Objective 9. Apply equilibrium principles to acid-base titrations and buffers.
Practice Problem solutions

1. Aspirin and salicylic acid are pain relievers. Salicylic acid is not used as a pain reliever anymore because it is hard on a person's stomach. Salicylic acid is used to make aspirin. Aspirin has a $\mathrm{pK}_{\mathrm{a}}$ of 3.5 ; salicylic acid has a $\mathrm{pK}_{\mathrm{a}}$ of 2.98 .
a. Aspirin is less hard on a person's stomach becuase it is a $\qquad$ acid than salicylic acid based on $\mathrm{pK}_{\mathrm{a}}$.
Answer: weaker. Lower pKa means higher Ka which means stronger acid
b. The purity of aspirin can be determined by titrating an aspirin solution with 0.1 M NaOH . Draw the titration curve ( pH vs. volume of NaOH added) of aspirin. Calculate the starting pH (assume you have 20 ml of 0.1 M solution), the pH at the half-way point, and the pH at the end point.
(i) pH at starting point, only acid is present so use $\mathrm{K}_{\mathrm{a}}$ of aspirin.
$\mathrm{pK}_{\mathrm{a}}=-\log \mathrm{K}_{\mathrm{a}}$.
Solve for $\mathrm{K}_{\mathrm{a}}=10^{-\mathrm{pKa}}=10^{-3.5}=3.2 \times 10^{-4}$.
Acid dissociation reaction: $\mathrm{C}_{9} \mathrm{H}_{8} \mathrm{O}_{4}-->\mathrm{H}^{+}+\mathrm{C}_{9} \mathrm{H}_{7} \mathrm{O}_{4}{ }^{-}$
Initial
Reacts
Equilibrium

| 0.1 | 0 | 0 |
| :---: | :---: | :---: |
| x | x | x |
| $0.1-\mathrm{x}$ | x | x |

Note: Since $K_{a}=3.2 \times 10^{-4}$ (very small), we can assume $0.1-x \approx 0.1$.
Set up your equilibrium constant equation and solve for $x=\left[\mathrm{H}^{+}\right]$.
Then, use $\mathrm{pH}=-\log \left[\mathrm{H}^{+}\right]$to calculate pH . (Answer: pH between 2.1 and 2.4)
Answer: $\mathrm{K}_{\mathrm{a}}=3.2 \times 10^{-4}=[\mathrm{x}][\mathrm{x}] /[0.1-\mathrm{x}] \approx[\mathrm{x}][\mathrm{x}] /[0.1]$
Solve for $x=\left[\left(3.2 \times 10^{-4}\right)(0.1)\right]^{\wedge} 0.5=5.7 \times 10^{-3}=\left[\mathrm{H}^{+}\right]$
$\mathrm{pH}=-\log \left[\mathrm{H}^{+}\right]=-\log \left(5.7 \times 10^{-3}\right)=2.2$
(ii) at half-way point, the concentration of base (the conjugate base of aspirin) $=$ the concentration of acid (the aspirin).

So use the Henderson-Hasselbach equation: $\mathrm{pH}=\mathrm{pK}_{\mathrm{a}}+\log$ [base]/[acid]
Since [base] = [acid], $\mathrm{pH}=\mathrm{pK}_{\mathrm{a}}$
Answer: $\mathrm{pH}=3.5$
(iii) at end point, only base (conjugate base of aspirin) is present so use $K_{b}$ of conjugate base of aspirin.

Calculate $\mathrm{K}_{\mathrm{b}}$ of conjugate base of aspirin: $\mathrm{K}_{\mathrm{a}} \mathrm{K}_{\mathrm{b}}=\mathrm{K}_{\mathrm{w}}$ or $\mathrm{K}_{\mathrm{b}}=\mathrm{K}_{\mathrm{w}} / \mathrm{K}_{\mathrm{a}}=1 \times 10^{-14} / 3.2 \times 10^{-4}=3.2 \times 10^{-11}$.
Base hydrolysis reaction:
Initial

| $\mathrm{C}_{9} \mathrm{H}_{7} \mathrm{O}_{4}{ }^{-}+\mathrm{H}_{2} \mathrm{O}-\mathrm{C} \mathrm{C}_{9} \mathrm{H}_{8} \mathrm{O}_{4}+$ | $\mathrm{OH}^{-}$ |  |
| :--- | :---: | :--- |
| 0.05 | 0 | 0 |
| $x$ | $x$ | $x$ |
| $0.05-x$ | $x$ | $x$ |

Equilibrium
0.05 -x

Note: the initial concentration of $\mathrm{C}_{9} \mathrm{H}_{7} \mathrm{O}_{4}{ }^{-}$is 0.05 M because 20 ml of 0.1 M NaOH is required to neutralize 20 ml of 0.1 M aspirin. The volume of solution at the endpoint is 40 ml so using the dilution equation $\left(\mathrm{C}_{1} \mathrm{~V}_{1}=\mathrm{C}_{2} \mathrm{~V}_{2}\right)$, the concentration of $\mathrm{C}_{9} \mathrm{H}_{7} \mathrm{O}_{4}{ }^{-}$is 0.05 M .
Set up your equilibrium constant equation and solve for $x=\left[\mathrm{OH}^{-}\right]$.
Then, use $\mathrm{pOH}=-\log \left[\mathrm{OH}^{-}\right]$to calculate pOH . (Answer: pOH between 5.7 and 6.0)
Last, use $\mathrm{pH}+\mathrm{pOH}=14$ to calculate pH . (Answer: pH between 7.9 and 8.2)
Answer: $\mathrm{K}_{\mathrm{b}}=3.2 \times 10^{-11}=[\mathrm{x}][\mathrm{x}] /[0.05-\mathrm{x}] \approx[\mathrm{x}][\mathrm{x}] /[0.05]$
Solve for $x=\left[\left(3.2 \times 10^{-11}\right)(0.05)\right]^{\wedge} 0.5=1.3 \times 10^{-6}=\left[\mathrm{OH}^{-}\right]$
$\mathrm{pOH}=-\log \left[\mathrm{OH}^{-}\right]=-\log \left(1.3 \times 10^{-6}\right)=5.9$
$\mathrm{pH}=14-\mathrm{pOH}=8.1$
Label the starting pH , half-way point pH , and endpoint pH values in your titration curve.

c. 20 ml of 0.1 M salicylic acid $\left(\mathrm{C}_{7} \mathrm{H}_{6} \mathrm{O}_{3}\right)$ is titrated with 0.1 M NaOH . Draw the titration curve ( pH vs. volume of NaOH added) of salicylic acid. Calculate the starting pH (assume you have 20 ml of 0.1 M solution), the pH at the half-way point, and the pH at the end point. (Answers: starting pH between 1.8 and $2.1, \mathrm{pH}$ at end point between 7.6 and 8.0)
Set up your equilibrium constant equation and solve for $x=\left[\mathrm{H}^{+}\right]$.
Then, use $\mathrm{pH}=-\log \left[\mathrm{H}^{+}\right]$to calculate pH . (Answer: pH between 2.1 and 2.4)

