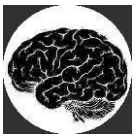
	SKILLS CENTER MODULE METHODS TASK ANSWER SHEET	**A BIOFIZZ**  **PRODUCTON**
Name: Iona Kelly	**Module:** Buffers and Stock Solutions	**Student ID:** XXXXXXXXX
Date: 5/14/25	**Class:** MCDB 1234/3456	

NOTES:

- You must use this form to submit your MMT as a word doc.
- Paste images, screen shots etc, from your MMT into this document
- Include Module title from the website
- In the space below, please copy/paste EACH MMT question and complete each prompt in full sentences with clean and clearly labelled data.
- Do not attach any data or responses that are not directly relevant to answering each individual MMT
- Please make sure to follow the rubric guidelines. **Universal module prompts** (those that are general to all modules) are indicated in bold font.
- Conclusions are required for EACH MMT. Please summarize the data used to draw conclusions. Discuss how the findings can be applied or relate to real-life scenarios. Clearly address the hypothesis, including discussing any sources of errors that might have influenced the results.

MMT QUESTIONS/ANSWERS

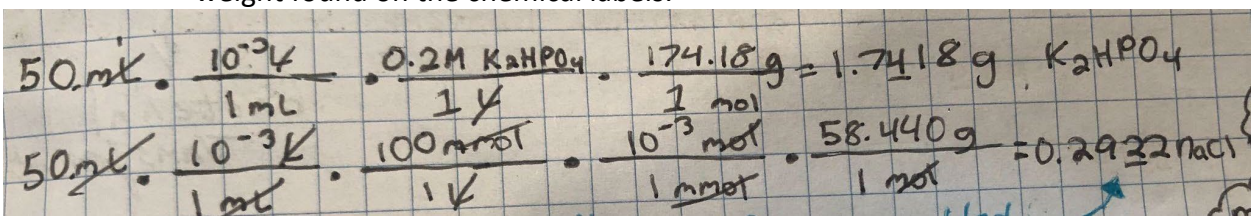
Planning/Organization)

This lab exercise involves preparing four standard laboratory buffers with the correct pH. This is valuable because the majority of biological experiments require the preparation of some variety of chemical solution. This makes preparing solutions an incredibly valuable skill in the lab setting.

Materials and Methods)

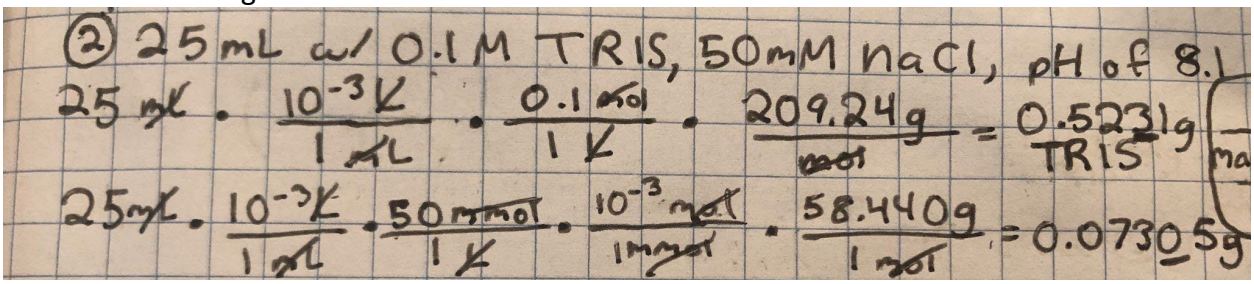
- Provide the step-by-step plan you used to execute the experimental procedure (must be organized in a way that the reader understands the logical flow of the lab).
- Show calculations where appropriate

- The first buffer to prepare is 50. mL of 0.2 M K_2HPO_4, 100 mM NaCl, pH 7.9
    - First, calculate the mass of K_2HPO_4 and NaCl necessary to prepare the solution using the provided final volume and molarities as well as the molecular/formula weight found on the chemical labels.



    - Then measure 50 mL of deionized water in a graduated cylinder and transfer to a beaker
        - Preferably a little bit less to account for added volume from other components (you can add more water at the end if you don't have the correct volume)
    - Measure the calculated masses of K_2HPO_4 and NaCl using a scale and weight boats
    - Mix the K_2HPO_4 and NaC in with the water in the beaker
    - Then calibrate the pH probe using the provided solutions at 4.0, 7.0, and 10.0 pH
        - Make sure to rinse the probe with deionized water before and after each time you use it and return it to the buffer solution when not in use
    - Measure the pH of the prepared solution and add drops of HCl or NaOH to lower or raise the pH to 7.9
        - Track the number of drops of each added
    - Label a 50 mL conical tube with your name, the date, and each of the components

