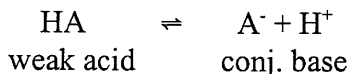


## APPENDIX II

BUFFERS: Explanation, Sample Problems and Problems To Be Done

A buffer is a mixture of a weak acid and its conjugate base.

Buffers can resist changes in pH when small amounts of acid or base are added to the system.



Adding  $\text{H}^+$  pushes the equilibrium to the left  $\text{HA} \rightleftharpoons \text{A}^- + \text{H}^+$

Adding  $\text{OH}^-$  pushes the equilibrium to the right  $\text{HA} \rightleftharpoons \text{A}^- + \text{H}^+$  since  $\text{OH}^- + \text{H}^+ \rightleftharpoons \text{H}_2\text{O}$ .

Note: Strong acids and bases do not have this ability since they dissociate completely in water, eg.  $\text{HCl} \rightarrow \text{H}^+ + \text{Cl}^-$ , and since this reaction is irreversible,  $\text{H}^+$  ions cannot be mopped up, neither can further dissociation occur to produce more  $\text{H}^+$  to remove  $\text{OH}^-$ .

Buffers are effective 1.0 pH unit either side of the pKa (in practice it is usual to use only 0.5 pH units either side of the pKa). This is called the buffering region, an area where a titration curve would be fairly flat. Outside of this region the pH changes rapidly with the addition of base or acid.

An example of a buffer system is acetic acid and acetate ion, with a pKa of 4.76 and a theoretical buffering region ranging from pH 3.76 to pH 5.76.

pH	3.76	4.76	5.76
Species Present	Mostly $\text{CH}_3\text{COOH} \rightleftharpoons$	Equal Amounts $\text{CH}_3\text{COOH} \rightleftharpoons \text{CH}_3\text{COO}^-$	Mostly $\text{CH}_3\text{COO}^-$

At the midpoint, i.e. the pKa, the buffer contains equal amounts of  $\text{CH}_3\text{COOH}$  and  $\text{CH}_3\text{COO}^-$  thus it is an equally effective buffer for  $\text{H}^+$  or  $\text{OH}^-$ . At the extremes of the buffering region it would only be effective against  $\text{H}^+$  (pH 5.75) or  $\text{OH}^-$  (pH 3.75) but not both. At other points in the buffering region it would be effective against both  $\text{H}^+$  and  $\text{OH}^-$  although it would be more effective against one or the other.

The Henderson-Hasselbalch equation relates the pKa, the pH and the buffering capacity of a weak acid - conjugate base pair

$$\text{pH} = \text{pKa} + \log \frac{[\text{Base}]}{[\text{Acid}]}$$

So this equation can be used to find the base to acid ratio required to make buffers of a particular pH for use in particular situations.

## Buffer Preparation

Buffers can be prepared in 3 ways.

1. Mix weak acid and conjugate base in the correct proportions to establish the required ratio and thus the required pH. (eg Acetic acid + sodium acetate.  $\text{CH}_3\text{COONa} \rightarrow \text{CH}_3\text{COO}^- + \text{Na}^+$ ).
2. Mix weak acid and strong base. Here the strong base converts some of the weak acid to conjugate base to establish the ratio. (eg Acetic acid + NaOH).
3. Mix weak base and strong acid. Here strong acid converts conjugate base to weak acid to establish the required ratio. (eg Sodium acetate + HCl).

## Sample Problems

1. Given that the pKa values for lysine are  $\text{pKa}_1=2.18$ ,  $\text{pKa}_2=8.95$ , and  $\text{pKa}_3=10.53$  draw an approximate curve for the titration of 10 mL of 0.01 M lysine with 0.01 M NaOH starting at  $\text{pH}=1$ . Identify the following on the curve: pKas, buffering regions, pI, and region where the solution would contain only positively charged molecules of amino acid.

