

Biology/Chemistry 302 -- Biochemistry Aqueous equilibria and buffer construction

(last revised 2006 Sep 14)

Goals:

Making solutions

- 1) Given a periodic table, you should be able to calculate the number of grams of solute needed to create an aqueous solution of any given concentration of any soluble substance.
- 2) You should be able to envision and calculate how to create a solution of any given concentration of any soluble substance, starting with a concentrated stock solution of that substance.

General aqueous equilibria

- 3) For any given chemical equation, you should have a deep-seated understanding of the relationship between the magnitude of the equilibrium constant and the concentration of species in solution at equilibrium.
- 4) Given a chemical equation, an equilibrium constant for that equation, and starting concentrations of the chemical species involved, you should be able to calculate the concentrations of all species at equilibrium.

Buffers

- 5) Given a table of weak acid K_a s or pK_a s, you should be able to choose an appropriate acid to use to make a buffer solution at any given pH.
- 6) Given a table of weak acid K_a s or pK_a s, you should be able to calculate the solution concentrations of the major chemical species in a buffer solution of any given concentration at any given pH.
- 7) Given a table of weak acid K_a s or pK_a s and stock solutions of relevant strong acids and bases, you should be able to envision and describe the steps required to create a buffer solution of any given concentration at any given pH.

a. Solution Stoichiometry

1. What is the molarity of a solution created by dissolving 20.0 g of NaOH in water to 2.0 liters?

0.25 M

2. If ten milliliters of the solution in #1 are transferred to a new flask and the volume is increased to 200 mL, what is the new concentration?

0.0125 M

3. If ten milliliters of the solution in #1 are transferred to a new flask, 0.585 grams of NaCl are added, and the volume is adjusted to 50 mL, what is the concentration of sodium ions?

0.25 M

4. What is the concentration of sodium ions in a solution that is made by dissolving 0.4 moles of sodium phosphate in water to 400 mL?

3 M

5. Suppose I want to take a portion of the solution in #1 and dilute it to 300 mL in order to create a 0.004 M solution of NaOH. How many milliliters of the solution in #1 would I have to use?

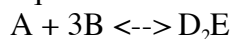
4.8 mL

A. Qualitative analysis of equilibrium, and interpreting K_{eq}

1. Consider two beakers containing equimolar concentrations of acid. A contains HCl, B contains oxalic acid. Both acids are titrated to their endpoints (equivalence points). Will the pH of beaker A be higher, will B be higher, or will they be equal at the equivalence point?

B

2. The equilibrium constant for the following reaction is 0.02.



If $[A] = 0.5$, $[B] = 1$, and $[D_2E] = 0.4$, is $Q > K$, $Q < K$, or $Q = K$?

$Q > K$

3. Consider an acetic acid/acetate buffer at pH 5.0. To beakers containing equal amounts of this buffer are added: (A) 0.002 moles NaOH and (b) 0.002 moles sodium acetate. Will A, B, both, or neither cause the pH of the buffer to rise?

both

4. In solution above a saturated solution of silver chloride at room temperature, the silver ion concentration is about 1×10^{-5} M. Two such solutions are put into beakers A and B, and to beaker A is added .005 moles of hydrochloric acid. If 0.005 moles of nitric acid are added to beaker B, would you expect that beaker A, beaker B, both, or neither would show an INCREASE in the molar solubility of silver chloride?

neither

5. Citric acid is a triprotic acid with three K_a values: 7.4×10^{-4} , 1.7×10^{-5} , and 4.0×10^{-7} . Ammonia is a weak base with $K_b = 1.8 \times 10^{-5}$. Which is a stronger base, ammonia or dihydrogen citrate?

K_b for dihydrogen citrate = 1.3×10^{-11} , so ammonia is stronger

6. If you dissolve disodium monohydrogen citrate in water, would you expect the resulting solution to be acidic, basic, or neutral? (refer to #5)

very weakly basic (remember to use the right K_a in computing K_b , which will be the K_a of its conjugate acid)

7. If you dissolve equal amounts of sodium nitrate and ammonium nitrate in water (that is, a mixture of the two), would you expect the resulting solution to be acidic, basic, or neutral?

acidic

8. Is the solution described in #7 a buffer?

no

9. True or false: if reaction A's rate constant is bigger than reaction B's, then reaction A's rate will always be larger than reaction B's.

false

10. For the equilibrium described in question #2, which of the following are true?

- The reactants are more stable than the products
- The products can be expected to be more plentiful at equilibrium
- The activation energy for the formation of D_2E from A and B is lower than the activation energy for the reverse reaction.

only (a) is true

B. Quantitative analysis of equilibrium

i. Preamble: terms and definitions

kinds of K_s

pX

11. What is the pH of an aqueous solution that has a $[H^+]$ of 6.65×10^{-10} M?

9.18

12. What is the pH of an aqueous solution that has a $[OH^-]$ of 3.45×10^{-5} M?

9.54

13. a. If in an aqueous solution $3[H^+] = [OH^-]$, what is the pH of the solution?

