بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

BioChemistry

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

MID | Lecture 4

PH and Buffers Pt.2

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Color coding used in the modified:

- *Black: the original slides*
- *Navy Blue: the doctor's explanation/words*
- *Gray: additional information and explanation*
- *Red: important information*

Titration curve of buffer

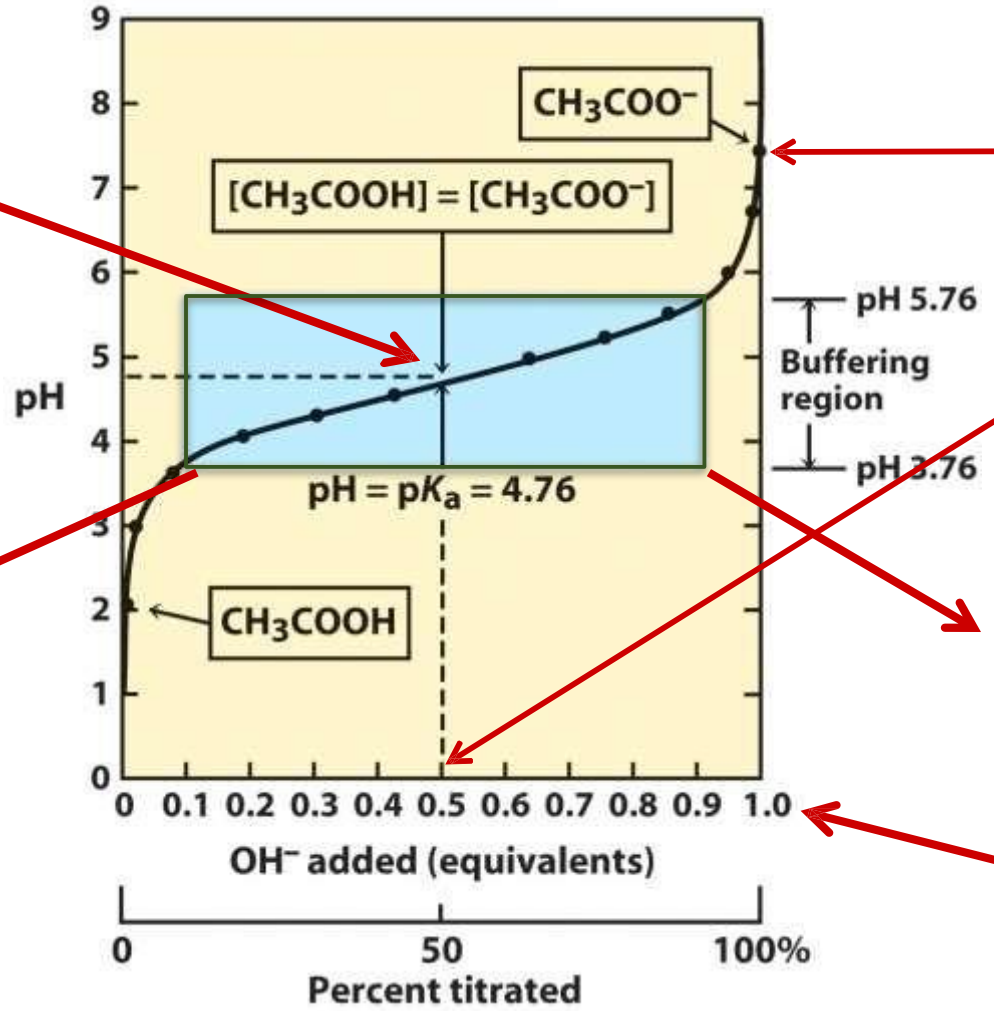
$$CH_3CO_2H(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + CH_3CO_2^-(aq)$$

Weak acid
Acetic acid

Conjugate base
($NaCH_3CO_2$)
Salt of the weak acid

What is the midpoint (inflection point)?

What is the ratio of the conjugate base:acid at the different points?



Equivalence point

Half equivalence point

Buffering range (pKa +/- 1)

Note the equivalents of the added base (or acid)

Regarding the titration curve:

At the very beginning the acetic acid is fully protonated 100%.