## Answer

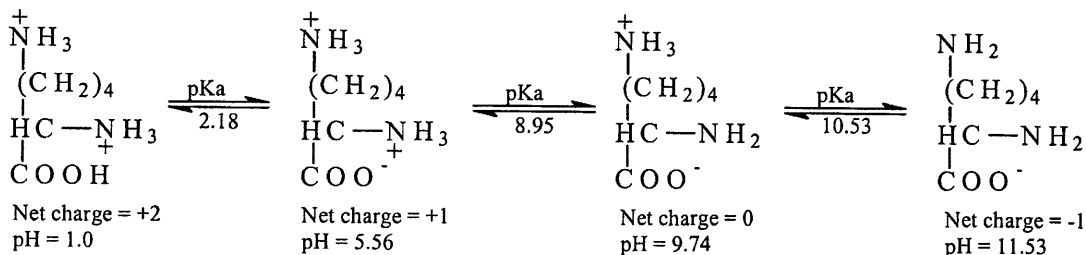
Since the extent of the buffering regions are  $\pm 1.0$  pH unit from the pKa values, for lysine the buffering regions should be pH 1.18 to 3.18, 7.95 to 9.95, and 9.53 to 11.53, with the last two buffering regions overlapping.

The theoretical length of the buffering region can be calculated as follows:

$$\begin{aligned} \text{Vol.}_{\text{a.a.}} \times \text{Conc.}_{\text{a.a.}} &= \text{Vol.}_{\text{BASE}} \times \text{Conc.}_{\text{BASE}} \\ 10 \text{ mL} \times 0.01 &= \text{Vol.}_{\text{BASE}} \times 0.01 \text{ M} \\ \text{Vol.}_{\text{BASE}} &= 10.0 \text{ mL} \end{aligned}$$

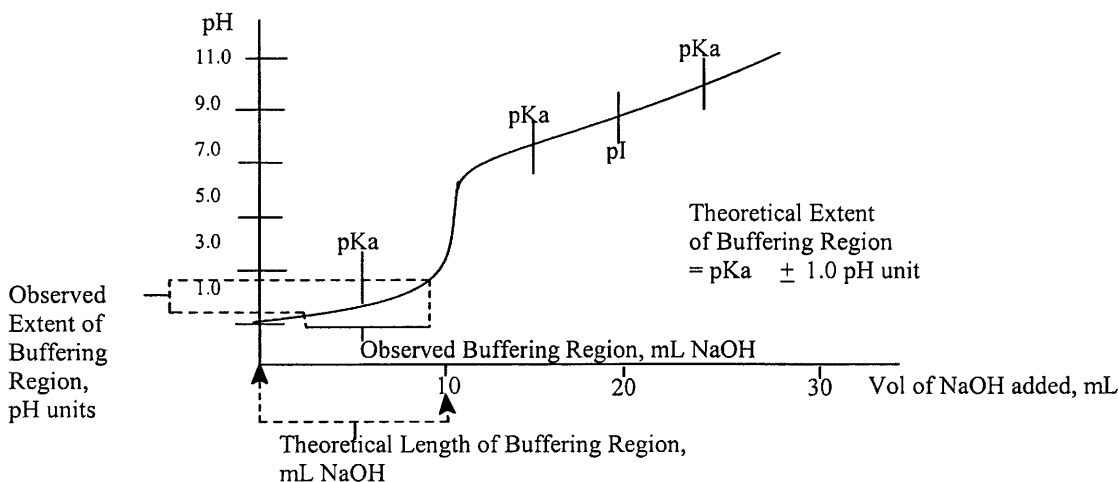
The pI or isoelectric point is the pH at which there is no net charge on the molecule and so for lysine

$$\text{pI} = \frac{8.95 + 10.53}{2} = 9.74$$



The solution would contain only molecules with a net positive charge from a pH of 1.0 to a pH of 5.56. Above pH 5.56 increasing amounts of the isoelectric species and then net negatively charged species would be found.

