pH Buffers Experiment Booklet

Thermo Scientific™ Orion™ Lab Star pH Bench Meter

thermo scientific


10.01
Buffer
pH 7.00
Buffer
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Buffer
thermo scientific
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-883
Measure (ESC)
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Lab Star PH111

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Pre-lab activities

Purpose

This document serves as an introduction on the correct procedures for pH electrode handling and meter calibration. It provides a framework on how to prepare and test phosphate buffers to determine buffering properties.

Background

Buffers

A buffer is a mixture of a weak acid and its conjugate base that resists pH changes when small amounts of acid or base are added. pH buffers stabilize the pH of biological systems and analytical systems. In the body, phosphates are an important buffer intracellularly and in tubules of the kidneys. The bicarbonate-carbonic acid buffer is another important buffer in the body. In the research lab, these buffers and others, such as Tris, HEPES, ACES, and more are commonly used.

A simple inorganic phosphate buffer consists of monobasic phosphate ion, $H_2PO_4^-$ (the weak acid) and dibasic phosphate ion, HPO_4^{2-}(the conjugate base). Together they form the phosphate buffer, and they balance the pH, protecting the living system or analytical system against large pH changes that can interfere with the proper function of the system.

When acid is added to the buffer, the conjugate base reacts to form the weak acid.

$$HPO_4^{2-} + H^+ \leftrightarrow H_2PO_4^-$$

When base (alkali) is added to the buffer, the weak acid reacts to form the conjugate base.

$$H_2PO_4^- + OH^- \leftrightarrow HPO_4^{2-} + H_2O$$

In this fashion, the buffer solution reacts with added acids or bases to buffer the pH and prevent the pH from changing significantly.

The Henderson-Hasselbalch equation describes the pH of a buffer solution using the acid ionization constant (pKa) of the acidic component and the ratio of the conjugate base and weak acid concentrations.

Henderson-Hasslebalch equation for buffers:

$$pH = pKa + \log\left(\frac{[\text{basic component}]}{[\text{acidic component}]}\right)$$

Note that the pH of the buffer solution is dependent on the pKa of the weak acid and the log of the concentration ratio of the basic component (conjugate base) to the acidic component (weak acid). If a small amount of acid is added to the buffer, it will combine with an equal amount of the base component to convert it to the acid form of the buffer. The change in the concentration ratio of basic/acidic components is small, so the pH change is small. The same effect applies when a small amount of base is added to the buffer. Thus, the solution is buffered to keep the pH steady. To some extent, the buffer pH is also concentration dependent.

The buffering capacity of the solution is related to both the concentration and the ratio. The amount of acid or base that can be added to the solution without causing a large change in pH is the buffering capacity. The higher the concentrations of acidic and basic components in the buffer, the more acid or base it can neutralize. The buffer capacity is highest when the ratio of basic/acid components is 1 and the H-H equation becomes pH = pKa. In general, the buffering capacity is suitable over a pH range of pKa +/- 1 pH

Buffer Capacity, B = n/Δ pH

Where n = number of mmoles of acid or base added per liter of buffer solution

n = conc acid or base (M) x vol acid or base (L) x 1000 (mmole/mol) ÷ volume of buffer (L)

Buffers may be prepared by different methods. One method involves creating separate solutions of the weak acid and the conjugate base. Amounts of the solutions are mixed together in the determined proportions to achieve the desired buffer pH. Another method involves creating one solution and titrating with acid or base to achieve the desired buffer pH. For example, a solution of the weak acid can be titrated with a strong base to convert some of the weak acid to the conjugate base until the desired pH is achieved and the solution is then buffered against undesired pH changes.

pH measurements

- When preparing and using pH calibration or biological buffers, it is important to have a good pH meter and electrode measuring system in your laboratory.
- Accurate, reliable, and quick pH readings depend on proper calibration, handling, maintenance, and storage of the electrode and meter system. Important factors include:
  - Storing the pH electrode in the proper electrode storage solution
  - Using the pH storage bottle correctly (if using a sealed storage bottle)
  - Opening the electrode fill hole and adding fill solution to the level of the fill hole every day before use (refillable electrodes only)
  - Using freshly poured, unexpired pH calibration buffers and using pH calibration buffers that will bracket the expected pH measurement range
  - Thoroughly rinsing the electrodes with generous amounts of purified water
  - Using a temperature sensor (automatic or manual) to correct for the effects of temperature on the calibration
  - Periodically maintaining the electrode, such as draining and refilling (refillable electrodes only) and cleaning

See Table 1 for best practices for pH measurement and electrode care.

