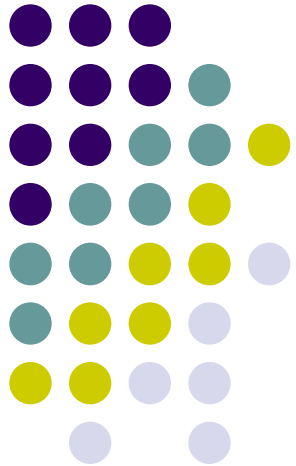
pH and Buffers



pH

- pH is commonly expressed as $-\log[H^+]$

It approximates the negative log (base 10) of the molar concentrations of hydrogen ions H+ (really hydronium ions H_30+) in solution

So a solution of HCl with a pH of 2.0 has a concentration of hydronium ions of $1x\ 10^{-2}$ (1/100!!)

Compared to a more dilute solution of HCl with a pH of 5.0, which has a hydronium ions concentration of 1×10^{-5} (1/100,000).

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- Pure water has $[H^+]=10^{-7}$ and thus pH=7.

pH

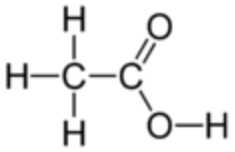
- pH is commonly expressed as $-\log[H^+]$
- Pure water has $[H^+]=10^{-7}$ and thus pH=7.
- Acids have a high $[H^+]$ and thus a low pH.
- Bases have a low $[H^+]$ and thus a high pH.

Bases contribute –OH ions when they dissociate. These bind to the H+ ions produced when water dissociates. Thus, these OH ions “suck up” the H+ ions in solution, reducing their concentration.

NaOH with a pH of 12.0 contributes so many –OH ions that almost all the H+ ions are bound into water molecules, reducing the free H+ (and hydronium) ion concentration to 1×10^{-12} (1,000,000,000,000 = 1/trillion)

pH

Acid	Normality	pH
Acetic	N	2.4
Acetic	0.1 N	2.9
Acetic	0.01 N	3.4
Hydrochloric	N	0.1
Hydrochloric	0.1 N	1.1
Hydrochloric	0.01 N	2.0
Sulfuric	N	0.3
Sulfuric	0.1 N	1.2
Sulfuric	0.01 N	2.1

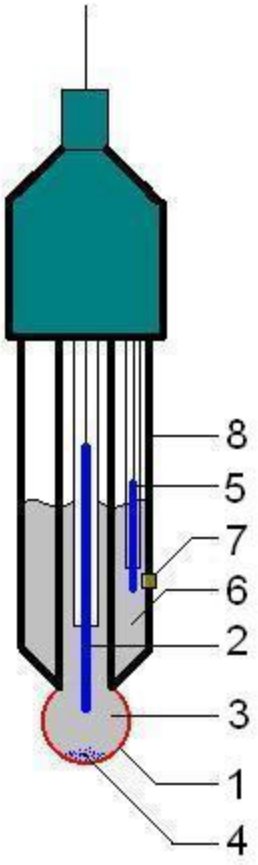


How do normality and molarity relate to pH??

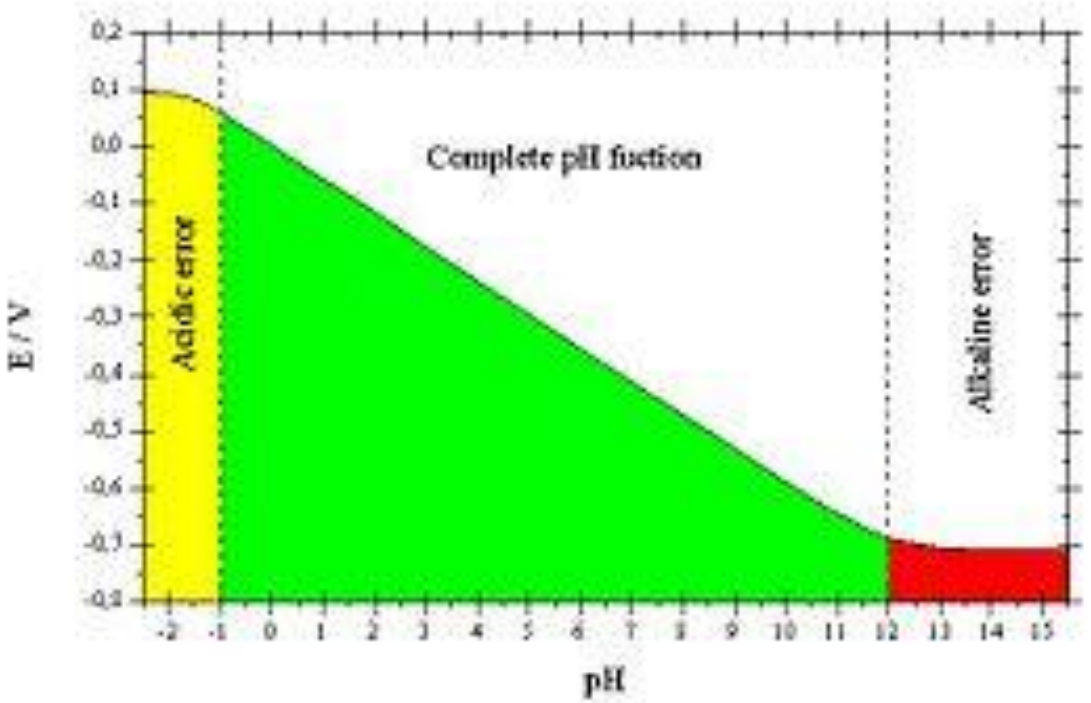
Molarity is the fractions of a mole in solution; normality is a measure of the concentration of reactive groups which may affect pH.