7.24

b. If in an aqueous solution $[H^+] = 7.5 [OH^-]$, what is the pH of the solution?

6.56

14. The K_{sp} for magnesium hydroxide is 9×10^{-12} at 25 °C. Write a balanced chemical equation that describes this equilibrium.



1. The simple case: starting with all reactant or all product

15. Magnesium phosphate is a soluble salt. If 0.5 moles of magnesium phosphate are added to 500 mL of water, what will be the solution concentration of magnesium ions?

3 M

16. Magnesium hydroxide is a sparingly soluble salt with $K_{sp} = 9 \times 10^{-12}$. If 0.5 moles of magnesium hydroxide are added to 500 mL of water, will the solution concentration of magnesium ions be greater than, less than, or equal to 1.0 M?

less

17. A 400 mL solution contains 845 mg of sodium hydroxide. What is the pH of this aqueous solution?

12.9

18. What is the pH of a saturated solution of magnesium hydroxide? (see #14 for the K_{sp})

10.4

19. A 0.01 M solution of a monoprotic organic acid has a pH of 3.67. What is the pKa of the acid?

5.33

20. The pKa of chloroacetic acid is 2.85. What is the pH of a 0.05 M solution of this acid?

2.11

21. A solution of sodium hypochlorite is made by dissolving the solid salt in 500 mL of water. The pH of the resulting solution is found to be 4.5. The K_a of hypochlorous acid is 3×10^{-8} . How much solid sodium hypochlorite (52.5 g/mol) must have been added to the water? (You may assume that it was completely soluble.)

0.875 g

2. Starting with a non-equilibrium mixture of reactants and products

22. Sodium malate (2.22 g; 186 g/mol) and sodium hydrogen malate (0.73 g; 162 g/mol) were dissolved in water and diluted to 0.5 L. What is the pH of the resulting buffer? Use pK_a values of 3.4 and 5.1 for malic acid.

5.5

23. A 1.00 g sample of propionic acid (74 g/mol) and 0.86 g of potassium propionate were combined with enough water to make 0.10 L of solution. If the pK_a of propionic acid is 4.87,

what is the pH of the solution?

4.65

3. "Alien influences": when an external chemical agent perturbs an equilibrium mixture

24. What is the pH of a 250 mL solution that is made by mixing 0.02 moles of potassium hydroxide and 0.10 moles of hydrofluoric acid ($K_a = 6 \times 10^{-4}$)?

2.62

25. What is the pH of a 500 mL solution that is made by mixing 200 millimoles of phosphoric acid and 130 millimoles of potassium hydroxide? Use pKa values of 2.12, 7.21, and 12.7 for phosphoric acid.

2.39

Groups of questions unified by chemical focus, but crossing problem "types"

Silver cyanide (AgCN) has a K_{sp} of 2.2×10^{-16} .

Hydrocyanic acid (HCN) is a weak acid with $\text{p}K_a = 9.14$.

26. Will the solubility of silver cyanide go up or down in acidic solution relative to pure water?

up

27. What is the molar solubility of silver cyanide in pure water?

$1.5 \times 10^{-8} \text{ M}$

28. What is the molar solubility of silver cyanide in 0.01 M sodium cyanide?

$2.2 \times 10^{-14} \text{ M}$

29. What is the pH of a 0.03 M solution of hydrocyanic acid?

5.33

30. What is the pH of a 0.03 M solution of hydrocyanic acid in 0.005 M sodium cyanide?

8.36

Benzoic acid ($\text{HC}_7\text{H}_5\text{O}_2$) is a weak acid with $\text{p}K_a = 4.18$

31. Will adding NaOH to a solution of benzoic acid increase or decrease the level of dissociation?

increase

32. Will adding sodium benzoate to a solution of benzoic acid increase or decrease the pH?

increase

*****AND MORE*****

1) What is the pH of a solution that is 0.01 M in hydroxide ions?