Answers: pH at starting point $=1.99$
Set up your equilibrium reaction and equilibrium constant equation:

$$
\mathrm{HA}<==>\mathrm{H}^{+}+\mathrm{A}^{-}
$$

At equilibrium: 0.1-x x $\quad x$
$K_{a}=10^{- \text {pKa }}=10^{-2.98}=1.05 \times 10^{-3}=[x][x] /[0.1-x] \approx[x][x] /[0.1]$. (REMEMBER: assume $0.1-x=x$ since $K_{a}$ is very small.)
Solve for $x=\left[\left(1.05 \times 10^{-3}\right)(0.1)\right]^{\wedge} 0.5=0.01023=\left[\mathrm{H}^{+}\right]$
$\mathrm{pH}=-\log \left[\mathrm{H}^{+}\right]=-\log (0.01023)=1.99$
pH at $1 / 2$ way point $=\mathrm{pK}_{\mathrm{a}}=2.98$
pH at end point $=7.8$
Set up your equilibrium reaction and equilibrium constant equation:

$$
\mathrm{A}^{-}+\mathrm{H}_{2} \mathrm{O}<==>\mathrm{OH}^{-}+\mathrm{HA}
$$

At equilibrium: 0.05-x $x$
$\mathrm{K}_{\mathrm{b}}=\mathrm{K}_{\mathrm{w}} / \mathrm{K}_{\mathrm{a}}=1 \times 10^{-14} / 1.05 \times 10^{-3}=9.5 \times 10^{-12}=[\mathrm{x}][\mathrm{x}] /[0.05-\mathrm{x}] \approx[\mathrm{x}][\mathrm{x}] /[0.05]$
REMEMBER: The volume of solution at the endpoint is 40 ml so using the dilution equation $\left(\mathrm{C}_{1} \mathrm{~V}_{1}=\mathrm{C}_{2} \mathrm{~V}_{2}\right)$, the concentration of $A^{-}$is 0.05 M .
Solve for $x=\left[\mathrm{OH}^{-}\right]=\left[\left(9.5 \times 10^{-12}\right)(0.05)\right]^{\wedge} 0.5=6.9 \times 10^{-7}$
Then, use $\mathrm{pOH}=-\log \left[\mathrm{OH}^{-}\right]$to calculate $\mathrm{pOH} . \mathrm{pOH}=-\log \left[\mathrm{OH}^{-}\right]=-\log \left(6.9 \times 10^{-7}\right)=6.2$
Last, use $\mathrm{pH}+\mathrm{pOH}=14$ to calculate $\mathrm{pH} . \mathrm{pH}=14-\mathrm{pOH}=7.8$
2. From Lab 5, you know a buffer contains a weak acid and its conjugate base (or a weak base and its conjugate acid). The buffer pH is approximately the $\mathrm{pK}_{\mathrm{a}}$ of the acid $\pm 2$. You can use the Henderson-Hasselbach equation to detemine the ratio of the conjugate base to acid: $\mathrm{pH}=\mathrm{pK}_{\mathrm{a}}+\log$ [base]/[acid]
Aspirin has a $\mathrm{pK}_{\mathrm{a}}$ of 3.5. Use the Henderson-Hasselbach equation to calculate the ratio of $\left[\mathrm{C}_{9} \mathrm{H}_{7} \mathrm{O}_{4}\right]$ to $\left[\mathrm{C}_{9} \mathrm{H}_{8} \mathrm{O}_{4}\right]$ to make a pH 3 buffer. Note: the buffer pH is less than the $\mathrm{pK}_{\mathrm{a}}$ so there should be more acid than base.
Answer: Henderson-Hasselbach equation: $\mathrm{pH}=\mathrm{pK}_{\mathrm{a}}+\log$ [base]/[acid]
$3=3.5+\log$ [base]/[acid]
[base]/[acid] $=10^{-0.5}=0.32$
This number tells you more acid $(\mathrm{HA})$ is needed than base $\left(\mathrm{A}^{-}\right)$to make this buffer because the buffer pH is lower than the $\mathrm{pK}_{\mathrm{a}}$.
3. Carbonic acid, $\mathrm{H}_{2} \mathrm{CO}_{3}$, is formed when an acid reacts with a carbonate or bicarbonate (gas forming reaction from Chem $1 \mathrm{~A})$. It is the acid that forms when $\mathrm{CO}_{2}(\mathrm{~g})$ is bubbled into water at $\qquad$ pressure or $\qquad$ temperature.
20 ml 0.1 M solution of $\mathrm{H}_{2} \mathrm{CO}_{3}$ is titrated with 0.1 M NaOH .
a. $\mathrm{H}_{2} \mathrm{CO}_{3}$ is a diprotic acid. Look up the $\mathrm{K}_{\mathrm{a} 1}$ and $\mathrm{K}_{\mathrm{a} 2}$ of $\mathrm{H}_{2} \mathrm{CO}_{3}$.