Ways to measure pH

- pH meter
  - Electrode measures H^+ concentration
  - Must standardize (calibrate) before using.



Actually measuring a voltage – a charge differential – between a control solution and the external fluid.



Ways to measure pH

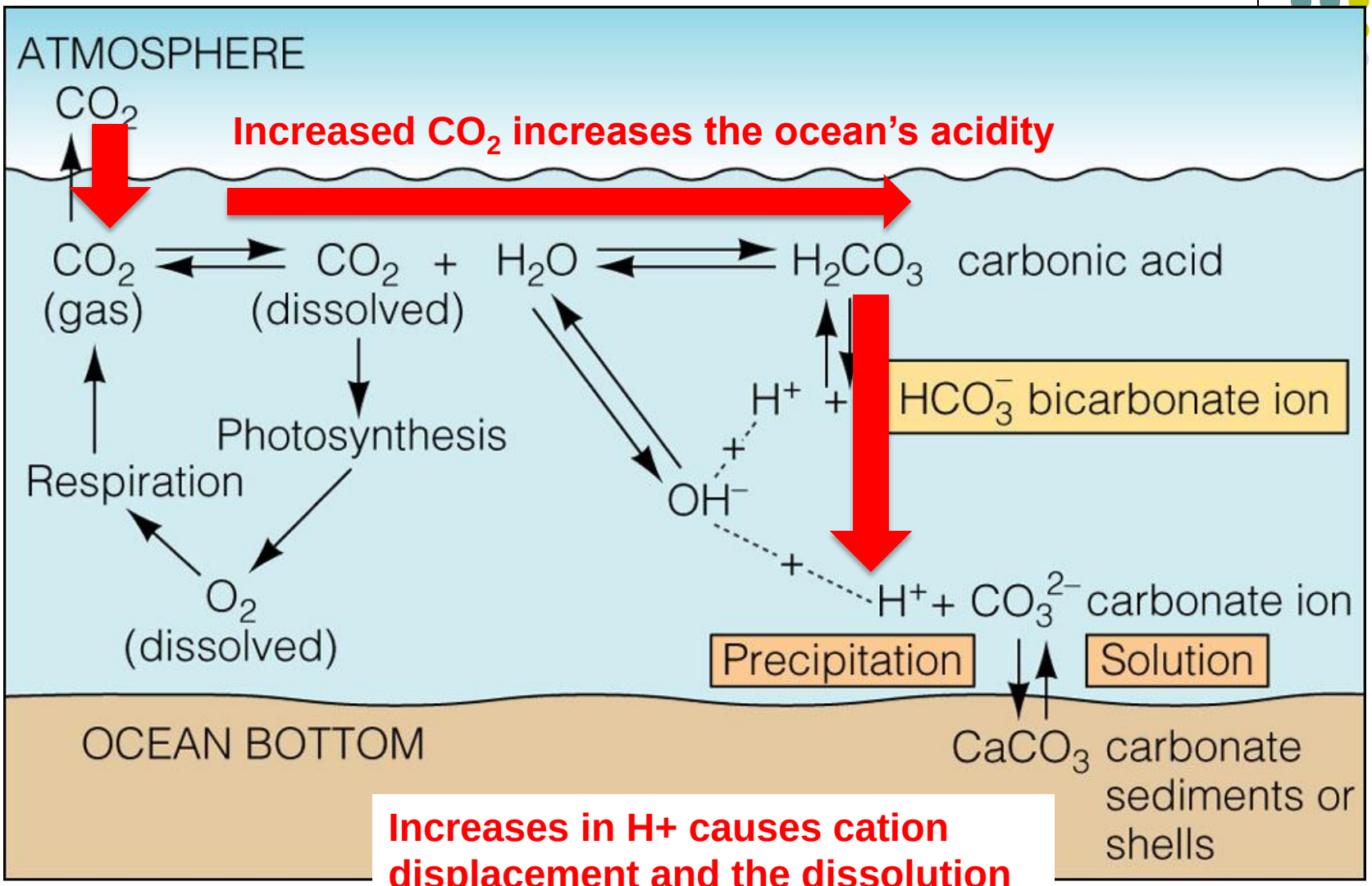
- Indicator dyes and test strips
  - Less precise
  - Each indicator is only good for a small pH range (1-2 pH units)
  - But may be good for field usage, or measuring small volumes, or dealing with noxious samples.

Why is pH important in biology?

- pH affects solubility of many substances.

[A] (mol/L)	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-10}
Initial pH	0.00	1.00	2.00	3.00	4.00	5.00	6.00	6.79	7.00
Final pH	6.75	7.25	7.75	8.14	8.25	8.26	8.26	8.26	8.27
Dissolved $CaCO_3$ (g per liter of acid)	50.0	5.00	0.514	0.0849	0.0504	0.0474	0.0471	0.0470	0.0470

More calcium carbonate dissolves as pH drops



Increases in H+ causes cation displacement and the dissolution of Calcium Carbonate (shell, limestone, etc.)