12

2) What is the pH of a solution made by dissolving 0.01 moles of nitric acid in water to a final volume of 300 mL?

1.5

3) What is the pH of a solution made by dissolving 0.02 moles of acetic acid in water to a final volume of 300 mL?

2.9

4) What is the pH of a solution made by dissolving 0.03 moles of sodium acetate in water to a final volume of 300 mL?

8.9

5) What is the pH of a solution made by dissolving 0.01 moles of nitric acid AND 0.03 moles of sodium acetate in water to 300 mL?

5.04

6) What is the pH of a solution made by dissolving 0.02 moles of acetic acid AND 0.03 moles of sodium acetate in water to 300 mL?

4.92

7) What is the pH of a solution made by dissolving 0.01 moles of nitric acid AND 0.03 moles of sodium acetate AND 0.02 moles of acetic acid in water to 300 mL?

4.56

*****AND EVEN MORE*****

1) what's the pH of a solution made by dissolving 0.003 moles of sodium benzoate in water to a volume of 300 mL?

(all K_a , K_b , and K_{sp} values are in the tables I handed out in class)

1) 8.09

2) what's the molar solubility of copper(I) iodide?

2) $1 \times 10^{-6} \text{ M}$

3) what's the pH of a solution made by dissolving 0.12 moles of monochloro acetic acid in water to a volume of 45 mL?

3) 1.21

4) Suppose I took the solution in #1, and added 0.001 moles benzoic acid to it. What would be the new pH?

4) 4.66

5) Suppose I took the copper(I) iodide, but instead of dissolving it in water, I dissolved it in 1 mM NaI. What would be the molar solubility in this solution?

5) $1 \times 10^{-15} \text{ M}$

6) How much sodium monochloro acetate would I have to add to the solution in #3 to bring it to pH 3.0?

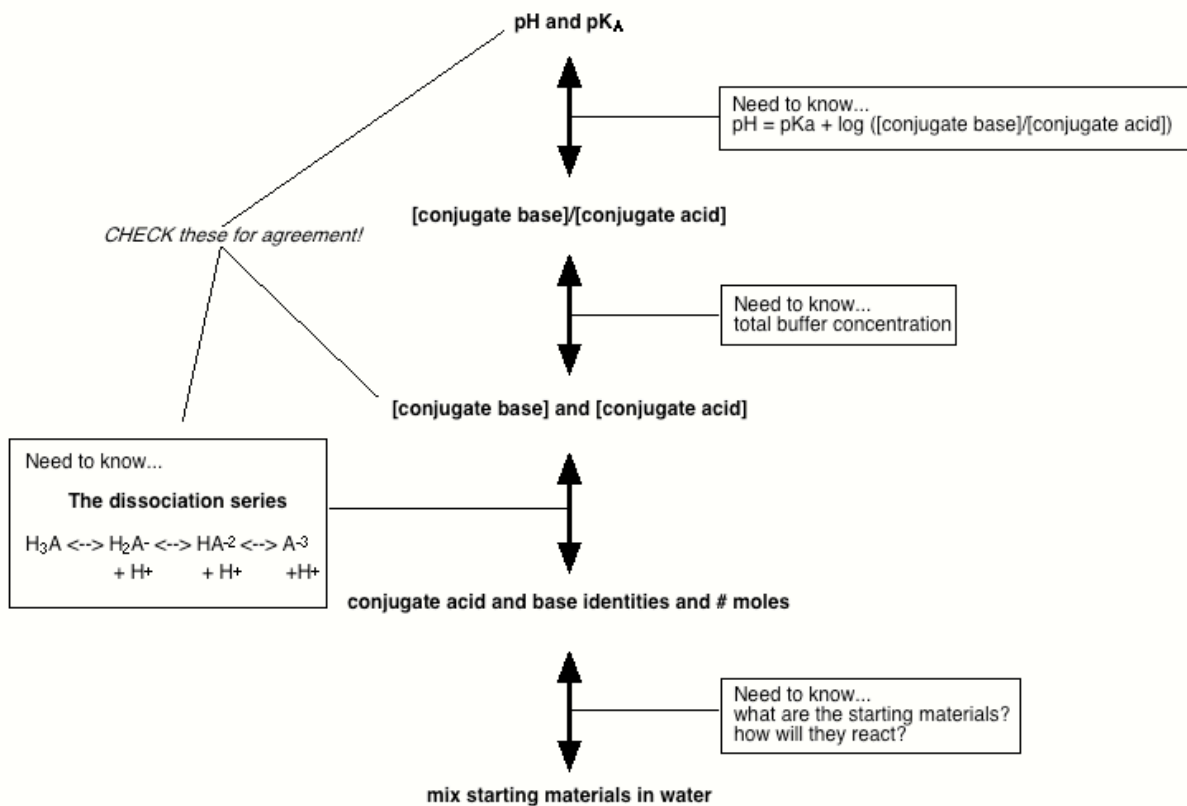
6) 0.17 moles

BUFFERS -- these fall under general category B2 above -- non-equilibrium mixtures of reactants (here, a weak acid) and products (its conjugate base). We treat them as special, but they really aren't.