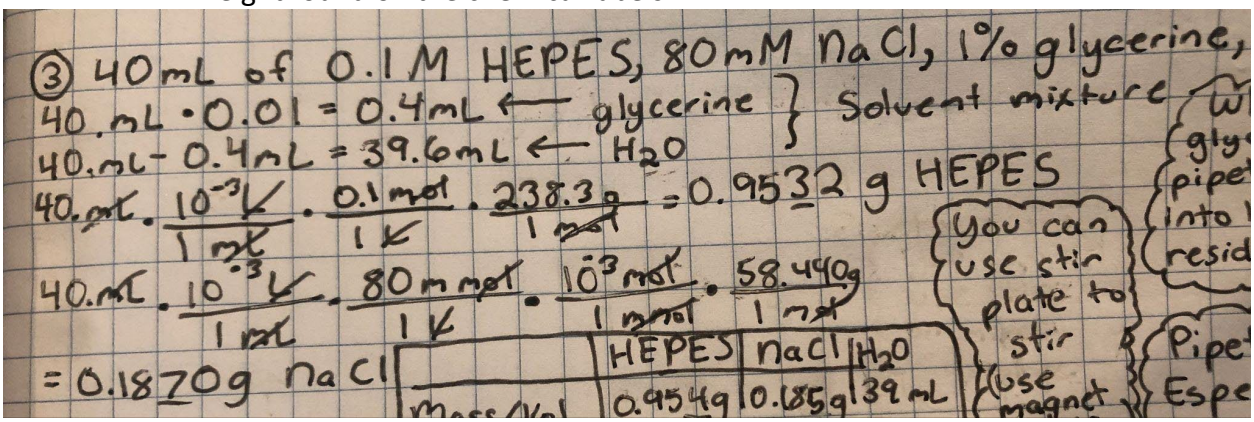
- The second buffer to prepare is 25. mL of 0.2 M TRIS, 50 mM NaCl, pH 8.1
    - First, calculate the mass of TRIS and NaCl necessary to prepare the solution using the provided final volume and molarities as well as the molecular/formula weight found on the chemical labels.



    - Then measure 25 mL of deionized water in a graduated cylinder and transfer to a beaker
        - Preferably a little bit less to account for added volume from other components (you can add more water at the end if you don't have the correct volume)

- Measure the calculated masses of TRIS and NaCl using a scale and weight boats
- Mix the TRIS and NaC in with the water in the beaker
- Then calibrate the pH probe using the provided solutions at 4.0, 7.0, and 10.0 pH
  - Make sure to rinse the probe with deionized water before and after each time you use it and return it to the buffer solution when not in use
- Measure the pH of the prepared solution and add drops of HCl or NaOH to lower or raise the pH to 8.1
  - Track the number of drops of each added
- Label a 50 mL conical tube with your name, the date, and each of the components

- The third buffer to prepare is 40 mL of 0.1 M HEPES, 80 mM NaCl, 1% glycerol, pH 7.4
  - First, calculate the volume of glycerol you need to prepare the solution by finding 1% of 40 mL
  - Then find the mass of HEPES and NaCl necessary to prepare the solution using the provided final volume and molarities as well as the molecular/formula weight found on the chemical labels.



  - Then measure the glycerol using a pipette and dispense into a graduated cylinder
    - Make sure to slowly aspirate the glycerol because it is very viscous and rinse the residual glycerol into the graduated cylinder using deionized water.
  - Then add deionized water to the graduated cylinder until the solution is a total of 40 mL, mix, and transfer to a beaker
    - Preferably a little bit less to account for added volume from other components (you can add more water at the end if you don't have the correct volume)
  - Measure the calculated masses of HEPES and NaCl using a scale and weight boats
  - Mix the TRIS and NaC in with the water in the beaker
  - Then calibrate the pH probe using the provided solutions at 4.0, 7.0, and 10.0 pH
    - Make sure to rinse the probe with deionized water before and after each time you use it and return it to the buffer solution when not in use

