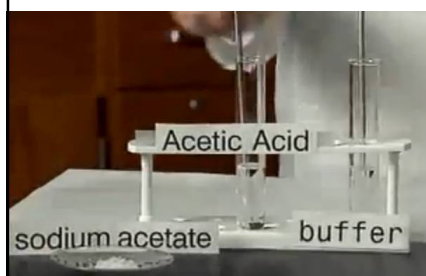


Preparation of Buffers



Preparation of Buffers

➤ Example: what are the concentrations of HOAc and OAc⁻ in 0.2M acetate buffer, pH=5, $k_a=1.7 \times 10^{-5}$, $pK_a=4.77$?

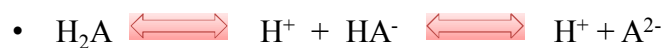
- Concentration of the buffer = $[HA] + [A^-]$
- $0.2 = [CH_3COOH] + [CH_3COO^-]$
- Assume $[A^-] = y$ thus, $[HA] = 0.2 - y$
- $pH = pK_a + \log \frac{[A^-]}{[HA]}$
- $5 = 4.77 + \log \frac{y}{0.2-y}$
- $\text{anti log } 0.23 = \frac{y}{0.2-y}$
- $1.7 = \frac{y}{0.2-y}$
- $y = 0.34 - 1.7y$
- $y = \frac{0.34}{2.7}$
- $y = 0.126 \text{ molar.}$
- Since $[HA] = 0.2 - y$ then $[HA] = 0.2 - 0.126 = 0.074 \text{ molar.}$

Preparation of Buffers Continue

➤ Example: Describe the preparation of 3liters of 0.2M acetate buffer, pH=5 starting from solid sodium acetate trihydrate MW=136 and a 1M solution of acetic acid, pka=4.77?

- From the previous example $[HA] = 0.074$ molar, $[A^-] = 0.126$ molar
- No. of moles of **HA** = $M * V_{(L)}$
- $= 0.074 * 3 = 0.222$ mole
- From HA molarity (1M), $M = \text{No. of moles} / V_{(L)}$ thus,
- $V = \text{No. of moles} / M = 0.222 / 1 = 0.222$ L
- No. of moles of **A⁻** = $M * V_{(L)}$
- $= 0.126 * 3 = 0.378$ mole
- No. of moles **A⁻** = w_{t_g} / MW
- $0.378 = w_{t_g} / 136$
- $Wt = 51.4$ g
- To prepare 3L buffer, dissolve 51.4g of the solid sodium acetate trihydrate with 222ml of the 1M acetic acid and make up the volume to 3 L with water.

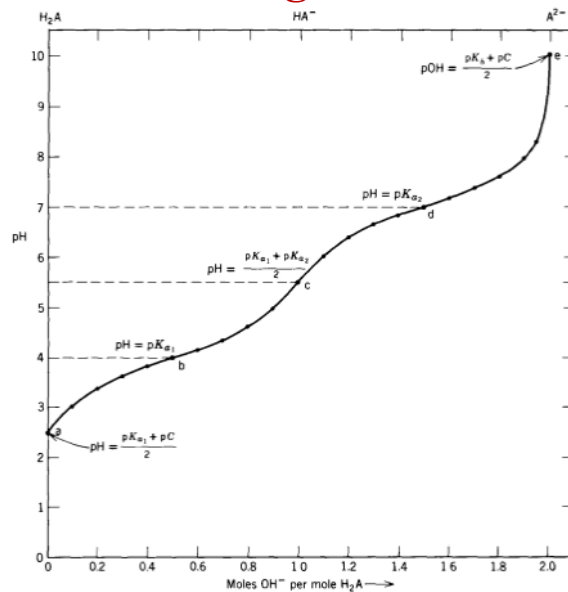
Polyprotic Acids



$$K_{a1} = \frac{[H^+][HA^-]}{[H_2A]} \quad K_{a2} = \frac{[H^+][A^{2-}]}{[HA^-]}$$

- Polyprotic acid ionizes in a successive steps.
- For most common weak diprotic acids, $K_{a1} > K_{a2}$
- The pH of a solution of H_2A is established almost exclusively by the 1st ionization.
- $pH = pK_{a1} + \log \frac{[HA^-]}{[H_2A]}$
- $pH = pK_{a2} + \log \frac{[A^{2-}]}{[HA^-]}$

