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 pK_a of 3.5; salicylic acid has a pK_a of 2.98. a. Aspirin is less hard on a person's stomach becuase it is a acid than salicylic acid based on pK_a .

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Answer: weaker. Lower pKa means higher Ka which means stronger acid
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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 K_a of aspirin. $pK_a = -\log K_a$. Solve for $K_a = 10^{-pKa} = 10^{-3.5} = 3.2 \times 10^{-4}$. Acid dissociation reaction: $C_9H_8O_4 \rightarrow H^+ + C_9H_7O_4^-$ Initial 0.1 0 0 Reacts Х Х х Equilibrium 0.1 - xх 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 $x = [H^{\dagger}]$. Then, use $pH = -\log[H^{\dagger}]$ to calculate pH. (Answer: pH between 2.1 and 2.4) Answer: $K_a = 3.2 \times 10^{-4} = [x] [x]/[0.1-x] \approx [x] [x]/[0.1]$ Solve for $x = [(3.2x10^{-4})(0.1)]^{0.5} = 5.7x10^{-3} = [H^{+}]$ $pH = -\log[H^+] = -\log(5.7 \times 10^{-3}) = 2.2$ (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: $pH = pK_a + \log [base]/[acid]$ Since [base] = [acid], $pH = pK_a$ Answer: 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 K_b of conjugate base of aspirin: $K_aK_b = K_w$ or $K_b = K_w/K_a = 1x10^{-14}/3.2x10^{-4} = 3.2x10^{-11}$. $C_9H_7O_4^{-} + H_2O --> C_9H_8O_4 + OH^{-}$ Base hydrolysis reaction: Initial 0.05 0 0 Reacts Х Х х 0.05 – x Equilibrium х х

Note: the initial concentration of $C_9H_7O_4^-$ 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 ($C_1V_1 = C_2V_2$), the concentration of $C_9H_7O_4^-$ is 0.05 M.

Set up your equilibrium constant equation and solve for x = [OH]. Then, use pOH = - log[OH] to calculate pOH. (Answer: pOH between 5.7 and 6.0) Last, use pH + pOH = 14 to calculate pH. (Answer: pH between 7.9 and 8.2) Answer: $K_b = 3.2 \times 10^{-11} = [x] [x]/[0.05-x] \approx [x] [x]/[0.05]$ Solve for x = [(3.2x10⁻¹¹)(0.05)]^0.5 = 1.3x10⁻⁶ = [OH] pOH = -log[OH] = -log (1.3x10⁻⁶) = 5.9 pH = 14 - 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 ($C_7H_6O_3$) 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, pH at end point between 7.6 and 8.0) Set up your equilibrium constant equation and solve for x = [H⁺]. Then, use pH = - log[H⁺] 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: $HA <==> H^+ + A^-$ At equilibrium: 0.1-x x x x $K_a = 10^{-pKa} = 10^{-2.98} = 1.05 \times 10^{-3} = [x] [x]/[0.1-x] \approx [x] [x]/[0.1]. (REMEMBER: assume 0.1-x = x since K_a is very small.)$ $Solve for x = [(1.05x10⁻³)(0.1)]^{0.5} = 0.01023 = [H^+]$ $pH = - log[H^+] = -log(0.01023) = 1.99$ $pH at ½ way point = pK_a = 2.98$ pH at end point = 7.8Set up your equilibrium reaction and equilibrium constant equation: $<math>A^- + H_2O <==> OH^- + HA$ At equilibrium: 0.05-x x x x $K_b = K_w/K_a = 1x10^{-14}/1.05x10^{-3} = 9.5x10^{-12} = [x] [x]/[0.05-x] \approx [x] [x]/[0.05]$ REMEMBER: The volume of solution at the endpoint is 40 mI so using the dilution equation (C₁V₁ = C₂V₂), the

concentration of A⁻ is 0.05 M. Solve for x = $[OH^-] = [(9.5 \times 10^{-12})(0.05)]^{0.5} = 6.9 \times 10^{-7}$ Then, use pOH = - log[OH⁻] to calculate pOH. pOH = -log[OH⁻] = -log (6.9 \times 10^{-7}) = 6.2 Last, use pH + pOH = 14 to calculate pH. pH = 14 - 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 pK_a of the acid ± 2. You can use the Henderson-Hasselbach equation to detemine the ratio of the conjugate base to acid: $pH = pK_a + \log [base]/[acid]$

Aspirin has a pK_a of 3.5. Use the Henderson-Hasselbach equation to calculate the ratio of $[C_9H_7O_4]$ to $[C_9H_8O_4]$ to make a pH 3 buffer. Note: the buffer pH is less than the pK_a so there should be more acid than base.