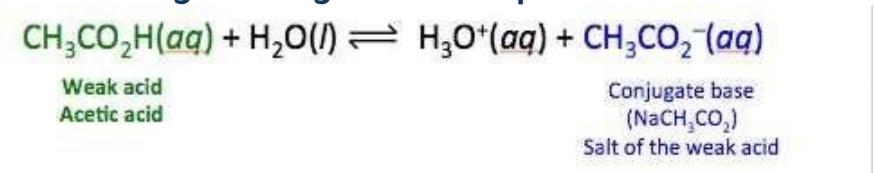
1) when the pH (y axis) is very low the acetic acid will be mainly protonated (CH3COOH)= No charge= In the acidic form.

2) While adding the strong base the pH will rise **dramatically** before reaching the buffering point.

3) When reaching the point where the acetic acid will start working as a buffer it'll start becoming unprotonated (CH3COO-/ acetate form).

4) When in the buffering range the resistance will start (pH will increase slightly/ change in pH will be subtle).

5) While adding strong base the pH will increase in this mechanism:



1) When adding the strong base, it'll dissociates completely to give OH-.
2) OH- will bind with H3O+ so protons amount will decrease Or OH- will react with acetic acid, producing acetate ion and water, as a result, the ratio [CH3COO-]/[CH3COOH] changes
3) Therefore, pH will increase but not dramatically (only slightly) and that's because of the acetate (sodium acetate) and the acetic acid (the buffer) .
4) **Compensation** happens; acetic acid dissociates to give protons and acetate, and to maintain the pH
5) So, acetic acid's concentration decreases and acetate's concentration increases.

Now going back to Henderson-Hasselbalch equation: $pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right)$

There is more than one factor that determines the pH of a solution containing a weak acid (not only the concentration of protons) but also:

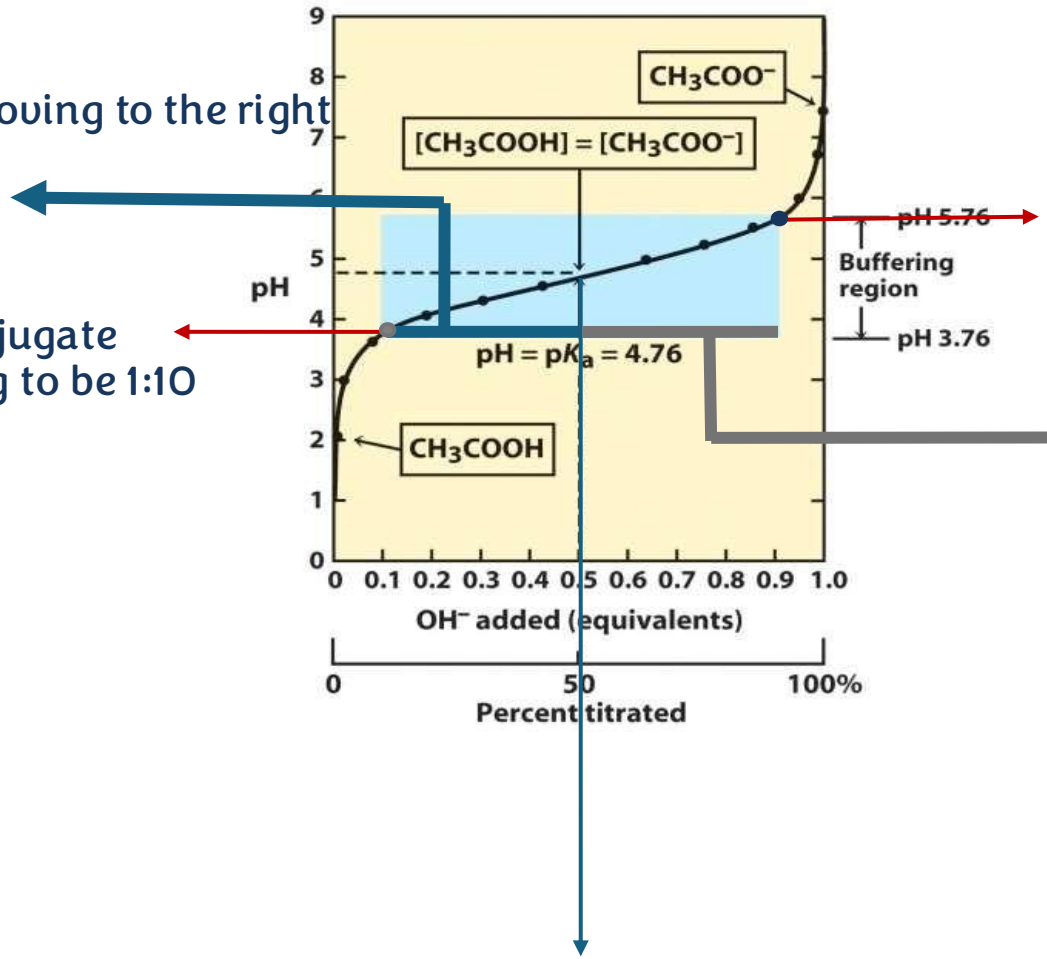
1) Concentration of the conjugate base

2) Concentration of the weak acid

1) In this range the Logarithm will be a fraction. It's going to be the logarithm of a number less than one and the logarithm of a number less than one is a negative fraction.

pH=pka-fraction

1) Fraction will decrease by moving to the right until it reaches 0.



1) The ratio of the conjugate base to the acid is going to be 1:10

2) Log(1/10)=-1

3) pH=pka-1

1) The ratio of the conjugate base to the acid is going to be 10:1

2) Log(10/1)=1

3) pH=pka+1

1) Conjugate base concentration is increasing, and the acid concentration is decreasing. So, it is going to be the logarithm of a number that is more than one. And the logarithm of a number more than one is a positive number.

2) pH=pka+fraction

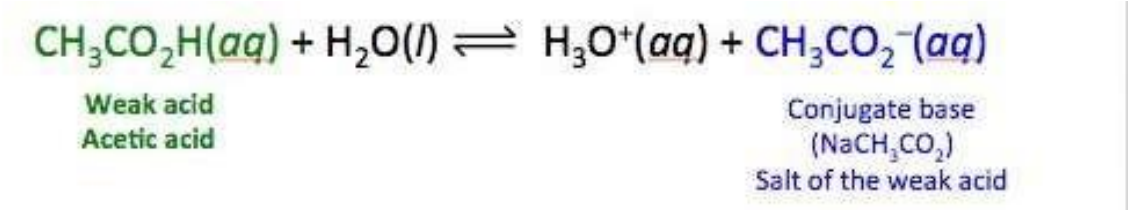
3) Fraction will increase by moving to the right until it reaches 1.

A buffer: Is a mixture of a weak acid and its conjugate base(salt). Or a mixture of a weak base with its conjugate acid(salt).

1) Log(1)=0

2) pH=pka

3) Conjugate base concentration and acid concentration are equal



If we are in the buffering range, and added HCl, what will happen:

1) HCl Is going to dissociate completely.

2) Proton's concentration will increase in the solution.

3) Acetate will start binding with those extra protons to decrease their number.

4) So, the concentration of the acetic acid will increase, and the concentration of the acetate will decrease.

5) So, it's going to be the logarithm of a number that is less than 1 .

