

BUFFER CALCULATIONS AND PREPARATION

Objective

To learn how to calculate reagents needed to prepare buffer solutions, prepare and check the pH of buffer solutions in the laboratory.

Safety

Do not ingest chemicals. Wash your hands prior to leaving lab. Clean up all spills immediately. Use the balances carefully and be careful not to spill chemicals in them. Do not move the balances.

Background

There are three definitions for acids and bases which you have covered in general chemistry. These include the Arrhenius (an acid is an H^+ donor and a base is an OH^- donor), Bronsted-Lowry (an acid is an H^+ donor and a base is an H^+ acceptor, e.g. NH_3), and Lewis (an acid donates a share in an electron pair and a base accepts a share in an electron pair) definitions. Strong acids and bases ionize completely. Strong acids include HNO_3 , H_2SO_4 (first proton only), HCl , HBr , HI , $HClO_4$. Strong bases include $LiOH$, $NaOH$, $RbOH$, $Mg(OH)_2$, $Ca(OH)_2$, $Sr(OH)_2$, and $Ba(OH)_2$. Adding a strong acid or a strong base to a salt creates weak acids and weak bases, respectively.

Water is an example of a solvent and a weak acid/base “amphoteric” solution as it can perform either function. It forms $1 \times 10^{-7} M H^+$ and OH^- in solution. This is the basis of the pH and pOH scales: by taking the $-\log$ of the H^+ and OH^- concentrations, we get the pH and pOH (both 7 in pure water). Since the concentrations of H^+ and OH^- are identical, the solution is neutral. Conversely, a solution is acidic if the $[H^+] > [OH^-]$ and the $pH < 7$ and is basic if the $[H^+] < [OH^-]$ and the $pH > 7$. The special equilibrium constant for water, K_w , is equal to $[H^+][OH^-]$ or 1×10^{-14} . The pK_w can be determined by taking the $-\log$ of the K_w or 14. Thus, the $pH + pOH = 14$.

Weak acids and bases dissociate $<10\%$ forming an equilibrium of the product and reactants all present in the final solution. Combining these with a conjugate acid or base (common ion salt), strong acid, or strong base creates a buffer.

A pH buffer is a solution that resists large changes in pH due to small additions of acid (H^+) or base (OH^-) (Arrhenius definition). It contains a mixture of a conjugate acid (H^+ donor) and a conjugate base (H^+ acceptor); usually a weak acid and a salt of its conjugate base or a weak base and a salt of its conjugate acid. Common examples include a solution of acetic acid (weak acid) and sodium acetate (salt of its conjugate base); or a solution of ammonia (weak base) and ammonium chloride (salt of its conjugate acid). Both conjugate components (weak acid, “HA;” weak base, “A $^-$ ”) must be present in comparable concentrations (within a factor of 10) to have a buffer system: $0.1 < [A^-] / [HA] < 10$. The sum of the buffer concentration is the sum of the concentrations of the conjugate components: **$[HA] + [A^-] = \text{concentration of common ion}$** . Buffers work best at a pH near the pK of the ionizing group and the pKa is often used in solving

pH problems with buffers. A useful equation for solving buffer problems is the Henderson-Hasselbalch equation:

$$\text{pH} = \text{pK}_a + \log([\text{A}^-] / [\text{HA}])$$

This lab is focused on practicing solving concentration and pH problems with strong and weak acids and bases, buffer solutions, and the effects of adding new components and disturbing the equilibrium and subsequently preparing buffer solution in the laboratory. The buffers prepared in this lab will be used for subsequent experiments involving chromatography, enzyme kinetics, electrophoresis, and protein characterization.

Example problems and solutions: Make sure you feel comfortable with these!

Calculate the pH of 0.35 M acetic acid. Weak acids and weak bases ionize < 5%. This is an equilibrium problem. Let x represent the number of moles or molarity ionized and the (initial concentration – x) to represent the amount of weak acid or base that remains in solution.