Titration of a weak diprotic acid with strong base



Dissociation of a H_3PO_4



$$K_{a1} = \frac{[H^+][H_2PO_4^-]}{[H_3PO_4]} \quad K_{a2} = \frac{[H^+][HPO_4^{2-}]}{[H_2PO_4^-]} \quad K_{a3} = \frac{[H^+][PO_4^{3-}]}{[HPO_4^{2-}]}$$

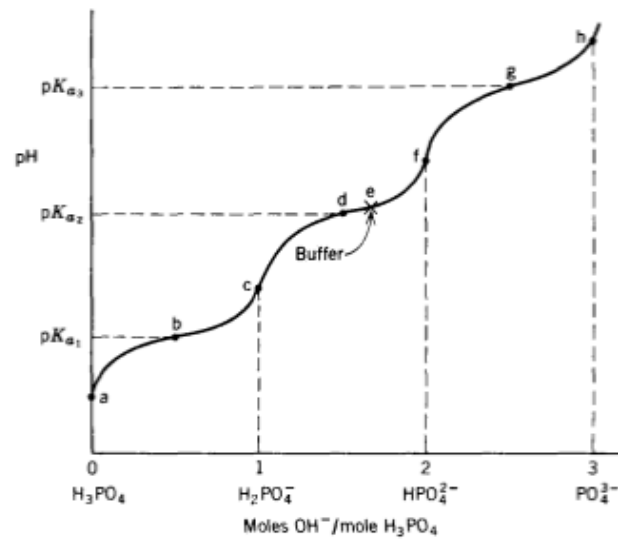
• For H_3PO_4 : $K_{a1} > K_{a2} > K_{a3}$

$$\bullet \quad pH = pK_{a1} + \log \frac{[H_2PO_4^-]}{[H_3PO_4]}$$

$$\bullet \quad pH = pK_{a2} + \log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

$$\bullet \quad pH = pK_{a3} + \log \frac{[PO_4^{3-}]}{[HPO_4^{2-}]}$$

Titration of a H_3PO_4 with strong base



Examples of Buffers

Table 2

Common buffers	pK_a
Phosphoric acid	2.15
Malonic acid	2.85
Formic acid	3.75
6-Aminocaproic acid	4.37
Acetic acid	4.76
MES	6.21
Triethanolamine	7.76
TRIS	8.06
Tricine	8.26
Borate	9.23

Physiological Buffers

- **Importance of Buffers in Physiological Systems:**
- Processes that take place in living organisms are called physiological processes. Like blood circulatory system, respiration etc. The internal pH of most living cells is close to 7.0. The pH of human blood is 7.4. A blood pH of below 7 or above 7.8 can cause death within minutes. So buffering of blood pH is very important to stabilize it around 7.4. pH plays an important role in almost all biological processes. Small change in pH i.e. decreased or high pH can cause metabolic implications in human body like acidosis and alkalosis. Where metabolism is involved there would be definitely a need of buffer as within cells metabolism is associated with the release of protons (H^+) i.e. decrease in pH or uptake of protons (H^+) i.e. increase in pH. Important buffers that are dominant in human body are:
 1. Bicarbonate buffers
 2. Phosphate buffers
 3. Protein buffers

Physiological Buffers

- **Bicarbonates buffers (Buffering in blood)**
- Blood is a biological fluid in which Carbonic acid and Hydrogen carbonate buffer system plays an important role in maintaining pH around 7.40. In this buffer, carbonic acid (H_2CO_3) act as a weak acid and hydrogen carbonate ion (HCO_3^-) act as conjugate base of a weak acid or salt of weak acid.
- $H_2CO_3 \leftrightarrow H^+ + HCO_3^-$
- When there is excessive amount of H^+ in the blood it is consumed by HCO_3^- forming carbonic acid that is a weak acid which does not alter the blood pH so much and when there is excessive amount of OH^- in the blood it is consumed by H_2CO_3 as it will release the H^+ ions upon excess amount of OH^- in the blood forming H_2O .
- Proportion of carbonic acid and hydrogen carbonate is also very much important in blood. Carbonic acid concentration is controlled by respiration through lungs while hydrogen carbonate concentration is controlled by urination through kidneys.
- Carbonic acid buffer system is a critical buffer for blood as in the absence of this buffer system the pH may fall below this normal value within blood producing a condition called **acidosis** (acidosis may be respiratory or metabolic acidosis) or the pH may rise above normal level producing a condition known as **alkalosis** (alkalosis may be respiratory or metabolic alkalosis).