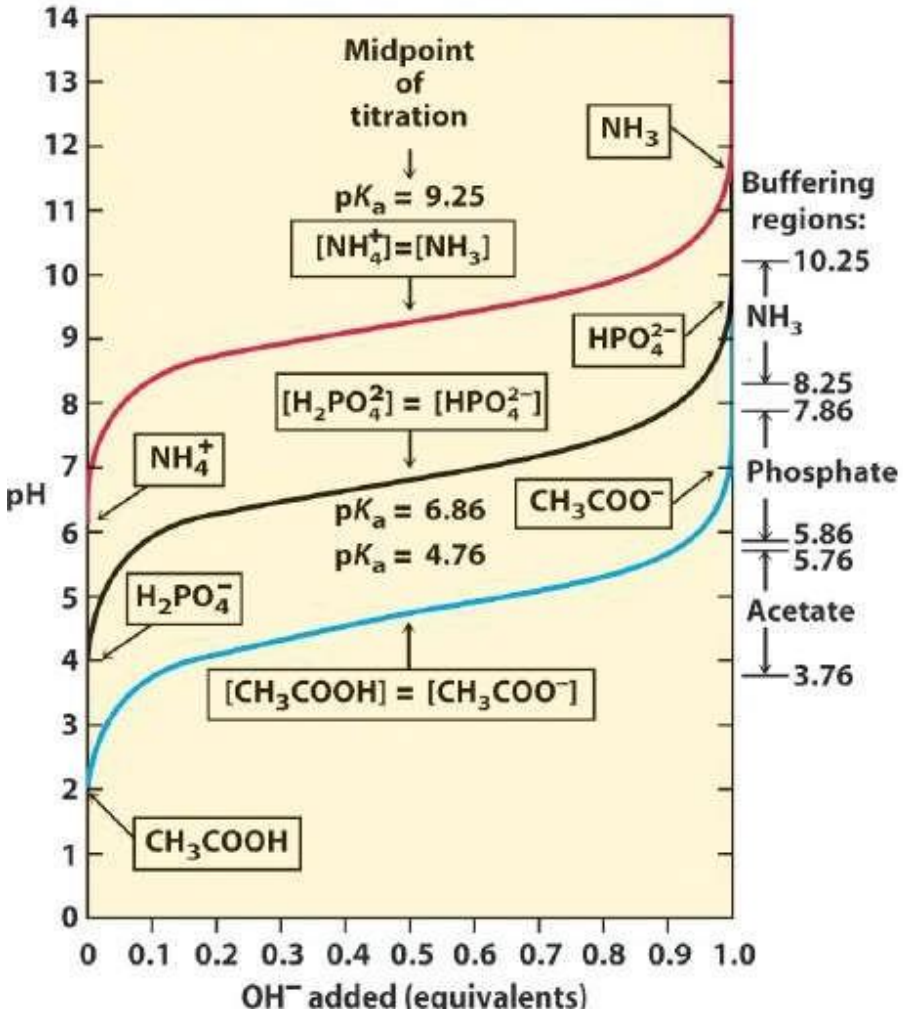
pH=pka-fraction

(Notice that the concentration of protons is relatively constant, but the concentration of the conjugate base is decreasing, so the PH will decrease, but **not dramatically** because we are within the buffering range (pka-1,pka+1).

How do we make/choose a buffer?

- A buffer is made by combining weak acid/base and its salt.
- The buffering capacity of a buffer to function depends on:
  - Buffer concentration
  - Buffering range
    - pKa of the buffer
    - The desired pH

Note: increasing the concentration does not change the buffering range but it increases buffering capacity or strength.



All buffers act in the same way, the buffering range is (pka-1,pka+1) but they differ in the region (midpoint/ pka value).

Looking at the diagram in the previous slide:

1) Acetate buffer (the blue line): the buffering range (4.76-1,4.76+1).

2) Phosphate buffer (the black line): a combination of dihydrogen phosphate and mono hydrogen phosphate, when their amounts are equal they'll be at the pka point of the buffer. The buffering range (6.86-1,6.86+1).

3) Ammonia buffer (the red line): a combination of ammonium NH4+ and ammonia NH3, the buffering range (9.25-1,9.25+1).

4) Notice that when looking at the X axis, the midpoint of all buffers will meet the same amount of the equivalents of the strong base (0.5), and that at the end of all the buffering regions, the whole solution will be undissociated (In the conjugate base form, unprotonated, the amount of the weak acid is the same amount as the strong base in equivalents).

The buffering capacity depends on:

1) The buffer concentration.

The capacity of a buffer whose concentration is 1 M is better than when it's with 0.1 M concentration. The 1 M concentration is going to handle a stronger base better, but the buffering range stays the same for both of them(pka is constant).

Equivalents for them are the same, what changes is the molarity.

2) The buffering range.

Let's say you are in a laboratory, and you want to study the function of a certain enzyme which works at a PH level of 5 (as in lysosomes), while adding some products like the enzyme's substrate, it might change the PH, so how do I make sure that the PH does not change? Use a buffer.

Now which buffer should I use? Because the desired PH is 5, we can't use ammonia buffer (it works at a range of 8-10) or the phosphate buffer (5.8-7.8) but I can use the acetate buffer as it works on the range (3.76-5.76).

