MEDICAL LABORATORY

NTQF Level III

Learning Guide -40

Unit of Competence	**Prepare Laboratory Solutions**
Module Title:	**Preparing Laboratory Solutions**
LG Code:	**HLT MLT3 M08 LO2-LG-40**
TTLM Code:	**HLT MLT3 TTLM 1019v1**

LO 2: Standardize solution

Instruction Sheet	Learning Guide 40

This learning guide is developed to provide you the necessary information regarding the following **content coverage** and topics –

- Assembling laboratory equipments for solution preparation
- Making serial dilution
- Standardization of solutions
- determining concentration of standard solution
- Labeling and storage of standard solution

This guide will also assist you to attain the learning outcome stated in the cover page.

Specifically, upon completion of this Learning Guide, **you will be able to –**

- Assemble appropriate laboratory equipment
- perform Serial dilutions as required
- standardize the solution to the required specified range and precision
- determine the concentration of standardize solutions
- label and store Solutions to maintain identity and stability and re-standardized if require

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below 3 to 6.
3. Read the information written in the information “Sheet 1, Sheet 2, Sheet 3 and Sheet 4,---”**in page ---, ---, --- and ---** respectively.
4. Accomplish the “Self-check 1, Self-check t 2, Self-check 3 and Self-check 4” ,---”**in page ---, ---, --- and ---** respectively
5. If you earned a satisfactory evaluation from the “Self-check” proceed to “Operation Sheet 1, Operation Sheet 2 and Operation Sheet 3 ”**in page ---.**
6. Do the “LAP test” **in page – ---**

Information Sheet-1	Assembling laboratory equipments for solution preparation

1.1. Introduction

- To prepare the solutions correctly the appropriate grade of glassware needs to be used.

1.2. Grades of Glassware

- There are many kinds and grades of glassware available in the Analytical (diagnostic) Laboratory. The following lists glassware that may be used.
  - ✓ Hard Glass - as in combustion tubes
  - ✓ Borosilicate Glass - most laboratory grade glassware is borosilicate glass
  - ✓ Low Alkali Borosilicate Glass
  - ✓ Heat Resistant Borosilicate Glass (eg Pyrex brand) - widely used in laboratories

A. Class A Glassware: for accuracy

burettes > volumetric flasks > pipettes > graduated cylinders > graduated beakers

(most accurate) (least accurate)

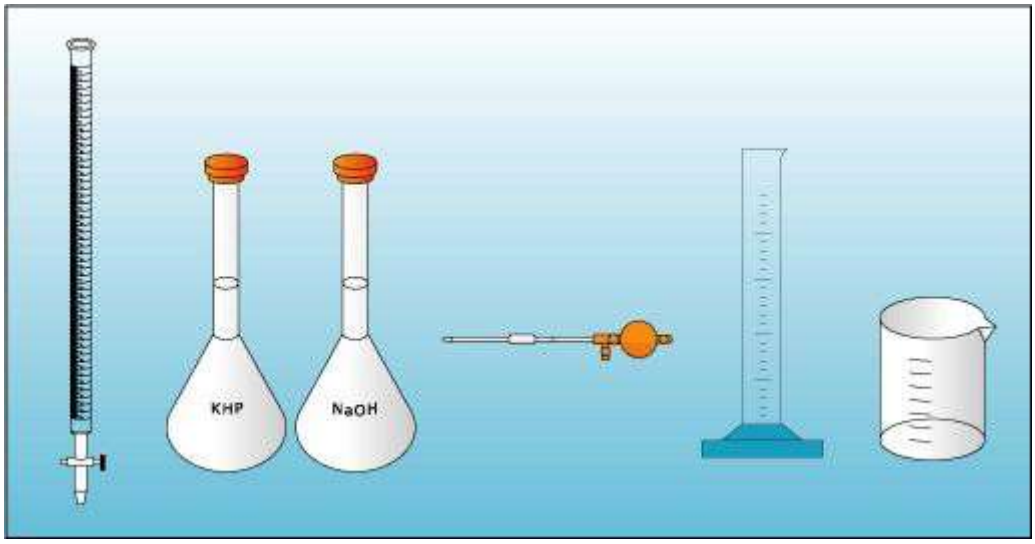


Fig:- 2.1 Class A Glassware

B. Class B Glassware: used when accuracy not critical

C. Specially Washed Glassware: for cleanliness

acid washed > Milli-Q water > distilled/deionised water > cold water > hot water

(most clean) (least clean)

Fig:- 1.2 **Specially Washed Glassware**: for cleanliness

D. Specially Treated Glassware: eg coated with silicone, Teflon, other coatings on inside; plastic film covering on outside to reduce risk of cracking. Sterile Glassware – autoclaved.