Answer: Henderson-Hasselbach equation: $pH = pK_a + log [base]/[acid] 3 = 3.5 + log [base]/[acid] [base]/[acid] = 10^{-0.5} = 0.32$

This number tells you more acid (HA) is needed than base (A^{-}) to make this buffer because the buffer pH is lower than the pK_a .

3. Carbonic acid, H_2CO_3 , is formed when an acid reacts with a carbonate or bicarbonate (gas forming reaction from Chem 1A). It is the acid that forms when CO_2 (g) is bubbled into water at _____ pressure or _____ temperature. 20 ml 0.1 M solution of H_2CO_3 is titrated with 0.1 M NaOH.

a. H_2CO_3 is a diprotic acid. Look up the K_{a1} and K_{a2} of H₂CO₃. $H_2CO_3 \xrightarrow{-->} H^+ + HCO_3 \xrightarrow{-} K_{a1} = HCO_3 \xrightarrow{-->} H^+ + CO_3^{-2} K_{a2} =$

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 K_a of H₂CO₃. (Answer: pH between 3.5 and 4.0)

(ii) at <u>first</u> half-way point, the concentration of base (HCO₃⁻) = the concentration of acid (H₂CO₃).

So use the Henderson-Hasselbach equation: $pH = pK_{a1} + log [base]/[acid]$

Since [base] = [acid], $pH = pK_{a1}$.

(iii) at <u>first</u> end point, only base (HCO₃⁻) is present but HCO₃⁻ also behaves like an acid (see K_{a2}). Use $pH = 0.5(pK_{a1} + pK_{a2})$.

(iv) at second half-way point, the concentration of base (CO_3^{-2}) = the concentration of acid (HCO_3^{-1}) .

So use the Henderson-Hasselbach equation: $pH = pK_{a2} + log [base]/[acid]$

Since [base] = [acid], $pH = pK_{a2}$.

(v) at second end point, only base (CO_3^{-2}) is present so use K_b of CO_3^{-2} .

Calculate K_b of CO_3^{-2} : $K_aK_b = K_w$ or $K_b = K_w/K_a = 1x10^{-14}/4.7x10^{-11} = 2.1x10^{-4}$. In this case, use K_a of HCO₃⁻² since HCO₃⁻³ is the conjugate acid of 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 H_2CO_3 , HCO_3^2 , or 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 H_2CO_3 , HCO_3^- , or CO_3^{2-2} ? Hint: see your titration curve.

Answers: $K_{a1} = 4.0 \times 10^{-7}$ (pK_{a1} = 6.4), $K_{a2} = 5.01 \times 10^{-11}$ (pK_{a2} = 10.3) Titration curve shows two end points.



REMEMBER: The volume of solution at the endpoint is 60 ml so using the dilution equation ($C_1V_1 = C_2V_2$), the concentration of A⁻ is 0.033 M.

Solve for $x = [OH] = [(0.0002)(0.033)]^{0.5} = 0.0026$ Then, use $pOH = -\log[OH]$ to calculate pOH. $pOH = -\log[OH] = -\log(0.0026) = 2.6$

Last, use pH + pOH = 14 to calculate pH. pH = 14 - pOH = 11.4

4. Phosphoric acid, H₃PO₄, is added to soda to give it a tart taste.

a. H_3PO_4 is a triprotic acid. Look up the K_{a1} , K_{a2} , and K_{a3} of H_3PO_4 .

$H_3PO_4> H' + H_2PO_4$	K _{a1} =
$H_2PO_4^{-}> H^+ + HPO_4^{-2}$	K _{a2} =
the acid dissociation reaction for K _{a3} ?	K _{a3} =

What is the acid dissociation reaction for Ka3?