You can also determine the concentration of a buffer depending on what products you're adding and what are the concentration of them.

(the more their concentration is the more you should add the buffer concentration to maintain the PH).

Exercise

- A solution of 0.1 M acetic acid and 0.2 M acetate ion. The pKa of acetic acid is 4.8. Hence, the pH of the solution is given by

$$\mathrm{pH} = 4.8 + \log(0.2/0.1) = 4.8 + \log 2.0 = 4.8 + 0.3 = 5.1$$

- Similarly, the pKa of an acid can be calculated

Since you are smart, you can estimate it.
No calculator is needed.

Base 10 Logarithms

$\mathrm{Log}_{10}(0.01)=-2$	since $10^{-2}=1/100$
$\mathrm{Log}_{10}(0.1)=-1$	since $10^{-1}=1/10$
$\mathrm{Log}_{10}(1)=0$	since $10^{0}=1$
$\mathrm{Log}_{10}(10)=1$	since $10^{1}=10$
$\mathrm{Log}_{10}(100)=2$	since $10^{2}=100$
$\mathrm{Log}_{10}(1000)=3$	since $10^{3}=1000$

Exercise

1. Predict then calculate the pH of a buffer containing
   - 0.1M HF and 0.12M NaF? ($K_a = 3.5 \times 10^{-4}$)
   - 0.1M HF and 0.1M NaF, when 0.02M HCl is added to the solution?

   pH = pKa + log ([NaF]/[HF])
   pH = 3.54 ..... Or you can solve it by finding the [H+] from Ka, then find the pH.

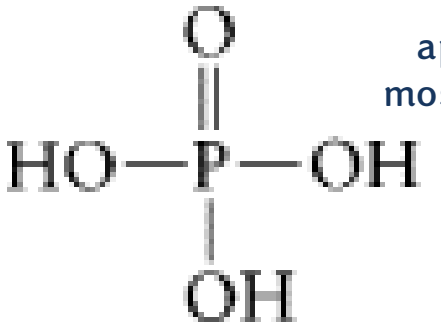
   pH = pKa + log (([NaF]-0.02)/([HF]+ 0.02))
   pH = 3.29
2. What is the pH of a lactate buffer that contains 75% lactic acid and 25% lactate? (pKa = 3.86)

   pH = 3.86 + log ([lactate]/[lactic acid])
   pH = 3.86 + log (0.25/0.75)
   pH = 3.38

3. What is the concentration of 5 ml of acetic acid that can be titrated completely by 44.5 ml of 0.1 M NaOH?

   Eq of base = Eq of acid
   n x M1 x Vol1 = n x M2 x Vol2
   1 x 0.1 x 0.0445 = 1 x M2 x 0.005
   M2 = 0.89 M

