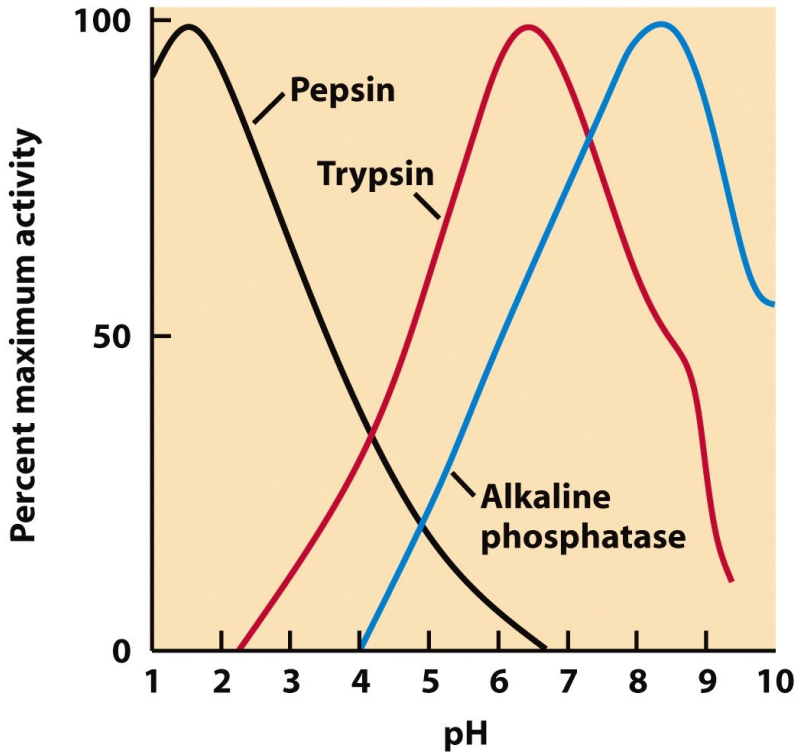
Bio375
Class 2:
(Ch. 2, pp. 58-69)

pH & biological buffers

Biological fluids are highly buffered

Fluid	pH
Blood plasma	7.4
Human milk	7.4
Saliva	6.4-7.0
Liver cytosol	6.9
Lysosomal matrix	5.5-6.5
Gastric juice	1.5-3.0



- Constant pH necessary because structure and function of macromolecules are profoundly affected by pH.

- Constant pH maintained by **buffers** (weak acid & conjugate base).

Sorensen's pH scale

TABLE 2–6	The pH Scale		
$[H^+]$ (M)	pH	$[OH^-]$ (M)	pOH*
10^0 (1)	0	10^{-14}	14
10^{-1}	1	10^{-13}	13
10^{-2}	2	10^{-12}	12
10^{-3}	3	10^{-11}	11
10^{-4}	4	10^{-10}	10
10^{-5}	5	10^{-9}	9
10^{-6}	6	10^{-8}	8
10^{-7}	7	10^{-7}	7
10^{-8}	8	10^{-6}	6
10^{-9}	9	10^{-5}	5
10^{-10}	10	10^{-4}	4
10^{-11}	11	10^{-3}	3
10^{-12}	12	10^{-2}	2
10^{-13}	13	10^{-1}	1
10^{-14}	14	10^0 (1)	0

- pH measures acidity or $[H^+]$

$$\mathbf{pH = - \log [H^+]}$$

- Negative relationship
- Logarithmic scale
- pH ranges from 0 to 14

$$[H^+]\,[OH^-] = 1 \times 10^{-14}\ M^2$$

$$pH + pOH = 14$$

What is $[OH^-]$ of a solution at pH 6.0?