Strategy for a general build-a-buffer scenario:

- 1) write out the dissociation equilibria involved
- 2) write down the relevant pK_a s
- 3) given target pH and pK_a s, figure out the dominant buffer species at equilibrium
- 4) given target pH and total buffer concentration, calculate equilibrium concentrations of the dominant species
- 5) given the available resources, figure out a way to get to the concentrations you've just calculated

Buffer Construction Flowchart



A monocarboxylic acid and its potassium salt were combined in a 2:1 mole ratio and dissolved in water. How might you mathematically define the pH of the resulting solution?

- A. $\text{pH} = \text{pKa} - 0.30$
- B. $\text{pH} = \text{pKa} + 0.30$
- C. $\text{pH} = \text{pKa} - 0.70$
- D. $\text{pH} = \text{pKa} + 0.70$
- E. $\text{pH} = 0.30$

A

At what pH's will the average charge on the phosphate species be: -0.5, -1.0, -1.5? Use pKa values of 2.12, 7.21, and 12.7 for phosphoric acid.

- A. 2.12, 7.21, 12.7
- B. 2.12, 4.67, 7.21
- C. 4.67, 9.96, 12.7
- D. 7.21, 9.96, 12.7
- E. none of the above

B

What is the pH of a 250 mL solution that contains 0.75 g of potassium hydroxide and 4.76 g of potassium dihydrogen phosphate? Use pKa values of 2.12, 7.21, and 12.7 for phosphoric acid.

7.00

What is the pH of a 250 mL solution that contains 3.17 g of potassium monohydrogen phosphate and 3.90 g of potassium dihydrogen phosphate?

7.41

We commonly refer to the "total buffer concentration" of a buffer solution -- the sum of the concentrations of the conjugate acid-base pair that produces the buffer. If 25.0 mL of a 0.25 M potassium monohydrogen phosphate was combined with 175 mL of 0.033 M potassium dihydrogen phosphate, what is the concentration of the resulting buffer? Assume the volumes to be additive.

0.060 M

In lab, you're having fun with buffers based on a 0.1 M solution of sodium phosphate at pH 7.0. In this system, there are three pK_{AS} : 12.7, 7.21, and 2.12.

A. You may recall that in the pre-lab lecture on the first day, you were shown a chart indicating the relative amounts of different forms of phosphate (H_3PO_4 , $H_2PO_4^-$, HPO_4^{2-} , PO_4^{3-}) needed to produce buffers of different pH. For your buffer conditions, please indicate to me the predominant phosphate species present, and the relative amounts of each at equilibrium.

62% $H_2PO_4^-$, 38% HPO_4^{2-} (H_3PO_4 and PO_4^{3-} are not present at meaningful levels)

B. What is the concentration of hydroxide ion in this buffer?

$[OH^-] = 1.0 \times 10^{-7} M$

C. Would it be possible for you to make this buffer, starting with only 1 M phosphoric acid and 6 M HCl? Or with 1 M phosphoric acid and 5 M NaOH? Explain.

We need a mix of diprotonated and monoprotonated phosphate. Since both of these have lower levels of protonation than phosphoric acid, to get to pH 7 we need to deprotonate the phosphoric acid stock. Thus the HCl wouldn't work, but the NaOH would.

D. If you were to make this buffer, starting with 1 M phosphoric acid and adding sodium hydroxide until the pH reached 7.0, how many moles of sodium hydroxide would it take?

0.138 moles NaOH -- enough to first convert all the H_3PO_4 to $H_2PO_4^-$ (100 mmol), plus enough to deprotonate 38% of the $H_2PO_4^-$ (38 mmol).