Safety notes

- Observe the safety protocols described by your laboratory safety program.
- Review the Safety Data Sheets for the chemicals that you will be working with. Be proactive- understand the correct actions for spills, contact with your skin or eyes, and other possible routes of exposure.
- Typical personal protective equipment (PPE) may include: safety glasses, a laboratory apron or lab coat, and disposable gloves. Check with your laboratory supervisor for the proper personal protective equipment (PPE).

Table 1. Best practices for pH measurement and electrode care

Daily care

- A three-point calibration at pH 4, 7, and 10 is a good option for covering a wide range of pH readings. Check the expiration date on the pH calibration buffer bottles to ensure integrity.
- Pour a fresh portion of each calibration buffer into a clean, dry beaker. Don't use yesterday's calibration buffers for today's calibration.
- If the pH electrode is immersed in a sealed storage bottle, properly remove the electrode without creating a vacuum. Completely unscrew the top of the storage bottle first, then slide the pH electrode out of the storage bottle.
- If the electrode is refillable, uncover the electrode fill hole. Add the correct electrode fill solution to the level of the fill hole. Leave the fill hole uncovered while in use.
- Before and after every pH measurement and calibration point, rinse the pH electrode (body and glass bulb) and temperature sensor with generous amounts of reagent grade water (RGW) such as distilled, deionized or otherwise purified water. Shake electrodes gently to remove excess water droplets.
- Between measurements, temporarily store the rinsed electrodes in pH 7 calibration buffer.
- When done with all the measurements, rinse and store the pH electrode properly. When using a pH storage bottle, avoid pushing bubbles into the electrode. Unscrew the bottle cap and slide onto the electrode body. Then place the electrode in the storage bottle and screw the bottle into the cap.
- If the electrode is refillable, cover the fill hole before storage.

Monthly care

- Empty the electrode storage bottle and refill with a fresh portion of Thermo Scientific™ Orion™ pH Electrode Storage Solution (Cat. #: 910001).
- If the pH electrode is refillable, drain the electrode fill solution and replace with new solution. Use the correct fill solution for your electrode. Soak for 15 minutes or overnight in pH electrode storage solution.
- The above routine maintenance steps can help prevent crystallization, contamination, and mold in the storage bottle and in the electrode itself. Consult the pH electrode user guide for more details on storage and maintenance.

As needed

- Perform routine care when pH response has slowed, results are variable, or calibration and verification do not meet criteria.
- Clean the pH electrode if routine care has not restored performance.
- A warm (not hot) solution of 1% lab detergent is a good mild cleaner for routine use. Soak for 15 minutes, rinse well, then perform routine care.
- If needed, next try a general acidic cleaner, like Thermo Scientific™ Orion™ pH Cleaning Solution C for General Cleaning (Cat. #: 900023). Follow package instructions for use.
- Orion pH electrode cleaning solutions are also available for proteins, microbes, and oil & grease.

Equipment list

- Thermo Scientific™ Orion™ Lab Star pH Bench Meter Kit (LSTAR1115, LSTAR1116, LSTAR1117, or LSTAR1118)*
- Thermo Scientific™ Orion™ pH Calibration Buffers 4, 7, 10 (910104, 910107, 910110)
- Thermo Scientific™ Orion™ pH Calibration Buffers 6.86 or 9.18 (910686 or 910918)
- Thermo Scientific™ Orion™ Electrode Fill Solution (2 oz/30 mL bottle comes with the electrode)
- Thermo Scientific™ Orion™ pH Electrode Storage Solution (910001)
- Reagent Grade Water (RGW) – distilled, deionized, or purified water
- Plastic rinse bottle filled w/ RGW
- 5 100 or 150 mL beakers
- 12 150 mL beakers
- 2 500 mL beakers
- 2 100 mL graduated cylinders
- 2 1 mL or graduated 10 mL pipets and pipet bulb
- Waste beaker
- Lint free lab wipes
- Reagents & solutions
    - 0.050 M sodium phosphate dibasic or potassium phosphate dibasic solution
    - 0.050 M potassium phosphate monobasic or sodium phosphate monobasic solution
    - 0.1 M HCl (Acid)
    - 0.1 M NaOH (base)
- Calculator or spreadsheet
- Results sheets (found at the end of this document)

Pre-lab activity

Before moving forward, please complete the pre-lab activities (pH buffer calculations and questions) on pages 16-18 of this document. Once completed, you may proceed to the next section titled “Lab activities & experiments”.

*The Thermo Scientific™ Orion™ Lab Star pH Bench Meter Kit includes a pH electrode with built-in temperature sensor or pH electrode with separate automatic temperature compensation (ATC) probe (927007MD).