Tritration Curve of 0.01 M Lysine (10 mL) with 0.01 M NaOH



2. To 20 mL of 0.1 M aspartate buffer,  $\text{pH} = \text{pI}$ , is added 10 mL of 0.3 M NaOH. What is the new pH? The  $\text{pK}_a$  values for aspartate are 1.88, 3.65 and 9.60.

Answer

20 mL 0.1 M asp  $\equiv$  2 mmoles

10 mL 0.3 M NaOH  $\equiv$  3 mmoles

i.e. 1.5 x the amount of  $\text{OH}^-$  compared to asp

Initially we are at  $\text{pI} = \frac{1.88 + 3.65}{2} = 2.76$

So with 1.5 x  $\text{OH}^-$   $\therefore$  we move through 1.5 buffering ranges ending up at  $\text{pK}_{a3} = \text{pH } 9.6$ .

pH and Buffer Problems

1. What is the pH of a 0.002 M solution of  $\text{HNO}_3$ ?
2. How many milliliters of 0.5 M HCl are required to neutralize 8.0 g of NaOH. MW of NaOH is 40.
3. A buffer solution is prepared by mixing 500 mL of 0.4 M  $\text{Na}_2\text{HPO}_4$  and 500 mL of 0.2 M  $\text{NaH}_2\text{PO}_4$ . What is the pH of the buffer solution prepared? What is the change in pH if 10 mL of 1.0 M NaOH is added to this buffer? ( $\text{pK}_{a2}$  for  $\text{H}_2\text{PO}_4^- \rightleftharpoons \text{HPO}_4^{2-} + \text{H}^+$  is 7.20).
4. If 100 mL of 0.2 M sodium acetate ( $\text{CH}_3\text{COONa}$ ) is mixed with 50 mL of 0.1 M HCl, what is the pH of the final solution? ( $\text{pK}_a$  for acetic acid = 4.74).
5. If 10 mL of 0.1 M HCl and 100 mL of 0.5 M TRIS buffer, pH 8.20, are mixed, what would the final pH of the buffer then be? ( $\text{pK}_a$  for TRIS is 8.08).

6. A tetracycline antibiotic containing one tertiary nitrogen atom and two hydroxyl groups has pKa values of 3.45, 7.92 and 9.81. The effective form of this antibiotic on cultures of E. coli is the zwitterion molecular species. If a concentration of  $1.87 \times 10^{-7}$  M of tetracycline causes a doubling of the mean generation time of a culture at pH 7.00, what concentration of the antibiotic would show the same degree of inhibition at pH = 7.8?
7. The E-amino group of Lysine has a pKa of 10.5. What fraction of this group will be protonated at pH of 9.5 and at a pH of 11.0?
8. 100 mL of valine (base) containing 5.5g/L (MW 117) were titrated with 0.1 M HCL. After the addition of 18.8 mL of acid to the amino acid solution the pH was found to be 2.50. Calculate the pKa<sub>1</sub> of valine.
9. Determine the pKa of phosphoric acid if blood, pH 7.4 contains 12.85 mg  $\text{HPO}_4^{=}$  (MW 96) and 3.21 mg  $\text{H}_2\text{PO}_4^-$  (MW 97) per 100 mL of plasma.
10. The acid dissociation constants of haemoglobin and oxyhaemoglobin are  $6.6 \times 10^{-9}$  and  $2.4 \times 10^{-7}$  respectively. If normal blood pH value is 7.4, determine which is the more efficient buffering system in blood. What is the ratio of free acid to conj base for oxyhaemoglobin and haemoglobin in such blood?

