT DILUTIONS
Accurately Diluting a Solution
Introduction
Whether performing a simple titration or calibrating a sophisticated instrument, the ability to prepare solutions of known concentration accurately and reproducibly is skill essential to all analytical chemists. These documents explain to show to use standard volumetric glassware to dilute a concentrated stock solution in order to prepare a more dilute solution in just such a manner. You will need:
• A volumetric transfer pipette
• A suitably sized volumetric flask
• A stock solution of known concentration
• A supply of solvent, normally distilled or deionised water
• A wash bottle containing the same solvent (distilled or deionised water)
• A large, clearly labelled waste container
• A small, labelled beaker to pipette from
• A pipette filler (single bulb, three-way valve, or pump)
• Some clean, contaminant-free wipes
• Some clean, dry, dropper (*i.e.* Pasteur) pipettes and bulbs

Equipment for preparing a dilute solution
Safety Information
Before undertaking *any* operation in the chemical laboratory, you should always make sure that you know what materials you are working with, what their chemical and physical properties are and what safety precautions you should take. Always wear appropriate clothing, including long pants, closed-toe shoes or boots, a lab coat and safety goggles or glasses. Fully enclosed, indirectly vented goggles provide the greatest protection for your eyes. You should *never* wear contact lenses in the laboratory; always wear goggles over prescription glasses. Make sure.

That any loose clothing is kept within your lab coat and tie back any long hair .You should *always* wear appropriate gloves when handling toxic or corrosive substances, such as solvents or strong acids and bases. Heavy-duty nitrile rubber gloves offer the best protection, although they make it difficult to handle glassware. Disposable nitrile gloves can also be used, but they do not offer protection against prolonged contact with either solvents or concentrated acids and bases and should generally be used only for dilute solutions. Finally, remember that no safety equipment is foolproof; the best safety precaution is to be aware of what you are doing and exercise common sense at all times.

3. Pipette Fillers
When using pipettes, you should *never* pipette by mouth; *always* use apipette filler of some kind. There are three basic types of filler: the pump, the single bulb, and the 3-valve bulb. All three have their advantages and disadvantages. One thing they all have in common, however, is that they do not function properly if solution is drawn inside them; proper technique is therefore essential for all three types. Only the 3-valve pipette filler will be described in this document.

4. Transfer Pipettes
Although they may not appear so at first glance, volumetric pipettes are Precision scientific instruments that, if used and cared for properly, can deliver specific liquid volumes with considerable accuracy and precision .There are various types and grades of volumetric pipette. Some are designed to deliver the calibrated volume by draining under gravity, while others must be 'blown out' in order to obtain the correct volume. The former are often marked 'TD' ("to deliver"), while the

latter are marked 'TC' ("to contain"); it is essential to know which type you have, as using the wrong technique for the pipette will introduce a systematic error into all your dilutions. Pipettes are further distinguished as being either transfer (i.e. bulb) or measuring (i.e. graduated, Mohr, or serological) pipettes. Generally, the former are more accurate for a fixed volume, while the latter are more versatile. The uncertainty in the volume delivered by a pipette can be determined from the manufacturer's stated tolerance. The table below lists values for typical class 'A' volumetric pipettes and flasks when used with aqueous solutions at a temperature of 20 °C. In practice, these values are only obtained if the glassware is properly clean, free of damage, and used correctly. Always inspect your pipettes before use, paying particular attention to the tip; if this is blocked or chipped, the pipette may not function correctly. If a solution beads on the inner surfaces of the pipette rather than draining smoothly, the pipette needs to be cleaned before use. Wash with an appropriate soap or detergent, and rinse thoroughly with distilled ordeionised water. Glassware for preparing low concentration solutions of metals should be soaked in metal-free nitric acid (10% by volume) and rinsed with high purity deionised water. Volumetric glassware should be allowed to dry by evaporation; do *not* dry pipettes and flasks in an oven! Acetone can be used to speed up the process, but be aware that many grades of acetone contain low levels of compounds that might well render the glass surface greasy again.

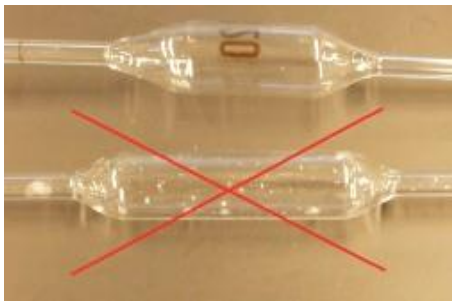


Fig. 2: a clean and a badly contaminated 20 ml transfer pipette

5. Using the 3-Valve Pipette Filler

The 3-valve pipette filler allows the operator to draw solution up into the pipette, lower the solution level to the calibration mark, and finally drain the solution from the pipette. The valves consist of small glass or metal balls sandwiched against a plastic membrane. They are located under the flat, round portions at the top, side and bottom of the bulb and are operated by squeezing them. The upper valve (1) allows you to expel the air from the bulb without squirting any solution from the pipette; the lower valve (2) allows you to draw solution up through the pipette to fill it; and the side valve (3) allows you to drain the pipette

When using the filler, it is important to keep a firm hold of both the filler *and* the pipette; *never* dangle the pipette from the filler! First, squeeze on the first (upper) valve and expel the air from the bulb. Now, gently slide the pipette filler over the end of the pipette. Do not force the bulb on - it should be on far enough that the bulb does not fall off when you let go of it, but not so tight that it cannot be removed easily. If you push the pipette too far, you may damage the filler and break the pipette when you try to remove it. If you are right-handed, hold the pipette in your left hand while you take a firm hold.