A few years ago, one of my research students came across an experimental protocol recommended by a biochemical reagent supplier that involved a Tris buffer at pH 6.0 ("Tris" is a nickname for Tris hydroxymethyl amino methane). Please consult the pK_A table given above and answer the questions below.

a) Is Tris a compound you would choose for a buffer at pH 6.0? Please carefully explain to me why it is or is not. If it is not a chemistry you'd choose for this buffer, please suggest an alternative chemistry and justify your choice.

Tris is NOT an optimal buffer chemistry for pH 6, as this is more than 2 pH units away from the pK_a for Tris. Thus, at this pH, Tris would be > 90% protonated and would be a lousy buffer against added acid. MES and carbonic acid both have pK_a values around pH 6 and would therefore be much better choices.

b) What will be the concentration of hydroxide ions in this buffer?

$[OH^-] = 1.0 \times 10^{-8} M$

c) Given the compound you selected in part (a) for this buffer, please tell me the equilibrium concentrations you'd expect for the conjugate acid and conjugate base species in the buffer at pH 6.0. Assume that you are dealing with 1.00 liters of buffer at a total buffer concentration of 10.0 mM.

For carbonic acid: $[HA] = 6.9 mM$, $[A^-] = 3.1 mM$

For MES: $[HA] = 5.5 \text{ mM}$, $[A^-] = 4.5 \text{ mM}$

d) Suppose I want to make this buffer by dissolving in water some amount of the compound that you selected from the table, and then adding either strong acid (e.g., 6.0 M HCl) or strong base (e.g., 5.0 M NaOH) to get to the correct pH. Please tell me how many moles of your chosen compound I should start with, and how many milliliters of strong acid or strong base you predict I would need to add. (If you are unsure of your answer to part (c), assume here that the conjugate acid and base concentrations are each equal to 5.0 mM)

For carbonic acid: start with 10.0 mmol carbonic acid in 800 mL water, add 0.62 mL (3.1 mmol) NaOH, bring the total volume to 1.0 liter.

For MES: start with 10.0 mmol MES (protonated form), add 0.90 mL (4.5 mmol) NaOH, bring to volume.

Bonnie the Biochem Goddess is ready to carry out an ion-exchange chromatography experiment, but she needs to mix up a liter of buffer first, at a concentration of 100 mM and a pH of 10.0. Being the geek/goddess that she is, she pulls out her handy pocket buffer chart, reproduced below.

Compound	pK
Oxalic acid	1.27
H ₃ PO ₄	2.15
Citric acid	3.13
Formic acid	3.75
Succinic acid	4.21
Oxalate ⁻¹	4.27
Acetic acid	4.76
Succinate ⁻¹	5.64
MES	6.09
Carbonic acid	6.35
PIPES	6.76
H ₂ PO ₄ ⁻	6.82
MOPS	7.15
HEPES	7.47
Tricine	8.05
Tris	8.08
Glycylglycine	8.25
Bicine	8.26
Boric acid	9.24
Bicarbonate ⁻¹	10.33
Piperidine	11.12
HPO ₄ ⁻²	12.38

(data from Dawson et al (1986), *Data for Biochemical Research* (3rd ed), pp. 424-425)

a) Which of these compounds would be reasonable choices for her to use for her buffer? Why? Assume that Bonnie has access to all of these compounds in her stockroom.

Boric acid, bicarbonate

b) Being the wonderful soul that you are, you offer to make Bonnie's buffer for her. Please describe how you would do this, using only the compounds listed in the chart plus NaOH (5 M solution) and HCl (6M solution). Do not worry about the molar masses; simply give me mole quantities.

This will depend on the buffer chemistry chosen, but for example: I want 1.0 liters of 100 mM bicarbonate buffer at pH 10.0. Thus I need to start with 100 mmol sodium bicarbonate and convert part of this weak acid into its conjugate base. To do this, I will add NaOH until the pH reaches 10. This will be just short of the pKa (to be specific, when I have added 32 millimoles of NaOH, giving me 32 mmol carbonate and 68 mmol bicarbonate).

More and more and more... The single best way to practise buffer problems is to make your own questions. For instance,

- pick a pH and buffer concentration. Choose a buffer chemistry from the table above, and determine the identities and equilibrium concentrations of the dominant buffer species.

...or...

- pick a weak acid from the pKa table, and identify its conjugate base. Choose arbitrary numbers of moles of each. Pick a random volume, and determine the resulting buffer pH.

Of course, questions like this have no answer key. Whatchya gonna do? Grab a classmate, and have them answer the same questions. Compare answers. Still not sure you know the right answer? Email me your questions, and I'll tell you my answers. Hopefully I'll get 'em right!