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 H_3PO_4 is present. Which K_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 ($H_2PO_4^{-}$) is present but $H_2PO_4^{-}$ also behaves like an acid (see K_{a2}). Use $pH = 0.5(pK_{a1} + pK_{a2})$.

(iv) pH at second half-way point, use Henderson-Hasselbach equation.

(v) pH at second end point, only base (HPO₄⁻²) is present but HPO₄⁻² also behaves like an acid (see K_{a3}). Use $pH = 0.5(pK_{a2} + pK_{a3})$.

(vi) pH at third half-way point, use Henderson-Hasselbach equation.

(vii) pH at third end point, only base (PO₄⁻³) is present so use K_b of PO₄⁻³. (Answer: pH between 12.7 and 13) c. Coca-Cola has a pH of 2.5. At this pH, does Coke contain mostly H₃PO₄, H₂PO₄, HPO₄⁻², or PO₄⁻³? 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 (H_3PO_4 or $H_2PO_4^-$ or HPO_4^{-2} or PO_4^{-3}) and base (H_3PO_4 or $H_2PO_4^-$ or HPO_4^{-2} or PO_4^{-3}) at this pH. Answers: $K_{a1} = 7.6 \times 10^{-3}$ (pK_{a1} = 2.12), $K_{a2} = 6.2 \times 10^{-8}$ (pK_{a2} = 7.21), $K_{a3} = 5.0 \times 10^{-13}$ (pK_{a2} = 12.3) Titration curve shows three end points.



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

 $H_{3}A <=> H^{+} + H_{2}A^{-}$ At equilibrium: 0.1-x x x $K_{a} = 10^{-pKa} = 7.6x10^{-3} = [x] [x]/[0.1-x] \approx [x] [x]/[0.1]$. (REMEMBER: assume 0.1-x = x since K_a is very small.) Solve for x = [(7.6x10^{-3})(0.1)]^{0.5} = 0.028 = [H^{+}] pH = - log[H^{+}] = -log(0.028) = 1.6 (Point A)

pH at 1^{st} ½ way point = pK_{a1} = 2.12 (Point B)

pH at 1^{st} end point = $0.5(pK_{a1} + pK_{a2}) = 0.5(2.12 + 7.21) = 4.67$ (Point C)

pH at 2^{nd} ½ way point = pK_{a2} = 7.21 (Point D)

pH at 2^{nd} end point = $0.5(pK_{a2} + pK_{a3}) = 0.5(7.21 + 12.3) = 9.76$ (Point E)

pH at 3^{rd} $\frac{1}{2}$ way point = pK_{a2} = 12.3 (Point F)

pH at 3rd end point = 11.4 (Point G) Set up your equilibrium reaction and equilibrium constant equation: $A^{3^{-}} + H_2O <=> OH^{-} + HA^{2^{-}}$ At equilibrium: $0.025 \cdot x = x = x$ $K_b = K_w/K_{a2} = 1x10^{-14}/5.0x10^{-13} = 0.020 = [x] [x]/[0.025 \cdot x] \approx [x] [x]/[0.025]$ REMEMBER: The volume of solution at the endpoint is 80 ml so using the dilution equation (C₁V₁ = C₂V₂), the concentration of A⁻ is 0.025 M. Solve for x = [OH] = [(0.020)(0.025)]^{0.5} = 0.022 Then, use pOH = - log[OH] to calculate pOH. pOH = -log[OH⁻] = -log (0.022) = 1.7 Last, use pH + pOH = 14 to calculate pH. pH = 14 - pOH = 12.3

NOTE: in this case, $K_b = 0.020$ is not very small so the assumption $0.025 \cdot x \approx 0.025$ may not a good assumption. Solve for x with the quadratic equation: x = 0.0145 and -0.035x = [OH] cannot be a negative concentration so use x = [OH] = 0.0145pOH = - log[0.0145] = 1.84pH = 14 - 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 1st half way point and 1st end point so Coke contains mostly H₂PO₄⁻¹ and a smaller amount of H₃PO₄. d. At the pH of Coke, phosphoric acid works as a buffer. Calculate the ratio of [base] to [acid]. Identify the phosphorus containing acid (H₃PO₄ or H₂PO₄⁻² or PO₄⁻³) and base (H₃PO₄ or H₂PO₄⁻¹ or HPO₄⁻² or PO₄⁻³) at this pH. Answer: Henderson-Hasselbach equation: pH = pK_a + log [base]/[acid] 2.5 = 2.12 + log [H₂PO₄⁻¹/[H₃PO₄]

Solve for $[H_2PO_4]/[H_3PO_4] = 2.37$

At pH 2.5, Coke contains mostly H₂PO₄ (approximately 70%) and a smaller amount of H₃PO₄ (approximately 30%).