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H2CO
Ka1 =
HCO}\mp@subsup{}{}{-}---> H+ + CO_ -- - -
K
```

b. Draw the titration curve. Calculate the pH at starting point, each half-way point, and each end point.
(i) pH at starting point, only acid is present so use $\mathrm{K}_{\mathrm{a}}$ of $\mathrm{H}_{2} \mathrm{CO}_{3}$. (Answer: pH between 3.5 and 4.0)
(ii) at first half-way point, the concentration of base $\left(\mathrm{HCO}_{3}{ }^{-}\right)=$the concentration of acid $\left(\mathrm{H}_{2} \mathrm{CO}_{3}\right)$.

So use the Henderson-Hasselbach equation: $\mathrm{pH}=\mathrm{pK}_{\mathrm{a} 1}+\log$ [base]/[acid]
Since [base] = [acid], $\mathrm{pH}=\mathrm{pK}_{\mathrm{a} 1}$.
(iii) at first end point, only base $\left(\mathrm{HCO}_{3}{ }^{-}\right)$is present but $\mathrm{HCO}_{3}{ }^{-}$also behaves like an acid (see $\mathrm{K}_{\mathrm{a} 2}$ ).

Use $\mathrm{pH}=0.5\left(\mathrm{pK}_{\mathrm{a} 1}+\mathrm{pK}_{\mathrm{a} 2}\right)$.
(iv) at second half-way point, the concentration of base $\left(\mathrm{CO}_{3}{ }^{-2}\right)=$ the concentration of acid $\left(\mathrm{HCO}_{3}{ }^{-}\right)$.

So use the Henderson-Hasselbach equation: $\mathrm{pH}=\mathrm{pK}_{\mathrm{a} 2}+\log$ [base]/[acid]
Since [base] = [acid], $\mathrm{pH}=\mathrm{pK}_{\mathrm{a} 2}$.
(v) at second end point, only base $\left(\mathrm{CO}_{3}{ }^{-2}\right)$ is present so use $\mathrm{K}_{\mathrm{b}}$ of $\mathrm{CO}_{3}{ }^{-2}$.

Calculate $\mathrm{K}_{\mathrm{b}}$ of $\mathrm{CO}_{3}^{-2}: \mathrm{K}_{\mathrm{a}} \mathrm{K}_{\mathrm{b}}=\mathrm{K}_{\mathrm{w}}$ or $\mathrm{K}_{\mathrm{b}}=\mathrm{K}_{\mathrm{w}} / \mathrm{K}_{\mathrm{a}}=1 \times 10^{-14} / 4.7 \times 10^{-11}=2.1 \times 10^{-4}$. In this case, use $\mathrm{K}_{\mathrm{a}}$ of $\mathrm{HCO}_{3}{ }^{-}$since $\mathrm{HCO}_{3}{ }^{-}$is the conjugate acid of $\mathrm{CO}_{3}{ }^{-2}$. (Answer: pH between 11.1 and 11.7)
(vi) Draw your titration curve. How does this titration curve (for a diprotic acid) look different that the titration curve in Question 1? Label the pH at starting point, each half-way point, and each end point.
c. Could you make a pH 7.4 buffer using $\mathrm{H}_{2} \mathrm{CO}_{3}, \mathrm{HCO}_{3}{ }^{-}$, or $\mathrm{CO}_{3}{ }^{2-}$ ? If so, which substance serves as the acid and which serves as the base?
d. At what pH can baking soda be used as a buffer?
e. The pH of carbonated soda or water is between 3 and 4. At this pH , does the solution contain mostly $\mathrm{H}_{2} \mathrm{CO}_{3}, \mathrm{HCO}_{3}{ }^{-}$, or $\mathrm{CO}_{3}{ }^{2-}$ ? Hint: see your titration curve.
Answers: $\mathrm{K}_{\mathrm{a} 1}=4.0 \times 10^{-7}\left(\mathrm{pK}_{\mathrm{a} 1}=6.4\right), \mathrm{K}_{\mathrm{a} 2}=5.01 \times 10^{-11}\left(\mathrm{pK}_{\mathrm{a} 2}=10.3\right)$
Titration curve shows two end points.

pH at starting point $=3.7($ Point A$)$
Set up your equilibrium reaction and equilibrium constant equation:

$$
\mathrm{H}_{2} \mathrm{~A}<==>\mathrm{H}^{+}+\mathrm{HA}^{-}
$$

At equilibrium: 0.1-x $x \quad x$
$\mathrm{K}_{\mathrm{a}}=10^{-\mathrm{pKa}}=4.0 \times 10^{-7}=[\mathrm{x}][\mathrm{x}] /[0.1-\mathrm{x}] \approx[\mathrm{x}][\mathrm{x}] /[0.1]$. (REMEMBER: assume $0.1-\mathrm{x}=\mathrm{x}$ since $\mathrm{K}_{\mathrm{a}}$ is very small.)
Solve for $x=\left[\left(4.0 \times 10^{-7}\right)(0.1)\right]^{\wedge} 0.5=0.0002=\left[\mathrm{H}^{+}\right]$
$\mathrm{pH}=-\log \left[\mathrm{H}^{+}\right]=-\log (0.0002)=3.7$ (Point $\left.A\right)$
pH at $1^{\text {st } 1 / 2}$ way point $=\mathrm{pK}_{\mathrm{a} 1}=6.4($ Point B$)$
pH at $1^{\text {st }}$ end point $=0.5\left(\mathrm{pK}_{\mathrm{a} 1}+\mathrm{pK}_{\mathrm{a} 2}\right)=0.5(6.4+10.3)=8.35($ Point C$)$
pH at $2^{\text {nd }} 1 / 2$ way point $=\mathrm{pK}_{\mathrm{a} 2}=10.3($ Point D$)$
pH at $2^{\text {nd }}$ end point $=11.4($ Point E$)$
Set up your equilibrium reaction and equilibrium constant equation:

$$
\mathrm{A}^{2-}+\mathrm{H}_{2} \mathrm{O}<==>\mathrm{OH}^{-}+\mathrm{HA}^{-}
$$

At equilibrium: 0.033-x $\quad \mathrm{x} \quad \mathrm{x}$
$\mathrm{K}_{\mathrm{b}}=\mathrm{K}_{\mathrm{w}} / \mathrm{K}_{\mathrm{a} 2}=1 \times 10^{-14} / 5.01 \times 10^{-11}=0.0002=[\mathrm{x}][\mathrm{x}] /[0.033-\mathrm{x}] \approx[\mathrm{x}][\mathrm{x}] /[0.033]$
REMEMBER: The volume of solution at the endpoint is 60 ml so using the dilution equation $\left(\mathrm{C}_{1} \mathrm{~V}_{1}=\mathrm{C}_{2} \mathrm{~V}_{2}\right)$, the concentration of $A^{-}$is 0.033 M .
Solve for $x=\left[\mathrm{OH}^{-}\right]=[(0.0002)(0.033)]^{\wedge} 0.5=0.0026$
Then, use $\mathrm{pOH}=-\log \left[\mathrm{OH}^{-}\right]$to calculate $\mathrm{pOH} . \mathrm{pOH}=-\log \left[\mathrm{OH}^{-}\right]=-\log (0.0026)=2.6$
Last, use $\mathrm{pH}+\mathrm{pOH}=14$ to calculate $\mathrm{pH} . \mathrm{pH}=14-\mathrm{pOH}=11.4$
4. Phosphoric acid, $\mathrm{H}_{3} \mathrm{PO}_{4}$, is added to soda to give it a tart taste.
a. $\mathrm{H}_{3} \mathrm{PO}_{4}$ is a triprotic acid. Look up the $\mathrm{K}_{\mathrm{a} 1}, \mathrm{~K}_{\mathrm{a} 2}$, and $\mathrm{K}_{\mathrm{a} 3}$ of $\mathrm{H}_{3} \mathrm{PO}_{4}$.

$$
\begin{array}{cl}
\mathrm{H}_{3} \mathrm{PO}_{4}-->\mathrm{H}^{+}+\mathrm{H}_{2} \mathrm{PO}_{4}^{-} & \mathrm{K}_{\mathrm{a} 1}= \\
\mathrm{H}_{2} \mathrm{PO}_{4}^{-}--->\mathrm{H}^{+}+\mathrm{HPO}_{4}^{-2} & \mathrm{~K}_{\mathrm{a} 2}= \\
\text { What is the acid dissociation reaction for } \mathrm{K}_{\mathrm{a} 3} ? & \mathrm{~K}_{\mathrm{a} 3}=
\end{array}
$$

b. Draw the titration curve. Calculate the pH at starting point, each half-way point, and each end point.
(i) pH at starting point, only $\mathrm{H}_{3} \mathrm{PO}_{4}$ is present. Which $\mathrm{K}_{\mathrm{a}}$ should you use? (Answer: pH between 1.5 and 1.7)
(ii) pH at first half-way point, use Henderson-Hasselbach equation.
