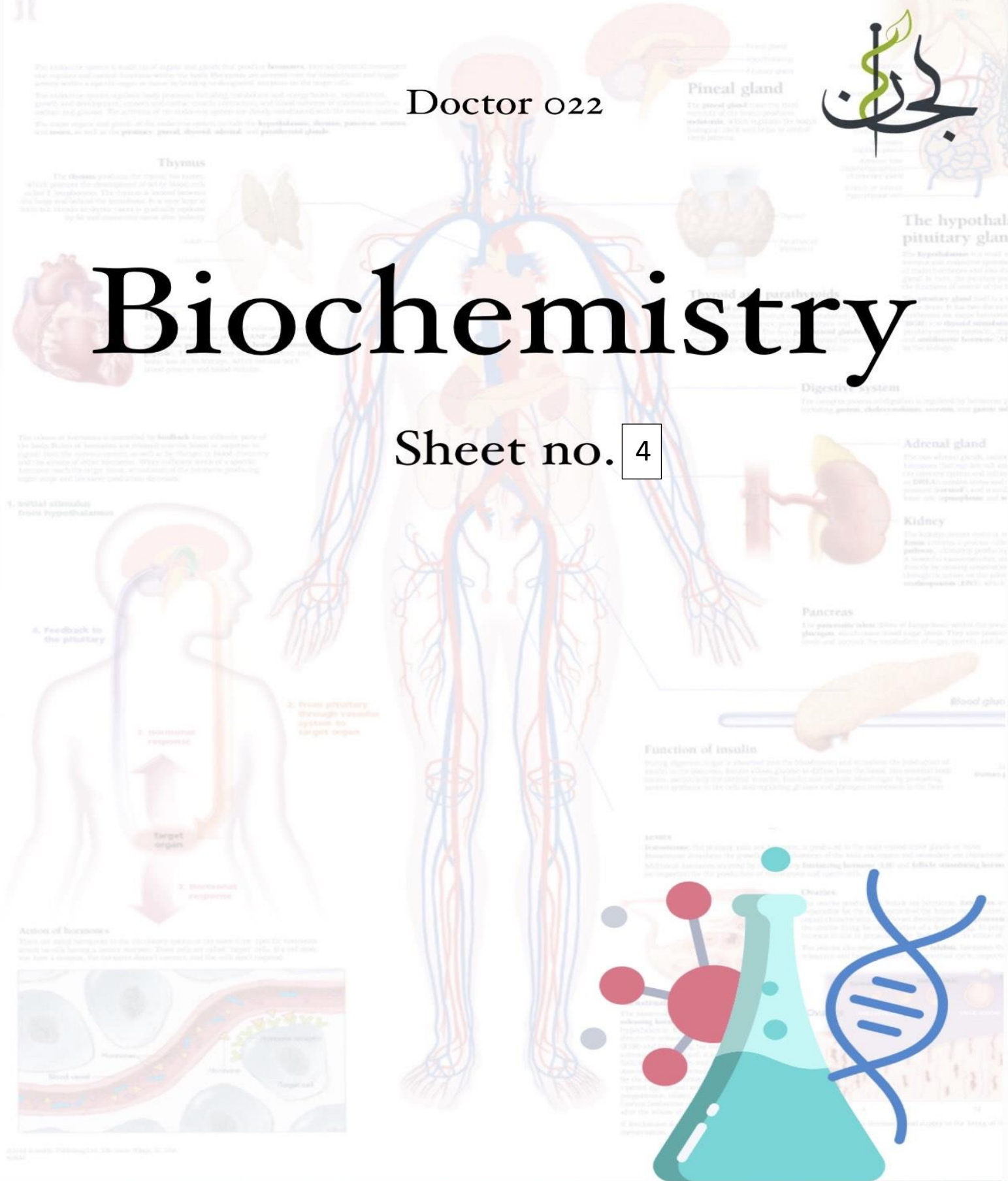


Biochemistry

Sheet no. 4



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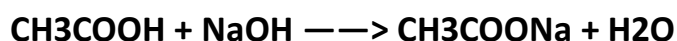
A Revision of Buffering and Titration

A buffer is solution of a weak acid/base and its conjugate base/acid (found in the form of a salt). In order to prepare a buffer, a weak acid/base (depending on the type of buffer that we want to prepare) reacts with a strong base/acid of less concentration in order to produce a salt.