5. Citric acid (pK_1 = 3.13, pK_2 = 4.76, pK_3 = 6.40) 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 pKa 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.

$$pK_{a1} = 3.13$$

 $pK_{a2} = 4.76$ $PK_{a3} = 6.4$

b. Use Henderson-Hasselbach equation: $pH = pK_a + \log [base]/[acid]$ pH 2.4 is lower than pK1 so the acid = $C_6H_8O_7$. and base = $C_6H_7O_7^-$. $2.4 = 3.13 + \log [C_6H_7O_7^-]/[C_6H_8O_7]$ $[C_6H_7O_7^-]/[C_6H_8O_7] = 0.19$ c. At pH 2.4, mostly citric acid and smaller amount of conjugate base of citric acid (citrate).

6. EDTA ($pK_1 = 2.0$, $pK_2 = 2.7$, $pK_3 = 6.2$, $pK_4 = 10.0$), oxalic acid ($pK_1 = 1.2$, $pK_2 = 4.2$), and citric acid ($pK_1 = 3.13$, $pK_2 = 4.76$, $pK_3 = 6.40$) bind to metals, are used as chelating agents, and are used as buffers.

a. Oxalic acid is the strongest acid because ____

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 pK_a, which means highest K_a.

b. EDTA titration curve.

EDTA has four pKa's so the EDTA titration curve shows four (4) end points. ADD A 4TH HALF WAY POINT (POINT H) AND 4TH END POINT (POINT I) TO THE TITRATION CURVE SHOWN BELOW. K_{a1} = 0.01 (pK_{a1} = 2.0), K_{a2} = 0.002 (pK_{a2} = 2.7), K_{a3} = 6.3×10^{-7} (pK_{a2} = 6.2), K_{a4} = 1.0×10^{-10} (pK_{a2} = 10.0),



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

 $H_4A <=> H^+ + H_3A^-$ At equilibrium: 0.1-x x x $K_a = 0.01 = [x] [x]/[0.1-x] \approx [x] [x]/[0.1].$ (REMEMBER: assume 0.1-x = x since K_a is very small.) Solve for x = [(0.01)(0.1)]^0.5 = 0.032 = [H^+] pH = - log[H^+] = -log(0.032) = 1.5 (Point A)

pH at $1^{st} \frac{1}{2}$ way point = pK_{a1} = 2.0 (Point B)

pH at 1^{st} end point = $0.5(pK_{a1} + pK_{a2}) = 0.5(2.0 + 2.7) = 2.34$ (Point C)

pH at 2^{nd} $\frac{1}{2}$ way point = pK_{a2} = 2.7 (Point D)

pH at 2^{nd} end point = $0.5(pK_{a2} + pK_{a3}) = 0.5(2.7 + 6.2) = 4.45$ (Point E)

pH at 3^{rd} ½ way point = pK_{a2} = 6.2 (Point F)

pH at 3^{rd} end point = $0.5(pK_{a2} + pK_{a3}) = 0.5(6.2 + 10.0) = 8.1$ (Point G)

pH at 4^{th} $\frac{1}{2}$ way point = pK_{a2} = 10.0 (Point H)

