

## pH and Buffers Problem Set

### Some tips for working pH problems

In most pH problems you have to calculate one of the four quantities,  $[H^+]$ ,  $[HA]$ ,  $[A^-]$  or  $K_a$ , for the equilibrium reaction:



The most general relationship between these quantities is

$$K_a = \frac{[H^+][A^-]}{[HA]} \quad [1]$$

1) If you know three of these quantities, you can use this equation to determine the fourth one. Often  $pK_a$  is known, but the rest is not, and the trick is to figure out the other quantities (the concentrations) from the information you have. Carefully read the problem! In some cases it helps to relate the concentrations of  $H^+$ ,  $HA$ , and  $A^-$  to each other and express them through a single unknown. This will then bring you to allow you to solve the equation [1].

2) The Henderson-Hasselbalch equation is very useful when you know the molar ratio for the conjugate acid-base pair,  $[A^-]/[HA]$  (a reminder:  $pH = -\log_{10}([H^+])$ ;  $pK_a = -\log_{10}(K_a)$ ):

$$pH = pK_a + \log_{10} \frac{[A^-]}{[HA]} \quad [2]$$

3) Alternatively, if you know the pH and  $pK_a$ , you can determine the base/acid ratio using the following equation (following from [2]):

$$\frac{[A^-]}{[HA]} = 10^{pH - pK_a} \quad [3]$$

4) The molar concentrations of  $[H^+]$  and  $[OH^-]$  are related to each other via the ion product of water,

$$[H^+][OH^-] = 10^{-14} M^2 \quad [4]$$

5) The concept of 'mass conservation' is often useful. If you dissolve, for example, one mole of acetic acid in one liter of water, you would get 1 M solution of acetic acid (HOAc) + acetate ion (OAc<sup>-</sup>). The relative concentrations of HOAc and OAc<sup>-</sup> could change, when, for example, you titrate the buffer with OH<sup>-</sup>, but the total amount of 'acetate' will always be one mole, and, if changes in the volume can be neglected, the total 'acetate' concentration will always be 1M:  $[HOAc] + [OAc^-] = 1 M$ .

6) Strong acids (HCl, HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, HBr, HI, HClO<sub>4</sub>) and strong bases (NaOH, KOH) ionize completely when they are dissolved in water. For example, 1M solution of HCl will contain 1M H<sup>+</sup> and 1M Cl<sup>-</sup>.

7) Most salts ionize completely when dissolved in water. For example, 1M solution of NaCl contains 1M Na<sup>+</sup> and 1M Cl<sup>-</sup>.

8) When you add H<sup>+</sup> or OH<sup>-</sup> to a buffered solution, the H<sup>+</sup> will react essentially completely with any base (A<sup>-</sup> or OH<sup>-</sup>) in the solution, and the OH<sup>-</sup> will react essentially completely with any acid (HA or H<sup>+</sup>) in the solution.

## Practice Problems

1. You have a buffer solution containing an acid HA whose  $pK_a$  is 6.5 and its conjugate base  $A^-$ .

- (a) What is the pH of a solution in which  $[HA] = [A^-]$  ?
  - (b) What is the pH of a solution in which  $[HA] = 0.1 \times [A^-]$  ?
  - (c) What is the pH of a solution in which  $[HA] = 10 \times [A^-]$  ?
  - (d) Calculate the ratio  $[A^-]/[HA]$  for pH = 4.5; pH = 5.5; pH = 6.5; pH = 7.5; pH = 8.5.
- Answer the questions (a)-(c) for acetic acid ( $pK_a = 4.76$ ) and for ammonium ion ( $pK_a = 9.25$ ).

2. From the results of problem 1, what general rules you can infer for the following:

- (a) The relative concentrations of acid and conjugate base when the  $pH = pK_a$
- (b) The predominant species (HA or  $A^-$ ) when the pH is less than the  $pK_a$
- (c) The predominant species (HA or  $A^-$ ) when the pH is higher than the  $pK_a$
- (d) The ratio of  $[HA]$  to  $[A^-]$  when the pH is 1.0 pH unit above or below the  $pK_a$ .
- (e) The ratio of  $[HA]$  to  $[A^-]$  when the pH is 2.0 pH unit above or below the  $pK_a$ .

3. Alanine (Ala) is a diprotic acid that can undergo two dissociation reactions with  $pK_a$ 's given in Table 5-1, p. 118 in *Lehninger*.