(iii) pH at first end point, only base $\left(\mathrm{H}_{2} \mathrm{PO}_{4}{ }^{-}\right)$is present but $\mathrm{H}_{2} \mathrm{PO}_{4}{ }^{-}$also behaves like an acid (see $\mathrm{K}_{\mathrm{a} 2}$ ).

Use $\mathrm{pH}=0.5\left(\mathrm{pK}_{\mathrm{a} 1}+\mathrm{pK}_{\mathrm{a} 2}\right)$.
(iv) pH at second half-way point, use Henderson-Hasselbach equation.
(v) pH at second end point, only base $\left(\mathrm{HPO}_{4}{ }^{-2}\right.$ ) is present but $\mathrm{HPO}_{4}{ }^{-2}$ also behaves like an acid (see $\mathrm{K}_{\mathrm{a} 3}$ ).

Use $\mathrm{pH}=0.5\left(\mathrm{pK}_{\mathrm{a} 2}+\mathrm{pK}_{\mathrm{a} 3}\right)$.
(vi) pH at third half-way point, use Henderson-Hasselbach equation.
(vii) pH at third end point, only base $\left(\mathrm{PO}_{4}^{-3}\right)$ is present so use $\mathrm{K}_{\mathrm{b}}$ of $\mathrm{PO}_{4}{ }^{-3}$. (Answer: pH between 12.7 and 13)
c. Coca-Cola has a pH of 2.5. At this pH, does Coke contain mostly $\mathrm{H}_{3} \mathrm{PO}_{4}, \mathrm{H}_{2} \mathrm{PO}_{4}{ }^{-}, \mathrm{HPO}_{4}{ }^{-2}$, or $\mathrm{PO}_{4}{ }^{-3}$ ? Hint: see your titration curve.
d. At the pH of Coke, phosphoric acid works as a buffer. Calculate the ratio of [base] to [acid]. Identify the phosphorus containing acid $\left(\mathrm{H}_{3} \mathrm{PO}_{4}\right.$ or $\mathrm{H}_{2} \mathrm{PO}_{4}^{-}$or $\mathrm{HPO}_{4}^{-2}$ or $\mathrm{PO}_{4}{ }^{-3}$ ) and base $\left(\mathrm{H}_{3} \mathrm{PO}_{4}\right.$ or $\mathrm{H}_{2} \mathrm{PO}_{4}^{-}$or $\mathrm{HPO}_{4}^{-2}$ or $\left.\mathrm{PO}_{4}^{-3}\right)$ at this pH .
Answers: $\mathrm{K}_{\mathrm{a} 1}=7.6 \times 10^{-3}\left(\mathrm{pK}_{\mathrm{a} 1}=2.12\right), \mathrm{K}_{\mathrm{a} 2}=6.2 \times 10^{-8}\left(\mathrm{pK}_{\mathrm{a} 2}=7.21\right), \mathrm{K}_{\mathrm{a} 3}=5.0 \times 10^{-13}\left(\mathrm{pK}_{\mathrm{a} 2}=12.3\right)$
Titration curve shows three end points.

pH at starting point $=1.6$ (Point A)
Set up your equilibrium reaction and equilibrium constant equation:

$$
\mathrm{H}_{3} \mathrm{~A}<==>\mathrm{H}^{+}+\mathrm{H}_{2} \mathrm{~A}^{-}
$$

At equilibrium: $0.1-\mathrm{x} \quad \mathrm{x} \quad \mathrm{x}$
$\mathrm{K}_{\mathrm{a}}=10^{-\mathrm{pKa}}=7.6 \times 10^{-3}=[\mathrm{x}][\mathrm{x}] /[0.1-\mathrm{x}] \approx[\mathrm{x}][\mathrm{x}] /[0.1]$. (REMEMBER: assume $0.1-\mathrm{x}=\mathrm{x}$ since $\mathrm{K}_{\mathrm{a}}$ is very small.)
Solve for $x=\left[\left(7.6 \times 10^{-3}\right)(0.1)\right]^{\wedge} 0.5=0.028=\left[\mathrm{H}^{+}\right]$
$\mathrm{pH}=-\log \left[\mathrm{H}^{+}\right]=-\log (0.028)=1.6$ (Point A)
pH at $1^{\text {st }} 1 / 2$ way point $=\mathrm{pK}_{\mathrm{a} 1}=2.12($ Point B$)$
pH at $1^{\text {st }}$ end point $=0.5\left(\mathrm{pK}_{\mathrm{a} 1}+\mathrm{pK}_{\mathrm{a} 2}\right)=0.5(2.12+7.21)=4.67($ Point C$)$
pH at $2^{\text {nd }} 1 / 2$ way point $=\mathrm{pK}_{\mathrm{a} 2}=7.21($ Point D$)$
pH at $2^{\text {nd }}$ end point $=0.5\left(\mathrm{pK}_{\mathrm{a} 2}+\mathrm{pK}_{\mathrm{a} 3}\right)=0.5(7.21+12.3)=9.76($ Point E$)$
pH at $3^{\text {rd }} 1 / 2$ way point $=\mathrm{pK}_{\mathrm{a} 2}=12.3($ Point F$)$
pH at $3^{\text {rd }}$ end point $=11.4$ (Point G)
Set up your equilibrium reaction and equilibrium constant equation:

$$
\mathrm{A}^{3-}+\mathrm{H}_{2} \mathrm{O}<==>\mathrm{OH}^{-}+\mathrm{HA}^{2-}
$$

At equilibrium: $0.025-x$
x
$\mathrm{K}_{\mathrm{b}}=\mathrm{K}_{w} / \mathrm{K}_{\mathrm{a} 2}=1 \times 10^{-14} / 5.0 \times 10^{-13}=0.020=[\mathrm{x}][\mathrm{x}] /[0.025-\mathrm{x}] \approx[\mathrm{x}][\mathrm{x}] /[0.025]$
REMEMBER: The volume of solution at the endpoint is 80 ml so using the dilution equation $\left(\mathrm{C}_{1} \mathrm{~V}_{1}=\mathrm{C}_{2} \mathrm{~V}_{2}\right)$, the concentration of $\mathrm{A}^{-}$is 0.025 M .
Solve for $x=\left[\mathrm{OH}^{-}\right]=[(0.020)(0.025)]^{\wedge} 0.5=0.022$
Then, use $\mathrm{pOH}=-\log \left[\mathrm{OH}^{-}\right]$to calculate $\mathrm{pOH} . \mathrm{pOH}=-\log \left[\mathrm{OH}^{-}\right]=-\log (0.022)=1.7$
Last, use $\mathrm{pH}+\mathrm{pOH}=14$ to calculate $\mathrm{pH} . \mathrm{pH}=14-\mathrm{pOH}=12.3$
NOTE: in this case, $\mathrm{K}_{\mathrm{b}}=0.020$ is not very small so the assumption $0.025-\mathrm{x} \approx 0.025$ may not a good assumption.
Solve for x with the quadratic equation: $\mathrm{x}=0.0145$ and -0.035
$x=\left[\mathrm{OH}^{-}\right]$cannot be a negative concentration so use $\mathrm{x}=\left[\mathrm{OH}^{-}\right]=0.0145$
$\mathrm{pOH}=-\log [0.0145]=1.84$
$\mathrm{pH}=14-\mathrm{pOH}=12.16$. This number is close to 12.3 using the simplifying assumption.

## c. Coca-Cola has a pH of 2.5.

Answer: From titration curve, this pH is between the $1^{\text {st }}$ half way point and $1^{\text {st }}$ end point so Coke contains mostly $\mathrm{H}_{2} \mathrm{PO}_{4}^{-}$ and a smaller amount of $\mathrm{H}_{3} \mathrm{PO}_{4}$.
d. At the pH of Coke, phosphoric acid works as a buffer. Calculate the ratio of [base] to [acid]. Identify the phosphorus containing acid $\left(\mathrm{H}_{3} \mathrm{PO}_{4}\right.$ or $\mathrm{H}_{2} \mathrm{PO}_{4}^{-}$or $\mathrm{HPO}_{4}^{-2}$ or $\left.\mathrm{PO}_{4}^{-3}\right)$ and base $\left(\mathrm{H}_{3} \mathrm{PO}_{4}\right.$ or $\mathrm{H}_{2} \mathrm{PO}_{4}^{-}$or $\mathrm{HPO}_{4}^{-2}$ or $\left.\mathrm{PO}_{4}^{-3}\right)$ at this pH .
Answer: Henderson-Hasselbach equation: $\mathrm{pH}=\mathrm{pK}_{\mathrm{a}}+\log$ [base]/[acid]
$\left.2.5=2.12+\log \left[\mathrm{H}_{2} \mathrm{PO}_{4}\right]\right] /\left[\mathrm{H}_{3} \mathrm{PO}_{4}\right]$
Solve for $\left[\mathrm{H}_{2} \mathrm{PO}_{4}\right] /\left[\mathrm{H}_{3} \mathrm{PO}_{4}\right]=2.37$
At pH 2.5, Coke contains mostly $\mathrm{H}_{2} \mathrm{PO}_{4}{ }^{-}$(approximately $70 \%$ ) and a smaller amount of $\mathrm{H}_{3} \mathrm{PO}_{4}$ (approximately $30 \%$ ).