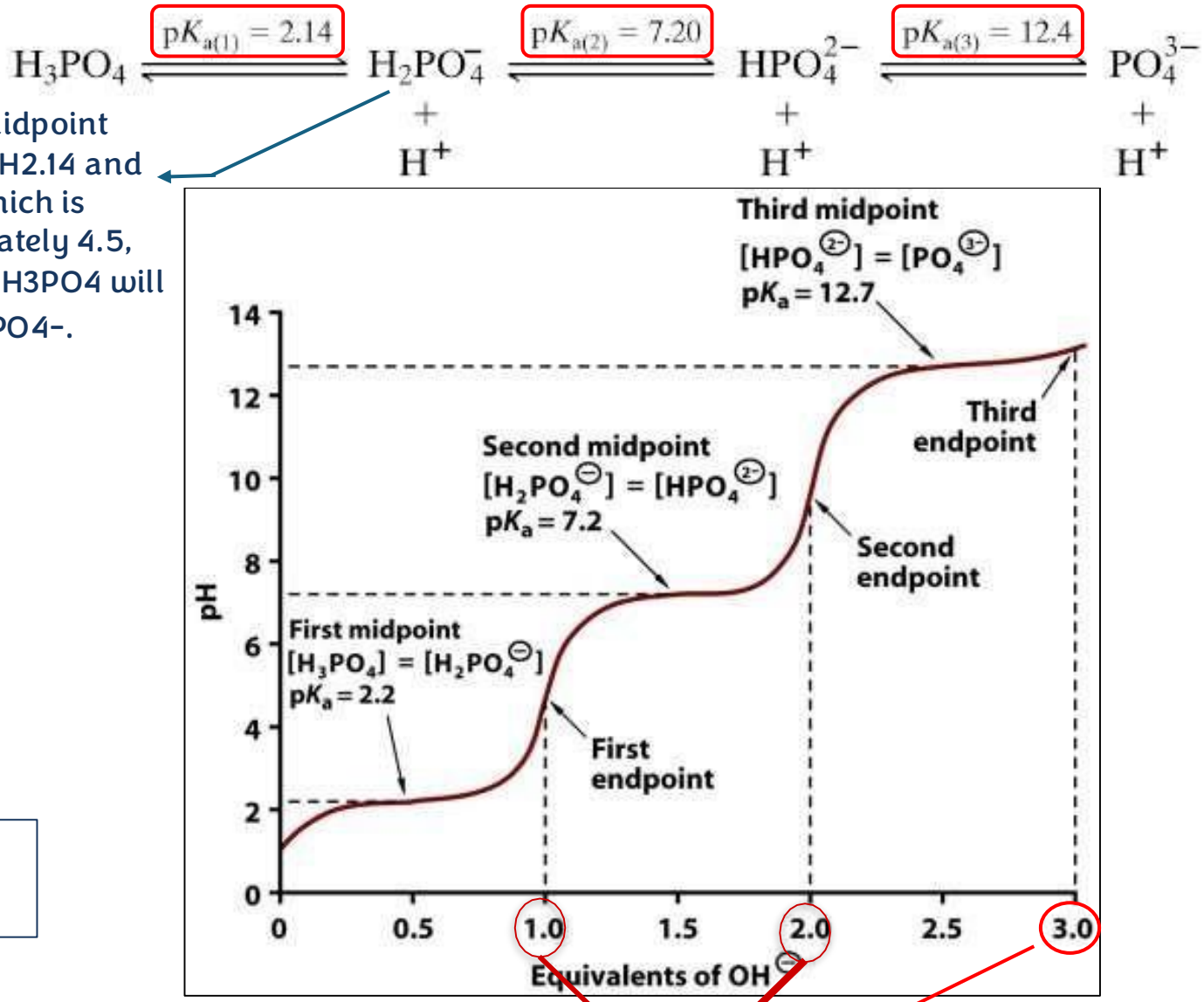
   - **The number of equivalents of OH^- required for complete neutralization is equal to the number of equivalents of hydrogen ion present as H^+ and HA.**



At the midpoint between pH2.14 and 7.2, which is approximately 4.5, most of the H3PO4 will be H2PO4-.

Titration curve of phosphate buffer

Dr.'s notes about the diagram are in the following slides.



Note values Equivalents Points

Phosphate Buffer System

Phosphoric acid (H_3PO_4) is a triprotic acid, meaning it can donate three protons. Therefore, it has three dissociation steps, each characterized by its own pKa value and buffering range.

The first dissociation is:

$H_3PO_4 \rightleftharpoons H^+ + H_2PO_4^-$, $pKa_1 \approx 2.2$

At pH = pKa_1 (2.2), phosphoric acid (H_3PO_4) and dihydrogen phosphate ($H_2PO_4^-$) are present in equal concentrations (50% each) and it represents the **midpoint at 0.5 equivalents**.

- Below pH 2.2, H_3PO_4 is the predominant form.
- Above pH 2.2, $H_2PO_4^-$ becomes the predominant form.

The effective buffering range extends approximately one pH unit above and below the pKa, i.e., from pH 1.2 to 3.2. Outside this range, the solution no longer resists changes in pH, so the pH rises rapidly (dramatically) until the next buffering region is reached.

What characterizes the **first equivalence point (at 1 equivalent strong base)**?

At this point, phosphoric acid (H_3PO_4) has been completely neutralized. It no longer exists as H_3PO_4. Instead, it all exists as $H_2PO_4^-$. Once neutralization is complete, every molecule of phosphoric acid has lost its first proton.

It's relatively easy for the first proton to leave.