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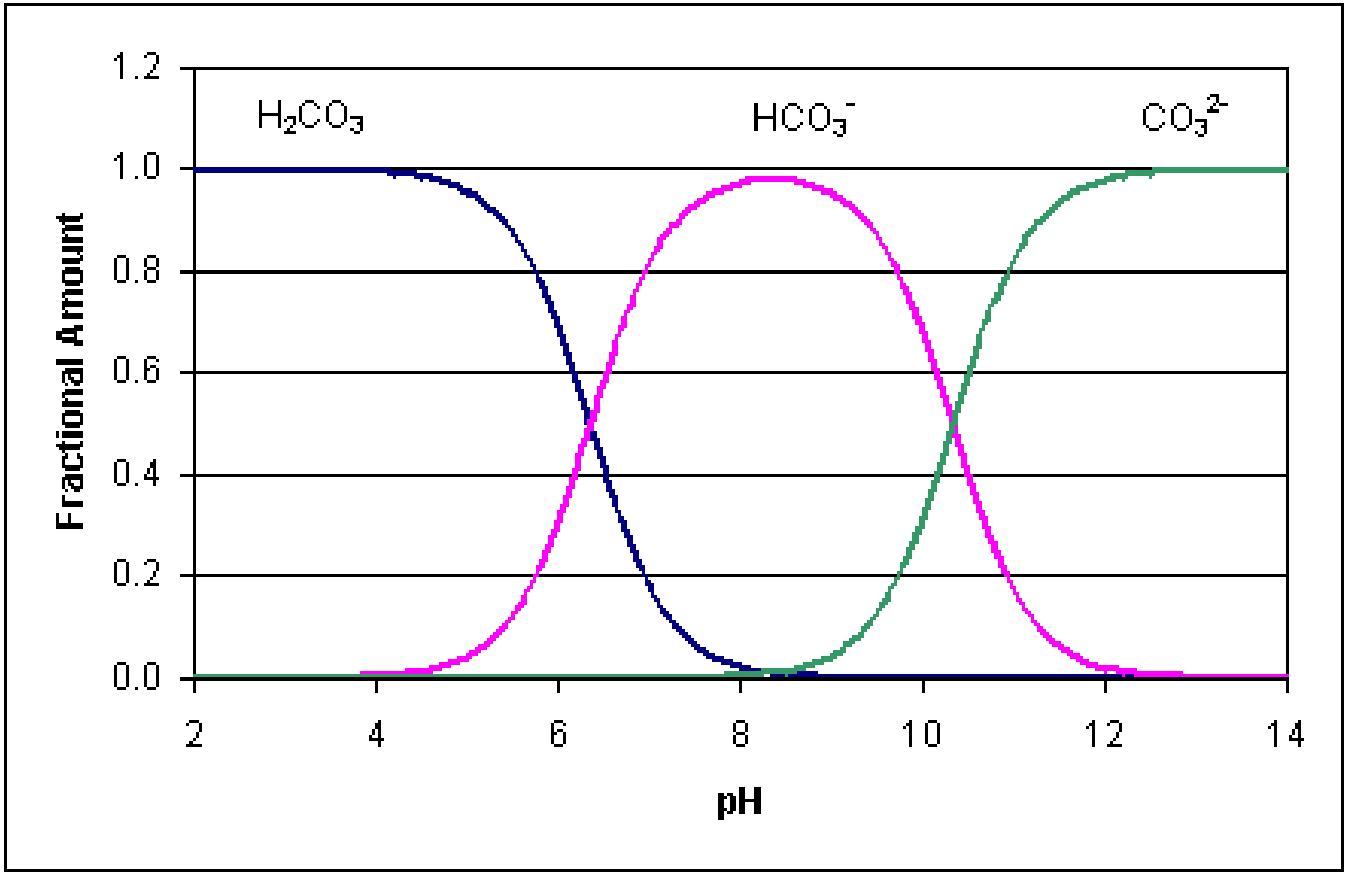
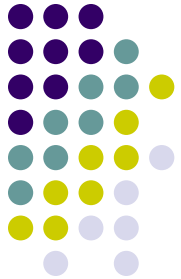
Drives equilibria and reversible states of compounds