Lab activities & experiments

Lab activity – preparation of test solutions and buffers

Instructions

1. Gather supplies and prepare labware

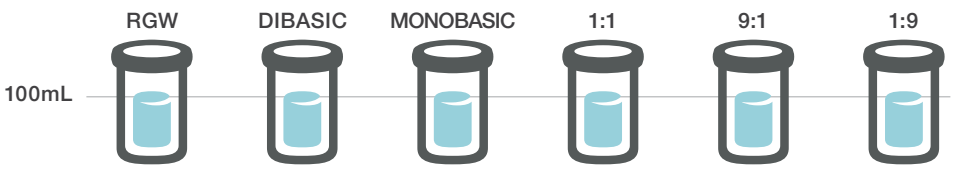
- Obtain 2 clean 500 mL beakers. Label one as “monobasic” and the other as “dibasic”.
  - Fill the “monobasic” beaker with ~ 300 mL of monobasic 0.05 M PO_4 solution.
  - Fill the “dibasic” beaker with ~ 300 mL of dibasic 0.05 M PO_4 solution.
- Obtain 2 clean 100 mL graduated cylinders. Label one pipet as “monobasic” and the other as “dibasic”.
- Obtain 2 clean pipets and a pipet bulb. Label one as “acid” and the other as “base”.
- Obtain 12 clean 150 mL beakers. Label the beakers according to the table below, with two beakers labeled for each test solution. Put aside the second set of labeled beakers for now.

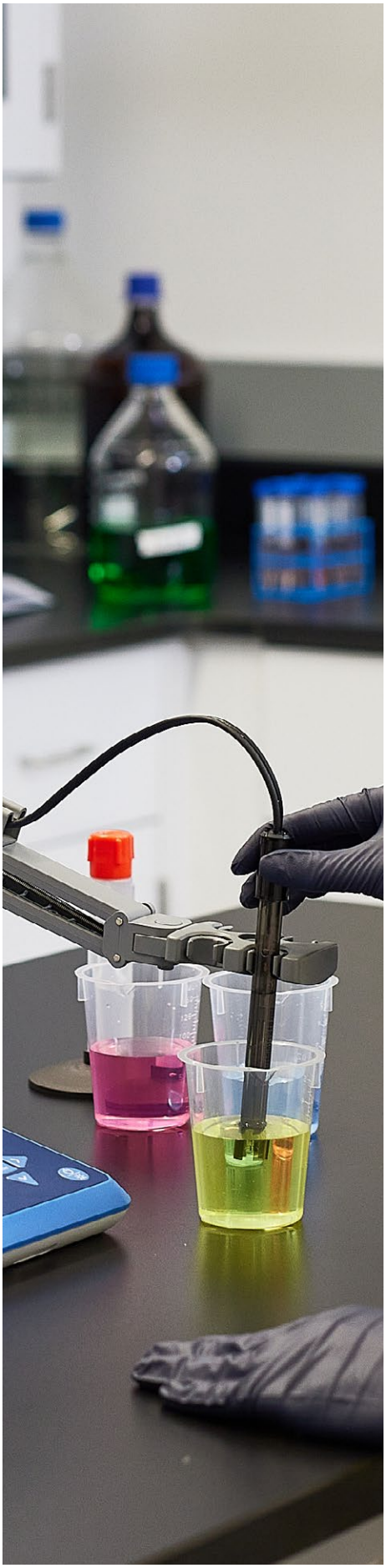
2. Prepare test solutions and buffers.

- Fill the 150 mL beaker labeled as “RGW” directly with RGW to the 100 mL line on the side of the beaker. Don’t use a graduated cylinder (unless it has been thoroughly rinsed with generous amounts of RGW first).
- Using Table 2 below as a guide, prepare a beaker of each of the five phosphate (PO_4) solutions by mixing the indicated volumes of dibasic and monobasic PO_4 together in the corresponding labeled beaker. Use the labeled graduated cylinders to measure the volumes. Swirl gently to mix or use a clean stir rod to mix each solution.
- Now there will be six beakers containing 100 mL of each of the six test solutions and six empty labeled beakers.
- Divide each solution between the two labeled beakers as follows: pour 50 mL of each test solution into the second labeled beaker.
- When finished, there will be two sets of beakers containing 50 mL of each of the six test solutions.
- Add “E3” to the labels on the second set of beakers and put them aside for use later in Experiment 3.