- (a) Draw the chemical structure of each of the three possible forms of Ala in aqueous solution, i.e. the fully protonated, zwitterionic, and the other ionic form.
- (b) Of the three possible forms of Ala, which form would be present at the highest concentration in a solution of
  - (1) pH = 1.0
  - (2) pH = 6.2
  - (3) pH = 8.02
  - (4) pH = 11.9
- (c) what fraction of Ala is in the zwitterionic form at the isoelectric point?

4. You want to prepare a solution that has  $pH = 2$  by adding some amount of 1M HCl to 250 mL of pure water. Calculate the amount of the acid you have to add? What is the  $[OH^-]$  before the HCl is added, and after the HCl is added? (ignore changes in the volume of the solution)

What would be the pH if instead of HCl you took a bottle containing 1M NaOH?

5. Acetate buffer is often used as a buffer solution for protein studies at acidic pH (from 3.6 to 5.6). The dissociation reaction is (see e.g. Fig. 4-14, p. 99 in *Lehninger*)



Here  $CH_3COOH$  (further abbreviated as HOAc) and the acetate anion  $CH_3COO^-$  (further OAc<sup>-</sup>) are the conjugate acid-base pair.

- (a) Suppose you prepared an acetate buffer containing 0.1 M of HOAc and 0.1 M of OAc<sup>-</sup>. What are the  $[H^+]$  and pH of this solution?
- (b) Now you add 0.01M HCl to your acetate buffer. What are the  $[H^+]$  and the pH after the HCl is added and the mixture reaches equilibrium?

- (c) Suppose you add 0.01M HCl to a solution containing 0.18 M of HOAc and 0.02M of  $\text{OAc}^-$ . What are the  $[\text{H}^+]$  and the pH before the HCl is added, and after the HCl is added?
- (d) Suppose you add 0.01M HCl to a solution containing 0.02 M of HOAc and 0.18M of  $\text{OAc}^-$ . What are the  $[\text{H}^+]$  and the pH before the HCl is added, and after the HCl is added?
- (e) Repeat questions (b)-(d), except now you add 0.15 M HCl.

## 6. Understanding how buffer solution works

In order to better understand how buffer solution works you want to calculate a titration curve. Some examples are presented in Figure 4-16 ( page 101 of *Lehninger*).

In order to do that, choose one of the weak acids shown in Figure 4-16, for example: acetic acid ( $\text{pK}_a = 4.76$ ), dihydrogen phosphate ( $\text{pK}_a = 6.86$ ), or ammonium ion ( $\text{pK}_a = 9.25$ ).

1) Calculate the pH of a 1M solution of that acid at the beginning of the titration, i.e. before any  $\text{OH}^-$  is added (this corresponds to zero moles of  $\text{OH}^-$  added).

2) Now assume you are titrating your buffer, i.e. you are successively adding certain molar amounts of  $\text{OH}^-$  per mole acid. Assume that  $\text{OH}^-$  reacts completely with an equal amount of the weak acid and converts it to the conjugate base. At each step calculate the pH of the solution. Use the Henderson-Hasselbalch equation. Ignore changes in the volume of the solution during the titration.

(a) First you add 0.05 mole  $\text{OH}^-$ /mole acid. Calculate the pH of the resulting solution and notice the change in the pH. Keep in mind that if some amount of  $\text{H}^+$  is present in the solution, it will react essentially completely with  $\text{OH}^-$ , and the remaining amount of  $\text{OH}^-$  will then react with HA.

(b) Repeat this step at least 2 more times, notice how the change in the pH alters with every titration point.

(c) Continue the titration by adding 0.1 moles  $\text{OH}^-$ /mole acid and recording the pH, till you reach the total added amount of  $\text{OH}^- = 0.5$  mole/mole acid. You are now at the midpoint of the titration. What is the pH at this point? Compare it with  $\text{pK}_a$ .

(d) Now add 0.05 mole  $\text{OH}^-$ /mole acid. Compare the change in pH with that at the beginning steps of the titration.

(e) Continue the titration by adding  $\text{OH}^-$  in steps of 0.1 mole  $\text{OH}^-$ /mole acid.

3) Make a table where the first column represents the total amount of  $\text{OH}^-$  equivalents, i.e. moles  $\text{OH}^-$  per mole acid, and the second column represents the pH of the solution. Draw a graph of pH vs.  $\text{OH}^-$  equivalents added. Compare your titration curve with that in Figure 4-16; for a more direct comparison see question 5 below.