The second dissociation is:

$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$, $pKa_2 = 7.2$

This is the most biologically important buffering system. The effective buffering range is pH 6.2–8.2. At pH= pKa_2 = 7.2 (**midpoint at 1.5 equivalents of strong base)**, equal concentrations of dihydrogen phosphate ($H_2PO_4^-$) and monohydrogen phosphate (HPO_4^{2-}) are present.

The second equivalence point is reached after the addition of two equivalents of a strong base, since two protons have been completely removed from phosphoric acid.

Because this pKa is close to physiological pH, the phosphate buffer is highly effective in maintaining a near-neutral pH. It plays an important role in buffering the cytosol of cells, where the intracellular pH is approximately 7.0. Many enzymes exhibit maximal activity near neutral pH, making this buffering system essential for normal cellular function.

- Below pH 7.2, $H_2PO_4^-$ is the predominant form.
- Above pH 7.2, HPO4-2 becomes the predominant form.

It is harder to pull the second proton away.

The Third dissociation is:

$HPO_4^{2-} \rightleftharpoons H^+ + PO_4^{3-}$, $pK_{a3} \approx 12.7$

The buffering range extends from approximated 11.7–13.7.

This buffering system is effective only under strongly alkaline conditions. As the solution becomes highly basic (high pH) and proton concentration decreases, HPO_4^{2-} releases its final proton to oppose further increases in pH.

At pH = pK_{a3}, equal concentrations of monohydrogen phosphate (HPO_4^{2-}) and phosphate (PO_4^{3-}) are present.

The third midpoint occurs after the addition of **2.5 equivalents of strong base**. By this stage, two equivalents have already removed the first two protons, while the additional half equivalent neutralizes half of the remaining third proton. Consequently, the concentrations of HPO_4^{2-} and PO_4^{3-} are equal, and the pH equals pK_{a3}.

It is extremely difficult to pull the third proton away.

Excercises

1. What is the **pKa** of a dihydrogen phosphate buffer when a **pH** of **7.2** is obtained when 100 ml of **0.1** M NaH_2PO_4 is mixed with 100 ml of **0.3** M Na_2HPO_4?

   pH = pKa + log (0.2/0.1), pKa = 6.72

2. A solution was prepared by dissolving 0.02 moles of acetic acid (pKa.= 4.8) in water to give 1 liter of solution.
   - What is the pH?

   Ka = 1.6×10^{-5} , [H+] = 5.65×10^{-4} ,pH = 3.25
   - To this solution, 0.008 moles NaOH were added. What is the new pH? (ignore changes in volume). [acetate]= [H+]

     pH = pKa + log ((5.65×10^{-4} +0.008)/ (0.02-0.008))

     pH = 4.8 – 0.146

     pH = 4.65

Biological buffers in human body

Let's discuss it in the following slides

- Carbonic acid-bicarbonate system (blood)
- Dihydrogen phosphate-monohydrogen phosphate system (intracellular)
  - ATP, glucose-6-phosphate, bisphsphoglycerate (RBC)
- Proteins (why?)
  - Hemoglobin in blood
  - Other proteins in blood and cells

Biological Buffers in the Human Body

Biological buffers are essential for maintaining a stable pH in the human body. It is very important that the pH of our cells, tissues, and body fluids remains within a narrow range. This stable pH allows proteins, lipids, cell membranes, carbohydrates, and other biological molecules to maintain their normal structure and function.

If the pH of a solution changes, especially inside the cell, proteins may lose both their structure and their biological activity. This occurs because changing the pH alters the protonation state of ionizable functional groups. For example, lowering the pH increases the concentration of protons (H^+), which can protonate different functional groups on proteins and other biomolecules.

Consider the **carboxyl group** as an example. Under physiological conditions, it usually exists in its deprotonated form/ conjugated form (COO^-), carrying a negative charge. Protein folding and molecular interactions are organized based on the presence of this negative charge. However, if the pH decreases, the carboxyl group becomes protonated (COOH), causing the negative charge to disappear. As a result, the electrostatic interactions that stabilize the protein are disrupted, leading to changes in protein structure and, consequently, loss of protein function. The same principle also applies to biological membranes, whose structure and stability depend on the proper ionization of their components.