Table 2. Preparation of test solutions and buffers

Test solution	mL 0.05 M dibasic PO_4	mL 0.05 M monobasic PO_4	mL RGW
RGW	0	0	100
Dibasic PO_4	100	0	0
Monobasic PO_4	0	100	0
1:1 PO_4 buffer	50	50	0
9:1 PO_4 buffer	90	10	0
1:9 PO_4 buffer	10	90	0





Experiment 1 – calibration and verification of the pH system (meter and electrode)

Instructions

1. Check meter settings and adjust if needed.

- Access Settings from the Menu key.
- Scroll right to the pH resolution setting. Set to 0.01 pH.
- Continue to scroll right to the Read type. Set to Cont. If you want to automatically log the data, set to Auto instead.
- When done with settings, press Menu to return to the home screen.

2. Prepare the calibration buffers.

- Check the expiration dates on the pH calibration buffers to ensure that they are all still good. Use a fresh pour of each calibration buffer each day. This provides the most accurate calibration. Don't use yesterday's calibration buffers for today's calibration.
- Pour about 50 mL of the pH 4.01 calibration buffer into a clean 100 or 150 mL beaker.
- Pour about 50 mL of the pH 7.00 calibration buffer into a clean 100 or 150 mL beaker.
- Pour about 50 mL of the pH 7.00 calibration buffer into a clean 100 or 150 mL beaker. Label beaker as "interim storage"
- Pour about 50 mL of the pH 10.01 calibration buffer into a clean 100 or 150 mL beaker.
- Pour about 50 mL of the pH 6.86 or pH 9.19 calibration buffer into a clean 100 or 150 mL beaker. This will be used to verify the calibration, after it has been completed.

3. Connect the pH and temperature sensors to the meter.

- Ensure that the pH electrode is connected to the BNC connector on the back of the meter and the temperature sensor is connected to the ATC connector on the back of the meter.
- Note – if your pH electrode is a triode, the temperature sensor will be the second plug on the electrode cable.

4. Prepare the pH electrode.

- If the pH electrode is immersed in a sealed storage bottle, properly remove the electrode without creating a vacuum. Unscrew the top of the storage bottle first, then slide the pH electrode out of the storage bottle.
- If the electrode is refillable, uncover the electrode fill hole. Add electrode fill solution to the level of the fill hole. Leave the fill hole uncovered while in use.
- Place the pH electrode and the ATC (temperature sensor) into the electrode holder. If the pH electrode is a triode with an integrated ATC, there will not be a separate ATC.
- Rinse thoroughly with RGW and place the pH electrode into the interim storage beaker of pH 7 buffer. This prevents the glass sensing bulb from drying out and equilibrates the electrode to the buffer matrix.