Bronsted-Lowry definition: acids & bases

- Acid = proton donor
- Base = proton acceptor

Strong acids and bases
undergo **complete** dissociation in solution

Calculate pH of 0.1 M HCl (strong acid)

Calculate pH of 0.1 M NaOH (strong base)

Dissociation of weak acids and bases (more relevant to biological systems)

- **Weak** acids and bases undergo **partial** dissociation until they reach equilibrium
- Extent of dissociation dictated by acid dissociation constant (K_a)

Stronger the acid, greater the tendency to give up protons

$$\mathbf{pK_a = -logK_a}$$

Larger K_a = lower pK_a (-ve, log relationship)

Which one is the strongest acid? Which one is the weakest?

	K_a	pK_a
CH_3COOH	1.7×10^{-5} M	4.8
H_3PO_4	7.1×10^{-3} M	2.2
H_2CO_3	4.5×10^{-7} M	6.4

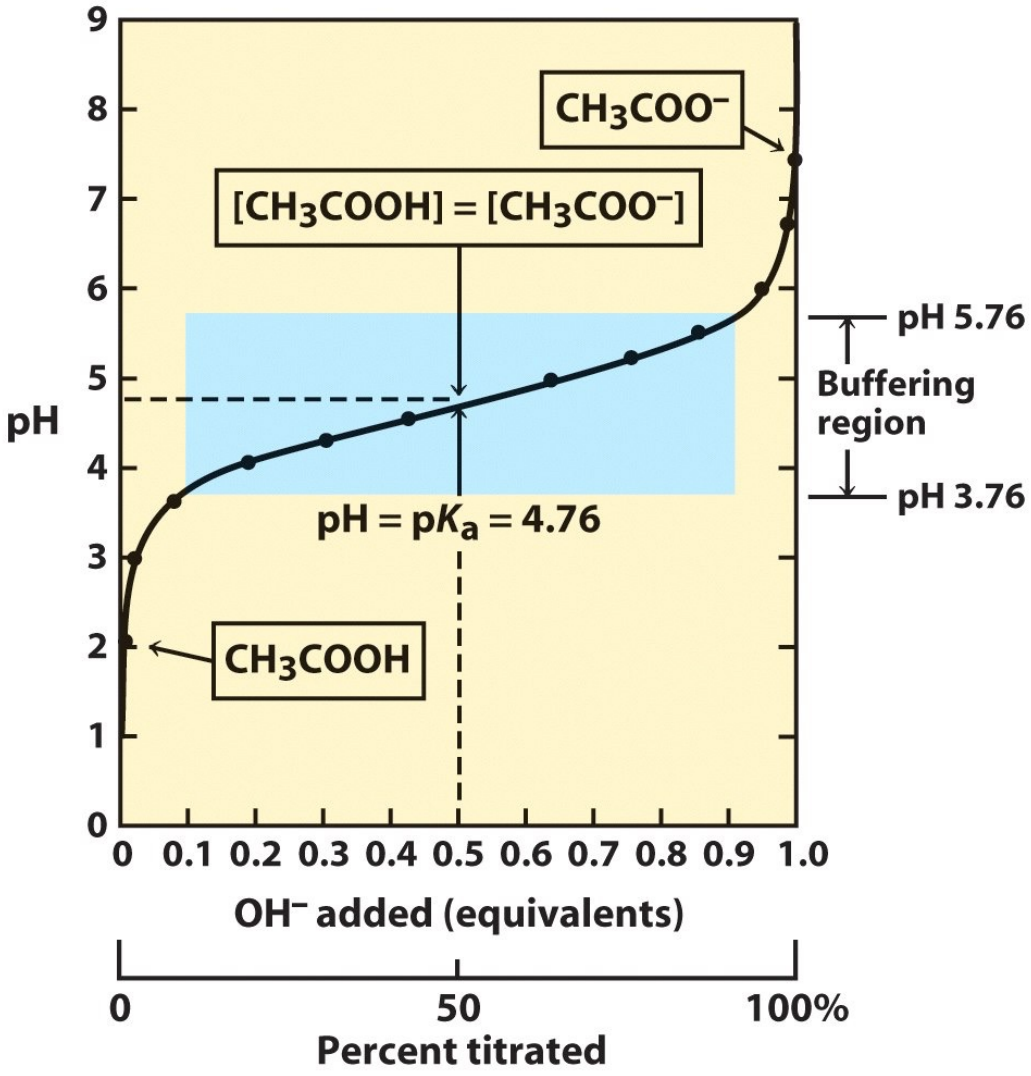
Calculate pH and % dissociation of a solution of 0.1 M CH_3COOH (weak acid)