Physiological Buffers

- **Phosphate buffer (Buffering of internal cell fluids)**
- The phosphate buffer system works in the internal fluid of all cells. This buffer system consists of dihydrogen phosphate ions (H_2PO_4^-) as a weak acid and hydrogen phosphate ions (HPO_4^{2-}) as a conjugate base of weak acid. These two ions are in equilibrium with each other as indicated by the chemical equation below.
- $\text{H}_2\text{PO}_4^- \leftrightarrow \text{H}^+ + \text{HPO}_4^{2-}$
- If additional hydrogen ions enter the cellular fluid, they are consumed in the reaction with HPO_4^{2-} , and the equilibrium shifts to the left. If additional hydroxide ions enter the cellular fluid, they react with H_2PO_4^- , producing HPO_4^{2-} , and shifting the equilibrium to the right. In the absence of phosphate buffer from cell fluid, sharp changes in pH of cell fluids may cause cell death or improper working of different proteins and cell organelles present within the cell.

Physiological Buffers

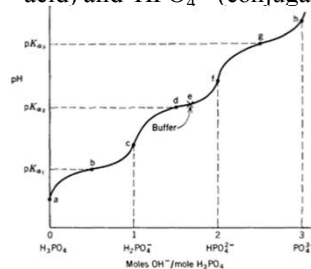
- **Protein buffer (Buffering in Cells and Tissues)**
- Proteins are mainly composed of amino acids. These amino acids contain functional groups that act as weak acid and bases when there are sharp changes in pH in order to stabilize the pH within the body cells. In short it can be said that proteins act as buffers themselves. Protein is a significant buffer the main buffering site for protein is cells and tissues but even in blood it act as a buffer consuming hydrogen ions produced due to the dissociation of the carbonic acid into hydrogen bicarbonate. To understand the proteins as a buffer we have to look into the structure of amino acids which consists of
- carboxyl group (COOH)
- amino group (NH_2)
- hydrogen atom
- R group
- From the above four groups COOH and NH_2 act as buffer systems for acidic and basic conditions.
- At a near neutral pH, like the pH of blood, the carboxyl group is actually COO^- instead of COOH . Then, if a protein finds itself in a more acidic solution, the carboxyl group will be able to take on the extra hydrogen ions and return to the COOH configuration.
- At a near neutral pH, like in blood, the amino group is actually NH_3^+ rather than just NH_2 . It actually tends to carry an extra hydrogen ion on it at a normal pH. Then, if a protein finds itself in a more basic environment, its amino groups on its amino acids can actually release their hydrogen ions and return to NH_2 . As all cells and tissues are composed of proteins mainly so in the absence of protein buffer the sharp changes in pH may cause cell death or tissue damage of a living organisms.

➤ Example: Describe the preparation of 10 liters of 0.045M potassium phosphate buffer, pH= 7.5?

- From the previous example $[HA] = 0.075$ molar, $[A^-] = 0.126$ molar
- No. of moles of **HA** = $M * V_{(L)}$
 $= 0.074 * 3 = 0.222$ mole
- From HA molarity (1M), $M = \text{No. of moles} / V_{(L)}$ thus,
- $V = \text{No. of moles} / M = 0.222 / 1 = 0.222$ L
- No. of moles of **A⁻** = $M * V_{(L)}$
 $= 0.126 * 3 = 0.378$ mole
- No. of moles **A⁻** = w_t_g / MW
 $0.378 = w_t_g / 136$
- $Wt = 51.4$ g
- To prepare 3L buffer, dissolve 51.4g of the solid sodium acetate trihydrate with 222ml of the 1M acetic acid and make up the volume to 3 L with water.