- In laboratories there are two main grades of reagents, used according to their purity.
  - ✓ **Analytical reagent grade (designated AR)** which is used for high precision work where accuracy is the most important outcome. For example, AR would be used when you wish to calculate the precise amount of a substance.
  - ✓ **Technical reagent grade** used for work where accuracy of results is secondary to the outcome. An example of use would be in the preparation of tissue stains, where the exact quantity of stain in solution is not critical for the result.
- The above information is usually found on the reagent label; however it is not a guarantee of the purity because:
  - ✓ tests for some impurities may not have been done by the manufacturer
  - ✓ the reagent may have been contaminated in the laboratory after being opened
  - ✓ The reagent may not be sufficiently dry, owing to absorption of moisture.

Self-Check -1	Written Test

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. Which of the following correct sequence of Glassware from most accurate to least accurate
   A. Burettes > volumetric flasks > pipettes > graduated cylinders > graduated beakers
   B. Graduated cylinders > graduated beakers> pipettes > Burettes > volumetric flasks
   C. Graduated beakers > graduated cylinders > pipettes > volumetric flasks > Burettes
   D. Pipettes > Burettes > volumetric flasks > graduated beakers > graduated cylinders
2. Analytical reagent grade is used for high precision work where accuracy is the most important outcome
   A. True B. False
3. information found on the reagent label is not a guarantee of the purity
   A. True B. False

Note: **Satisfactory rating – 1.5 points** **Unsatisfactory - below 1.5 points**

Answer Sheet

Score = _____

Rating: _____

Name: _____ Date: _____

Short Answer Questions

Information Sheet-2	Making serial dilution

2.1. **Materials used for serial dilution**

- Buffer used to dissolve the sample
- The sample
- Multiple tubes
- Pipette or graduated cylinder
- Stirring rod
- Test tube rack
- Funnel
- Stationery
- Labeling materials

2.2. **Procedure**

- Shake the solution by hand or use the stirring rod to swirl the solution.
- Make sure the solution is uniformly mixed.
- Determine initial dilution
- Take half of the solution out to a new tube and add equal amount of buffer into it.
- Take half of the newly made solution to another new tube and add equal amount of buffer into it.
- Calculate the dilution at each point

2.3. Considerations for making a serial dilution

- Depending on circumstances you do not necessarily have to set up the first tube containing the undiluted material
- The last tube will contain 10 mL. Usually this is not a problem as more reagent is made up than required.
- If it is a problem, simply remove exactly 1 mL of the final dilution and discard it according to the appropriate laboratory procedures.

Self-Check -2	Written Test

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. the volume of solution is increase in a series of test tubes through Serial dilution
   A. true
   B. false
2. it is necessarily have to set up the first tube containing the undiluted material in serial dilution
   A. true
   B. false

Note: **Satisfactory rating - 1 points** **Unsatisfactory - below 3 points**

Answer Sheet

Score = _____

Rating: _____

Name: _____ Date: _____

Short Answer Questions

Information Sheet-3	Standardization of solutions

3.1 Introduction

- Standardization is the process of determining the accurate concentration of a standard solution by titrating it against a solution of accurate concentration with high degree of purity.