(biochemists need to know algebra)

Acid-base titration

How? Neutralize acid with a strong base

Base forces more acid to dissociate (reaction forced to the right)



Relationship between pH & pKa

- Start of titration:
  $[HA] >>> [A^-]$

- End of titration, acid is neutralized

- **Midpoint of titration:**
  $[HA] = [A^-]$ &
  $pH = pK_a$

- Buffering range for any weak acid = $pK_a \pm 1$

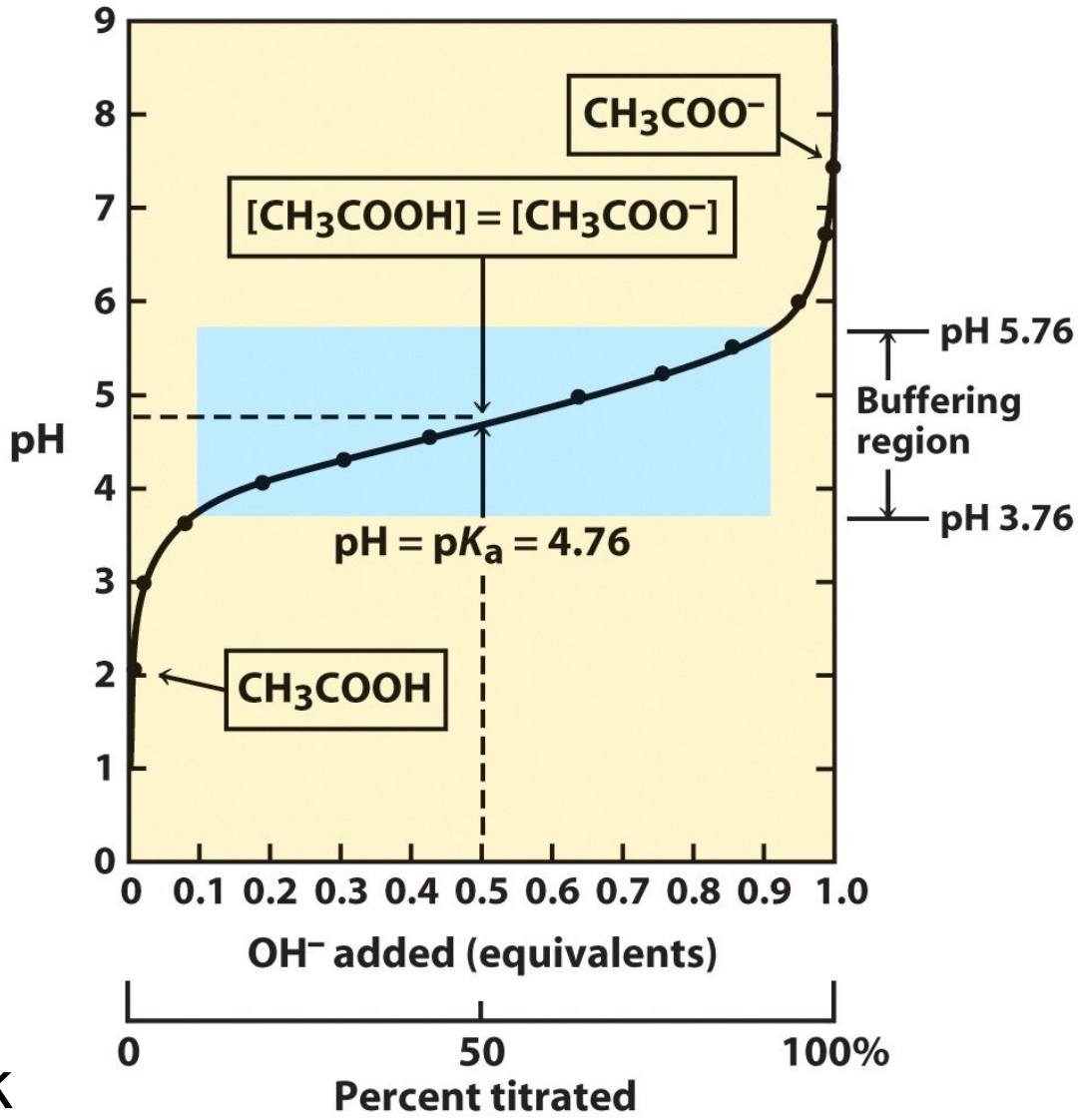


Figure 2-17

Titration curves for all weak acids have similar shapes

- Similar shape but displaced along vertical (pH) axis; because different acids have different pKa values

- All weak acids buffer best in the range of $pK_a \pm 1$

- What is buffering range for:
  - Acetic acid
  - Dihydrogen phosphate
  - Ammonium