A common clinical example is **heartburn** (acid reflux). In this condition, gastric acid refluxes from the stomach into the esophagus, producing a burning sensation and pain. To relieve these symptoms, patients take **antacids**, which are weak bases or basic salts, such as aluminum hydroxide or magnesium hydroxide. These compounds neutralize excess gastric acid and increase the pH in the stomach, providing symptomatic relief.

However, increasing the gastric pH has a disadvantage. Digestive enzymes, particularly pepsin, require an acidic environment for optimal activity. When the stomach pH rises, these enzymes become less active and are no longer able to digest proteins efficiently. Consequently, patients may experience **indigestion (dyspepsia)** after taking antacids.

Different regions of the body require different pH values to function properly. For example, the **lysosomes** maintain an acidic pH of approximately 5.0–5.5, which is necessary for the activity of their hydrolytic enzymes. Altering the lysosomal pH impairs their normal function. This principle is also utilized in the treatment of certain diseases, such as malaria, where some antimalarial drugs interfere with the acidic environment required by the parasite, thereby reducing its survival.

Therefore, maintaining the appropriate pH in body fluids, cells, and intracellular organelles is **essential** for normal physiology. This regulation is achieved by several physiological buffer systems, the most important of which is the carbonic acid–bicarbonate buffer system, followed by the phosphate buffer system and protein buffer systems.

The most important physiological buffer system is the **carbonic acid–bicarbonate buffer system**, which is the primary buffering system in the blood. Blood pH must be maintained very precisely at approximately 7.4, with a **normal range of 7.35–7.45**. Even a small deviation from this range can significantly affect normal physiological processes. We will discuss the carbonic acid–bicarbonate buffer system in more details in the next lecture.

Another important physiological buffer is the **dihydrogen** phosphate–**monohydrogen phosphate buffer system**, which functions primarily inside cells, particularly in the cytosol. This buffer has a pKa of approximately **7.2**, making it highly effective at buffering near neutral pH. Therefore, it serves as the major intracellular buffer system.

In addition to phosphate, several intracellular molecules also contribute to pH regulation. These include **ATP**, the cell's energy currency, **glucose-6-phosphate**, and **2,3-bisphosphoglycerate** (2,3-BPG) in red blood cells. Although their primary functions are not buffering, they contain ionizable groups that can undergo protonation and deprotonation. Depending on the pH, these molecules can either bind protons (H^+) or release them, thereby **contributing to the maintenance of intracellular pH**.

Proteins also play a major role in buffering. Amino acid side chains contain ionizable functional groups that can accept or donate protons as the pH changes. Consequently, proteins act as important physiological buffers. One of the most significant protein buffers is **hemoglobin**. Red blood cells contain enormous amounts of hemoglobin. Since hemoglobin is composed of amino acids with ionizable groups, it can continuously bind and release protons. This extensive proton exchange makes **hemoglobin one of the major contributors to maintaining blood pH within its range of 7.35–7.45.**

Therefore, blood pH is maintained through the combined actions of the carbonic acid–bicarbonate buffer system, the phosphate buffer system, and protein buffers, particularly hemoglobin. Together, these buffering systems ensure that pH remains remarkably stable despite continuous metabolic acid and base production.

QUIZ :)

https://share.gemini.google/TZAHXEqpsVIV

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slide 15 Slide 4		Correct the answer of excercise 2 Correct point 2
V1 → V2			

Additional Resources:

Reference Used:
(numbered in order as cited in the text)

1. Prof. Mamoun Ahram's live lecture

رسالة من الفريق العلمي:

تأمل هذه الساعات التي فاتت من ظهر اليوم، أو من عصر اليوم، هذه الساعات التي فاتت، ذهبت علي و عليك، هذه الساعات سلخت من أعمارنا ولن تعود أبداً، فإن كنا عمرناها بتسبيح أو تحميد أو تكبير أو سجدة أو نفع للمسلمين فإنها ستكون شاهدة غداً في صحائفنا، وإن ذهبت هذه الساعات من ظهر أو عصر اليوم سدى !

فيا حسرتنا ويا غبننا في فرصة أعطيت لنا ثم سحبت ولم نستغلها .

-إبراهيم السكران