pH at 3rd end point = 11.2 (Point I) Set up your equilibrium reaction and equilibrium constant equation: $A^{4^{+}} + H_2O <=> OH^{-} + HA^{3^{-}}$ At equilibrium: 0.020-x x x $K_b = K_w/K_{a2} = 1x10^{-14}/1.0x10^{-10} = 0.0001 = [x] [x]/[0.020-x]$ REMEMBER: The volume of solution at the endpoint is 100 ml so using the dilution equation (C₁V₁ = C₂V₂), the concentration of A⁻ is 0.020 M. Solve for x = [OH⁻] = [(0.0001)(0.020)]^{0.5} = 0.0014 Then, use pOH = - log[OH⁻] to calculate pOH. pOH = -log[OH⁻] = -log (0.0014) = 2.8 Last, use pH + pOH = 14 to calculate pH. pH = 14 - pOH = 11.2 c. pH 6 buffer is between the 2nd endpoint and 3rd half-way point. So to make a pH 6 buffer with EDTA, use EDTA²⁻ (H₂A²⁻) as the acid and EDTA³⁻ (HA³⁻) as the base. Use Henderson-Hasselbach equation: pH = pK_a + log [base]/[acid]

 $6 = 6.2 + \log [HA^{3}]/[H_{2}A^{2}]$ $[HA^{3}]/[H_{2}A^{2}] = 0.63$

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 $pK_2 = 4.07$ $PK_3 = 9.47$ $pK_1 = 2.10$

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: $K_{a1} = 7.9 \times 10^{-3}$ (pK_{a1} = 2.1), $K_{a2} = 8.51 \times 10^{-5}$ (pK_{a2} = 4.07), $K_{a3} = 3.39 \times 10^{-10}$ (pK_{a2} = 9.47) Titration curve shows three end points.

pH A B C A D E C A D E C A O 10 20 30 40 50 60

volume of NaOH

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

charge = +1

pH at 1^{st} $\frac{1}{2}$ way point = pK_{a1} = 2.1 (Point B). 50% acid (charge = +1) and 50% base (charge = 0)



pH at 1^{st} end point = $0.5(pK_{a1} + pK_{a2}) = 0.5(2.1 + 4.07) = 3.09$ (Point C). Charge = 0. This is the ISOELECTRIC POINT of glutamic acid = the pH at which the charge = 0.

 $\frac{1}{2}$ way point = pK_{a2} = 4.07 (Point D). 50% acid (charge = 0) and 50% base (charge = -1)

charge = 0

pH at 2nd

charge = 0charge = -1pH at 2nd end point = $0.5(pK_{a2} + pK_{a3}) = 0.5(4.07 + 9.47) = 6.77$ (Point E). Charge = -1 ŇΗ₃ charge = -1pH at 3rd ½ way point = pK_{a2} = 9.47 (Point F). 50% acid (charge = -1) and 50% base (charge = -2) NH₃ NHa charge = -1 charge = -2pH at 3rd end point = 10.9 (Point G). Charge = -2 ŃHcharge = -2Set up your equilibrium reaction and equilibrium constant equation: $A^{3-} + H_2O <==> OH^- + HA^{2-}$ At equilibrium: 0.025-x Х $K_b = K_w/K_{a2} = 1 \times 10^{-14}/2.95 \times 10^{-5} = 0.020 = [x] [x]/[0.025 - x] \approx [x] [x]/[0.025]$

 $K_b = K_w/K_{a2} = 1x10^{-14}/2.95x10^{-5} = 0.020 = [x] [x]/[0.025-x] \approx [x] [x]/[0.025]$ REMEMBER: The volume of solution at the endpoint is 80 ml so using the dilution equation (C₁V₁ = C₂V₂), the concentration of A⁻ is 0.025 M. Solve for x = [OH⁻] = [(2.95x10⁻⁵)(0.025)]^0.5 = 0.00086 Then, use pOH = - log[OH⁻] to calculate pOH. pOH = -log[OH⁻] = -log (0.00086) = 3.1 Last, use pH + pOH = 14 to calculate pH. pH = 14 - 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.