4) Analyze your plot. Find the range of pH values where the pH of the solution changes least with each amount of  $\text{OH}^-$  added. Find the range(s) where it changes most. How are these pH values related to the  $\text{pK}_a$ ?

5\*) For those of you who might want to make a more quantitative comparison of your titration curve. Using the Henderson-Hasselbalch equation, the titration curve can be described as follows:

$$pH = pK_a + \log_{10} \frac{x}{1-x}$$

where  $x$  represents the amount of  $\text{OH}^-$  equivalents added:  $x$  ranges from 0 (the starting point of the titration) to 1 (the end point). For example, if  $C$  is the molar amount of HA initially present, and  $X$  is the total molar amount of  $\text{OH}^-$  then  $x = X/C$ . Plot this curve and compare it with the one you just calculated.

## 7. How to prepare a buffer

There are two principal methods for preparing buffers:

Method 1. Both components of the conjugate acid-base pair are weighed out separately to obtain the desired ratio and then dissolved in water.

Method 2. Both components are obtained from a prescribed amount of only one component, with the second being produced by a specified amount of strong acid or strong base to yield the desired ratio.

Consider the following two examples.

### *Method 1.*

Phosphate buffer (pH range 5.8 – 8.0).

Assume you have prepared two separate stock solutions:

Solution A: 0.1M solution of monobasic potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) and

Solution B: 0.1M solution of dibasic potassium phosphate ( $\text{K}_2\text{HPO}_4$ )

The equilibrium:  $\text{H}_2\text{PO}_4^- \rightleftharpoons \text{H}^+ + \text{HPO}_4^{2-}; \quad pK_a = 6.86$

In order to get 200 mL of the desired buffer, you take 50 mL of solution A, add to it some amount of solution B, and then adjust the total volume to 200 mL by adding pure water.

- 1) What amounts of solution B you have to add in order to have the final pH of 6.0; 7.0; 7.3?
- 2) What will be the pH if you mix 50 mL of both solutions A and B?
- 3) What will be the pH if you add 5 mL of B? 100 mL of B?

**Method 2.**

Tris-HCl buffer is often used as a buffer for biochemical reactions in the pH range from 7.2 to 9.0. It is made by dissolving in water Tris(hydroxymethyl)aminomethane, a weak base, and then adding HCl.

The conjugate acid-base pair for Tris is  $(\text{HOCH}_2)_3\text{C-NH}_3^+$  (here TRIS $\cdot\text{H}^+$ ) and  $(\text{HOCH}_2)_3\text{C-NH}_2$  (here TRISbase); its equilibrium is



Assume you have to prepare 200 mL of the buffer. To do so, you take 100 mL of 0.1 M TRISbase, add HCl and then adjust the volume with pure water. How many mL of 0.1M HCl you have to add to it in order to have the final pH =9.0? pH = 8.0? pH =7.2 ?

**8.** Assume you have successfully prepared the phosphate buffer in Problem 7, Method 1 example (2). What will be the pH after you add 1 mL of 1M HCl? Answer the same question if you added 10 mL of 1M HCl instead.

**Answers to pH and Buffers Problem Set**

1. Here I will provide answers for the  $pK_a = 6.5$  example only

(a) 6.5; (b) 7.5; (c) 5.5;

(d)

pH	$[A^-]/[HA]$
4.5	1/100
5.5	1/10
6.5	1/1
7.5	10/1
8.5	100/1

2. (a) when  $pH = pK_a$ , the molar concentrations of acid and conjugate base are equal

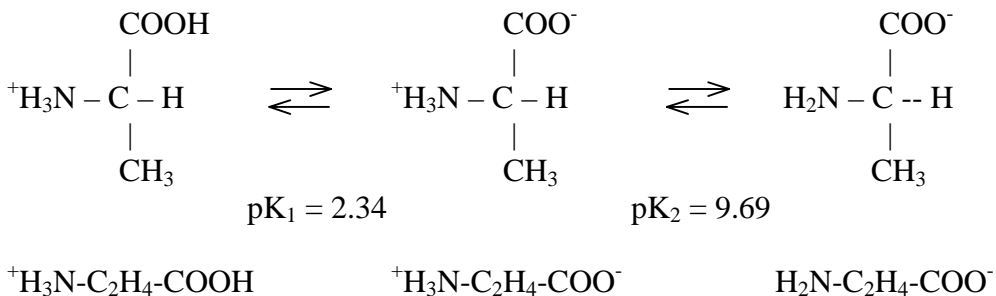
(b) HA is the predominant species when the pH is less than the  $pK_a$