5. Citric acid $\left(\mathrm{pK}_{1}=3.13, \mathrm{pK}_{2}=4.76, \mathrm{pK}_{3}=6.40\right)$ is the acid in lemon juice. Lemon juice has a pH of 2.4.
a. Draw the structure of citric acid. Circle the acidic protons and wrte the $\mathrm{pK}_{\mathrm{a}}$ of that proton next to your circle.
b. At pH 2.4 , calculate the ratio of the acid and its conjugate base present.
c. Draw the structure and show the charge of the acid and base at this pH .

Answers: a.

b. Use Henderson-Hasselbach equation: $\mathrm{pH}=\mathrm{pK}_{\mathrm{a}}+\log$ [base]/[acid]
pH 2.4 is lower than pK 1 so the acid $=\mathrm{C}_{6} \mathrm{H}_{8} \mathrm{O}_{7}$. and base $=\mathrm{C}_{6} \mathrm{H}_{7} \mathrm{O}_{7}{ }^{-}$.
$2.4=3.13+\log \left[\mathrm{C}_{6} \mathrm{H}_{7} \mathrm{O}_{7}{ }^{-}\right] /\left[\mathrm{C}_{6} \mathrm{H}_{8} \mathrm{O}_{7}\right]$
$\left[\mathrm{C}_{6} \mathrm{H}_{7} \mathrm{O}_{7}^{-7}\right] /\left[\mathrm{C}_{6} \mathrm{H}_{8} \mathrm{O}_{7}\right]=0.19$
c. At pH 2.4 , mostly citric acid and smaller amount of conjugate base of citric acid (citrate).

citric acid, $\mathrm{C}_{6} \mathrm{H}_{8} \mathrm{O}_{7}$
charge $=0$

conjugate base of citric
acid, $\mathrm{C}_{6} \mathrm{H}_{7} \mathrm{O}_{7}^{-}$(citrate)
charge $=-1$
6. EDTA ( $\mathrm{pK}_{1}=2.0, \mathrm{pK}_{2}=2.7, \mathrm{pK}_{3}=6.2, \mathrm{pK}_{4}=10.0$ ), oxalic acid ( $\mathrm{pK}_{1}=1.2, \mathrm{pK}_{2}=4.2$ ), and citric acid ( $\mathrm{pK}_{1}=3.13$, $\mathrm{pK}_{2}=$ $4.76, \mathrm{pK}_{3}=6.40$ ) bind to metals, are used as chelating agents, and are used as buffers.
a. Oxalic acid is the strongest acid because $\qquad$ -.
b. Draw a titration curve ( pH vs. volume of base) of EDTA. Calculate the starting pH (assume a 0.1 M solution), the pH at each half-way point, and the pH at each end point. Label each pH on your titration curve.
c. Which substance would you use to make a pH 6 buffer? Calculate the ratio of [base]/[acid] to make this buffer.

Answers:
a. Oxalic acid has the lowest $\mathrm{pK}_{\mathrm{a}}$, which means highest $\mathrm{K}_{\mathrm{a}}$.
b. EDTA titration curve.

EDTA has four pKa's so the EDTA titration curve shows four (4) end points. ADD A $4{ }^{\text {TH }}$ HALF WAY POINT (POINT H)
AND $4^{\text {TH }}$ END POINT (POINT I) TO THE TITRATION CURVE SHOWN BELOW.
$\mathrm{K}_{\mathrm{a} 1}=0.01\left(\mathrm{pK}_{\mathrm{a} 1}=2.0\right), \mathrm{K}_{\mathrm{a} 2}=0.002\left(\mathrm{pK}_{\mathrm{a} 2}=2.7\right), \mathrm{K}_{\mathrm{a} 3}=6.3 \times 10^{-7}\left(\mathrm{pK}_{\mathrm{a} 2}=6.2\right), \mathrm{K}_{\mathrm{a} 4}=1.0 \times 10^{-10}\left(\mathrm{pK}_{\mathrm{a} 2}=10.0\right)$,

A

pH at starting point $=1.5($ Point A$)$
Set up your equilibrium reaction and equilibrium constant equation:

$$
\mathrm{H}_{4} \mathrm{~A}<==>\mathrm{H}^{+}+\mathrm{H}_{3} \mathrm{~A}^{-}
$$

At equilibrium: 0.1-x $x \quad x$
$\mathrm{K}_{\mathrm{a}}=0.01=[\mathrm{x}][\mathrm{x}] /[0.1-\mathrm{x}] \approx[\mathrm{x}][\mathrm{x}] /[0.1]$. (REMEMBER: assume $0.1-\mathrm{x}=\mathrm{x}$ since $\mathrm{K}_{\mathrm{a}}$ is very small.)
Solve for $x=[(0.01)(0.1)]^{\wedge} 0.5=0.032=\left[\mathrm{H}^{+}\right]$
$\mathrm{pH}=-\log \left[\mathrm{H}^{+}\right]=-\log (0.032)=1.5($ Point A$)$
pH at $1^{\text {st } 1 / 2}$ way point $=\mathrm{pK}_{\mathrm{a} 1}=2.0($ Point B$)$
pH at $1^{\text {st }}$ end point $=0.5\left(\mathrm{pK}_{\mathrm{a} 1}+\mathrm{pK}_{\mathrm{a} 2}\right)=0.5(2.0+2.7)=2.34($ Point C$)$
pH at $2^{\text {nd }} 1 / 2$ way point $=\mathrm{pK}_{\mathrm{a} 2}=2.7($ Point D$)$
pH at $2^{\text {nd }}$ end point $=0.5\left(\mathrm{pK}_{\mathrm{a} 2}+\mathrm{pK}_{\mathrm{a} 3}\right)=0.5(2.7+6.2)=4.45($ Point E$)$
pH at $3^{\text {rd } 1 / 2}$ way point $=\mathrm{pK}_{\mathrm{a} 2}=6.2($ Point F$)$
pH at $3^{\text {rd }}$ end point $=0.5\left(\mathrm{pK}_{\mathrm{a} 2}+\mathrm{pK}_{\mathrm{a} 3}\right)=0.5(6.2+10.0)=8.1($ Point G $)$
pH at $4^{\text {th }} 1 / 2$ way point $=\mathrm{pK}_{\mathrm{a} 2}=10.0($ Point H$)$
pH at $3^{\text {rd }}$ end point $=11.2($ Point I$)$
Set up your equilibrium reaction and equilibrium constant equation:

$$
\mathrm{A}^{4-}+\mathrm{H}_{2} \mathrm{O}<==>\mathrm{OH}^{-}+\mathrm{HA}^{3-}
$$

At equilibrium: $0.020-x$
x
X
$\mathrm{K}_{\mathrm{b}}=\mathrm{K}_{\mathrm{w}} / \mathrm{K}_{\mathrm{a} 2}=1 \times 10^{-14} / 1.0 \times 10^{-10}=0.0001=[\mathrm{x}][\mathrm{x}] /[0.020-\mathrm{x}]$
REMEMBER: The volume of solution at the endpoint is 100 ml so using the dilution equation $\left(\mathrm{C}_{1} \mathrm{~V}_{1}=\mathrm{C}_{2} \mathrm{~V}_{2}\right)$, the concentration of $A^{-}$is 0.020 M .
Solve for $x=\left[\mathrm{OH}^{-}\right]=[(0.0001)(0.020)]^{\wedge} 0.5=0.0014$
Then, use $\mathrm{pOH}=-\log \left[\mathrm{OH}^{-}\right]$to calculate $\mathrm{pOH} . \mathrm{pOH}=-\log \left[\mathrm{OH}^{-}\right]=-\log (0.0014)=2.8$
Last, use $\mathrm{pH}+\mathrm{pOH}=14$ to calculate $\mathrm{pH} . \mathrm{pH}=14-\mathrm{pOH}=11.2$
c. pH 6 buffer is between the $2^{\text {nd }}$ endpoint and $3^{\text {rd }}$ half-way point. So to make a pH 6 buffer with EDTA, use EDTA ${ }^{2-}\left(\mathrm{H}_{2} \mathrm{~A}^{2-}\right)$ as the acid and EDTA ${ }^{3-}\left(\mathrm{HA}^{3-}\right)$ as the base.
Use Henderson-Hasselbach equation: $\mathrm{pH}=\mathrm{pK}_{\mathrm{a}}+\log$ [base]/[acid]
$6=6.2+\log \left[\mathrm{HA}^{3-}\right] /\left[\mathrm{H}_{2} \mathrm{~A}^{2}\right]$
$\left[\mathrm{HA}^{3-}\right] /\left[\mathrm{H}_{2} \mathrm{~A}^{2-}\right]=0.63$
7. (From Lab 5) MSG (monosodium glutamate) is used as a flavor enhancer in foods. MSG can be made from glutamic acid (an amino acid).
glutamic acid

a. Draw a titration curve of glutamic acid if titrated with 0.1 M NaOH . Calculate the pH at each half-way point and each end point. Show the charge of the amino acid at each half-way point and each end point.