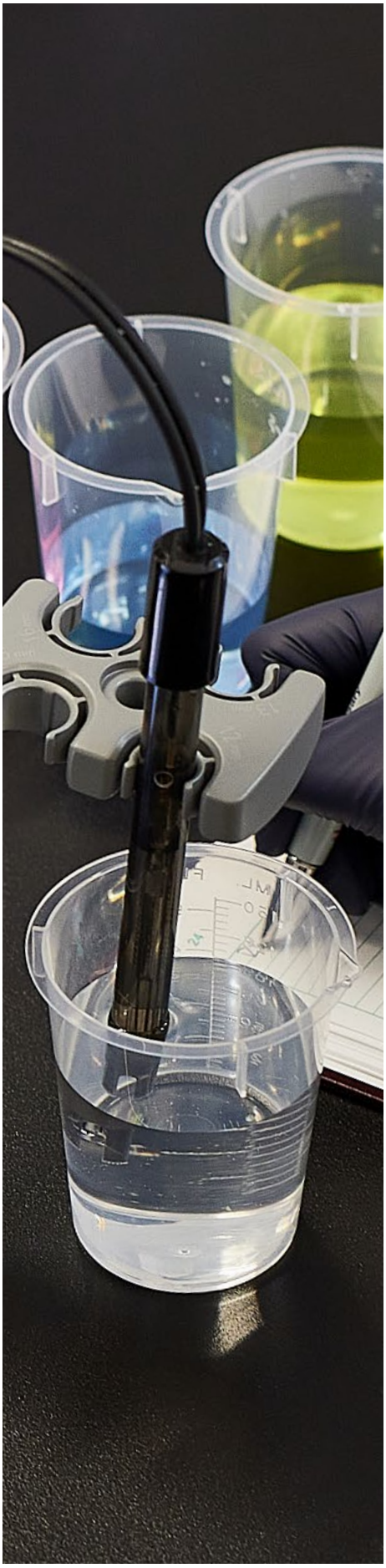


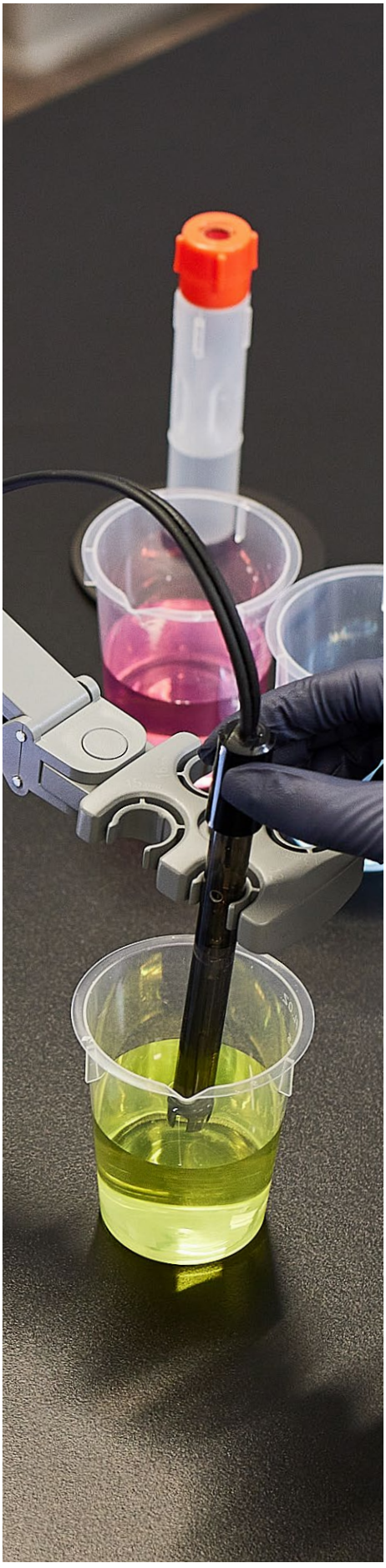
Table 3. pH electrode testing protocols

Protocols for best results – testing with the pH electrode

- **Rinse before every calibration point or measurement.**
  - Remove electrode from the interim storage beaker of pH 7 and place in the electrode holder with the ATC (if separate ATC). Position a waste beaker under the electrodes in the holder and use a squirt (rinse) bottle to rinse the pH electrode (body and glass pH bulb) and ATC with generous amounts of RGW. After rinsing, shake holder gently to remove excess water droplets. (Or gently touch a wiper to the water droplets – do not rub or apply pressure).
- **Swirl when taking a measurement or calibrating.**
  - Lower the holder into the beaker so that the electrodes (pH and ATC) are immersed about 1 inch into the solution.
  - Swirl the beaker around the electrodes for 10 seconds then allow the pH reading to stabilize.
  - Wait for the readings to stabilize. The meter displays a check mark when stable. Log or record the stable pH and temperature.
- **Rinse after every calibration point or measurement.**
  - Raise the holder and position a waste beaker under the electrodes. Rinse the electrodes with generous amounts of RGW from the squirt bottle, ensuring that the electrode bodies and pH glass bulb are completely rinsed. Shake gently to remove excess water droplets.
- **Store between measurements in pH 7 buffer.**
  - Temporarily store the pH electrode by lowering the clean electrodes into the interim storage beaker of pH 7 buffer. Do not allow the pH electrode glass sensing bulb to dry out.

5. Calibrate the meter and electrode.

- Review Table 3 “Protocols for Best Results – Testing with the pH Electrode”.
- Press the Cal button on the meter and follow the onscreen prompts to calibrate the pH system (electrode and meter) at pH 4, 7, and 10.
  - Remember to rinse with generous amounts of RGW before and after each calibration buffer.
  - Remember to swirl the beaker around the electrodes for 10 seconds and then let rest. Wait for the stable reading.
- When the calibration is completed, use the results sheet to record the average slope of the calibration as displayed on the meter. Evaluate against criteria:
  - Criteria – 95 to 100% slope (best); 92 to 102% (good); not 92 to 102% (failed).
  - If the slope fails criteria, take corrective action. Consult Table 1 for proper setup, usage, and care of electrodes. Consult Table 2 for protocols for best results. Then repeat the calibration for a better result.
  - If the slope meets good or best criteria, perform a calibration verification by measuring a pH 6.86 or 9.18 calibration buffer.
  - Record the verification pH and the temperature readings on the results sheet and evaluate your calibration against criteria.
    1. Consult the temperature table to find the expected pH at the measured temperature.
    2. Criteria – +/- 0.05 pH (best); +/- 0.10 pH (good); not +/- 0.10 pH (failed).
    3. If the verification fails criteria, take corrective action. Consult Table 1 for proper setup, usage, and care of electrodes. Consult Table 2 for protocols for best results. Then repeat the calibration and verification for a better result.
    4. If the verification meets good or best criteria, continue with the experiment.
  - After calibration and verification, remember to rinse and place into interim storage according to Table 3.