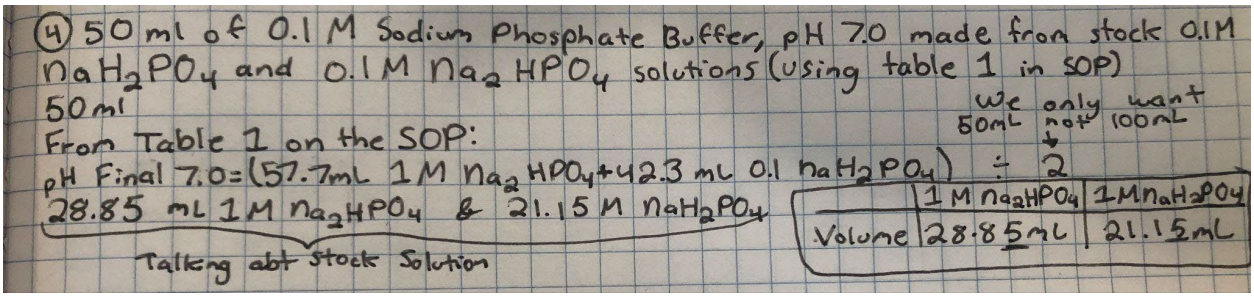
- Measure the pH of the prepared solution and add drops of HCl or NaOH to lower or raise the pH to 7.4
  - Track the number of drops of each added
- Label a 50 mL conical tube with your name, the date, and each of the components

- The fourth buffer to prepare is 50 mL of 0.1 M Sodium Phosphate buffer, pH 7.0 made from stock 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 solutions.
  - First, determine the volumes of stock 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 solutions necessary to obtain a buffer with a pH of 7.0 using Table 1 in the SOP

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

  - Divide each volume by two in order to prepare 50 mL of solution instead of 100 mL
  - Then convert the decimal portion of the volume to µL so that you can measure it with a pipette



- Measure out the volumes of each stock solution in a graduated cylinder to the nearest whole number of mL
- Use a pipette to accurately measure the remaining solution
- Transfer to a beaker and mix
- Then calibrate the pH probe using the provided solutions at 4.0, 7.0, and 10.0 pH
  - Make sure to rinse the probe with deionized water before and after each time you use it and return it to the buffer solution when not in use
- Measure the pH of the prepared solution to check that the pH is 7.0
- Label a 50 mL conical tube with your name, the date, and each of the components

Data Analysis & Discussion)

My first buffer of 50. mL of 0.2 M K_2HPO_4, 100 mM NaCl had the goal of pH 7.9 and my completed buffer measured at 7.91 pH which was within the accepted range.

My second buffer of 25. mL of 0.2 M TRIS, 50 mM NaCl had the goal of pH 8.1 and my completed buffer measured 8.16 after some correction, which was within the accepted range

My third buffer of 40 mL of 0.1 M HEPES, 80 mM NaCl, 1% glycerol had the goal of pH 7.4 and my completed buffer measured at 7.42 pH which was within the accepted range.

My fourth buffer of 50 ml of 0.1 M Sodium Phosphate buffer, made from stock 0.1M NaH2PO4 and 0.1M Na2HPO4 solutions had the goal of a pH of 7.1 and my completed buffer had a measured pH of 7.13 which was within the accepted range

Conclusion)

I was able to prepare my buffers effectively with the exception of the second buffer where there was an error with the second base because there was a calculation error where I used Bis-Tris which has a molecular weight of 209.24 g/mol instead of the TRIS base which has a molecular weight of 121.1 g/mole. This changed the calculation and ultimately caused issues with titrating the buffer to the correct pH. This is why I used so many drops of HCl and NaOH. This was a valuable skill to learn because buffers are used in various biochemical experiments and are used in many research procedures.

Sources Cited)

Stowell, M., Guise, M., & Hazlett, Z. (2023). Making Buffers. *Skills Center Standard Operating Procedure.* Pages 1-6.
https://skillscenter.colorado.edu/assets/files/Buffers_SOP.pdf

Please include the exact wording for each MMT prompt within the module, and answer each in order below:

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

- Buffer 1. 50 mL of 0.2M K2HPO4, 100mM NaCl, measured pH of 7.91

	K_2HPO_4	**NaCl**	**H_2O**	**HCl**	**NaOH**
Mass/Volume	1.7g	0.29g	50. mL	7 drops	1 drop

- Buffer 2. 25 mL of 0.1M TRIS, 50 mM NaCl, measured pH of 8.16
    - Note: Two drops of HCl and 4 drops of NaOH were added after procter check for correction

	TRIS	**NaCl**	**H_2O**	**HCl**	**NaOH**
Mass/Volume	0.52g	0.073g	24. mL	11 drops	19 drops

- Buffer 3. 40 mL of 0.1M HEPES, 80mM NaCl, 1% glycerol, measured pH 7.42

	HEPES	**NaCl**	**H_2O**	**HCl**	**NaOH**
Mass/Volume	0.95 g	0.19 g	39. mL	15 drops HCl	0 drops

- Buffer 4. 50 ml of 0.1 M Sodium Phosphate buffer, made from stock 0.1M NaH2PO4 and 0.1M Na2HPO4 solutions using Table 1, measured pH of 7.13

	1 M Na_2HPO_4	**1 M NaH_2PO_4**
Volume	28.85 mL	21.15 mL

11.2) 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.