volume of NaOH

	histidine	lysine
pK _{a1}	1.82	2.18
pK _{a2}	6.0	8.95
pK _{a3}	9.17	10.5
K _b to calculate pH	1.48x10 ⁻⁵	0.00032
at 3 rd endpoint		
pH at starting	1.41 / +1	1.59 / +1
point / charge		
pH at 1 st half-way	1.82 / +1 and 0	2.18 / +1 and 0
point / charge		
pH at 1 st end	3.91 / 0 (ISOELECTRIC	5.56 / 0 (ISOELECTRIC
point / charge	POINT)	POINT)
pH at 2 nd half-way	6.0 / 0 and -1	8.95 / 0 and -1
point / charge		
pH at 2 nd end	7.58 / -1	9.72 / -1
point / charge		
pH at 3 rd half-way	9.17 / -1 and -2	10.5 / -1 and -2
point / charge		
pH at 3 rd end	10.8 / -2	11.4 / -2
point / charge		

c. To make pH 5 buffer:

histidine – pH 5 is between the 1st endpoint and 2nd half-way point. Use histidine with 0 charge as acid and -1 charge as base. Use Henderson-Hasselbach equation: $pH = pK_a + \log [base]/[acid]$ $5 = 6.0 + \log [histidine with -1 charge]/[histidine with 0 charge] [histidine with -1 charge]/[histidine with 0 charge] = 0.1$ 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 chargeand 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 5buffer.

lysine – pH 5 is between the 1st half-way point 1st endpoint. Use lysine with +1 charge as acid and 0 charge as base. Use Henderson-Hasselbach equation: pH = pK_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} = 661This 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 chargeand 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_a. 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 HCO₃⁻ /CO₂ (aq) blood buffer *in vivo* is an *open system* in which the concentration of dissolved CO₂ is maintained constant. Any excess CO₂ produced by the reaction H⁺ + HCO₃⁻ ---> H₂O + CO₂ is expelled by the lungs. Note that a typical laboratory buffer is a *closed system*. The concentration of conjugate acid increases when H⁺ reacts with the conjugate base. a. Calculate the K_{eg} and pK of Reaction (4) from the following reactions and K values.

	•	-
$CO_2(g) \longrightarrow CO_2(aq)$		$K_1 = 3 \times 10^{-5} \text{ at } 37^{\circ}\text{C}$
$CO_2 (aq) + H_2O (I) \implies H_2CO_3 (aq)$		$K_2 = 5 \times 10^{-3} \text{ at } 37^{\circ}\text{C}$
H_2CO_3 (aq) $=$ H^+ (aq) + HCO_3^- (aq)		pK _a = 3.8 at 37°C
$CO_2 (aq) + H_2O (I) \longrightarrow H^+ (aq) + HCO_3 (aq)$		K ₄ = ? at 37°C

b. The $[HCO_3] = 0.024$ M in blood at pH 7.4. Calculate the $[CO_2 (aq)]$ in blood at this pH.

c. 0.01 M H⁺ is added to blood. Calculate the pH of blood under conditions such that the increased [CO₂ (aq)] can <u>not</u> be released as CO₂ (g). In other words, assume that the blood buffer is a closed system.

d. 0.01 M H⁺ is added to blood. Calculate the pH of blood under conditions such that the increased [CO₂ (aq)] can be released as CO₂ (g). In other words, assume that the blood buffer is an open system. Remember that the [CO₂ (aq)] 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 HCO₃⁻ should be quickly depleted in an open system when acid is added. How is HCO₃⁻ replenished in blood?

Answers:

a. add Equation 2 to Equation 3 to get Equation 4. Convert pK_a to K_a for Equation 3. Ka = $10^{-pKa} = 10^{-3.8} = 1.58 \times 10^{-4}$. So K₄ = K₂ x K_a = $(5 \times 10^{-3})(1.58 \times 10^{-4}) = 7.92 \times 10^{-7}$. For next question, convert this K to pK = -log (7.92 \times 10^{-7}) = 6.1 b. See Equation 4 and use Henderson-Hasselbach equation: pH = pK_a + log [base]/[acid]

 $7.4 = 6.1 + \log [HCO_3^{-} (aq)]/[CO_2 (aq)]$ 7.4 = 6.1 + log [0.024]/[CO₂ (aq)]

Solve for $[CO_2 (aq)] = 0.0012 \text{ M}$

c. 0.01 M H⁺ is added to blood. The increased [CO₂ (aq)] CAN NOT be released as CO₂ (g). In other words, assume that the blood buffer is a closed system.