b. At what pH is glutamic acid in the form of glutamate? Give reasons.
c. What is the isoelectric point of glutamic acid?

Answers: $\mathrm{K}_{\mathrm{a} 1}=7.9 \times 10^{-3}\left(\mathrm{pK}_{\mathrm{a} 1}=2.1\right), \mathrm{K}_{\mathrm{a} 2}=8.51 \times 10^{-5}\left(\mathrm{pK}_{\mathrm{a} 2}=4.07\right), \mathrm{K}_{\mathrm{a} 3}=3.39 \times 10^{-10}\left(\mathrm{pK}_{\mathrm{a} 2}=9.47\right)$
Titration curve shows three end points.

pH at starting point $=1.55($ Point A$)$
Set up your equilibrium reaction and equilibrium constant equation:

$$
\mathrm{H}_{3} \mathrm{~A}<==>\mathrm{H}^{+}+\mathrm{H}_{2} \mathrm{~A}^{-}
$$

At equilibrium: 0.1-x $x \quad x$
$\mathrm{K}_{\mathrm{a}}=10^{-\mathrm{pKa}}=7.9 \times 10^{-3}=[\mathrm{x}][\mathrm{x}] /[0.1-\mathrm{x}] \approx[\mathrm{x}][\mathrm{x}] /[0.1]$. (REMEMBER: assume $0.1-\mathrm{x}=\mathrm{x}$ since $\mathrm{K}_{\mathrm{a}}$ is very small.)
Solve for $x=\left[\left(7.9 \times 10^{-3}\right)(0.1)\right]^{\wedge} 0.5=0.028=\left[H^{+}\right]$
$\mathrm{pH}=-\log \left[\mathrm{H}^{+}\right]=-\log (0.028)=1.55($ Point A$)$. Charge $=+1$

charge $=+1$
pH at $1^{\text {st } 1 / 2}$ way point $=\mathrm{pK}_{\mathrm{a} 1}=2.1($ Point $B) .50 \%$ acid $($ charge $=+1)$ and $50 \%$ base $($ charge $=0)$

pH at $1^{\text {st }}$ end point $=0.5\left(\mathrm{pK}_{\mathrm{a} 1}+\mathrm{pK}_{\mathrm{a} 2}\right)=0.5(2.1+4.07)=3.09($ Point C$)$. Charge $=0$. This is the ISOELECTRIC POINT of glutamic acid $=$ the pH at which the charge $=0$.

charge $=0$
pH at $2^{\text {nd }} 1 / 2$ way point $=\mathrm{pK}_{\mathrm{a} 2}=4.07($ Point D). $50 \%$ acid $($ charge $=0)$ and $50 \%$ base $($ charge $=-1)$

pH at $2^{\text {nd }}$ end point $=0.5\left(\mathrm{pK}_{\mathrm{a} 2}+\mathrm{pK}_{\mathrm{a} 3}\right)=0.5(4.07+9.47)=6.77($ Point E$)$. Charge $=-1$

charge $=-1$
pH at $3^{\text {rd }} 1 / 2$ way point $=\mathrm{pK}_{\mathrm{a} 2}=9.47($ Point F$) .50 \%$ acid $($ charge $=-1)$ and $50 \%$ base $($ charge $=-2)$

pH at $3^{\text {rd }}$ end point $=10.9($ Point G $)$. Charge $=-2$

charge $=-2$
Set up your equilibrium reaction and equilibrium constant equation:

$$
\mathrm{A}^{3-}+\mathrm{H}_{2} \mathrm{O}<==>\mathrm{OH}^{-}+\mathrm{HA}^{2-}
$$

At equilibrium: 0.025-x $\quad x \quad x$
$\mathrm{K}_{\mathrm{b}}=\mathrm{K}_{\mathrm{w}} / \mathrm{K}_{\mathrm{a} 2}=1 \times 10^{-14} / 2.95 \times 10^{-5}=0.020=[\mathrm{x}][\mathrm{x}] /[0.025-\mathrm{x}] \approx[\mathrm{x}][\mathrm{x}] /[0.025]$
REMEMBER: The volume of solution at the endpoint is 80 ml so using the dilution equation $\left(\mathrm{C}_{1} \mathrm{~V}_{1}=\mathrm{C}_{2} \mathrm{~V}_{2}\right)$, the concentration of $A^{-}$is 0.025 M .
Solve for $x=\left[\mathrm{OH}^{-}\right]=\left[\left(2.95 \times 10^{-5}\right)(0.025)\right]^{\wedge} 0.5=0.00086$
Then, use $\mathrm{pOH}=-\log \left[\mathrm{OH}^{-}\right]$to calculate $\mathrm{pOH} . \mathrm{pOH}=-\log \left[\mathrm{OH}^{-}\right]=-\log (0.00086)=3.1$
Last, use $\mathrm{pH}+\mathrm{pOH}=14$ to calculate $\mathrm{pH} . \mathrm{pH}=14-\mathrm{pOH}=10.9$
8. You are given a 0.1 M mixture of two amino acids, histidine and lysine, and want to separate the amino acids by electrophoresis. The Lewis structures of each amino acid, the acidic protons, and pKa's are shown.

a. Draw a titration curve of each amino acid if titrated with 0.1 M NaOH . Calculate the pH at each half-way point and each end point. Show the charge of each amino acid at each half-way point and each end point.
b. What is the isoelectric point of histidine? Lysine?
c. Identify the amino acid and salt (conjugate base) combination you would use to make a pH 5 buffer. Describe how you would make this buffer.
d. What pH would you use in an electrophoresis experiment to separate these two amino acids? Give reasons. Answers: The titration curves for histidine and lysine show three end points.


|  | histidine | lysine |
| :---: | :---: | :---: |
| $\mathrm{pK}_{\mathrm{a} 1}$ | 1.82 | 2.18 |
| $\mathrm{pK}_{\mathrm{a} 2}$ | 6.0 | 8.95 |
| $\mathrm{pK}_{\mathrm{a} 3}$ | 9.17 | 10.5 |
| $\mathrm{K}_{\mathrm{b}}$ to calculate pH at $3^{\text {rd }}$ endpoint | $1.48 \times 10^{-5}$ | 0.00032 |
| pH at starting point / charge | 1.41 / +1 | 1.59 / +1 |
| pH at $1^{\text {st }}$ half-way point / charge | $1.82 /+1$ and 0 | $2.18 /+1$ and 0 |
| pH at $1^{\text {st }}$ end point / charge | $\begin{aligned} & 3.91 / 0 \text { (ISOELECTRIC } \\ & \text { POINT) } \end{aligned}$ | $\begin{aligned} & 5.56 / 0 \text { (ISOELECTRIC } \\ & \text { POINT) } \end{aligned}$ |
| pH at $2^{\text {nd }}$ half-way point / charge | 6.0 / 0 and -1 | 8.95 / 0 and -1 |
| pH at $2^{\text {nd }}$ end point / charge | 7.58 / -1 | 9.72 / -1 |
| pH at $3^{\text {rd }}$ half-way point / charge | 9.17 / -1 and -2 | 10.5 / -1 and -2 |
| pH at $3^{\text {rd }}$ end point / charge | 10.8 / -2 | 11.4 / -2 |

c. To make pH 5 buffer:
histidine -pH 5 is between the $1^{\text {st }}$ endpoint and $2^{\text {nd }}$ half-way point.
Use histidine with 0 charge as acid and -1 charge as base.
Use Henderson-Hasselbach equation: $\mathrm{pH}=\mathrm{pK}_{\mathrm{a}}+\log$ [base]/[acid]
$5=6.0+\log$ [histidine with -1 charge]/[ histidine with 0 charge]
[histidine with -1 charge]/[ histidine with 0 charge] $=0.1$
This means the ratio of histidine with -1 charge to histidine with 0 charge is $0.1: 1$.
So $\%$ histidine with -1 charge $=0.1 /(0.1+1)=9.1 \%$ histidine with -1 charge
and $100-9.1=90.9 \%$ histidine with 0 charge.
E.g., use 0.91 ml 0.1 M histidine with -1 charge and 9.09 ml of 0.1 M histidine with 0 charge to make a 10 ml of pH 5 buffer.
lysine -pH 5 is between the $1^{\text {st }}$ half-way point $1^{\text {st }}$ endpoint.