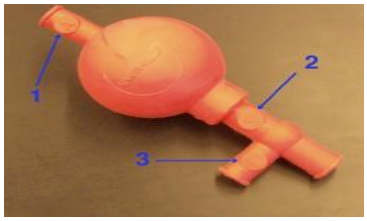


Fig. 3: the 3-valve pipette filler

When using the filler, it is important to keep a firm hold of both the filler *and* the pipette; *never* dangle the pipette from the filler! First, squeeze on the first (upper) valve and expel the air from the bulb. Now, gently slide the pipette filled over the end of the pipette. Do not force the bulb on - it should be on far enough that the bulb does not fall off when you let go of it, but not so tight that it cannot be removed easily. If you push the pipette too far, you may damage the filler and break the pipette when you try to remove it. If you are right-handed, hold the pipette in your left hand while you take a firm hold of both the pipette and the filler with your right. Use your ring (3rd) and little (4th) fingers to grasp the stem of the pipette firmly while supporting the filler and pipette from behind with your middle (2nd) finger. This leaves your thumb and index (1st) finger free to operate the valves. It is a good idea to practice operating the valves with one hand, while holding the lower part of the pipette with the other.

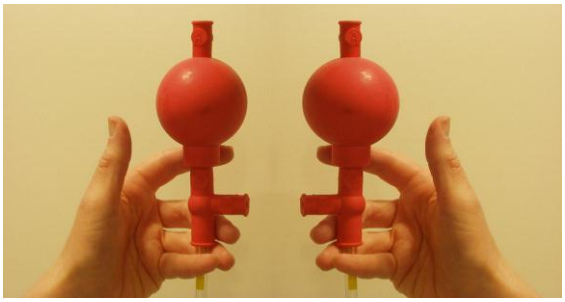


Fig. 4: left-handed and right-handed grip of the filler and pipette

6. Rinsing the Pipette

The first step is to rinse the pipette; this ensures that any water or solution from previous use is completely removed. It also leaves a thin film of your solution on the wall of the pipette, which ensures that it drains properly and delivers the correct volume. If the pipette previously held a different solution (or a different concentration of the same solution), first rinse the pipette with the solvent used in the solution before rinsing with that solution. To

rinse the pipette, first pour a small volume of the solution to be dispensed into a small, clean, dry beaker.

• Never pipette directly from a stock bottle; you may contaminate your solution, compromising all your subsequent Experiments

• Only remove enough solution for your needs; you cannot pour the solution back into the stock bottle, and you may not have enough for later if you use too much now

• It is essential to keep the tip of the pipette below the surface of the liquid at all times while drawing solution into the pipette; if the tip breaks the surface, air will be drawn in and the liquid already in the pipette will shoot up into the pipette filler

• It is a good idea to practice the following procedures with water before attempting to use your actual stock solution making sure you have a firm hold of the pipette and filler as described above, lower the pipette into the beaker so that the tip of the pipette is position well below the surface of the liquid. You can anchor the pipette in place with your other hand in two ways: either pinch the pipette to the side of the beaker, or grip the stem of the pipette between your index (1^{st}) and middle (2^{nd}) fingers while gripping the rim of the beaker between your remaining fingers and thumb. The advantage of this latter grip is that you can tip the beaker so as to draw the last portion of solution up into the pipette; this helps keep the volume of solution required to a minimum.

Fig. 5: anchoring the pipette in the beaker

With the pipette held vertically, pinch the lower (2^{nd}) valve on the pipette. Filler to draw solution up into the lower stem of the pipette; you do *not* want to fill the pipette at this time, since this would waste your stock solution. In general, you only need to draw up enough so that the liquid level just reaches the lower end of the centre bulge on a transfer pipette. Once the solution reaches this point, release the valve and raise the pipette up out of the solution. With the tip of the pipette positioned over your waste container, tip the pipette into a horizontal position and slip the filler off. Using your index (1st) finger over the end of the pipette to control the flow of liquid, allow the solution to drain through the centre portion and into the upper stem of the pipette; you want the solution to run past the calibration Mark on the neck of the pipette, but not all the way to the end. Tip the pipette and rotate it while allowing the rinse solution to drain into the waste container.

CONCLUSION

The key laboratory skills such as safe handling of glassware, glassware cleaning, glassware labeling, accurate transfer, accurate weighing and making stock solutions were developed and by using this skills quality preparatory work is done.

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