

Laboratory Exercise on weak acids/conjugate bases and buffers

Problems

1. Water has a concentration of 55.6 M. Demonstrate mathematically that the weak ionization of water does not appreciably affect the molarity of the solution.

Ch. 4 text - $K_d = 1.8 \times 10^{-16} \text{ M} = \frac{[H^+][OH^-]}{[H_2O]} = \frac{[H^+][OH^-]}{55.6}$

$1.0 \times 10^{-14} = [H^+][OH^-]$. @ neutrality $[H^+] = [OH^-] = 10^{-7} \text{ M}$

So the change in concentration ≈ 0 , i.e. $55.6 - 10^{-7} \approx 55.6 \text{ M}$

2. Phosphate has three ionizable groups, and thus can serve as a buffer at various ranges of pH.

a) What are the pH ranges over which phosphate-derived buffers could be useful? (see Appendix III of the Laboratory Manual)

H_3PO_4	$pK_a = 2.1$	pH Range	1.1 - 3.1
$H_2PO_4^-$	$pK_a = 7.2$	"	6.2 - 8.2
HPO_4^{2-}	$pK_a = 12.3$	"	11.3 - 13.3

b) Which of the buffers listed would be best for solutions of pH 6.5? Why?

MES, $pK_a = 6.2$ and is closest to pH 6.5

3. While several compounds used as biochemical buffers are largely unaffected by changes in temperature, Tris (tris(hydroxymethyl)-aminomethane) - a commonly used buffer, is a fairly temperature sensitive. The pK_a at 20°C is 8.3, but it changes $-0.031/^\circ\text{C}$ temperature increase.

a) If you made a Tris buffer at room temperature (25°C) such that the pH was 8.0, what would the change in pH be if you used the buffer at 10°C?

$pK_a @ 25^\circ\text{C} = 8.3 + 5(-0.031) = 8.145$
 $pK_a @ 10^\circ\text{C} = 8.3 + 10(-0.031) = 8.61$

$pH - pK_a$ must equal $\log \frac{[A^-]}{[HA]}$ which is constant. So $\Delta pH = \Delta pK_a$
 $x - 8.0 = 8.61 - 8.145$
 $x = 8.465$

b) Over what temperature range would it serve as a good buffer for a solution of pH 7.5?

If you want a pK_a within $\frac{1}{2}$ pH unit for effective buffering -
 The pK_a range 7.0 - 8.0 Range 29.7 - 61.9°C
 $pK_a = 7 = 8.3 + x(-0.031)$ ($x = 41.9^\circ\text{C} + 20^\circ\text{C} = 61.9^\circ\text{C}$)
 $pK_a = 8 = 8.3 + x(-0.031)$ ($x = 9.7^\circ\text{C} + 20^\circ\text{C} = 29.7^\circ\text{C}$)

4. Glutamic acid has three ionizable groups, an amino terminus with pK_a of 9.67, a carboxyl terminus with pK_a 2.19, and a side chain carboxylate with pK_a of 4.25.

a) Quantitatively, how will each of these groups appear at a pH of 4.0 (use percentages)?

N-term $4.0 = 9.67 + \log \frac{-NH_2}{-NH_3^+}$ $\frac{-NH_2}{-NH_3^+} = \frac{2.14 \times 10^{-6}}{1}$ charged

C-term $4.0 = 2.19 + \log \frac{-CO_2^-}{-COOH}$ $\% -NH_3^+ = \frac{2.14 \times 10^{-6}}{1 + 2.14 \times 10^{-6}} = 99.99\%$

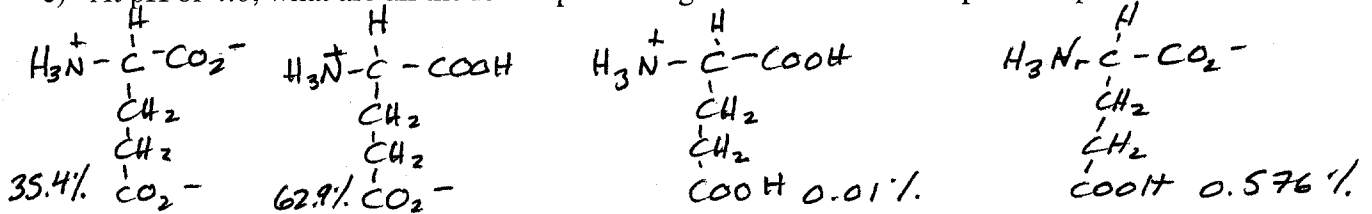
$\frac{-CO_2^-}{-COOH} = \frac{64.6}{1}$ $\% = \frac{64.6}{64.6 + 1} = 98.4\%$

R chain $4.0 = 4.25 + \log \frac{-CO_2^-}{-COOH}$ $\% = \frac{0.56}{1 + 0.56} = 36\%$

b) What will the net charge of the amino acid be at pH 4.0?

