	SKILLS CENTER STANDARD OPERATING PROCEDURE	A BIOFIZZ PRODUCTON
Making Buffers **Module Hours: 4.0**	**Effective Date: 02/10/2024** **PRQs** **Lab Safety** **Pipetting**	**Revision # 2.0** **M. Stowell** **Checked: M. Guise, Z.** **Hazlett-Klein**

BACKGROUND

Most biological experiments require preparation of chemical solutions in their procedure. This is to ensure biomaterials are maintained at physiological conditions for measurements, as well as to alter the charge properties of biomolecules to allow efficient isolation using methods such as ion exchange chromatography or centrifugation. There are two principal methods for the preparation of these chemical solutions; by weight volume (w/v) and by volume volume (v/v). It is important

Figure 1: pH scale comparing some commons solutions and their observed pH values.
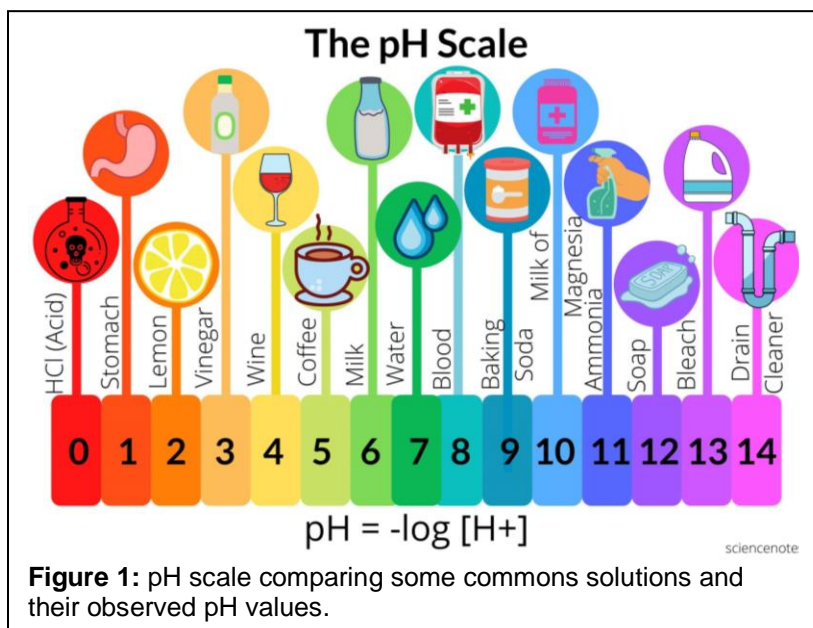
to recognize the difference between these methods and the manner by which such solutions are prepared. Many experiments involving chemicals call for their use in solution form at a defined pH, Figure 1. That is, two or more substances are mixed together in known quantities and the pH is adjusted to a desired value. This may involve weighing a precise amount of dry material or measuring a precise amount of liquid and adjusting the pH accordingly. Preparing solutions accurately improves an experiment's safety and reproducibility.

1. PURPOSE

The purpose of this procedure is to understand the basic principles of weighing solid, measuring liquids and adjusting the pH of solutions and to be able to conduct the process in the lab.

2. SCOPE

This procedure applies to qualified skills center users.

3. RESPONSIBILITY

3.1.   It is the responsibility of the user to understand and perform the procedure described in this document.

3.2.   It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure.

3.3.   It is the responsibility of the user to become trained on the procedure.

4. DEFINITIONS

4.1. Solvent - The substance which dissolves another to form a solution. For example, in a sugar and water solution, water is the solvent; sugar is the solute.

4.2. Solution - A mixture of two or more pure substances. In a solution, one pure substance is dissolved in another pure substance homogeneously. For example, in a sugar and water solution, the solution has the same concentration throughout, ie. it is homogenous.

4.3. Mole - A fundamental unit of mass (like a "dozen" to a baker) used by chemists. This term refers to a large number of elementary particles (atoms, molecules, ions, electrons, etc) of any substance. 1 mole is 6.02×10^{23} molecules of that substance. (Avogadro's number).