3.2. Standard solutions

- These are solutions in which the concentration of a given chemical is precisely known
- They are used to determine the value of an identical chemical with unknown concentration of a given solution.
- Chemicals that are used to prepare these solutions should be of analytical grade.
- Since poor standard solutions cause errors in the estimation of the intended substances, their accurate preparation is of utmost importance in order to obtain accurate and precise laboratory findings in medical laboratories

3.3. Classification of standard solutions

3.3.1. Primary standard solution

- Primary standard solution is a chemical solution that has the highest purity and can be used directly for the exact measurement of substances of unknown concentration in a given solution. These solutions include sodium chloride, sodium bicarbonate, potassium iodide, etc.
- Primary standard solution should be made of substances that are:
  - ✓ Free of impurities,
  - ✓ Stable on keeping in solid state and in solution,
  - ✓ Able to be accurately weighed or measured to give a solution of exactly known concentration,
  - ✓ Not hygroscopic (does not absorb moisture) and vaporize at 20^{O}c.

3.3.2. Secondary standard solutions

- Secondary standard solutions are solutions of lower purity and their concentrations are determined by comparison to primary standard solutions. Secondary standard solutions are used for analytical procedures after their concentration is already determined. Some examples of these solutions are nitric acid, hydrochloric acid, sulfuric acid, etc.

- In the preparation of secondary standard solutions, the following points should be taken into consideration:
  - ✓ Using analytical balance for weighing
  - ✓ Dissolving the weighted substance in the prescribed volume of solvent
  - ✓ Determining the exact concentration by comparison against a primary standard solution
  - ✓ Diluting stock secondary standard solutions using exact measurements.

3.4. Prepare standard solution

- Your ability to prepare reagents (solutions) containing the correct constituents in the correct concentrations is a competency that is critical to laboratory performance. Many other activities of the laboratory rely on the correct preparation of laboratory reagents.
- There are two methods of preparing **standard solutions**.

1. By direct weighing of a pure reagent and making up a known volume of solution.
2. By preparing a solution of approximately the required concentration and standardizing against a reference material.

- While the first method is simple and can be used in many cases, many standard solutions cannot be prepared in this way. If, for example, we attempt to prepare a standard sodium hydroxide (NaOH) solution by weighing out pure sodium hydroxide, dissolving it in water and making up the volume, we would find that solid NaOH reacts rapidly with moisture and carbon dioxide in the atmosphere, making it difficult to handle and keep pure. The result is an uncertainty in the concentration of the solution. In such cases it is necessary to prepare a solution of approximate concentration as best we can, then standardize the solution against a reference material. A pure reference material that can be readily titrated is required for the standardization.
- Examples of activities that rely on appropriately prepared reagents include:
  - ✓ sample preparation
  - ✓ sample storage
  - ✓ mobile phases in liquid chromatography
  - ✓ dilution of cells such as bacteria or red blood cells

- ✓ titration of unknown samples
- ✓ Staining of specimens.

- In the modern laboratory many reagents can be purchased 'off-the-shelf' in a ready-to-use format. However, you may still be asked to make up solutions or even to test that the ready-to-use reagents are indeed at the correct concentration.

Self-Check -3	Written Test

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

Secondary standard solutions are used for analytical procedures before their concentration is determined true false

1. Which of the following is true about Primary standard solution?
   A. Free of impurities
   B. Stable in solid state and in solution
   C. Able to be accurately weighed
   D. Should be hygroscopic
2. Standardization is the process of determining the accurate concentration of a standard solution
   A. True B. false
3. Primary standard solution is a chemical solution that has the highest purity
   A. True B. false
4. Secondary standard solutions are solutions of lower purity and their concentrations are determined by comparison to primary standard solutions
   A. True B. false

Note: **Satisfactory rating - 5 points** **Unsatisfactory - below 5points**

Answer Sheet

Score = _____

Rating: _____

Name: _____ Date: _____

Short Answer Questions

Information Sheet-4	determining concentration of standard solution

4.1. Standardizing solutions

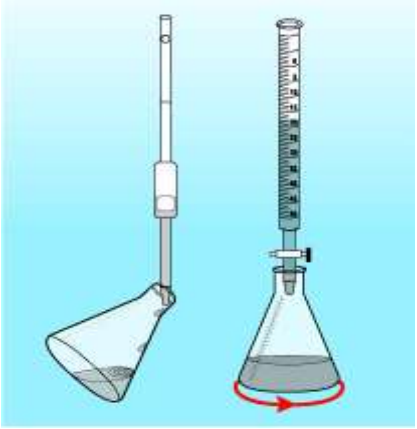
- The process of determining the unknown's concentration
- Why standardizing a solution?
  - ✓ To find the precise concentration of the solution.
  - ✓ To quantify the purity of chemicals or double check it.
- A volumetric analysis (standardization) often can be done through **titrations** between two different chemicals, usually an acid or a base