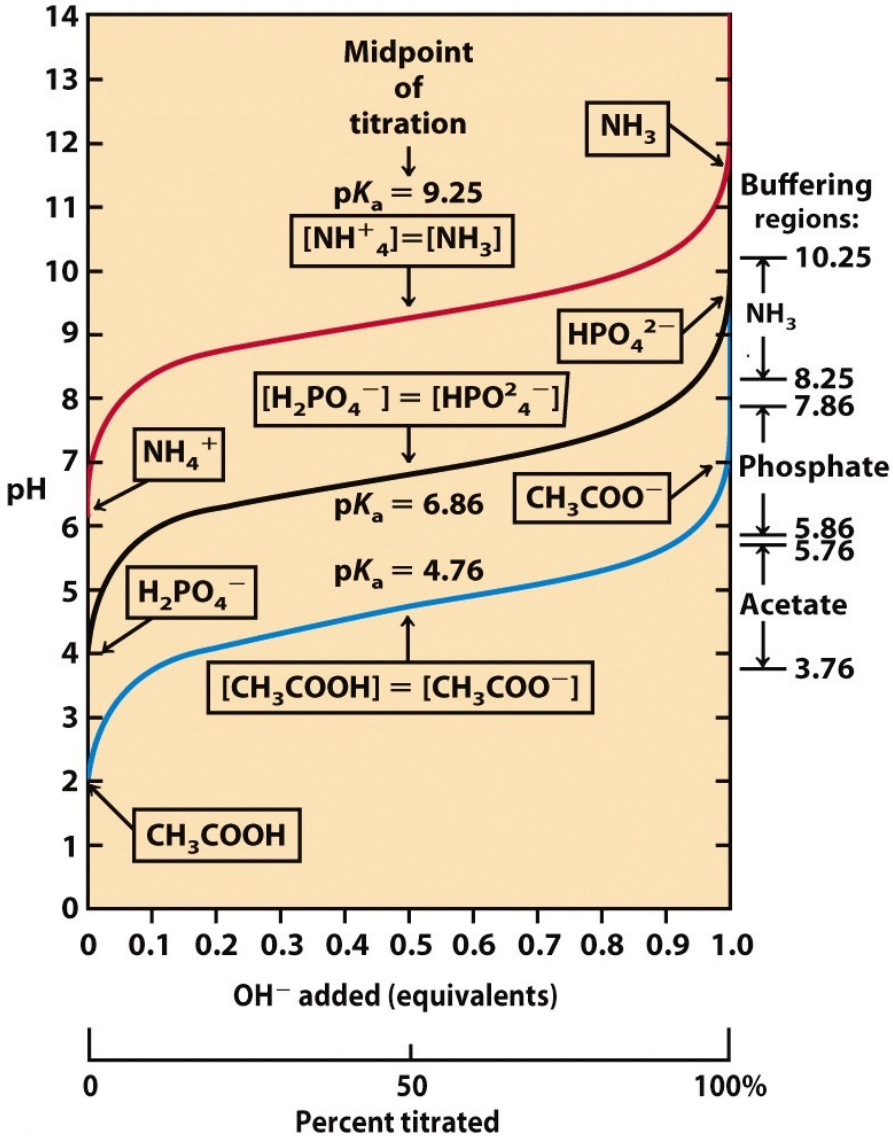


Figure 2-18

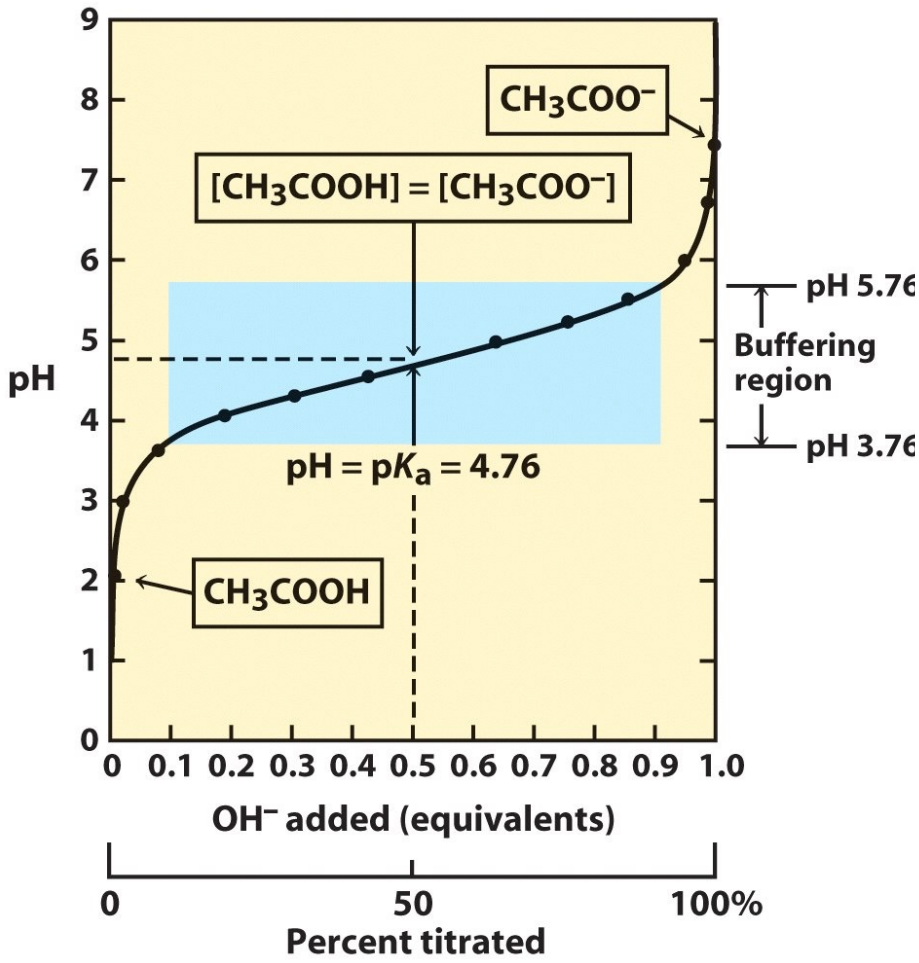
Henderson-Hasselbach equation

Dissociation of a weak acid: $HA \underset{}{\overset{K_a}{\rightleftharpoons}} H^+ + A^-$

Henderson – Hasselbalch:

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

pH of a solution depends on its pKa and the extent to which it has dissociated.