Carbonic Acid Bicarbonate Carbonate

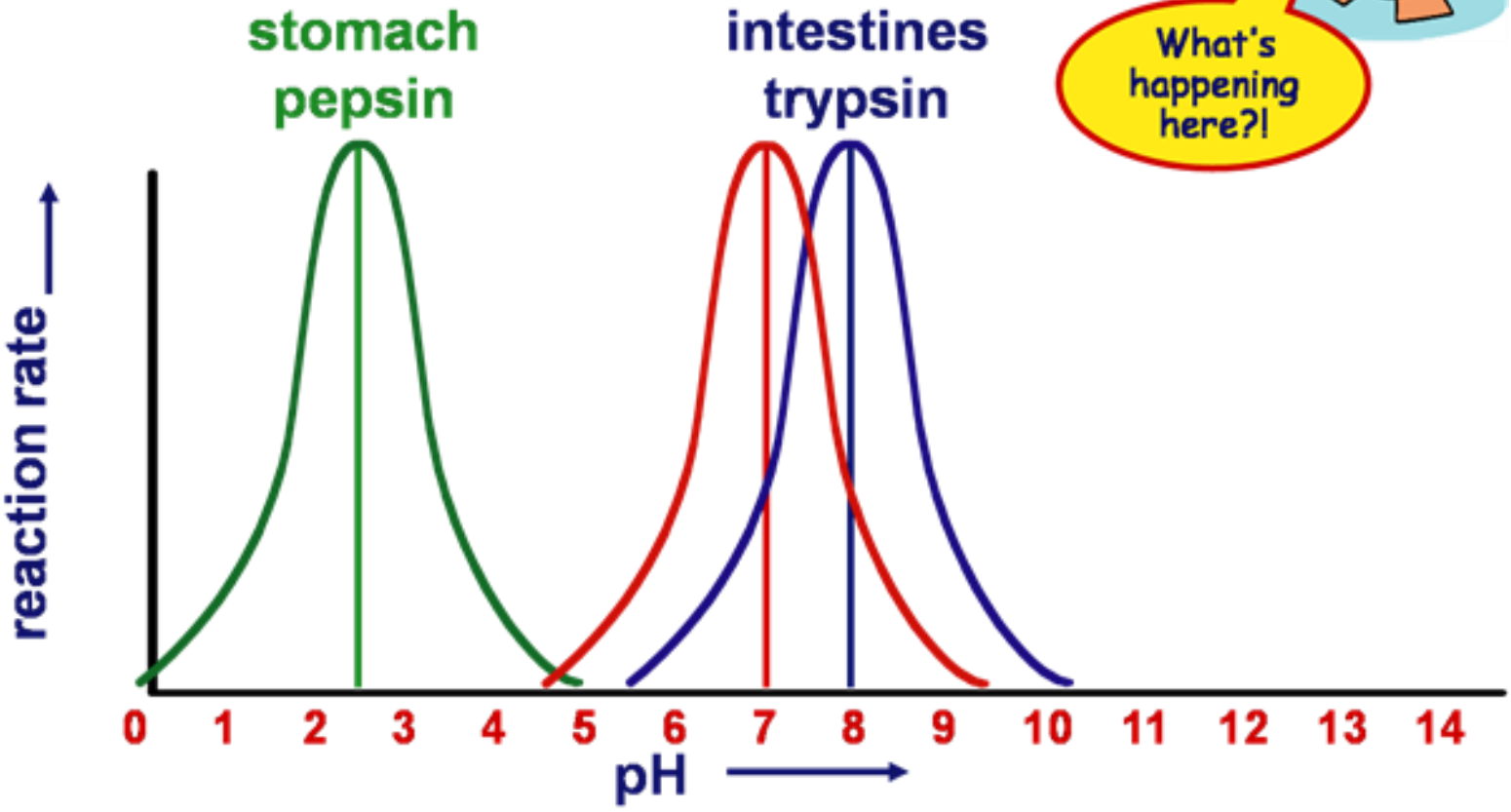


Why is pH important in biology?

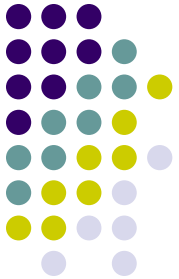


- pH affects solubility of many substances.
- pH affects structure and function of most proteins - including enzymes.

pH

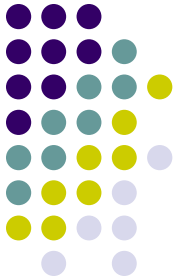

stomach
pepsin
intestines
trypsin
What's happening here?!
reaction rate
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
pH

Why is pH important in biology?



- pH affects solubility of many substances.
- pH affects structure and function of most proteins - including enzymes.
- Many cells and organisms (esp. plants and aquatic animals) can only survive in a specific pH environment.

Why is pH important in biology?



- pH affects solubility of many substances.
- pH affects structure and function of most proteins - including enzymes.
- Many cells and organisms (esp. plants and aquatic animals) can only survive in a specific pH environment.
- Important point -
  - pH is dependent upon temperature

Buffers

- Definition: a solution that resists change in pH
  - Typically a mixture of the acid and base form of a chemical
  - Can be adjusted to a particular pH value

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

Blood: pH = 7.35-7.45

Too acidic? Increase respiration rate expelling CO2, driving reaction to the left and reducing H+ concentration.

Excretory system – excrete more or less bicarbonate

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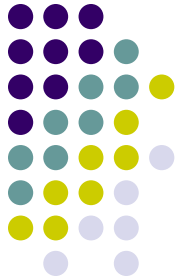
pH below 7.4 in rats – $CaCO_3$ in BONE dissociates, carbonates soak up extra H+ to buffer blood. But bones weakened.