4.4. pH - The pH scale can tell if a liquid is more acidic or basic. The range of the pH scale is from 0 to 14 from very acidic to very basic. A pH of 7 is neutral. A pH less than 7 is acidic and greater than 7 is basic. pH is a log scale. Each whole pH value below 7 is ten times more acidic than the next higher value. For example, a pH of 4 is ten times more acidic than a pH of 5 and a hundred times (10 X 10) more acidic than a pH of 6. This holds true for pH values above 7, each of which is ten times more basic (also called alkaline) than the next lower whole value. An example would be a pH of 10 is ten times more alkaline than a pH of 9.

5. MATERIALS/EQUIPMENT

5.1.   Deionized water
5.2.   Solid Buffer Salts
5.3.   NaOH solution
5.4.   HCl solution
5.5.   Graduated cylinders
5.6.   Digital scale
5.7.   pH meter

6. COMMON LAB RECIPES

6.1. 1.5 M Tris-HCl (pH 8.8)
Tris base 181.7g in 1L H_2O, adjust pH to 8.8 using HCl.

6.2. 0.5 M Tris-HCl (pH 6.8)
Tris base 60.6g in 1L H_2O, adjust pH to 6.8 using HCl.

6.3. 10% (w/v) SDS
SDS (sodium dodecyl sulfate) 100g in 1L H_2O.

6.4. 2X SDS Protein Sample Buffer
1.25M Tris HCl (pH 6.8), 4% (w/v) SDS, 20% (v/v) glycerol, trace bromophenol blue. Note: For reducing conditions use 950 ul 2x SDS sample buffer plus 50µL 2-mercaptoethanol.

6.5. 10% (w/v) Ammonium Persulfate (APS)
10g ammonium sulfate in 100ml H_2O, aliquot into 1ml vials and stored at -20^oC until needed.

6.6. Acrylamide/ bisacrylamide stock solution
For 1L, 30% (w/v) acrylamide 300g, 0.8 % (w/v) bisacrylamide 8g. Add H_2O to the final volume and filter through the 0.22 µm membrane. Store at 4^oC in the dark.

6.7. Coomassie Brilliant Blue Staining Solution (1 liter)
Coomassie Brilliant Blue R250 2.5g, Methanol 400ml, Glacial Acetic Acid 100ml, H_2O 500 ml

6.8. High Methanol Destain Solution
For 1L, Methanol 400ml, Glacial Acetic Acid 100ml, H_2O 500ml

6.9. 4% (v/v) Glycerol Solution
For 1L, Glycerol 40ml, H_2O 960ml

6.10. Electrophoresis Buffer (TAE)
50X stock solution pH ~8.5, 242g Tris base, 57.1ml glacial acetic acid, 37.2g $Na_2EDTA.2H_2O$, Add distilled water to 1 liter.

6.11.   10X DNA Loading Buffer
20% (w/v) Ficoll 400, 0.1M Na_2EDTA, pH 8.0, 1.0% SDS, 0.25% bromophenol blue, 0.25% xylene cyanol

6.12.   50 mM $CaCl_2$ solution, ice cold

6.13.   0.1 M NaH_2PO_4 (monobasic)
Dissolve 13.8 g of $NaH2PO4 \cdot H2O$ (monobasic; m.w. = 138) in sufficient H_2O to make a final volume of 1 L.

6.14.   0.1 M Na_2HPO_4 (dibasic)
Dissolve 14.2 g of $Na2HPO4$ (dibasic; m.w. = 142) in sufficient H_2O to make a final volume of 1 L.

7. PREPARATION OF SOLUTIONS

7.1. <u>Solution 1</u>. Using percentage by weight (w/v)

7.2. Formula, the formula for weight percent (w/v) is: [Mass of solute (g) / Volume of solution (ml)] x 100.

7.3. Example. A 10% NaCl solution has 10g of sodium chloride dissolved in 100ml of solution.

7.4. Procedure Weigh 10g of sodium chloride. Pour it into a graduated cylinder or volumetric flask containing about 80ml of water. Once the sodium chloride has dissolved completely (swirl the flask gently if necessary), add water to bring the volume up to the final 100ml. Caution: Do not simply measure 100ml of water and add 10g of sodium chloride. This will introduce error because adding the solid will change the final volume of the solution and throw off the final percentage.