SHORT ANSWERS

1. pH = 2.699
2. 400 ml
3. pH = 7.50 to pH 7.57
4. pH = 5.22
5. pH = 8.15
6.  $2.93 \times 10^{-7}$  M
7. pH 9.5 91%  
pH 11.0 24%
8. pKa = 2.32
9. pKa 6.8
10. Equally efficient  
oxy  $\frac{[\text{A}]}{[\text{B}]}$   $\frac{1}{6.02}$   
Haem  $\frac{[\text{A}]}{[\text{B}]}$   $\frac{6.02}{1}$

## LONG ANSWERS TO BUFFER PROBLEMS

- 1.
- $\text{HNO}_3$
- is a strong acid

$$[\text{H}^+] = 0.002 \text{ M} = 2 \times 10^{-3} \text{ M}$$

$$\therefore \text{pH} = \log \frac{1}{[\text{H}^+]} = \log \frac{1}{2 \times 10^{-3}} = \underline{2.699}$$

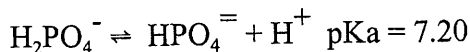
2. At the equivalence point, the no. of moles
- $\text{H}^+$
- added equals the no. of moles of
- $\text{OH}^-$
- present.

$$\begin{aligned} \text{no. of moles of OH}^- &= \text{volume}_{\text{acid}} \times \text{conc}_{\text{acid}} \\ &= \text{volume}_{\text{acid}} \times 0.5 \text{ M} \end{aligned}$$

$$\text{no. of moles of OH}^- = \frac{\text{wt}_{\text{NaOH}}}{\text{MW}_{\text{NaOH}}} = \frac{8.0}{40.}$$

$$\therefore \text{volume}_{\text{acid}} = \frac{8.0}{40 \times 0.5 \text{ M}} = \underline{.4 \text{ L or } 400 \text{ mL}}$$

- 3.
- $\text{Na}_2\text{HPO}_4 \rightarrow 2 \text{Na}^+ + \text{HPO}_4^{=}$
- 
- $\text{NaH}_2\text{PO}_4 \rightarrow \text{Na}^+ + \text{H}_2\text{PO}_4^-$



$$\text{pH} = 7.20 + \log \frac{[\text{HPO}_4^{=}]}{[\text{H}_2\text{PO}_4^-]}$$

$$\text{pH} = 7.20 + \log \frac{\left( \frac{0.5 \text{ L} \times 0.4 \text{ M}}{1 \text{ L}} \right)}{\left( \frac{0.5 \times 0.2 \text{ M}}{1 \text{ L}} \right)}$$

$$\text{pH} = 7.20 + \log 2 = 7.20 + (.30)$$

$$\text{pH} = 7.50$$

Initially the no. of moles of  $\text{HPO}_4^{=}$  equals 0.2 and the no. of moles of  $\text{H}_2\text{PO}_4^-$  equals 0.1.

When we add 0.01 moles of  $\text{NaOH}$ ,  $\text{H}_2\text{PO}_4^- + \text{OH}^- \rightarrow \text{HPO}_4^{=} + \text{H}_2\text{O}$  the no. of moles of  $\text{HPO}_4^{=}$  will increase by 0.01 and the no. of moles of  $\text{H}_2\text{PO}_4^-$  decrease by 0.01.

$$\therefore \text{pH} = 7.2 + \log \frac{\left( \frac{0.2 + 0.01}{1.01 \text{ L}} \right)}{\left( \frac{0.1 - 0.01}{1.01 \text{ L}} \right)} = 7.2 + .37 \quad \text{pH} = 7.57$$

4.  $\text{CH}_3\text{COONa} \rightarrow \text{Na}^+ + \text{CH}_3\text{COO}^-$   
 $\text{CH}_3\text{COO}^- + \text{HCl} \rightarrow \text{CH}_3\text{COOH} + \text{Cl}^-$   
 Final volume =  $100 + 50 = 150 \text{ mL}$  or  $.150 \text{ L}$

$$[\text{CH}_3\text{COO}^-]_{\text{final}} = \frac{.100 \times 0.2 \text{ M}}{.150} - \frac{0.05 \times 0.1 \text{ M}}{.150}$$

$$[\text{CH}_3\text{COOH}]_{\text{final}} = \frac{0.05 \times 0.1 \text{ M}}{.150}$$