Buffers

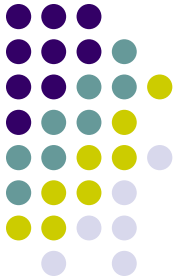


- Definition: a solution that resists change in pH
  - Typically a mixture of the acid and base form of a chemical
  - Can be adjusted to a particular pH value
- Why use them?
  - Enzyme reactions and cell functions have optimum pH's for performance
  - Important anytime the structure and/or activity of a biological material must be maintained

How buffers work

- Equilibrium between acid and base.
- Example: Acetate buffer
  - $CH_3COOH \leftrightarrow CH_3COO^- + H^+$
- If more H^+ is added to this solution, it simply shifts the equilibrium to the left, absorbing H^+, so the $[H^+]$ remains unchanged.
- If H^+ is removed (e.g. by adding OH-) then the equilibrium shifts to the right, releasing H^+ to keep the pH constant

Limits to the working range of a buffer

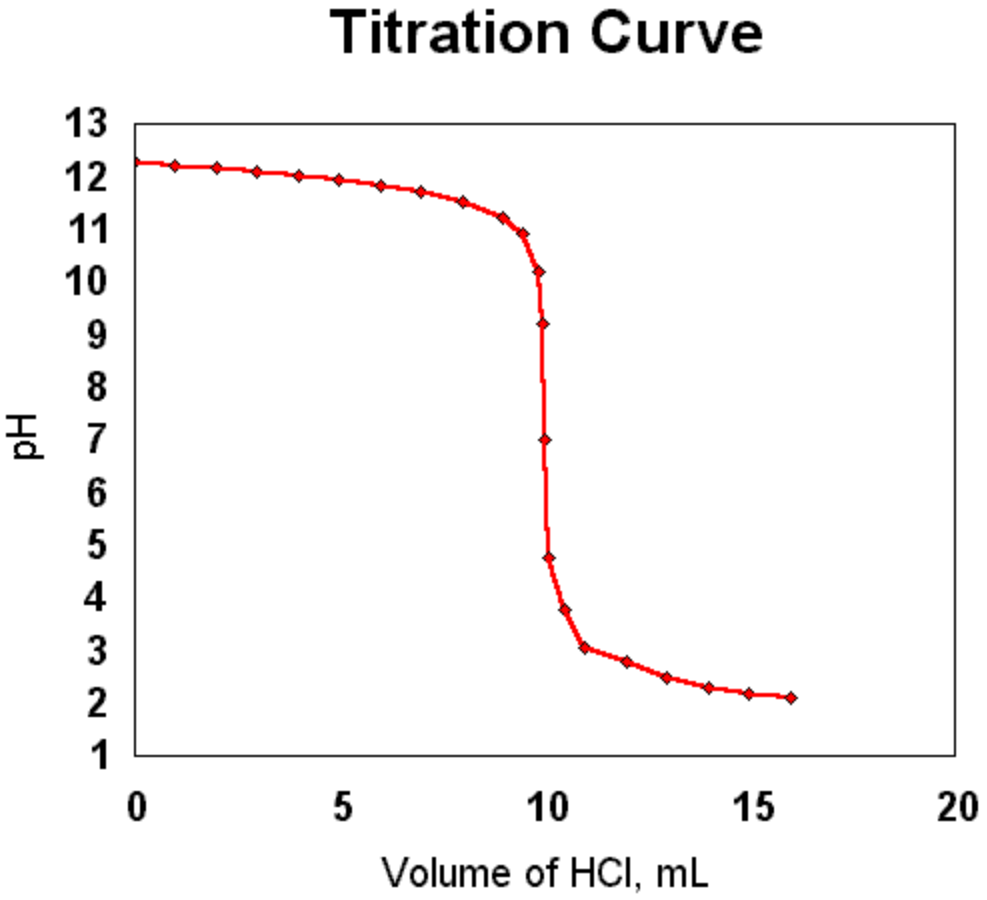


- Consider the previous example:
  - $CH_3COOH \leftrightarrow CH_3COO^- + H^+$
- If <u>too much</u> H^+ is added, the equilibrium is shifted all the way to the left, and there is no longer any more CH_3COO^- to "absorb" H^+.
- At that point the solution no longer resists change in pH; it is useless as a buffer.
- A similar argument applies to the upper end of the working range.

Chemistry of buffers

- Lets look at a titration curve

Titration is used to determine the concentration of an acid or base by adding the OTHER and finding an equivalency point…