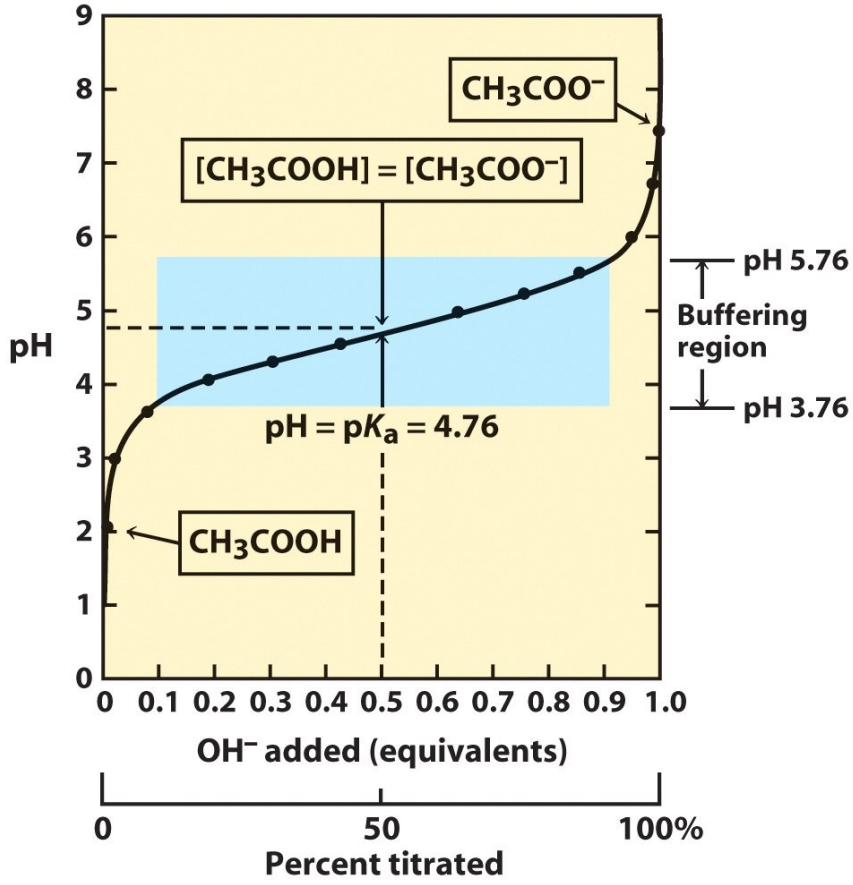
What does H-H equation tell us?