$$\text{pH} = \text{pK}_a + \log \frac{[\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]}$$

$$= 4.74 + \log \frac{0.015}{0.005} = 4.74 + (.477)$$

$$\text{pH} = 5.22$$

5. Initially  $\text{pH} = 8.2$   
 and  $[\text{TRIS}]_{\text{B}} + [\text{TRIS}]_{\text{A}} = 0.5 \text{ M}$

Applying the H-H equation  $\text{pH} = \text{pK}_a + \log \frac{[\text{TRIS}]_{\text{B}}}{[\text{TRIS}]_{\text{A}}}$

$$8.2 = 8.08 + \log \frac{[\text{TRIS}]_{\text{B}}}{[\text{TRIS}]_{\text{A}}}$$

$$0.12 = \log \frac{[\text{TRIS}]_{\text{B}}}{[\text{TRIS}]_{\text{A}}} \quad \text{or} \quad 1.32 = \frac{[\text{TRIS}]_{\text{B}}}{[\text{TRIS}]_{\text{A}}}$$

but  $[\text{TRIS}]_{\text{B}} + [\text{TRIS}]_{\text{A}} = 0.5$  or  $[\text{TRIS}]_{\text{B}} = 0.5 - [\text{TRIS}]_{\text{A}}$

$$\frac{1.32}{1.00} = \frac{0.5 - [\text{TRIS}]_{\text{A}}}{[\text{TRIS}]_{\text{A}}}$$

So  $1.32[\text{TRIS}]_{\text{A}} = 0.5 - 1.0[\text{TRIS}]_{\text{A}}$   
 and  $2.32 [\text{TRIS}]_{\text{A}} = 0.5$

$$[\text{TRIS}]_{\text{A}} = \frac{0.5}{2.32} = 0.22$$

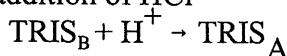
$$\therefore [\text{TRIS}]_{\text{B}} = 0.5 - 0.22 = 0.28$$

In the initial solution (100 mL)

$$\text{moles of TRIS}_{\text{A}} = 0.022$$

$$\text{moles of TRIS}_{\text{B}} = 0.028$$

Upon addition of HCl



moles of  $\text{TRIS}_{\text{B}}$  will decrease by no. of moles of HCl added.

$$\text{no. of moles of HCl added} = M \times V = 0.10 \times .01 \text{ L} = 0.001 \text{ moles}$$

$$\therefore \text{moles of TRIS}_{\text{B}} = 0.028 - .001 = 0.027$$

moles of  $\text{TRIS}_{\text{A}}$  will increase by no. of moles of HCl added.

$$\therefore \text{moles of TRIS}_{\text{A}} = 0.022 + .001 = 0.023$$

Again TRIS<sub>A</sub> and TRIS<sub>B</sub> occur in the SAME volume so

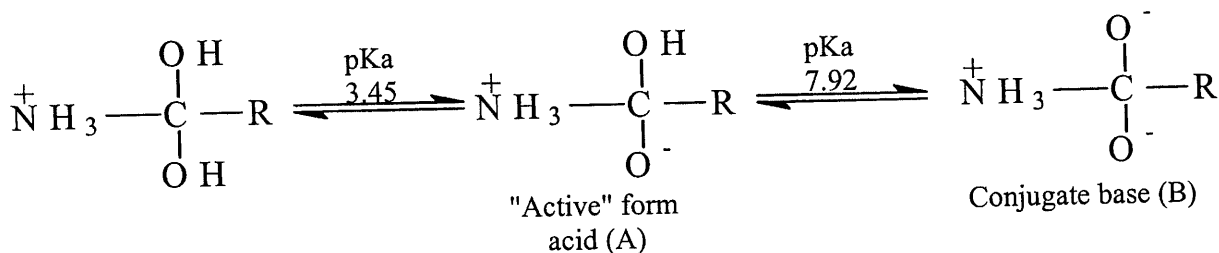
$$\frac{[\text{TRIS}]_B}{[\text{TRIS}]_A} = \frac{\text{moles of TRIS}_B}{\text{moles of TRIS}_A}$$

$$\therefore \text{pH} = 8.08 + \log \frac{0.027}{0.023}$$

$$= 8.08 + \log 1.173$$

$$= \underline{8.15}$$

6.