Use lysine with +1 charge as acid and 0 charge as base.
Use Henderson-Hasselbach equation: $\mathrm{pH}=\mathrm{pK}_{\mathrm{a}}+\log$ [base]/[acid]
$5=2.18+\log$ [lysine with 0 charge]/[ histidine with +1 charge]
[lysine with 0 charge]/[ histidine with +1 charge] $=10^{2.82}=661$
This means the ratio of lysine with 0 charge to lysine with +1 charge is 661:1.
So $\%$ lysine with 0 charge $=661 /(661+1)=99.8 \%$ lysine with 0 charge
and $100-99.8=0.2 \%$ lysine with +1 charge.
E.g., use 9.98 ml 0.1 M lysine with 0 charge and 0.2 ml of 0.1 M lysine with -1 charge to make a 10 ml of pH 5 buffer.
d. An electrophoresis experiment uses a difference in charge to separate two amino acids. Choose a pH at which the charge on histidine is different than the charge on lysine.
Example: at pH 3.91 , the charge on histidine $=0$ and charge on lysine is part +1 and part 0 .
At pH 5.56, the charge on histidine is part 0 and part -1 and charge on lysine is 0 .
9. Based on your knowledge of acids and bases, you know that a buffer is effective only in the region of its pK . Blood has a normal pH of 7.35-7.45 and contains two major buffer systems. It is important that the pH of blood remains relatively constant because at pH below 6.8 or greater than 8.0 , cells cannot function properly and death may result. The $\mathrm{HCO}_{3}{ }^{-}$ $/ \mathrm{CO}_{2}(\mathrm{aq})$ blood buffer in vivo is an open system in which the concentration of dissolved $\mathrm{CO}_{2}$ is maintained constant. Any excess $\mathrm{CO}_{2}$ produced by the reaction $\mathrm{H}^{+}+\mathrm{HCO}_{3}^{-}--->\mathrm{H}_{2} \mathrm{O}+\mathrm{CO}_{2}$ is expelled by the lungs. Note that a typical laboratory buffer is a closed system. The concentration of conjugate acid increases when $\mathrm{H}^{+}$reacts with the conjugate base.
a. Calculate the $\mathrm{K}_{\mathrm{eq}}$ and pK of Reaction (4) from the following reactions and K values.
$\mathrm{CO}_{2}(\mathrm{~g}) \rightleftharpoons \mathrm{CO}_{2}(\mathrm{aq})$
$\mathrm{CO}_{2}(\mathrm{aq})+\mathrm{H}_{2} \mathrm{O}(\mathrm{I}) \rightleftharpoons \mathrm{H}_{2} \mathrm{CO}_{3}(\mathrm{aq})$
$\mathrm{H}_{2} \mathrm{CO}_{3}(\mathrm{aq}) \rightleftharpoons \mathrm{H}^{+}(\mathrm{aq})+\mathrm{HCO}_{3}^{-}(\mathrm{aq})$
$\mathrm{CO}_{2}(\mathrm{aq})+\mathrm{H}_{2} \mathrm{O}(\mathrm{I}) \rightleftharpoons \mathrm{H}^{+}(\mathrm{aq})+\mathrm{HCO}_{3}^{-}(\mathrm{aq})$
$\mathrm{K}_{1}=3 \times 10^{-5}$ at $37^{\circ} \mathrm{C}$
b. The $\left[\mathrm{HCO}_{3}{ }^{-}\right]=0.024 \mathrm{M}$ in blood at pH 7.4 . Calculate the $\left[\mathrm{CO}_{2}(\mathrm{aq})\right]$ in blood at this pH .
c. $0.01 \mathrm{M} \mathrm{H}^{+}$is added to blood. Calculate the pH of blood under conditions such that the increased $\left[\mathrm{CO}_{2}(\mathrm{aq})\right]$ can not be released as $\mathrm{CO}_{2}(\mathrm{~g})$. In other words, assume that the blood buffer is a closed system.
d. $0.01 \mathrm{M} \mathrm{H}^{+}$is added to blood. Calculate the pH of blood under conditions such that the increased $\left[\mathrm{CO}_{2}(\mathrm{aq})\right]$ can be released as $\mathrm{CO}_{2}(\mathrm{~g})$. In other words, assume that the blood buffer is an open system. Remember that the [ $\left.\mathrm{CO}_{2}(\mathrm{aq})\right]$ remains constant in this open buffer system.
e. Your pH calculations should show a large decrease in pH in part c and a small decrease in pH in part d. Based on these calculations, it would appear that $\mathrm{HCO}_{3}{ }^{-}$should be quickly depleted in an open system when acid is added. How is $\mathrm{HCO}_{3}{ }^{-}$replenished in blood?
Answers:
a. add Equation 2 to Equation 3 to get Equation 4.

Convert $\mathrm{pK}_{\mathrm{a}}$ to $\mathrm{K}_{\mathrm{a}}$ for Equation 3. $\mathrm{Ka}=10^{-\mathrm{pKa}}=10^{-3.8}=1.58 \times 10^{-4}$.
So $\mathrm{K}_{4}=\mathrm{K}_{2} \times \mathrm{K}_{\mathrm{a}}=\left(5 \times 10^{-3}\right)\left(1.58 \times 10^{-4}\right)=7.92 \times 10^{-7}$.
For next question, convert this K to $\mathrm{pK}=-\log \left(7.92 \times 10^{-7}\right)=6.1$
b. See Equation 4 and use Henderson-Hasselbach equation: $\mathrm{pH}=\mathrm{pK}_{\mathrm{a}}+\log$ [base]/[acid]
$7.4=6.1+\log \left[\mathrm{HCO}_{3}^{-}(\mathrm{aq})\right] /\left[\mathrm{CO}_{2}(\mathrm{aq})\right]$
$7.4=6.1+\log [0.024] /\left[\mathrm{CO}_{2}(\mathrm{aq})\right]$
Solve for $\left[\mathrm{CO}_{2}(\mathrm{aq})\right]=0.0012 \mathrm{M}$
c. $0.01 \mathrm{M} \mathrm{H}^{+}$is added to blood. The increased $\left[\mathrm{CO}_{2}(\mathrm{aq})\right]$ CAN NOT be released as $\mathrm{CO}_{2}(\mathrm{~g})$. In other words, assume that the blood buffer is a closed system.
According to LeChatelier's principle, reaction shifts to reactant side.
Equation 4: $\quad \mathrm{CO}_{2}(\mathrm{aq})+\mathrm{H}_{2} \mathrm{O}-->\mathrm{H}^{+}+\mathrm{HCO}_{3}$
$\begin{array}{lll}\text { Initial } & 0.0012 & 0.024\end{array}$
Reacts $\quad 0.01 \quad 0.01 \quad 0.01$
Equilibrium $\quad 0.0012+0.01 \quad 0.024-0.01$
Use Henderson-Hasselbach equation to calculate pH :
$\mathrm{pH}=\mathrm{pK}_{\mathrm{a}}+\log$ [base]/[acid]
$\mathrm{pH}=6.1+\log \left[\mathrm{HCO}_{3}^{-}(\mathrm{aq})\right] /\left[\mathrm{CO}_{2}(\mathrm{aq})\right]=6.1+\log [0.014] /[0.0112]=6.2$
Note the pH drops from 7.4 to 6.2 if $\mathrm{CO}_{2}$ cannot escape under closed system conditions. This is a large drop in pH .
d. $0.01 \mathrm{M} \mathrm{H}^{+}$is added to blood. The increased $\left[\mathrm{CO}_{2}(\mathrm{aq})\right] \mathrm{CAN}$ be released as $\mathrm{CO}_{2}(\mathrm{~g})$. In other words, assume that the blood buffer is an open system.
According to LeChatelier's principle, reaction shifts to reactant side.
Equation 4: $\quad \mathrm{CO}_{2}(\mathrm{aq})+\mathrm{H}_{2} \mathrm{O}->\quad \mathrm{H}^{+}+\mathrm{HCO}_{3}{ }^{-}$