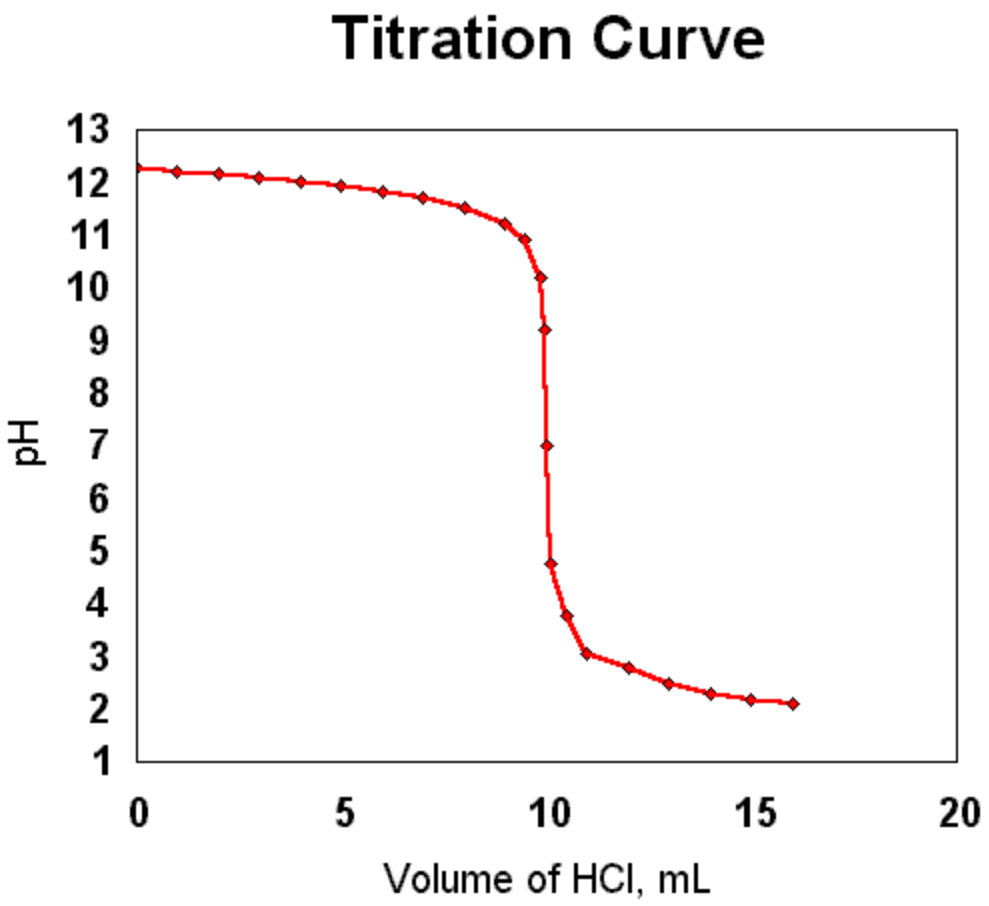


Titration is used to determine the concentration of an acid or base by adding the OTHER and finding an equivalency point…

Suppose you have a KOH solution, and you want to know its concentration (molarity).

Slowly add an acid (HCl) with a known concentration (0.1 M) and find the equivalency point…in this case it will be at pH = 7… and we use an indicator that changes color at that pH determine when that point has been reached.

So, suppose it takes 10ml of 0.1 M HCl to buffer 50 ml of the KOH.



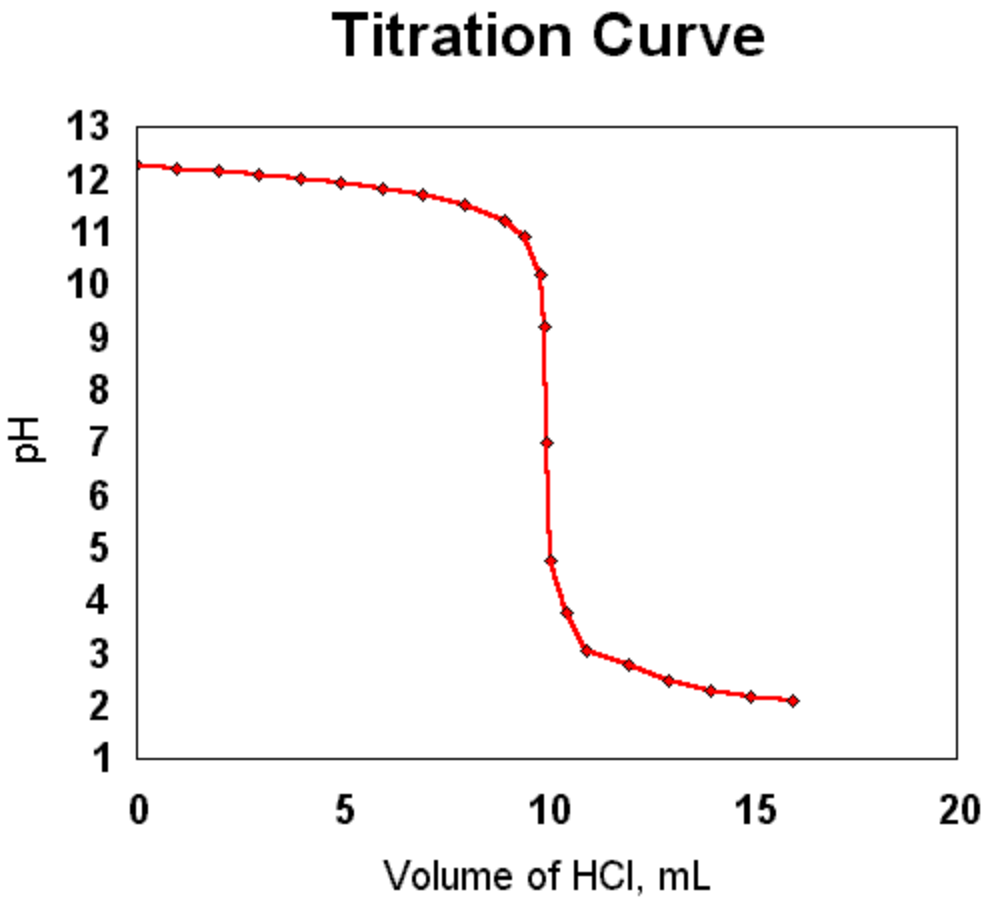
Titration is used to determine the concentration of an acid or base by adding the OTHER and finding an equivalency point…

So, suppose it takes 10ml of 0.1 M HCl to buffer 50 ml of the KOH.