at pH 7

$$\text{pH} = \text{pK}_a + \log \frac{[\text{B}]}{[\text{A}]}$$

$$7.00 = 7.92 + \log \frac{[\text{B}]}{[\text{A}]}$$

$$-0.92 = \log \frac{[\text{B}]}{[\text{A}]}$$

$$\frac{0.12}{1} = \frac{[\text{B}]}{[\text{A}]}$$

Total parts of B + A = 0.12 + 1 = 1.12 parts of which 0.12 part is B i.e. 0.12/1.12 and 1.00 part is A i.e. 1.00/1.12

Since  $[\text{B}] + [\text{A}] = 1.87 \times 10^{-7} \text{ M}$

$$[\text{A}] = \frac{1.00}{1.12} \times 1.87 \times 10^{-7} \text{ M (i.e. 1.00/1.12 of the total amount)}$$

$$= 1.67 \times 10^{-7} \text{ M "active" conc.}$$

at pH 7.8

$$7.8 = 7.92 + \log \frac{[\text{B}]}{[\text{A}]}$$

$$-0.12 = \log \frac{[\text{B}]}{[\text{A}]}$$

$$0.76 = \frac{[\text{B}]}{[\text{A}]}$$

since [A] must equal  $1.67 \times 10^{-7}$  M to be active

$$0.76 = \frac{[B]}{1.67 \times 10^{-7} \text{ M}}$$

$$\therefore [B] = 0.76 \times 1.67 \times 10^{-7} \text{ M}$$

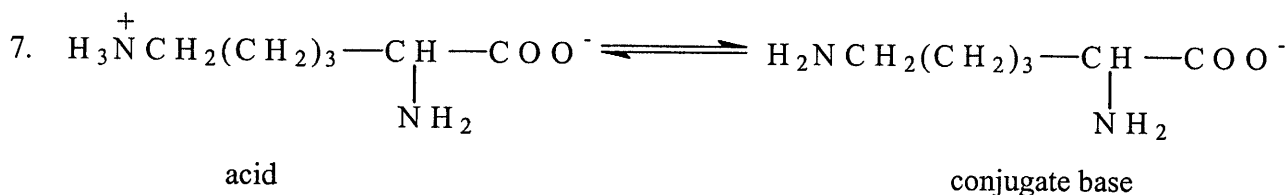
$$\therefore [B] = 1.26 \times 10^{-7} \text{ M}$$

$\therefore$  Total conc. of antibiotic

$$= [B] + [A]$$

$$= 1.26 \times 10^{-7} \text{ M} + 1.67 \times 10^{-7} \text{ M}$$

$$= 2.93 \times 10^{-7} \text{ M}$$



at pH = 9.5

$$9.5 = 10.5 + \log \frac{[\text{NH}_2]}{[\text{NH}_3^+]}$$

$$-1.0 = \log \frac{[\text{NH}_2]}{[\text{NH}_3^+]}$$

$$\frac{[\text{NH}_2]}{[\text{NH}_3^+]} = 0.1 \text{ or } 1/10$$

the fraction protonated =

$$\therefore \frac{10}{10+1} = \frac{10}{11} \text{ or } \underline{91\%}$$

at pH = 11.0

$$11.0 = 10.5 + \log \frac{[\text{NH}_2]}{[\text{NH}_3^+]}$$

$$0.5 = \log \frac{[\text{NH}_2]}{[\text{NH}_3^+]} \quad \text{so } \frac{3.2}{1} = \frac{[\text{NH}_2]}{[\text{NH}_3^+]}$$