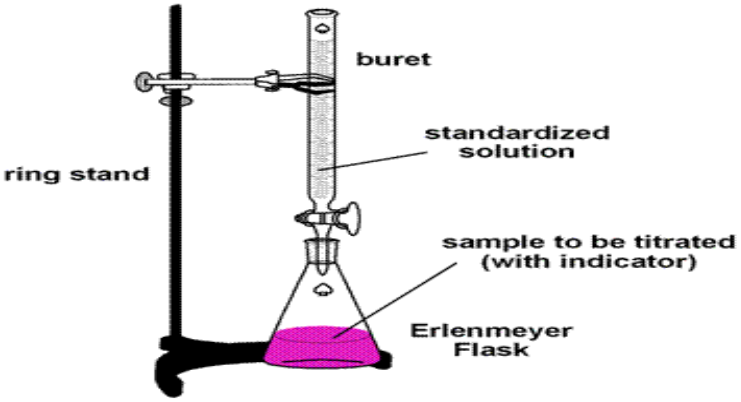
4.2. Titration

- Titrations are very valuable laboratory procedures, the main purpose of which is to determine the concentration of some type of unknown solution. The most common type of titrants is acids and bases. Concentrations of other types of solutions may also be calculated by means of titration, but this method is more advanced and beyond the scope of this unit. One can determine the concentration of a known acid or base in a solution and, from this, determine the pH of the unknown solution. This is very useful, especially in the analytical laboratory.
- In a titration, a solution of known concentration (titrant) is added to the solution of unknown concentration. This is done in such a way that the volume of solution that is added can be measured very accurately. The known solution should react with the unknown solution. For example, if the unknown solution is a base, the titrant should be an acid. The reaction is carried out to completion (until all of the unknown solution is reacted). The technician knows the reaction is complete when the solution changes colour, because of the addition of an indicator. By using stoichiometry, the technician can calculate how much of the unknown was present, and therefore calculate the concentration of the unknown solution.
- The simplest titrations are monoprotic acids and bases, such as:

$$NaOH(aq) + HCl(aq) \rightarrow NaCl(aq) + H2O(aq)$$

- Indicators are special chemicals that change colour, depending on the pH of the solution they are in. Indicators are a vital part of the titration process, since they signal the completion of the titration. Different indicators are used, depending on the pH range of the titration.
- The titrant should be (if possible) a strong acid or strong base to ensure a large pH change at the neutral point so that the indicator will change colour for a sharp end point.





pH Range	Indicator
0 - 1.6	Methyl Violet
2.9 - 4.0	Methyl Yellow
3.0 - 4.6	Bromophenol Blue
3.2 - 4.4	Methyl Orange
4.8 - 6.0	Methyl Red
5.5 - 8.0	Litmus
6.0 - 7.6	Bromothymol Blue
6.6 - 8.0	Phenol Red
8.2 - 10.6	Phenolphthalien
9.4 - 10.6	Thymolphthalien
10.0 - 12.0	Alizarin Yellow

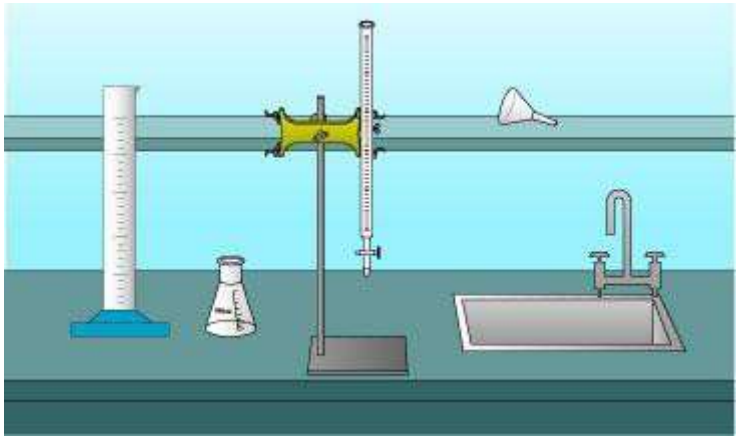
- The following diagrams show the different colours of a solution containing phenolphthalien, at stages doing a titration.