Experiment 2 – effect of acid on pH buffers and solutions

Instructions

1. Use the first set of prepared solutions (six beakers) for this experiment.

2. Recall the criteria for accuracy: +/- 0.05 pH (best); +/- 0.1 pH (good); not +/- 0.1 pH (failed).

3. Test RGW.

- Following the protocols from Table 3, take the pH and temperature readings of the RGW beaker.
- Wait for a stable reading or take a reading after 2 minutes, if not yet stable.
  - Record the readings in the results table.
  - If agreement between the expected pH and the measured pH is in range, move to the next step. If not good, rinse the pH electrode with generous amounts of RGW and prepare a new beaker of RGW for testing. Repeat the testing and compare the expected and measured pH values for agreement.
- Using your pipet labeled “acid”, add 1 mL of 0.1 N acid (hydrochloric acid, HCl) to the RGW beaker.
- Swirl the beaker around the electrodes for 10 seconds to mix, then allow the pH reading to stabilize. Take the pH and temperature readings.
  - Record the readings in the results sheet and answer the listed questions.
  - Use the buffer capacity equation on the results sheet to calculate the buffer capacity of the RGW. Record the value.

4. Test 1:1 buffer.

- Following the protocols from Table 3, wait for a stable reading, then take the pH and temperature readings of the 1:1 buffer beaker.
  - Record the readings in the results table and calculate the difference between the expected (calculated) pH at 20° C and the measured pH.
  - If agreement between the expected pH and the measured pH is best (+/- 0.05 pH) or good (+/- 0.10 pH), move to the next step. If failed, rinse the pH electrode with generous amounts of RGW and prepare a new beaker of 1:1 buffer for testing. Repeat the testing and compare the calculated and measured pH values for agreement.
- Add 1 mL of 0.1 N acid to the 1:1 buffer beaker. Swirl the beaker around the electrodes for 10 seconds to mix, then allow the pH reading to stabilize. Take the pH and temperature readings.
  - Record the readings in the results sheet.
  - Calculate the buffer capacity of the 1:1 buffer and record the value.
- Add another 1 mL of 0.1 N acid to the 1:1 buffer beaker and swirl as before. Take the pH and temperature readings. clean 100 or 150 mL beaker.
  - Record the readings in the results sheet.

5. Test 9:1 buffer.

- Following the protocols from Table 3, wait for a stable reading, then take the pH and temperature readings of the 9:1 buffer beaker.
  - Record the readings in the results sheet and calculate the difference between the expected (calculated) pH at 20° C and the measured pH.
  - If agreement between the expected pH and the measured pH is best (+/- 0.05 pH) or good (+/- 0.10 pH), move to the next step. If failed, rinse the pH electrode with generous amounts of RGW and prepare a new beaker of 9:1 buffer for testing. Repeat the testing and compare the calculated and measured pH values for agreement.
- Add 1 mL of 0.1 N acid to the 9:1 buffer beaker. Swirl the beaker around the electrodes for 10 seconds to mix, then allow the pH reading to stabilize. Take the pH and temperature readings.
  - Record the readings in the results sheet.
  - Calculate the buffer capacity of the 9:1 buffer and record the value.
- Add another 1 mL of 0.1 N acid to the 9:1 buffer beaker and swirl as before. Take the pH and temperature readings.
  - Record the readings in the results sheet.

6. Test 1:9 Buffer.

- Repeat the same steps for the 1:9 buffer as for the 9:1 Buffer and record the readings in the results sheet.

7. Test Dibasic solution.

- Following the protocols from Table 3, wait for a stable reading, then take the pH and temperature readings of the dibasic beaker.
  - Record the readings in the results sheet and answer the listed questions.
  - If agreement between the expected pH and the measured pH is in range, move to the next step. If not good, rinse the pH electrode with generous amounts of RGW and prepare a new beaker of dibasic solution for testing. Repeat the testing and compare the expected and measured pH values for agreement.
- Using your pipet labeled “acid”, add 1 mL of 0.1 N acid to the RGW beaker. Swirl the beaker around the electrodes for 10 seconds to mix, then allow the pH reading to stabilize. Take the pH and temperature readings.
  - Record the readings in the results sheet and answer the listed questions.
  - Use the buffer capacity equation on the results sheet to calculate the buffer capacity of the RGW. Record the value.
- Test Monobasic solution.
  - Repeat the same steps for the monobasic solution as for the Dibasic solution and record the readings in the results sheet.
- Answer the post-experiment 2 questions found at the end of this document. If running short on time, complete questions after the lab.