For example, if we were to prepare an acidic buffer to be used to resist changes in the pH of a solution to maintain it around 5 pH, the ideal buffer would consist of a weak acid with a pK_a value around 5 (a weak acid is used so that it doesn't dissociate completely into ions). This acid isn't enough on its own to act as a buffer however, as it is required to react it with a strong base with less equivalents than the weak acid, in order to produce the weak acid's conjugate base in the form of a salt, in desired amounts (a strong base is used so that all of it reacts with the weak acid to produce the salt). This solution of a weak acid and its conjugate base in an aqueous environment is what's known as the buffer.

-It is important to note that titrating a weak acid/base is only necessary for preparing a buffer. Titration as a whole however, can be applied to strong acids and bases as well.

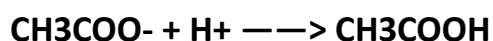
-An example of what happens when adding a strong base (ex. NaOH) on an acidic buffer (ex. Acetic acid buffer)



(The products are: salt and water)

salt refers to the conjugate base

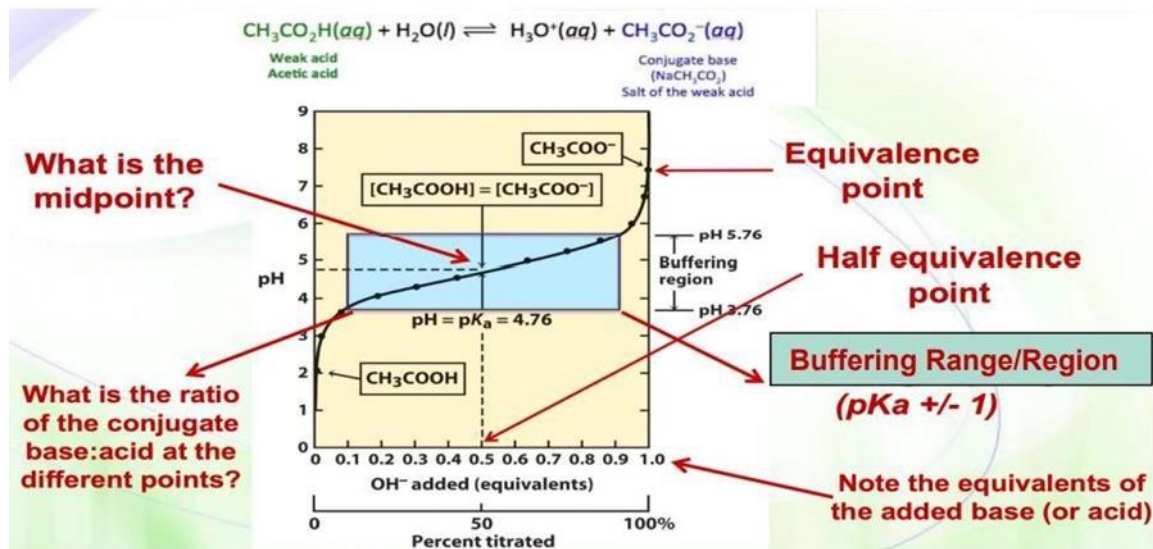
-Another example, let's test this buffer by adding a strong acid (ex. HCl) on the same acetate buffer.



The proton's source is the HCl and CH_3COO^- is the conjugate base of the Acetic Acid.

By that we have converted the strong acid (HCl) to a weak one (CH_3COOH). Thus, reducing the decrease in pH that would have happened if there wasn't a buffer.

The following image presents how an acidic buffer is prepared via titration using a strong base (the titration curve of an acidic buffer):