Ionization of Acetic acid: $\text{HCH}_3\text{COO}(\text{aq}) + \text{HOH}(\text{l}) \leftrightarrow \text{CH}_3\text{COO}^-(\text{aq}) + \text{H}_3\text{O}^+(\text{aq})$

Or: $\text{HCH}_3\text{COO}(\text{aq}) \leftrightarrow \text{CH}_3\text{COO}^-(\text{aq}) + \text{H}^+(\text{aq})$

I	0.35 M	0	0
C	-x	+x	+x
E	0.35-x	+x	+x

Forms H^+ so write $K_a = K_a = [\text{H}^+][\text{CH}_3\text{COO}^-] / [\text{HCH}_3\text{COO}] = 1.8 \times 10^{-5} = [\text{x}][\text{x}] / [0.35-\text{x}]$. If the value of the K_a is at least 100 times smaller than the concentration of the weak acid, ignore loss of x from 0.35 M and avoid solving the problem using the quadratic formula. $\text{x}^2 = 6.3 \times 10^{-6}$, take square-root of both sides, $\text{x} = 2.51 \times 10^{-3}$, solve for $\text{pH} = -\log[2.51 \times 10^{-3}] = 2.60$

Calculate the pH of 0.35 M sodium acetate. This is an equilibrium problem of the pH of a salt of a weak acid.

Ionization of Acetate anion: $\text{CH}_3\text{COO}^-(\text{aq}) + \text{HOH}(\text{l}) \leftrightarrow \text{CH}_3\text{COOH}(\text{aq}) + \text{OH}^-(\text{aq})$

I	0.35 M	0	0
C	-x	+x	+x
E	0.35-x	+x	+x

Forms OH^- so find $\text{pK}_b = 14 - \text{pK}_a (4.74)$, so $K_b = 10^{-\text{pK}_b} = 5.56 \times 10^{-10}$. Then solve $5.56 \times 10^{-10} = [\text{OH}^-][\text{CH}_3\text{COOH}] / [\text{CH}_3\text{COO}^-] = [\text{x}][\text{x}] / [0.35-\text{x}]$. $\text{x} = [\text{OH}^-]$, solve for pOH then pH as in the previous example. You should find a pH of 9.14.

Calculate the pH of a solution which is 0.35 M acetic acid and 0.35 M sodium acetate. This is an equilibrium problem of the pH of a buffer which contains a weak acid or weak base and their salt which can product the conjugate base or conjugate acid, respectively. These have initial concentrations in the reactants and products.

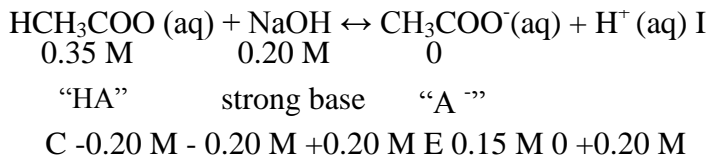
$\text{HCH}_3\text{COO}(\text{aq}) \leftrightarrow \text{CH}_3\text{COO}^-(\text{aq}) + \text{H}^+(\text{aq})$

E	0.35 M	0.35 M	0
"HA"		"A"	

Solve for the pH using the Henderson-Hasselbalch equation: $\text{pH} = \text{pK}_a + \log [\text{A}^-/\text{HA}]$, $\text{pH} = 4.74 + \log (0.35 / 0.35)$, $\text{pH} = 4.74 + 0$, $\text{pH} = 4.74$

Calculate the pH of a solution resulting from initial concentrations of 0.35 M acetic acid and 0.20 M NaOH. The equilibrium is changed by the addition of a strong acid or strong base. Determine how much, if any weak acids or weak bases are left and if there is excess strong acid

or strong base. If the strong acid or strong base is completely consumed, use the Henderson-Hasselbalch equation to compute the pH. If the strong acid or strong base is in excess (not completely neutralized), compute the pH using $\text{pH} = -\log [\text{H}^+]$ or $\text{pOH} = -\log [\text{OH}^-]$ and $14 - \text{pOH} = \text{pH}$. The strong bases (e.g. NaOH) will react with weak acids (e.g. HCH_3COO) and the conjugate bases (e.g. CH_3COO^-) will react with strong acids (e.g. HCl). Example:



Alternatively, solve the system of equations $\text{pH} = \text{pK}_a + \log ([\text{A}^-] / [\text{HA}])$ and $[\text{A}^-] + [\text{HA}] =$ concentration of common ion. Here, common ion = 0.35 M, $\text{pK}_a = 4.74$, and the common ion is initially in the acid ($[\text{A}^-]$) form. So $[\text{A}^-]$ was 0.35 M, but the added base will turn it into $[\text{A}^-] = 0.15 \text{ M}$ and $[\text{HA}] = 0.2 \text{ M}$. Then, use Henderson-Hasselbalch: $\text{pH} = 4.74 + \log (0.15/0.2) = 4.62$.

The last step is the “reality check”, which is perhaps the most important step. Above, we saw that 0.35 M acetic acid has a pH of 2.60. To that solution, we added base (NaOH). Our computed pH was higher than 2.60, which is consistent with the effects of adding base. Always realities check your answers!