7.5. <u>Solution 2</u>: Using percentage by volume (v/v). When the solute is a liquid, it is sometimes convenient to express the solution concentration as a volume percent.

7.6. Formula. The formula for volume percent (v/v) is: [Volume of solute (ml)/ Volume of solution (ml)] x 100.

7.7. Example. Make 1000ml of a 5% by volume solution of ethylene glycol in water.

7.8. Procedure. First, express the percent of solute as a decimal: 5% = 0.05. Multiply this decimal by the total volume: 0.05 x 1000ml = 50ml (ethylene glycol needed). Subtract the volume of solute (ethylene glycol) from the total solution volume:

1000ml (total solution volume) - 50ml (ethylene glycol volume) = 950ml (water needed). Dissolve 50ml ethylene glycol in a little less than 950ml of water. Now bring the final volume of solution up to 1000ml with the addition of more water. (This eliminates any error because the final volume of the solution may not equal the calculated sum of the individual components). So, 50ml ethylene glycol/ 1000ml solution x100 = 5% (v/v) ethylene glycol solution.

7.9. <u>Solution 3</u>: Molar Solutions. Molar solutions are the most useful in chemical reaction calculations because they directly relate the moles of solute to the volume of solution.

7.10. Formula. The formula for molarity (M) is: moles of solute / 1 liter of solution or gram-molecular masses of solute / 1 liter of solution.

7.11. Example. The molecular weight of a sodium chloride molecule (NaCl) is 58.44, so one gram-molecular mass (=1 mole) is 58.44g. We know this by looking at

TABLE 1. VOLUMES OF 0.1 M SODIUM PHOSPHATE MONOBASIC (NaH_2PO4) AND 0.1 M DIBASIC (Na_2HPO4) THAT MUST BE COMBINED FOR THE DESIRED PHS VALUE.

PH FINAL	VOLUME (ML) 0.1 Na_2HPO_4	VOLUME (ML) 0.1 NaH_2PO_4
5.8	7.9	92.1
6.0	12.0	88.0
6.2	17.8	82.2
6.4	25.5	74.5
6.6	35.2	64.8
6.8	46.3	53.7
7.0	57.7	42.3
7.2	68.4	31.6
7.4	77.4	22.6
7.6	84.5	15.5
7.8	89.6	10.4
8.0	93.2	6.8

the periodic table. The atomic mass (or weight) of Na is 22.99, the atomic mass of Cl is 35.45, so 22.99 + 35.45 = 58.44. Or more simply, just look at the container the chemical is sold in or on the Material Safety Data Sheet (MSDS) for molecular weight. If you dissolve 58.44g of NaCl in a final volume of 1 liter, you have made a 1M NaCl solution, a 1 molar solution.

7.12. Procedure. To make molar NaCl solutions of other concentrations dilute the mass of salt to 1000ml of solution as follows: 0.1M NaCl solution requires 0.1 x 58.44 g of NaCl = 5.844g. 0.5M NaCl solution requires 0.5 x 58.44 g of NaCl = 29.22g. 2M NaCl solution requires 2.0 x 58.44 g of NaCl = 116.88g

7.13. <u>Solution 4</u>: Sodium Phosphate buffer. Some buffers are prepared by adding two equal concentration solutions of similar types together to reach the proper

buffering range and pH. The preparation of a sodium phosphate buffer is one example of this method.

7.14. Formula. 0.1M monobasic (NaH_2PO_4) and 0.1M dibasic (Na_2HPO_4) sodium phosphate solutions must be prepared first (See recipes above). To reach the desired pH of a 0.1M sodium phosphate buffer, follow recipe in **Table 1**.