Acidic Solution

Endpoint

Basic Solution

4.3. Equipment used



- In a titration, a burette is used to deliver a measurable amount of a solution with a known concentration to a known volume of the solution with an unknown concentration contained in an Erlenmeyer flask. The Erlenmeyer flask is used because it can be swirl without spilling any of the contents.
- An indicator with a suitable pH range must be selected and added to the flask before the titration begins, so that the reaction completion may be detected.
- A graduated cylinder or bulb pipette is used t o measure a known volume of the solution with the unknown concentration to the flask. The unknown solution is then reacted with just enough titrant to react completely. The volume of titrant used is noted, and the technician uses the stoichiometry of the reaction and the SOP to do the calculations and determine the concentration of the unknown solution.

4.4. Titration- Reaction and Calculation

- Performing a titration is completely pointless if you do not understand the stoichiometry of the reaction. Understanding the reaction is the starting point for correct calculations.
- Look at the following reaction:

  Strong Acid + Strong Base→ Neutral solution (pH = 7.0)

  $HCl(aq) + NaOH(aq) \rightarrow NaCl(aq) + H_2O(l)$

- It is evident from the equation that one mole of HCl reacts with one mole of NaOH producing one mole of common salt and one mole of water as products. Thus a simple calculation:

- ✓ E.g. If 20 ml of 0.1M HCL reacts with exactly 20 ml of NaOH what is the molarity of the NaOH?

- A useful equation that can be used when calculations are not as simple as this example is:

$$C = \frac{n}{V} = \frac{m}{MrV}$$

- This equation can be modified to suit a number of different purposes.

A more complex calculation

Weak Acid + Strong Base → Weak Base + Water

(pH > 7.0; in these situations equilibrium will be achieved slightly above neutrality)

- ✓ For example, in a titration:10.25 mL of 0.25M NaOH neutralises 25 mL of a solution of CH3COOH. What is the molarity of the CH3COOH? This example is worked through in the following five steps.

Step1.

What is the reaction? Choose the correct reaction from those listed below.

a. 2NaOH(aq) + CH3COOH(aq) CH3COONa(aq) + H2O(l)
b. NaOH(aq) + CH3COOH(aq) CH3COONa(aq) + H2O(l)
c. NaOH(aq) + 2CH3COOH(aq) CH3COONa(aq) + H2O(l)
d. NaOH(aq) + CH3COOH(aq) CH3COOH2O(aq) + Na
e. NaOH(aq) + CH3COONa(aq) CH3COOH(aq) + H2O(l)

Step2.

In the previous reaction, how many moles of NaOH react with how many moles of CH3COOH? Choose the correct ratio from those listed below.

a. 2:1
b. 1:2
c. 1:1.5
d. 1.5:1
e. 1:1

Step3.

Calculate the number of moles that were present in the titrant NaOH.

$$C = \frac{n}{—}$$

V

Therefore n = C × V

n (NaOH) = C (NaOH) x V (NaOH) C (NaOH) = 0.25M

V (NaOH) = 10.25 mL = 0.01025 L n (NaOH) = 0.25 x 0.01025

= 0.00256 Moles

Step4.

Each mole of NaOH reacted with one mole of CH3COOH. Therefore there were 0.00256 moles of CH3COOH in the 25 mL volume.

Step5.

Calculate the number of moles of CH3COOH in one litre of the CH3COOH solution and this gives the molarity of the CH3COOH.

n (CH3COOH) = 0.00256 mol

V (CH3COOH) = 25 mL = 0.025 L

$$C\ (CH3COOH) = \frac{n\ (CH3COOH)}{V\ (CH3COOH)} = \frac{0.00256}{0.025\ L} = 0.10M$$

- ✓ NOTE: when there is a 1:1 molar ratio between the reactants the following equation can be used to simplify the calculation. C1V1 = C2V2
- ✓ Thus as long as you have three of the four values, the fourth can be calculated rapidly.
- ✓ One final example:

Strong acid + Weak BaseWeak Acid + Water

- (pH < 7.0; in these situations equilibrium will be achieved slightly below neutrality)
- In a titration, 72 mL of 2.2M NH4OH neutralises 15.74 mL of HCl (the strong acid titrant). Calculate the molarity of the HCl solution. Can you calculate the molarity of the HCl? The answer is **10.1**M, a concentrated acid indeed!