Prepare a 100 mL of 0.20 M acetate buffer, pH 4.40 from sodium acetate trihydrate ($\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$, MW = 136 g/mol), available 0.500 M HCl and 0.500 M NaOH.

Calculate how much salt(s) or solution and how much HCl or NaOH to add to prepare each of the following buffer solutions. Use the pK_a closest to the desired pH given for your calculations. Then write a recipe for each.

In preparing buffers, first determine the number of moles of the common ion you have (e.g. acetate). Determine total moles of common ion: $0.20 \text{ mol/L} \times 0.100 \text{ L} = 0.0200 \text{ mol}$ acetate which is equal to the total acetate to be used for the production of weak acid and the rest that remains as salt so $\text{HA} + \text{A}^- = 0.0200 \text{ mol}$ and $\text{A}^- = 0.0200 \text{ mol} - \text{HA}$

Then determine the mass of solid needed from the source given: $0.0200 \text{ mol acetate} \times 136 \text{ g/mol}$ $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O} = 2.72 \text{ g NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$.

Then determine the ratio of $[\text{A}^-]/[\text{HA}]$ using the Henderson-Hasselbalch equation remembering that the $\text{A}^- = 0.0200 \text{ mol} - \text{HA}$: $4.40 = 4.74 + \log [(0.0200 - \text{HA})/\text{HA}]$, ratio $\text{A}^-/\text{HA} = 0.457 = (0.0200 - \text{HA})/\text{HA}$ so $0.457 \cdot \text{HA} = 0.0200 - \text{HA}$, add HA to both sides: $1.457\text{HA} = 0.0200 \text{ mol}$, $\text{HA} = 0.0137 \text{ mol}$ and $\text{A}^- = 0.0063 \text{ mol}$

Determine if you need to add HCl or NaOH to create the buffer. In this case, you weighed out the solid salt, sodium acetate trihydrate so you need to create some acetic acid. To do this you must add some HCl to the salt. How much? $\text{HA} = 0.0137 \text{ mol}$ so $0.0137 \text{ mol} \times 1 \text{ L}/0.500 \text{ mol HCl} = 0.0274 \text{ L HCl} = 27.4 \text{ mL HCl}$.

So the final recipe: Weight out 2.72 g $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$ on an analytical balance, add to a 100.0 mL volumetric flask, dissolve in ~25 mL deionized water to dissolve the solid, add 27.4 mL of 0.500 M HCl add more deionized water to the line on the volumetric flask and then check the

pH.

Finally, reality checks. You are adding acetate, which should have a high pH. So you need to add a strong acid (HCl) to reduce the pH. If you add more strong acid than the weak base, the pH will not be in the buffering range (and will get very low), so you need to add fewer moles of HCl than the total moles of common ion. Everything checks out!

Materials

- Various variable volume micropipettes (10 μ L, 100 μ L, 1000 μ L)
- Various constant volume micropipettes (5 μ L, 25 μ L, 50 μ L)
- Volumetric flasks
- Balance
- Weigh Boats
- Deionized water and water bottles
- Salts (Tris base, sodium acetate, phosphoric acid or phosphate salts)
- Acids and bases (0.1 M and 1.0 M and 12 M HCl & 0.1 M and 1.0 M NaOH)
- Tips
- Erlenmeyer flasks
- pH meters

Procedure:

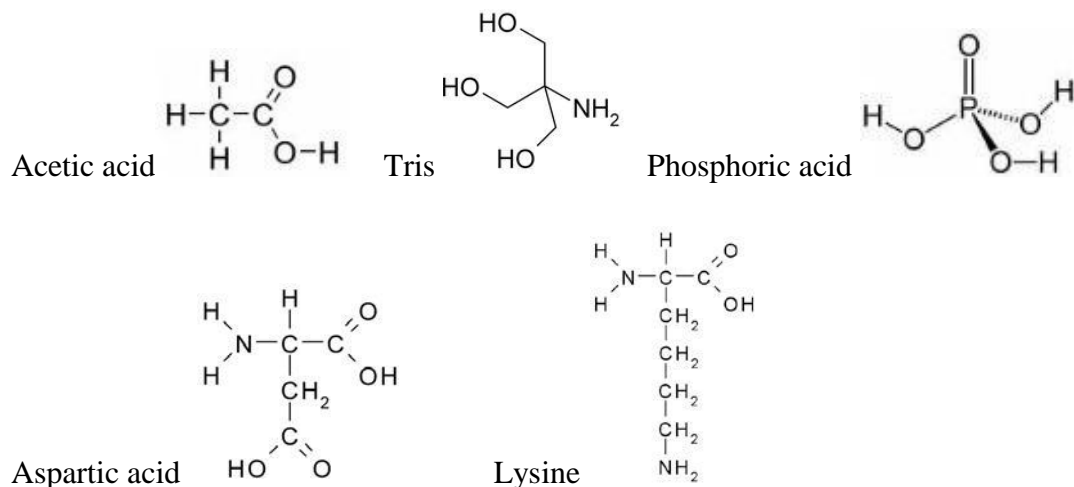
Part A.: Complete the pH of the following buffer problems. (Consult your Biochemistry textbook or a General Chemistry textbook as necessary to review pH calculations.)