Experiment 3 – effect of base on pH buffers and solutions

Instructions

1. Set aside the six beakers that were used in Experiment 2.
2. Set aside the 0.1 N acid and the “acid” labeled pipet.
3. Use the second set of prepared solutions for this experiment. They are labeled as “E3”.
4. Use the 0.1 N NaOH base solution and the “base” labeled pipet.
5. Repeat Experiment 2, but make additions of 0.1 M of NaOH instead of acid. Record the results in the results table for experiment 3 and make calculations.

Wrap up and station clean up

Instructions

1. Properly place the pH electrode back into the storage bottle. To avoid introducing air bubbles into the electrode or junction, unscrew the bottle cap and slide onto the electrode body. Then place the electrode in the bottle and screw the bottle into the cap. If the electrode is refillable, cover the fill hole.
2. Dispose of the calibration and test solutions as indicated by the laboratory supervisor.
3. Clean your beakers, graduated cylinders, and other lab ware by rinsing 3x with tap water and 3x with RGW.
4. Alternately, clean lab ware as indicated by the laboratory supervisor.
5. Leave your workstation clean and dry, ready for the next person who will use it. It may be you!

Results & questions

Pre-lab activity – pH buffer calculations

Instructions

Calculate the expected pH of the 1:1 PO_4 buffer, the 1:9 PO_4 buffer and the 9:1 PO_4 buffer at 20 and 25°C using the Henderson-Hasslebalch equation and the listed pKa values found below. Use the results table below to record your results. Once you have recorded your results, answer the pre-lab activity questions.

Henderson-Hasslebalch equation for buffers:

$$pH = pKa + \log\left(\frac{[\text{basic component}]}{[\text{acidic component}]}\right)$$

pH = pKa + log (conc ratio of basic to acidic component)

Test solution	5° C	20° C	25° C	37° C
Monobasic phosphate pKa*	6.92	6.89	6.88	6.85
Optimal pH buffering range	+/- 1 pH unit of the pKa			

*At 0.05 M

Phosphate buffers

Calculate component ratios and expected pH.

Test solution	1:1 Buffer	1:9 Buffer	9:1 Buffer
Ratio	1/1 = 1	1/9 = _____	9/1 = _____
Expected pH at 20° C	6.89 + log (1) = _____	6.89 + log (_____) = _____	6.89 + log (_____) = _____
Expected pH at 25° C	6.88 + log (1) = _____	6.88 + log (_____) = _____	6.88 + log (_____) = _____

Pre-lab activity – questions

1. How many mmoles of acid are present in 1 mL of 0.1 N (0.1 M) HCl?

2. How many liters is 1 mL of volume?

3. We will prepare our buffers by mixing solutions of dibasic and monobasic phosphate. What is another method for preparing buffers?

4. How many mmoles of dibasic PO_4 are present in 50 mL of 1.0 M dibasic PO_4 solution?

5. How many mmoles of acid are present in 2.5 mL of 1 M acid?

6. If we added 2.5 mL of 1 M acid (H^+) to the dibasic PO_4 solution and converted that number of mmoles of dibasic to monobasic, how many mmoles of dibasic PO_4 will be left?

7. What would the new concentration ratio (dibasic: monobasic) of the solution be?

8. What would the concentration of dibasic phosphate be in the final solution?

9. What would the expected pH be?

10. Would this be a good buffer?

Experiment 1 – calibration and verification of the pH system (meter and electrode)

Instructions

Calibrate the pH equipment (meter and electrode) at pH 4, 7, and 10. Use the results table below to record your results.

	Result	Best	Good
Average slope	_____%	95 to 101%	92 to 102%
Measured pH – calibration verification		+/- 0.05 pH of expected	+/- 0.10 pH of expected
Measured temperature – calibration verification			
Corrective action needed?*	Yes / No	If yes, describe the corrective action.	

*If corrective action is needed, consult Table 1 for proper setup, usage and care of electrodes. Consult Table 2 for protocols to obtain best results. Then repeat calibration for a better result.

Expected validation buffer values	15° C	20° C	25° C	30° C
pH 9.18	9.28	9.23	9.18	9.14
pH 6.86	6.90	6.88	6.86	6.85

Borate Buffer (pH 9.18) pH Dependence: $\Delta pH/\Delta T = -0.010$ pH/°C
Phosphate Buffer (pH 6.86) pH Dependence: $\Delta pH/\Delta T = -0.0022$ pH/°C

Experiment 2 – effect of acid on pH buffers and solutions

Instructions

Capture pH readings and record the effect of acid additions and buffer capacity. Use the results table below to record your results and answer the experiment 2 questions. If running short on time, complete questions after the lab.