According to LeChatelier's prin	ciple, reaction shifts to re	eactant s	ide.		
Equation 4:	CO ₂ (aq) + H ₂ O>	H ⁺ +	HCO ₃ ⁻		
Initial	0.0012		0.024		
Reacts	0.01	0.01	0.01		
Equilibrium	0.0012 + 0.01		0.024 – 0.01		
Use Henderson-Hasselbach equation to calculate pH:					
pH = pK _a + log [base]/[acid]					
pH = 6.1 + log [HCO3 ⁻ (aq)]/[C	CO ₂ (aq)] = 6.1 + log [0.0	14]/[0.01	112] = 6.2		

Note the pH drops from 7.4 to 6.2 if CO₂ cannot escape under closed system conditions. This is a large drop in pH.

d. 0.01 M H⁺ is added to blood. The increased [CO₂ (aq)] CAN be released as CO₂ (g). In other words, assume that the blood buffer is an open system.

According to LeChatelier's pr	rinciple, reaction shifts to re	eactant s	side.
Equation 4:	CO ₂ (aq) + H ₂ O>	H^+ +	HCO ₃ ⁻
Initial	0.0012		0.024
Reacts	0.01	0.01	0.01
Equilibrium	0.0012 + 0.01		0.024 – 0.01

HOWEVER, since CO2 can escape, the [CO₂] does NOT increase but remains the same.

Use Henderson-Hasselbach equation to calculate pH:

 $pH = pK_a + log [base]/[acid]$

pH = 6.1 + log [HCO₃⁻ (aq)]/[CO₂ (aq)] = 6.1 + log [0.014]/[0.0012] = 7.16

Note the pH drops from 7.4 to 7.16 if CO_2 can escape under open system conditions. This is a small drop in pH. The CO_2 escapes when we exhale.

e. See Question 10. When O_2 is released by hemoglobin in our tissues, the O_2 reacts with food (fuel in respiration reactions) to produce CO_2 and water. CO_2 and water produces $H^+ + HCO_3^-$ (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", 2nd 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, H Hgb O₂ forms. Draw a titration curve of H Hgb O₂. 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 H Hgb O₂ present or Hgb O₂? Calculate the ratio of Hgb O₂ to H Hgb O₂.

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, H Hgb ($pK_a = 7.7$) and H Hgb O₂ ($pK_a = 6.2$), and two base forms, Hgb and Hgb O₂. 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 H Hgb/Hgb and H Hgb O_2 /Hgb 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. H Hgb O₂ is a monoprotic acid. The titration curve shows one endpoint. pKa = 6.2 so pH at starting point = 3.6 pH at ½ way point = pK_a = 6.2 K_b = K_w/K_a = $1 \times 10^{-14}/10^{-6.2} = 1.58 \times 10^{-8} = [x] [x]/[0.05-x] \approx [x] [x]/[0.05]$ x = [OH] = 2.8×10^{-5} pH at end point = 9.4

H Hgb is a monoprotic acid. The titration curve shows one endpoint. pKa = 7.7 so pH at starting point = 4.3 pH at ½ way point = pKa = 7.7 $K_b = K_w/K_a = 1x10^{-14}/10^{-7.7} = 5.01x10^{-7} = [x] [x]/[0.05-x] \approx [x] [x]/[0.05]$ $x = [OH] = 1.5.8x10^{-4}$ pH at end point = 10.2

b. H Hgb O₂: pH 7.4 is higher than pKa of 6.2 so there will be more Hgb O₂ (base) than H Hgb O₂ (acid). Use Henderson-Hasselbach equation: $pH = pK_a + \log [base]/[acid]$ 7.4 = 6.2 + log [Hgb O₂]/[H Hgb O₂] $[Hgb O_2]/[H Hgb O_2] = 10^{1.2} = 15.8$

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: pH = $pK_a + \log [base]/[acid]$ 7.4 = 7.7 + log [Hgb]/[H Hgb] [Hgb]/[H 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 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 Hgb 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 $O_2 <=>$ H Hgb + O_2 $K_{O2} = 1$

Hgb $O_2 <=>$ Hgb + O_2 K' $_{O2} = 0.032$

Give reasons. The equilibrium constant for the acid form (H Hgb) is higher ($K_{O2} = 1$) – more products means H Hgb wants to give up O_2 .

The equilibrium constant for the base form (Hgb) is lower ($K'_{O2} = 0.032$) – more reactants means Hgb wants to hold onto O_2 .