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

1. At midpoint of titration when
   [HA] = [A^-], pH = pKa

2. When pH > pK_a,
   [A^-] > [HA] &
   the system can absorb more H^+

3. When pH < pK_a,
   [HA] > [A^-] &
   the system can absorb more OH^-



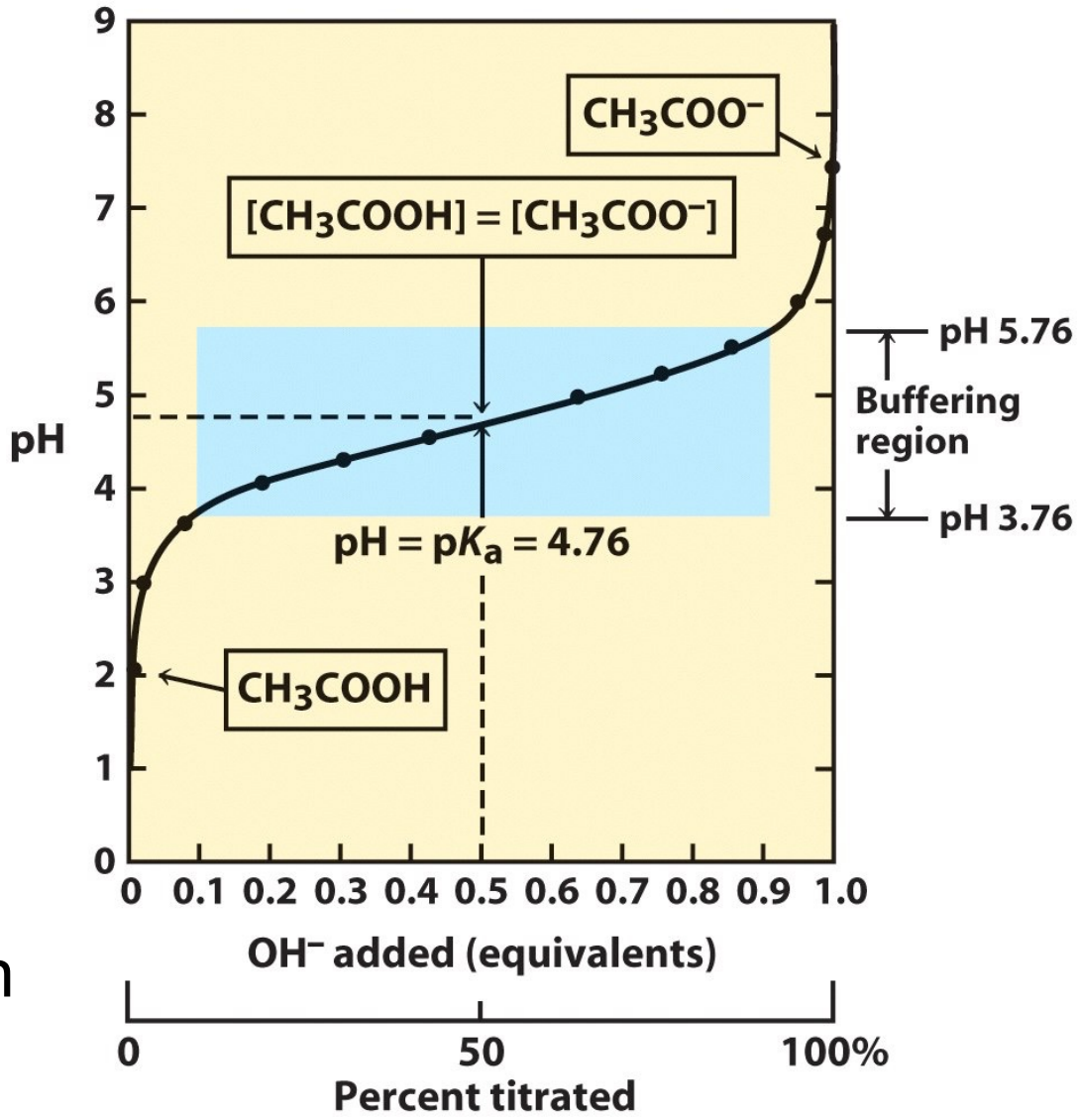
What does H-H equation allow us to do?