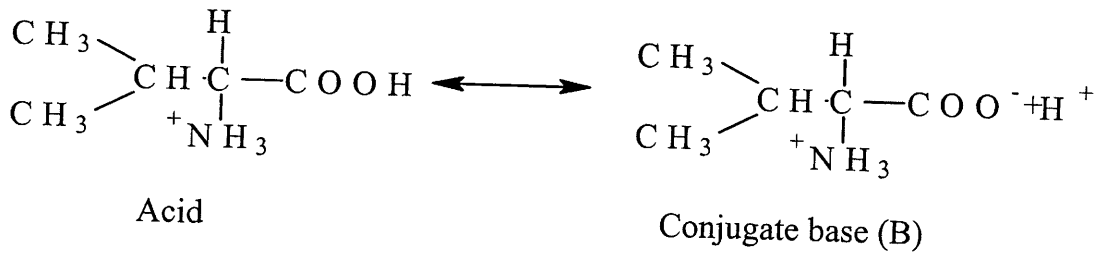
the fraction protonated =

$$\therefore \frac{1}{3.2+1} = \frac{1}{4.2} \text{ or } \underline{24\%}$$

Total parts = 3.2 + 1

$$\text{So prot } \frac{1}{4.2}$$

8.



Initially only base present

$$[\text{B}]_{\text{int}} = \frac{550\text{mg}}{117(\text{mw})} = 4.7 \text{ mmoles}$$

$$\text{HCl added} = \frac{0.1\text{M} \times 18.8}{1000} = 1.88 \times 10^{-3} \text{ moles} = 1.88 \text{ mmoles}$$

$$[\text{A}]_{\text{f}} = \frac{1.88\text{mmoles}}{118.8\text{mL}} \quad (\text{mmoles/mL} \vee \text{moles/L})$$

(total volume 100mL + 18.8 mL)

$$[\text{B}]_{\text{f}} = \frac{4.7 - 1.88\text{mmoles}}{118.8\text{mL}}$$

$$\text{pH} = \text{pKa} + \log \frac{[\text{B}]}{[\text{A}]}$$

$$\therefore \text{pKa} = \text{pH} - \log \frac{[\text{B}]}{[\text{A}]}$$

$$\therefore \text{pKa} = 2.50 - \log \frac{2.82}{1.88}$$

$$\therefore \text{pKa} = 2.32$$

9.  $\text{HPO}_4$ , MW 96  $[\text{B}] = 12.85 \text{ mg/MW}/100 \text{ mL}$

$\text{H}_2\text{PO}_4$ , MW 97  $[\text{A}] = 3.21 \text{ mg/MW}/100 \text{ mL}$

(mMoles/mL or moles/L)

$$\text{pH} = \text{pK}_a + \log \frac{[\text{B}]}{[\text{A}]}$$

$$\text{pKa} = \text{pH} - \log \frac{[\text{B}]}{[\text{A}]}$$

$$= 7.4 - \log \frac{1.34}{.33}$$

$$\text{pK}_a = 6.8$$

10.  $pK_{a_{oxy}} = -\log(2.4 \times 10^{-7}) = 6.82$   
 $pK_{a_{haem}} = -\log(6.6 \times 10^{-9}) = 8.18$   
both equidistant from pH 7.4 thus equally efficient

$$\text{for oxy } 7.4 = 6.62 + \log \frac{[B]}{[A]}$$
$$0.78 = \log \frac{[B]}{[A]}$$

$$\frac{[B]}{[A]} = 6.02 \text{ or } = \frac{[A]}{[B]} = \frac{1}{6.02}$$

$$\text{for haem } 7.4 = 8.18 + \log \frac{[B]}{[A]}$$
$$\therefore 0.78 = \log \frac{[A]}{[B]}$$

$$-0.78 = \log \frac{[B]}{[A]}$$
$$\text{and } \frac{[A]}{[B]} = 6.02$$