The original concentration of the base =

$$\frac{\text{Vol Acid x conc. Of acid}}{\text{Volume of Base}}$$

$$\frac{\text{10 ml x 0.1 M}}{\text{50 ml}} = 0.02\ \text{M}$$



Chemistry of buffers

- K_a = equilibrium constant for H+ transfer… also described as the dissociation constant…the tendancy of an acid to dissociate. AH → A- (base conjugant) + H+
- K_a = [A-] [H+]/ [AH] = [base] [H+] / [acid]
- Weak acids have low values… contribute few H+ ions…
- Because we are usually dealing with very small concentrations, log values are used…
- The log constant = $pK_a = -\log_{10} K_a$

Chemistry of buffers

- K_a = [A-] [H+]/ [AH] = [base] [H+] / [acid]
- Weak acids have low values… contribute few H+ ions…
- Because we are usually dealing with very small concentrations, log values are used…
- The log constant = $pK_a = -\log_{10} K_a$
- SO! Since pK is the negative log of K, weak acids have high values … (-2 – 12).
- HCl = -9.3 – very low ~complete dissociation

Chemistry of buffers

- First rearrange the first equation and solve for [H+]
  - $[H+] = K_a \times [acid]/[base]$
- Then take the log of both sides
  - $\log_{10}[H+] = \log_{10}K_a + \log_{10} [acid]/[base]$

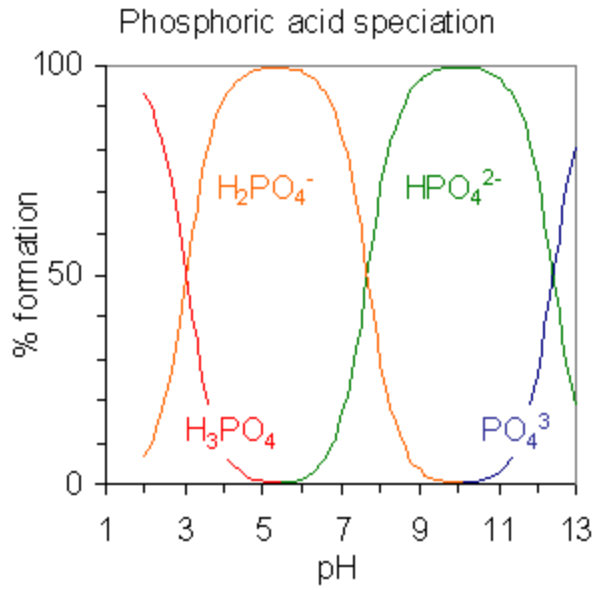
Chemistry of buffers

- -pH = -pKa + $\log_{10}$ [acid]/[base]
- Multiply both sides by –1 to get the Henderson-Hasselbach equation
  - **pH = pKa - $\log_{10}$ [acid]/[base]**

Chemistry of buffers

- What happens when the concentration of the acid and base are equal?
  - Example: Prepare a buffer with 0.10M acetic acid and 0.10M acetate
    - pH = pKa - $\log_{10}$ [acid]/[base]
    - pH = pKa - $\log_{10}$ [0.10]/[0.10]
    - pH=pKa
    - Thus, the pH where equal concentrations of acid and base are present is defined as the pKa

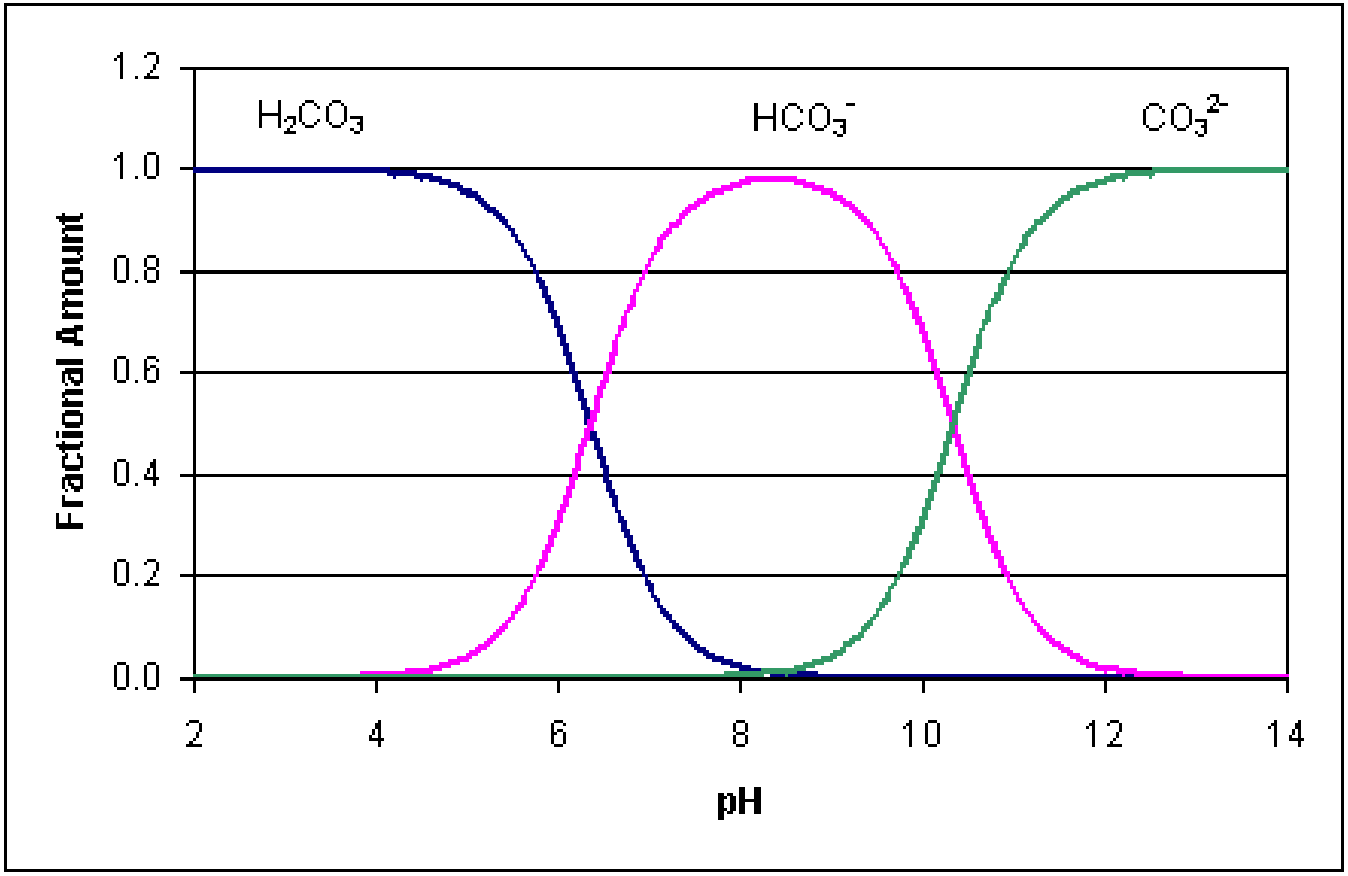
- A buffer works most effectively at pH values that are $\pm$ 1 pH unit from the pKa (the buffer range)