Step1. Assemble appropriate laboratory equipment

- Volumetric analysis involves the use of standard solutions and standardised solutions. A standard solution has an accurately determined concentration, whereas a standardised solution is one that has its concentration determined by titration against a standard solution.
- The chemical used to make up the standard solution must be cheap, readily available and stable, or it will not be suitable as a standard solution.

- The standardisation and use of volumetric solutions involves weighing, dilution and titration, using an appropriate end-point indicator.

Step2. Perform serial dilutions as required

- Serial dilutions are made in a sequential manner with the dilution factor between each sample in the series equal. The dilution factor may be any magnitude but the most often used are ½ and 1/10.

Step3. Standardize the solution to the required specified range and precision

A widely used procedure for preparing and standardizing solutions for titrations is to prepare a solution of approximately the desired concentration and then to titrate it against an accurately measured quantity of a compound of known purity. The compound of known purity is called a primary standard. Nearly all standard sodium hydroxide solutions can be standardized against a known mass of potassium hydrogen phthalate (KHP), $KHC_8H_4O_4$. You have collected the equipment and prepared the solutions you need to carry out the first titration to standardize the solution of 0.1M sodium hydroxide.

Self-Check -4	Written Test

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. Performing a titration is completely pointless if you do not understand the stoichiometry of the reaction.

   A. True B. False

2. _____ is/are determination of the concentration of a solution by comparing it with a standard solution?

   A. Calculation C. Titration

   B. Dilution D. Precipitation

3. _____ is/are special chemicals that change color, depending on the pH of the solution they are in.

   A. Indicators C. Staining day

   B. Molar solution D. Standard solution

Note: **Satisfactory rating - 5 points** **Unsatisfactory - below 5points**

Answer Sheet

Score = _____

Rating: _____

Name: _____ Date: _____

Information Sheet-5	Labeling and storage of standard solution

5.1. Labeling of Reagents

- Purpose of labeling. Workplace reagent labeling primarily serves two purposes, to:
  - ✓ identify the contents of the container
  - ✓ Warn of hazards.
- Reagent labeling is a complement to other sources of information such as the MSDS and other labeling requirements. It aims to assist with the safer use of a substance by identifying hazards likely to be associated with the use of the substance.
- **Responsibility**:- Chemical suppliers and employers have the primary responsibility to ensure that in the workplace, hazardous substances are correctly labeled.
- Employers must ensure that:
  - ✓ chemicals are appropriately and correctly labeled
  - ✓ libeling is not removed or modified
  - ✓ Decanted substances are labeled
  - ✓ There are prescribed measures for lost labels and unknown substances.
- **Workplace labels:** Hazardous substances must be labeled to show
  - ✓ contents
  - ✓ significant hazards
  - ✓ complementing other information (including MSDS information such as directions for use, first aid and emergency procedures)
  - ✓ Date opened.
- **Scope:** Workplace labels are required for containers containing:
  - ✓ hazardous substances
  - ✓ drugs and poisons
- And labeling is also required for:
  - ✓ decanted hazardous substances, not for immediate use
  - ✓ items (and substances) that can produce hazardous substances in use
  - ✓ Containers not cleaned.
- **Lost Labels:-** If the label is lost and the contents are unknown, the container should be:
  - ✓ marked ***CAUTION DO NOT USE: UNKNOWN SUBSTANCE***
  - ✓ stored in isolation until the contents can be identified

- ✓ If contents cannot be identified, the contents should be suitably disposed of (with advice from relevant authorities).

- **Replacement of labels:** A new label must be issued and placed on the container when:
  - ✓ the substance changes (including new ingredients)
  - ✓ new information becomes available that affects the information provided on the label (often instigated through a change of MSDS)
  - ✓ A new expiry date (if used) is required

Each solution is identified as either a primary or a secondary standard in the Laboratory Solutions Register.