(c)  $A^-$  is the predominant species when the pH is higher than the  $pK_a$

(d) When the pH is 1.0 pH unit above the  $pK_a$ , the ratio of [HA] to  $[A^-]$  is 1/10, when the pH is 1.0 pH unit below the  $pK_a$ , the ratio of [HA] to  $[A^-]$  is 10/1;

(e) When the pH is 2.0 pH units above the  $pK_a$ , the ratio of [HA] to  $[A^-]$  is 1/100, when the pH is 2.0 pH units below the  $pK_a$ , the ratio of [HA] to  $[A^-]$  is 100/1;

3. (a)



(b)

(1)  $^+\text{H}_3\text{N}-\text{C}_2\text{H}_4-\text{COOH}$ ; (2)  $^+\text{H}_3\text{N}-\text{C}_2\text{H}_4-\text{COO}^-$ ; (3)  $^+\text{H}_3\text{N}-\text{C}_2\text{H}_4-\text{COO}^-$ ; (4)  $\text{H}_2\text{N}-\text{C}_2\text{H}_4-\text{COO}^-$

(c) 99.96%

4. 2.5 mL;  $[\text{OH}^-] = 10^{-7} \text{ M}$  (before) and  $10^{-12} \text{ M}$  (after)

If you take NaOH instead, the pH would be 12.0.

5. (a)  $[\text{H}^+] = 1.74 \cdot 10^{-5} \text{ M}$ ; pH = 4.76;

(b)  $[\text{H}^+] = 2.12 \cdot 10^{-5} \text{ M}$ ; pH = 4.67;

(c) before:  $[\text{H}^+] = 1.56 \cdot 10^{-4} \text{ M}$ ; pH = 3.81; after:  $[\text{H}^+] = 3.3 \cdot 10^{-4} \text{ M}$ ; pH = 3.48

(d) before:  $[\text{H}^+] = 1.93 \cdot 10^{-6} \text{ M}$ ; pH=5.71; after:  $[\text{H}^+] = 3.07 \cdot 10^{-6} \text{ M}$ ; pH = 5.51

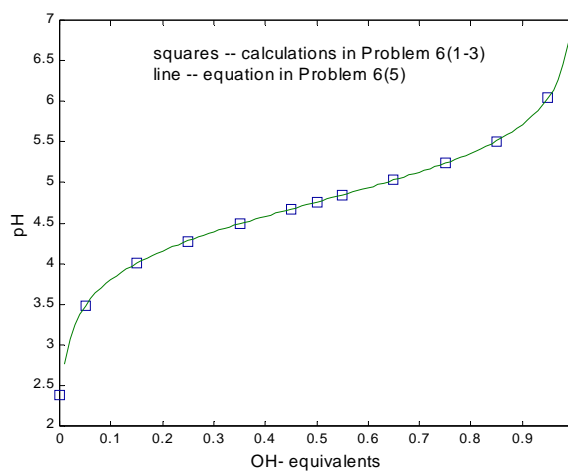
(e) the initial concentrations of protons and the pH are same. The final numbers are:

(b)  $[H^+] = 0.05 \text{ M}$ ;  $\text{pH} = 1.30$ ; (c)  $[H^+] = 0.13 \text{ M}$ ;  $\text{pH} = 0.89$ ; (d)  $\text{pH} = 4.01$ ;  
 $[H^+] = 9.85 \cdot 10^{-5} \text{ M}$

6. (1)  $\text{pH} = 2.38$  for acetic acid, 3.43 for dihydrogen phosphate, and 4.63 for ammonium ion.

The titration curve is calculated below only for acetic acid:

Total OH-equivalents added	pH
0	2.38
0.05	3.48
0.15	4.01
0.25	4.28
0.35	4.49
0.45	4.67
0.50	4.76
0.55	4.85
0.65	5.03
0.75	5.24
0.85	5.51
0.95	6.04



7. *Method 1.*

(1) 6.9 mL; 69 mL; 137.7 mL

(2) 6.86

(3) 5.86; 7.16.

*Method 2.*

10.7 mL; 54.6 mL; 88.4 mL.

8. 6.68; 1.6.