Test solution	Expected pH at 20° C	Measured pH*	Temp (°C)	Measured pH vs. expected (ΔpH)	pH After 1 mL Acid	pH now vs. starting pH (ΔpH)	pH after 2 mL Acid	pH now vs. starting pH (ΔpH)	Buffer capacity (mmole/L)
RGW	~5.5 to 8.0								
1:1 buffer									
9:1 buffer									
1:9 buffer									
0.05 M dibasic PO_4	~8.7 to 9.3								
0.05 M monobasic PO_4	~4.2 to 4.6								

* If the measured pH falls in range or the agreement between the expected pH and the measured pH is best (+/- 0.05 pH) or good (+/- 0.10 pH), move to the next step. If not, take corrective action as described in the lab protocol.

Buffer Capacity, B = n/Δ pH

Where n = number of mmoles of acid or base added per liter of buffer solution

n = conc acid or base (M) x vol acid or base (L) x 1000 (mmole/mol) ÷ volume of buffer (L)

Experiment 2 – questions

1. Did the pH reading in RGW stabilize quickly? Why or why not?

2. How does the buffer capacity of RGW compare to the other solutions?

3. What happened to the pH of the RGW when the acid was added?

4. What happened to the pH of the 1:1 buffer when the acid was added? How does that compare to what happened when acid was added to RGW?

5. What happens to the concentration of dibasic PO_4 (HPO_4^{2-}) in a solution when acid is added to a phosphate buffer?

6. What happens to the concentration of monobasic PO_4 (HPO_4^{-}) i in a solution when acid is added to a phosphate buffer?

7. Compare the pH of the 1:1 buffer after 1 mL of acid and after 2 mL of acid. Did it change much?

8. Adding acid changes the1:1 buffer ratio to a new ratio. What buffer ratio would lead to a new pH that is 1 pH lower than the starting pH? Use the Henderson-Hasslebalch equation for this.

9. What effect did the acid additions have on the 9:1 and 1:9 buffers? Was it same as for the 1:1 buffer?

10. Which of these two buffers stabilized the pH better against acid addition? Why was that?

11. What effect did the acid addition have on the dibasic and monobasic solutions? How did it compare to RGW? How did it compare to the buffers?

Experiment 3 – effect of base on pH buffers and solutions

Instructions

Prior to the experiment, complete the prediction questions. Capture pH readings and record the effect of base (alkaline) additions and buffer capacity. Use the results table below to record your results. Once you have recorded your results, answer the experiment 3 questions at the conclusion of the experiment.

Prediction questions

- Which solution do you expect to stabilize the pH best when alkali (base) is added?
- Which solution do you expect to do the second best job of resisting a pH change when alkali (base) is added?

Test solution	Expected pH at 20° C	Measured pH*	Temp (°C)	Measured pH vs. expected (ΔpH)	pH After 1 mL Acid	pH now vs. starting pH (ΔpH)	pH after 2 mL Acid	pH now vs. starting pH (ΔpH)	Buffer capacity (mmole/L)
RGW	~5.5 to 8.0								
1:1 buffer									
9:1 buffer									
1:9 buffer									
0.05 M dibasic PO_4	~8.7 to 9.3								
0.05 M monobasic PO_4	~4.2 to 4.6								

* If the measured pH falls in range or the agreement between the expected pH and the measured pH is best (+/- 0.05 pH) or good (+/- 0.10 pH), move to the next step. If not, take corrective action as described in the lab protocol.

Experiment 3 – questions

1. Which solution did the best job of resisting a pH change when acid was added?

2. Which was the second-best acid-resistant solution?

3. Why did these buffers work best?

4. Which solution did the best job of resisting a pH change when base was added?

5. Was this the same buffer that was the best when acid was added? Why or why not?

6. Which was the second-best base-resistant buffer?

7. Was the second-best base-resistant buffer the same as the second-best acid-resistant buffer? Why or why not?

_____

_____

_____

8. What happens to the concentration of dibasic PO_4 (HPO_4^{2-}) in a solution when base is added?

_____

_____

_____

9. What happens to the concentration of monobasic PO_4 (HPO_4^{-}) in a solution when base is added?

_____

_____

_____

10. If we had only monobasic PO_4 acid and base solution on hand, how would we prepare a 1:1 PO_4 buffer?

_____

_____

_____

11. Which solution had the least buffering capacity (acid and base) and why?

_____

_____

_____

12. Which solution did the best job of stabilizing the pH against both acid and base additions?

13. When preparing a PO_4 buffer solution, what buffer pH is best for the stabilizing the solution against both acid and base additions? How does that compare to the pKa of monobasic phosphate?

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