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

1. Calculate pH, given pK_a and $[A^-]/[HA]$

2. Calculate pK_a, given pH and $[A^-]/[HA]$

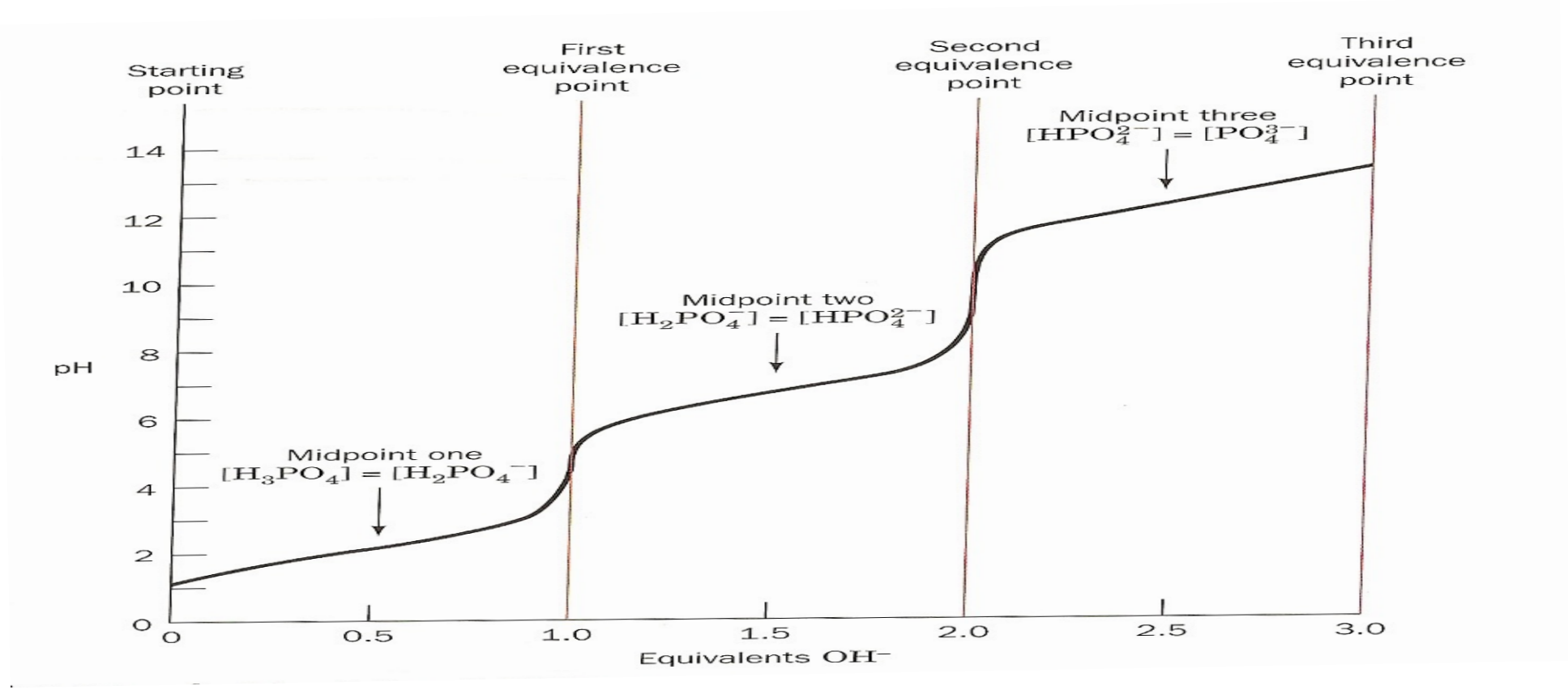
3. Calculate $[A^-]/[HA]$, given pH and pK_a



Biological buffers

- Buffer = mixture of weak acid-conjugate base pair
- Resists pH changes when small amounts of H^+ or OH^- are added
- Biological buffers -- polyprotic acids (more than 1 dissociable H^+)
- Ex. phosphoric acid

To make a buffer at pH 7.5, which acid-conjugate base pair would you use?



How to make a buffer?

- Fulfill two criteria:
    1. Molarity
    2. pH

Steps:

1. Choose weak acid with pKa around pH you want

2. Calculate $[A^-]/[HA]$ ratio using H-H equation

3. Molarity = $[HA] + [A^-]$

Sample problem:
How to make 300 mL of 0.1 M phosphate buffer at pH 7.5 from 1M stocks of H_3PO_4, NaH_2PO_4, Na_2HPO_4, Na_3PO_4?

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How to make 300 mL of 0.1 M phosphate buffer at pH 7.5 from 1M stocks of H_3PO_4, NaH_2PO_4, Na_2HPO_4, Na_3PO_4?

Step 1: Which acid/base pair to use? Look at pKa.

Sample problem:

How to make 300 mL of 0.1 M phosphate buffer at pH 7.5 from 1M stocks of H_3PO_4, NaH_2PO_4, Na_2HPO_4, Na_3PO_4?

Step 2: Calculate ratio of $[A^-]/[HA]$ using Henderson-Hasselbach

Sample problem:

How to make 300 mL of 0.1 M phosphate buffer at pH 7.5 from 1M stocks of H_3PO_4, NaH_2PO_4, Na_2HPO_4, Na_3PO_4?

Step 3: Calculate the appropriate Molar amounts of acid and conjugate base (and don't forget about the **final volume** of the buffer).

pH and buffers homework due Thurs 9/10 in class *(always look at useful information provided) !!*

Normality (N) = Molarity (M) X n (where n = number of protons exchanged)

What is normality of 1M HCl?
What is normality of 1M H_2SO_4?

$N_1V_1 = N_2V_2$