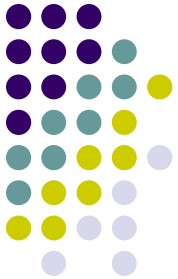
equilibrium	pK_a value
$H_3PO_4 \rightleftharpoons H_2PO_4^- + H^+$	pK_{a1} = 2.15
$H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+$	pK_{a2} = 7.20
$HPO_4^{2-} \rightleftharpoons PO_4^{3-} + H^+$	pK_{a3} = 12.37

Drives equilibria and reversible states of compounds

Carbonic Acid Bicarbonate Carbonate

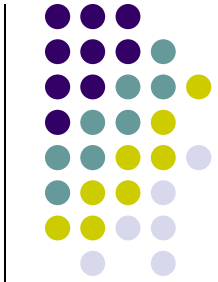


Factors in choosing a buffer



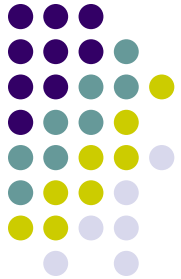
- Be sure it covers the pH range you need
  - Generally: pK_a of acid $\pm$ 1 pH unit
  - Consult tables for ranges or pK_a values
- Be sure it is not toxic to the cells or organisms you are working with.
- Be sure it would not confound the experiment (e.g. avoid phosphate buffers in experiments on plant mineral nutrition).

What to report when writing about a buffer:



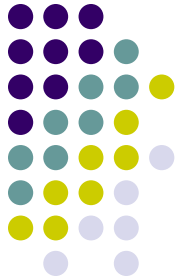
- The identity of the buffer (name or chemicals)
- The molarity of the buffer
- The pH of the buffer
- Examples:
  - “We used a 0.5M Tris buffer, pH 8.0.”
  - “The reaction was carried out in a 0.1M boric acid – sodium hydroxide buffer adjusted to pH 9.2.”

Three basic strategies for making a buffer



1. Guesswork – mix acid and base at the pH meter until you get the desired pH.
   - Wasteful on its own, but should be used for final adjustments after (2) or (3).
2. Calculation using the Henderson-Hasselbach equation.
3. Looking up recipe in a published table.

Calculating buffer recipes

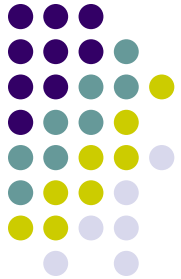


- Henderson-Hasselbach equation
  - **$pH = pKa - \log_{10} [acid]/[base]$**
- Rearrange the equation to get
  - **$10^{(pKa-pH)} = [acid]/[base]$**
- Look up pKa for acid in a table. Substitute this and the desired pH into equation above, and calculate the approximate ratio of acid to base.
- Because of the log, you want to pick a buffer with a pKa close to the pH you want.

Example

- You want to make about 500 mL of 0.2 M acetate buffer (acetic acid + sodium acetate), pH 4.0.
- Look up pKa and find it is 4.8.
- **$10^{(4.8 - 4.0)} = 10^{0.8} = 6.3 = [acid]/[base]$**
- If you use 70 mL of base, you will need 6.3X that amount of acid, or 441 mL. Mix those together and you have 511 mL (close enough).

Tables



- Tables are available to avoid doing this calculation for most buffers.
- tables

Titration

- Whether you use the formula or the tables, you will have to make fine adjustments to the final solution at the pH meter.
- This is unavoidable; therefore, you can be rather approximate about the amounts of acid and base that you mix. It's a waste of time to try to be super-precise in mixing, because you will need to make adjustments anyway.