Initial $0.0012 \quad 0.024$
Reacts $\quad 0.01 \quad 0.01 \quad 0.01$
Equilibrium $\quad 0.0012+0.01 \quad 0.024-0.01$
HOWEVER, since CO2 can escape, the $\left[\mathrm{CO}_{2}\right]$ does NOT increase but remains the same.
Use Henderson-Hasselbach equation to calculate pH :
$\mathrm{pH}=\mathrm{pK}_{\mathrm{a}}+\log$ [base]/[acid]
$\mathrm{pH}=6.1+\log \left[\mathrm{HCO}_{3}^{-}(\mathrm{aq})\right] /\left[\mathrm{CO}_{2}(\mathrm{aq})\right]=6.1+\log [0.014] /[0.0012]=7.16$
Note the pH drops from 7.4 to 7.16 if $\mathrm{CO}_{2}$ can escape under open system conditions. This is a small drop in pH .
The $\mathrm{CO}_{2}$ escapes when we exhale.
e. See Question 10. When $\mathrm{O}_{2}$ is released by hemoglobin in our tissues, the $\mathrm{O}_{2}$ reacts with food (fuel in respiration reactions) to produce $\mathrm{CO}_{2}$ and water. $\mathrm{CO}_{2}$ and water produces $\mathrm{H}^{+}+\mathrm{HCO}_{3}{ }^{-}$(see Equation 4) under blood pH 7.4 conditions.
10. The diagram below represents a simplified version of the buffering action of hemoglobin as a buffer and the uptake and release of oxygen (Reference: I.H. Segel, "Biochemical Calculations", $2^{\text {nd }}$ ed., Wiley, 1976, p. 88). Hemoglobin is the oxygen carrier in blood that transports oxygen from our lungs to tissues. Diffusion due to partial pressure differences is one mechanism by which oxygen transport occurs.


In lecture, we will discuss the relevant equilibrium reactions involving the four different forms hemoglobin.
a. When hemoglobin is oxygenated, $\mathrm{H} \mathrm{Hgb} \mathrm{O}_{2}$ forms. Draw a titration curve of H Hgb O . Assume you have 20 ml of 0.1 M acid and 0.1 M NaOH as the base. Calculate the pH at the starting point, half-way point, and end point.
b. At pH 7.4 , will there be more $\mathrm{H} \mathrm{Hgb} \mathrm{O}_{2}$ present or $\mathrm{Hgb} \mathrm{O}_{2}$ ? Calculate the ratio of $\mathrm{Hgb} \mathrm{O}_{2}$ to H Hgb O 2 .
c. When the acid form of oxygenated hemoglobin dissociates into the base form of oxygenated hemoglobin, what happens to the pH ? How is the pH buffered when the pH changes?

Since hemoglobin is a protein, it exists in two acid forms, $\mathrm{H} \mathrm{Hgb}\left(\mathrm{pK}_{\mathrm{a}}=7.7\right)$ and $\mathrm{H} \mathrm{Hgb} \mathrm{O} 2\left(\mathrm{pK}_{\mathrm{a}}=6.2\right)$, and two base forms, Hgb and $\mathrm{Hgb} \mathrm{O}_{2}$. At the blood pH of 7.4, which form of the oxygenated hemoglobin is present in the higher amount? Give reasons.
a. Calculate the ratio of $\mathrm{H} \mathrm{Hgb} / \mathrm{Hgb}$ and $\mathrm{H} \mathrm{Hgb} \mathrm{O} 2 / \mathrm{Hgb} \mathrm{O} 2$ present at pH 7.4 . (You want to calculate two ratios.) Of the four forms of hemoglobin present, which form is present in the highest amount at pH 7.4 ?
c. Does the acid form of hemoglobin (H Hgb) have a higher, lower, or same affinity for oxygen than its conjugate base (Hgb)? Give reasons.
Answers:
a. $\mathrm{H} \mathrm{Hgb} \mathrm{O}_{2}$ is a monoprotic acid. The titration curve shows one endpoint.
$\mathrm{pKa}=6.2 \mathrm{so} \mathrm{pH}$ at starting point $=3.6$
pH at $1 / 2$ way point $=\mathrm{pK}_{\mathrm{a}}=6.2$
$\mathrm{K}_{\mathrm{b}}=\mathrm{K}_{\mathrm{w}} / \mathrm{K}_{\mathrm{a}}=1 \times 10^{-14} / 10^{-6.2}=1.58 \times 10^{-8}=[\mathrm{x}][\mathrm{x}] /[0.05-\mathrm{x}] \approx[\mathrm{x}][\mathrm{x}] /[0.05]$
$\mathrm{x}=\left[\mathrm{OH}^{-}\right]=2.8 \times 10^{-5}$
pH at end point $=9.4$
H Hgb is a monoprotic acid. The titration curve shows one endpoint.
$\mathrm{pKa}=7.7$ so pH at starting point $=4.3$
pH at $1 / 2$ way point $=\mathrm{pK}_{\mathrm{a}}=7.7$
$\mathrm{K}_{\mathrm{b}}=\mathrm{K}_{\mathrm{w}} / \mathrm{K}_{\mathrm{a}}=1 \times 10^{-14} / 10^{-7.7}=5.01 \times 10^{-7}=[\mathrm{x}][\mathrm{x}] /[0.05-\mathrm{x}] \approx[\mathrm{x}][\mathrm{x}] /[0.05]$
$x=\left[\mathrm{OH}^{-}\right]=1.5 .8 \times 10^{-4}$
pH at end point $=10.2$
b. $\mathrm{H} \mathrm{Hgb} \mathrm{O}_{2}$ : pH 7.4 is higher than pKa of 6.2 so there will be more $\mathrm{Hgb} \mathrm{O}_{2}$ (base) than $\mathrm{H} \mathrm{Hgb} \mathrm{O}_{2}$ (acid).

Use Henderson-Hasselbach equation: $\mathrm{pH}=\mathrm{pK}_{\mathrm{a}}+\log$ [base]/[acid]
$7.4=6.2+\log \left[\mathrm{Hgb} \mathrm{O}_{2}\right] /\left[\mathrm{H} \mathrm{Hgb} \mathrm{O}_{2}\right]$
$\left[\mathrm{Hgb} \mathrm{O}_{2}\right] /\left[\mathrm{H} \mathrm{Hgb} \mathrm{O} \mathrm{O}_{2}\right]=10^{1.2}=15.8$
H Hgb: pH 7.4 is lower than pKa of 7.7 so there will be more H Hgb (acid) than Hgb (base).
Use Henderson-Hasselbach equation: $\mathrm{pH}=\mathrm{pK}_{\mathrm{a}}+\log$ [base]/[acid]
$7.4=7.7+\log [\mathrm{Hgb}] /[\mathrm{H} \mathrm{Hgb}]$
$[\mathrm{Hgb}] /[\mathrm{H} \mathrm{Hgb}]=10^{-0.3}=0.50$
c. When the acid form of oxygenated hemoglobin dissociates into the base form of oxygenated hemoglobin, the pH decreases (more $\mathrm{H}^{+}$is produced so pH decreases).
How is the pH buffered when the pH changes? See LeChatelier's principle - if blood gets acidic, reaction produces more H Hgb O 2 . If blood gets basic, reaction produces more $\mathrm{Hgb} \mathrm{O}_{2}$.
c. The acid form of hemoglobin (H Hgb) have a higher, lower, or same affinity for oxygen than its conjugate base (Hgb).

H Hgb O 2 <==> $\mathrm{H} \mathrm{Hgb}+\mathrm{O}_{2} \quad \mathrm{~K}_{\mathrm{O} 2}=1$
Hgb O 2 <==> $\mathrm{Hgb}+\mathrm{O}_{2} \quad \mathrm{~K}_{\mathrm{O} 2}=0.032$
Give reasons. The equilibrium constant for the acid form ( H Hgb ) is higher ( $\mathrm{K}_{\mathrm{0} 2}=1$ ) - more products means H Hgb wants to give up $\mathrm{O}_{2}$.
The equilibrium constant for the base form $(\mathrm{Hgb})$ is lower $\left(\mathrm{K}^{\prime}{ }_{\mathrm{O} 2}=0.032\right)$ - more reactants means Hgb wants to hold onto $\mathrm{O}_{2}$.