➤ Example: Describe the preparation of 10 liters of 0.045M potassium phosphate buffer, pH= 7.5? $pK_{a1} = 2.12$, $pK_{a2} = 7.21$, $pK_{a3} = 12.32$?

- $H_3PO_4 \rightleftharpoons H^+ + H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-} \rightleftharpoons H^+ + PO_4^{3-}$
- The $pH > K_{a2}$ thus, the 2 major ionic species present are $H_2PO_4^-$ (conjugate acid) and HPO_4^{2-} (conjugate base) with the HPO_4^{2-} predominating.

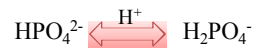


- The buffer can be prepared by several ways as:
 1. by mixing KH_2PO_4 and K_2HPO_4 in proper proportions.
 2. by starting with KH_2PO_4 and converting a portion of it into K_2HPO_4 by adding KOH.
 3. by starting with K_2HPO_4 and converting a portion of it into KH_2PO_4 by adding strong acid as HCl.

- Regardless of which method is used, first calculate the proportion and amounts of the 2 ionic species in the buffer.
- No. of moles = $M \times V_{(L)} = 0.045 \times 10$
- $= 0.45 \text{ moles}$
- $\text{pH} = \text{pK}_{a2} + \log \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}$
- $7.5 = 7.2 + \log \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}$
- $0.3 = \log \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}$
- $\text{Antilog } 0.3 = \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}$
- $2 = \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}$
- So the ratio is $\frac{2}{1}$ that is $\frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} = \frac{2}{1}$
- $\frac{2}{3} \times 0.45 \text{ mole} = 0.3 \text{ mole of } \text{HPO}_4^{2-} \text{ is needed and } \frac{1}{3} \times 0.45 \text{ mole} = 0.15 \text{ mole of } \text{H}_2\text{PO}_4^- \text{ is needed.}$

- **From mixing KH_2PO_4 and K_2HPO_4 :**
- $\text{wt}_g \text{ of } \text{K}_2\text{HPO}_4 = \text{no. of moles} \times \text{MW}$
- $= 0.3 \times 174 = 52.2\text{g}$
- $\text{wt}_g \text{ of } \text{KH}_2\text{PO}_4 = \text{no. of moles} \times \text{MW}$
- $= 0.15 \times 136 = 20.4\text{g}$
- Dissolve the two solutes in some water and make up the volume to 10L by water.
- **From KH_2PO_4 and KOH :**
- Start with KH_2PO_4 (position c) and add sufficient KOH to convert $\frac{2}{3}$ of the H_2PO_4^- to HPO_4^{2-} (position e). $\text{H}_2\text{PO}_4^- \xrightleftharpoons{\text{OH}^-} \text{HPO}_4^{2-}$
- $\text{wt}_g \text{ of } \text{KH}_2\text{PO}_4 = \text{no. of moles} \times \text{MW}$
- $= 0.45 \times 136 = 61.2\text{g.}$
- $\text{wt}_g \text{ of } \text{KOH} = \text{no. of moles} \times \text{MW}$
- $= 0.3 \times 56 = 16.8\text{g.}$
- Dissolve the two solutes in some water and make up the volume to 10L by water.

- **From K_2HPO_4 and HCl:**



- As the ratio we want to end up with is $\frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = \frac{2}{1}$ thus we want to convert only $\frac{1}{3}$ of the HPO_4^{2-} to $H_2PO_4^-$
- Suppose we have solid K_2HPO_4 and a 2M HCl
- $wt_g \text{ of } K_2HPO_4 = \text{no. of moles} * MW$
- $= 0.45 * 174 = 78.3g.$
- $\text{No. of moles of HCl} = \frac{1}{3} * 0.45 \text{ mole} = 0.15 \text{ mole of HCl}$
- $M = \text{No. of moles} / V_{(L)}$ thus, $V_{(L)} = \text{No. of moles} / M$
- $V_{(L)} = \text{No. of moles} / M$
- $= 0.15 / 2$
- $= 0.075L$
- Dissolve 78.3g of the K_2HPO_4 in a little water then add 75ml of the 2M HCl and make up the volume up to 10 liters with water.