Operation Sheet 1	Make serial dilution

To carry out successive 1/10 dilutions, 5 times

Step1. Set up seven clean tubes that hold about 20 mL as you will need to leave some room for mixing.

Step2. Carefully label each tube with the concentration and name of the reagent.

Step3. Leaving the first tube empty carefully pipette 9 mL of the diluent into each of the remaining five tubes

Step4. Carefully pipette 10 mL of the solution to be diluted into the first tube.

Step5. Using a 1 mL pipette carefully transfer 1 mL of the starting solution into the second tube.

Step6. Discard the pipette and using a fresh pipette, mix the contents in the second tube by pipetting up and down ten times. You now have a solution that is 1/10 the strength of the starting solution in the second tube.

Step7. Using the same pipette carefully transfer 1 mL from the second tube to the third tube and repeat step 6. You now have a 1/100 dilution of the starting solution in the third tube.

Repeat steps 6 and 7 until you have finished the dilution series.

Operation Sheet 2	Standardization of 0.1M Sodium Hydroxide Solution

Procedures

Preparation of a 0.1 Molar Sodium Hydroxide Solution

- This procedure describes the make-up of a sodium hydroxide (NaOH) solution to approximately molar concentration (0.1M).
- All volumetric glassware used in this procedure is Class A. Standard PPE should be worn when performing this work.

Step1- Locate the 6M sodium hydroxide stock solution in the laboratory store (**CAUTION: sodium hydroxide is very corrosive**)

Step2. Prepare an approximate 0.1M solution by diluting 20 mL of the 6M stock solution to 1 liter with distilled water. This can be safely done using a 50 mL measuring cylinder and a 1 liter graduated beaker.

Step3. Transfer the contents to a plastic bottle for storage and mix the contents well.

Preparation of Potassium Hydrogen Phthalate Standard Solution:

Step1. Place 50.1 g of KHP (Analytical Reagent Grade) in a weighing bottle and dry in an oven at 110 C for 2 hours. Store the KHP in a desiccator after this time.

Step2. Weigh the weighing bottle to 4 decimal places on the analytical balance.

Step3. Quantitatively transfer the KHP to a 250 mL volumetric flask.

Step4. Reweigh the weighing bottle and find the weight of the KHP to 4 decimal places, m (KHP).

Step5. To the volumetric flask, add 100 to 150 mL of distilled water (you may use a wash bottle) and swirl until the KHP dissolves.

Step6. Dilute to the mark with distilled water, replace the stopper and mix the contents by inverting and swirling the flask a number of times.

Step7. Calculate the molarity of the standard KHP solution, M (KHP), to 4 decimal places: M (KHP) = m (KHP) x 0.0196

Standardization of 0.1M Sodium Hydroxide Solution

Step1. Using a bulb pipette, quantitatively transfer a 25 mL aliquot of the standard KHP solution to a 250 mL Erlenmeyer flask. Wash down the inside of the flask with about 50 mL of distilled water delivered from a wash bottle.

Step2. Add 2 drops of phenolphthalein indicator and mix well.

Step3. Carefully fill a 50 mL burette with the prepared sodium hydroxide solution

Step4. Use a magnetic stirrer to stir the solution during the titration process.

Step5. Titrate the KHP solution to the first sign of a permanent pink end point (use a white tile beneath the Erlenmeyer flask during the titration). Record the titre to the nearest 0.01 mL.)

Step6. Repeat the titration (steps 1 to 3) until three titers are obtained that agree within 0.10 mL. Average these readings, T (NaOH).

Step7. Calculate the molarity of the sodium hydroxide solution M (NaOH) to 4 decimal places:

$$M(NaOH) = \frac{M(KHP) \times 25}{T(NaOH)}$$

Where T (NaOH) is the average of the 3 titres in mL.

LAP Test	Practical Demonstration

Name: _____ Date: _____

Time started: _____ Time finished: _____

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks within --- hour.

Task 1. Perform successive 1/2 dilutions, 5 times

Task 2. Perform Standardization of 0.1M Sodium Hydroxide Solution

List of Reference Materials

1- BOOKS

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