$-NH_3^+$ @ 99.99% balanced by 98.4% - C-term + 36% R-chain
 So charge $\approx \frac{1}{3}$ formal negative charge

c) At pH of 4.0, what are all the ionic species of glutamic acid that are possible/present?



5. You desire a 100.0 ml solution of HCl that has a pH of 2.1.

a) How much HCl (5.0 M) is needed to make this solution?

$$\text{pH} = 2.1 = -\log [\text{H}^+] \quad [\text{H}^+] = 7.9 \times 10^{-3} \text{ M}$$

$$0.1 \text{ L} \times \frac{7.9 \times 10^{-3} \text{ mol}}{\text{L}} \times \frac{\text{L}}{5.0 \text{ mol}} = 0.000159 \text{ L} \approx 0.159 \text{ mL}$$

b) If the solution contains 1 mmol of monosodium citrate, how much 5.0 M HCl is needed to make the solution under these circumstances?

pK_a of salt is 4.7, and so ~~and~~ dihydrogen citrate will have to be ~100% protonated @ pH 2.1 ($\Delta 2.6$ pH units)
 So $1 \text{ mmol} \times \frac{\text{mL}}{5 \text{ mM}} = 0.2 \text{ mL more or } 0.359 \text{ mL total}$

6. Glutamate has an isoelectric point of 3.22. Show work using the Henderson-Hasselbalch equation to demonstrate that glutamate is really neutral at pH 3.22. (see #4 for pK_a values)

N term $3.22 = 9.67 + \log \frac{A^-}{HA} \quad \frac{A^-}{HA} = \frac{3.5 \times 10^{-7}}{1} \quad \% \text{ HA} \approx 99.99\%$

C term $3.22 = 2.19 + \log \frac{A^-}{HA} \quad \frac{A^-}{HA} = \frac{10.715}{1} \quad \% \text{ A}^- = 91.46\%$

R group $3.22 = 4.25 + \log \frac{A^-}{HA} \quad \frac{A^-}{HA} = \frac{0.0933}{1} \quad \% \text{ A}^- = 8.53\%$

$\therefore -NH_3^+ = -COO^-$ Sum A⁻ = 99.99%
R-COO⁻

7. What is the pH at which the buffer HEPES will be 67% in its conjugate base form (see Appendix III of the Laboratory Manual)?

$$\text{pH} = 7.6 + \log \frac{0.67}{0.33}$$

$$\text{pH} = 7.6 + \log 2.03$$

$$\text{pH} = 7.6 + 0.307 = 7.907$$

8. You have just made a solution by combining 50 ml of 0.1 M sodium dihydrogen phosphate with 100 ml of 0.1 M disodium hydrogen phosphate. What is the pH of the resulting solution? (see Appendix III of the Laboratory Manual)

$$\begin{aligned} \text{pH} &= 7.2 + \log \frac{100}{50} \\ &= 7.2 + 0.3 \\ &= 7.5 \end{aligned}$$

Issue of relative ratio of conjugate base to acid

9. The imidazole ring of histidine can coordinate to chelated metal cations when in its uncharged state due to the lone pair of electrons on the tertiary amine. A recombinant protein engineered to contain multiple (6-10) adjacent histidines can be purified through this affinity interaction using a stationary phase of chelated metal ions.

- What pH would you use to insure a 90% probability that the interaction will occur?
- Based on the Laboratory Manual Appendix III list, what buffer would you choose for your purification procedures (in light of your answer to part a)?

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$$\begin{aligned} \text{a) } \text{pH} &= 6.0 + \log \frac{9}{1} \\ \text{pH} &= 6.95 \\ \text{So, } &\geq 6.95 \text{ pH} \end{aligned}$$

9 parts A⁻
to 1 part HA
= 90%

b) NaH_2PO_4 or MOPS, pKa closest to 6.95