Useful equations and information: $\text{pH} = -\log[\text{H}^+]$, $10^{-\text{pH}} = [\text{H}^+]$, $\text{pOH} = -\log[\text{OH}^-]$, $\text{pK}_a = -\log K_a$, $\text{pK}_b = -\log K_b$, $\text{pK}_w = -\log K_w$, $\text{pH} = \text{pK}_a + \log [\text{A}^-/\text{HA}]$, in neutral water: $[\text{H}^+] = [\text{OH}^-] = 1 \times 10^{-7} \text{ M}$, $K_w = [\text{H}^+][\text{OH}^-] = 1 \times 10^{-14} \text{ M}$, $\text{pH} + \text{pOH} = \text{pK}_w = 14$, $K_a * K_b = K_w$

pKa values: phosphate: 1.8, 6.90, 12.5; acetate: 4.74; tris: 8.00; aspartate: 2.00, 3.90, 10.0; lysine: 2.20, 9.20, 10.8

1. pH of 0.45 M HNO_3
2. $[\text{H}^+]$ of solution pH = 3.23
3. pH of 0.45 M KOH
4. pH of 0.45 M acetic acid
5. pH of 0.45 M sodium acetate
6. pH of a solution which is 0.45 M acetic acid and 0.45 M sodium acetate
7. pH of a solution resulting from initial concentrations of 0.45 M acetic acid and 0.2 M NaOH
8. pH of a solution resulting from initial concentrations of 0.45 M sodium acetate and 0.2 M HCl
9. Prepare a 100. mL of 0.45 M acetate buffer, pH 4.75 from sodium acetate trihydrate ($\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$), 0.500 M HCl and 0.500 M NaOH
10. Prepare 500. mL of 0.15 M Tris buffer, pH 8.25 from Tris monohydrochloride (Tris-HCl) ($\text{C}_4\text{H}_{11}\text{NO}_3\text{ClH}$), 0.400 M HCl and 0.350 M NaOH

Part B: Circle the acidic protons or add the basic ionizable proton(s) in each of the following molecules.



Part C: Preparing the Buffer Solution

1. Prepare 100 mL of 0.100 M phosphate buffer, pH 7.70 from solid KH_2PO_4 and available (0.1 M and 1.0 M) HCl and NaOH solutions. Show all of your work in your lab report.
2. Prepare 100 mL of 0.100 M phosphate buffer, pH 6.95 from solid KH_2PO_4 and solid K_2HPO_4 . Show all of your work in your lab report.

Part D: Checking the pH using a pH meter

1. The pH meter should be standardized with pH 4, 7, and 10 standards prior to use. Your instructor will demonstrate the use of the Vernier pH meter.
2. Check the pH values for your 2 buffers. The pH should be within 0.1 pH units of the theoretical value. If it is not, try preparing the buffers again.

Part E: Effects of Adding Acid or Base

1. To 9 mL of water, add 1 mL 0.1 M HCl. Record the pH.
2. To 9 mL of water, add 1 mL of 0.1 M NaOH. Record the pH.
3. To 9 mL of each of your two buffers, add 1 mL 0.1 M HCl. Record the pH for each.
4. To 9 mL of each of your two buffers, add 1 mL 0.1 M NaOH. Record the pH for each.

Part F: Effects of Dilution

1. To 1 mL of each of your two buffers, add 9 mL water. (Note: this is a 1:10 dilution). Record the pH.
2. Add 1 mL 0.1 M HCl to your diluted buffers. Record the pH for each.
3. Add 1 mL 0.1 M NaOH to your diluted buffers. Record the pH for each.

Questions/Analysis

Calculate the expected pH values for the measurements in Part E. and Part F. Are the values what you expected? Explain your answer.