7.15. Example. You need 100mL of a sodium phosphate buffer pH 6.7. Prepare 0.1M solutions of both mono- and di-basic sodium phosphate (See recipes). Add 565 ml 0.1 M NaH_2PO_4 to 435 ml 0.1 M Na_2HPO_4 in a sterile flask.

7.16. Procedure. Prepare 0.1M solutions of both mono- and di-basic sodium phosphate as directed in recipes. Determine desired pH of final buffer. Add appropriate amounts of mono- and di- basic sodium phosphate to final volume of 1L in sterile flask according to the the lookup table.

8. MEASURING PH

8.1. With test papers - Commercial calibrated test papers which are impregnated with pH indicators. The pH is determined by immersing the strip in the liquid to be tested and comparing its color with a standard color chart provided with the pH paper.

8.2. With digital readout via electronic meter - Electronic, bench top meters are available that read pH to resolutions of 0.001. These easy-to-use meters feature built-in memorized buffer values for quick, "automatic" calibration and automatic temperature compensation which eliminates errors in pH measurement caused by solution temperature variations.

8.3. Electronic pH meters require periodic calibration using standard value calibration solutions (sometimes called buffer solutions). Typical standards are pH 4.01, pH 7.01 and pH 10.01.

8.4. To calibrate the pH meter, begin by removing the protective cover from the electrode and immersing the electrode in a container containing the pH 7.0 buffer solution.

    8.4.1. Use the "pH/mv" button to change measurement modes; change to pH mode.

    8.4.2. Make sure to clear existing buffers (displayed as small numbers beneath pH value) when doing a new calibration. Select the "setup" button until "Clear Buffers" is displayed, and then select the "enter" button to clear the buffers.

    8.4.3. Now, once the pH has reached a steady number, select "standardize" and then "enter".

8.4.4. Make sure to spray electrode with DI water between measurements. Do not wipe electrode.

9. TROUBLESHOOTING

9.1. pH is not correct: Make sure that the pH meter is calibrated.

9.2. When titrating with HCl or NaOH I keep overshooting the target pH: Try using a lower concentration of HCl or NaOH as you titrate closer to the target pH.

9.3. I cannot weigh out very small quantities of buffer components: Make a more concentrated stock solution and then pipette the stock solution to achieve your target concentration.

9.4. Some solutions are too viscous to pipette: Consider using the molecular weight and density of the solution to weigh the appropriate quantity of solution.

10. REFERENCES

1. Short Protocols in Molecular Biology. 4th Edition. Editors Frederick M. Ausubel, Roger Brent, Robert E. Kingston, David D. Moore, J.G. Seidman, John A Smith and Kevin Struhl. John Wiley & Sons, Inc. 1999

2. 1 M sodium phosphate buffer (pH 6.0–7.2). Cold Spring Harbor Protocols. Retrieved from: http://cshprotocols.cshlp.org/content/2006/1/pdb.rec8303

3. Overview of acids, bases and the Henderson-Hasselbalch Equation. https://www.sciencedirect.com/science/article/abs/pii/B9780323034104500079

11. MODULE METHODS TASK

This task will test your ability to make several standard laboratory buffers with the correct pH.

1. Create a table showing the quantities (volume/ weight) of every component of the following buffers

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Buffer 1. 50 mL of 0.2M K_2HPO4, 100mM NaCl, pH 7.9
Buffer 2. 25 mL of 0.1M TRIS, 50 mM NaCl, pH 8.1
Buffer 3. 40 mL of 0.1M HEPES, 80mM NaCl, 1% glycerol, pH 7.4
Buffer 4. 50 ml of 0.1 M Sodium Phosphate buffer, pH 7.0 made from stock 0.1M NaH_2PO_4 and 0.1M Na_2HPO_4 solutions using Table 1.

2. Make each of the buffers listed above and store in a 50 mL conical tube, label them with your name, date and the buffer components. Paste a table Provide a listing the components and the various amounts in each buffer. Once you submit your MMT with your table of calculated components a proctor will test your buffers.