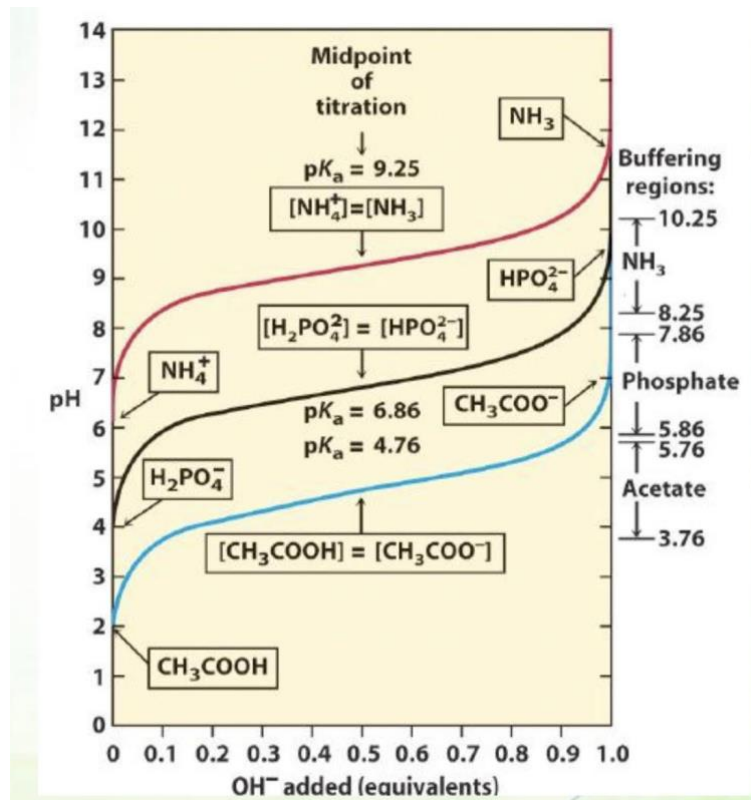
There are a few important things to mention considering this graph:

- **The buffering range/region** is the pH range at which the buffer is most effective and functions optimally. This range is constant for each type of buffer depending on the weak acid's pK_a and is always about 2 pH units, ranging from $pK_a - 1$ to $pK_a + 1$. This region isn't affected by the concentration of the weak acid.
- **Buffering capacity** is the term used to describe the ability of a solution to resist changes in pH functioning as a buffer. This term is used during the process of testing a buffer's effectiveness. The greater the concentration of the weak acid used in the buffer, the greater the buffering capacity is, meaning that a buffer that with 2 M of acetic acid can withstand more equivalents of a strong base than a buffer with 1 M. The titration curve doesn't provide any information on the concentration of the weak acid in the buffer or the buffer's capacity.
- **The Endpoint or Equivalence point** is the point at which all the equivalents of the weak acid (in this case) have reacted to produce the acid's conjugate base. This means that at this point there is almost no presence of the original weak acid. Make sure to note that this point is not necessarily around 7 pH. This value of this point only depends on the pK_a value of the weak acid.

How Do We Make/Choose a Buffer?

The ability of a buffer to function depends on:

- The buffer's concentration -> The buffering capacity
- The optimal pH of the solution so that it is close to the value of the pK_a of the weak acid in the buffer.



The figure just shows a comparison between three different buffers, showing their buffering regions and pK_a . The lower the pK_a of the acid used in the buffer, the more acidic that buffer is going to be, and the buffer is chosen depending on the pH of the environment that it is going to be used in.

EXERCISES ON BUFFER QUESTIONS:

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

$$pH = 4.8 + \log (0.2/0.1) = 4.8 + \log 2 = 4.8 + 0.3 = 5.1$$

- Predict then calculate the pH of a buffer containing:
-0.1M HF and 0.12M NaF? ($K_a = 3.5 \times 10^{-4}$)

Because the K_a value of the acid is given, we can calculate the concentration of H^+ and all that's left afterwards is to take the $-\log$ of the concentration to calculate the pH of the buffer.

The final answer: pH = 3.535

- -0.1M HF and 0.1M NaF, when 0.02M HCl is added to the solution?

The strong acid HCl will react with the conjugate base F^- to produce more HF. The concentration of HF will increase to 0.12M while the 0.08M of the conjugate base will be left. By using the value of K_a from the previous exercise, the pH of the buffer can be calculated.

The final answer: pH = 3.28

- What is the pH of a lactate buffer that contains 75% lactic acid and 25% lactate? ($pK_a = 3.86$)

Since the value of pK_a is given, it is easier to solve the problem using the Henderson-Hasselbalch equation, while keeping in mind that the ratio of weak acid to conjugate base in this case is 3:1.

The final answer: pH = 3.38

- What is the concentration of 5 ml of acetic acid knowing that 44.5 ml of 0.1 N of NaOH are needed to reach the end of the titration of acetic acid? Also, calculate the normality of acetic acid.

Both the weak acid and the strong base added have 1 equivalent for each mole, this means that their concentrations are equal to their normalities. The number of equivalents of OH^- required for complete neutralization/reaching the end of titration is equal to the number of equivalents of H^+ , therefore the first step is to calculate the number of equivalents of NaOH which is equal to that of acetic acid, and afterwards calculating the normality of the acid by dividing the equivalents by the acid's volume.

The final answer: 0.89N = 0.89M

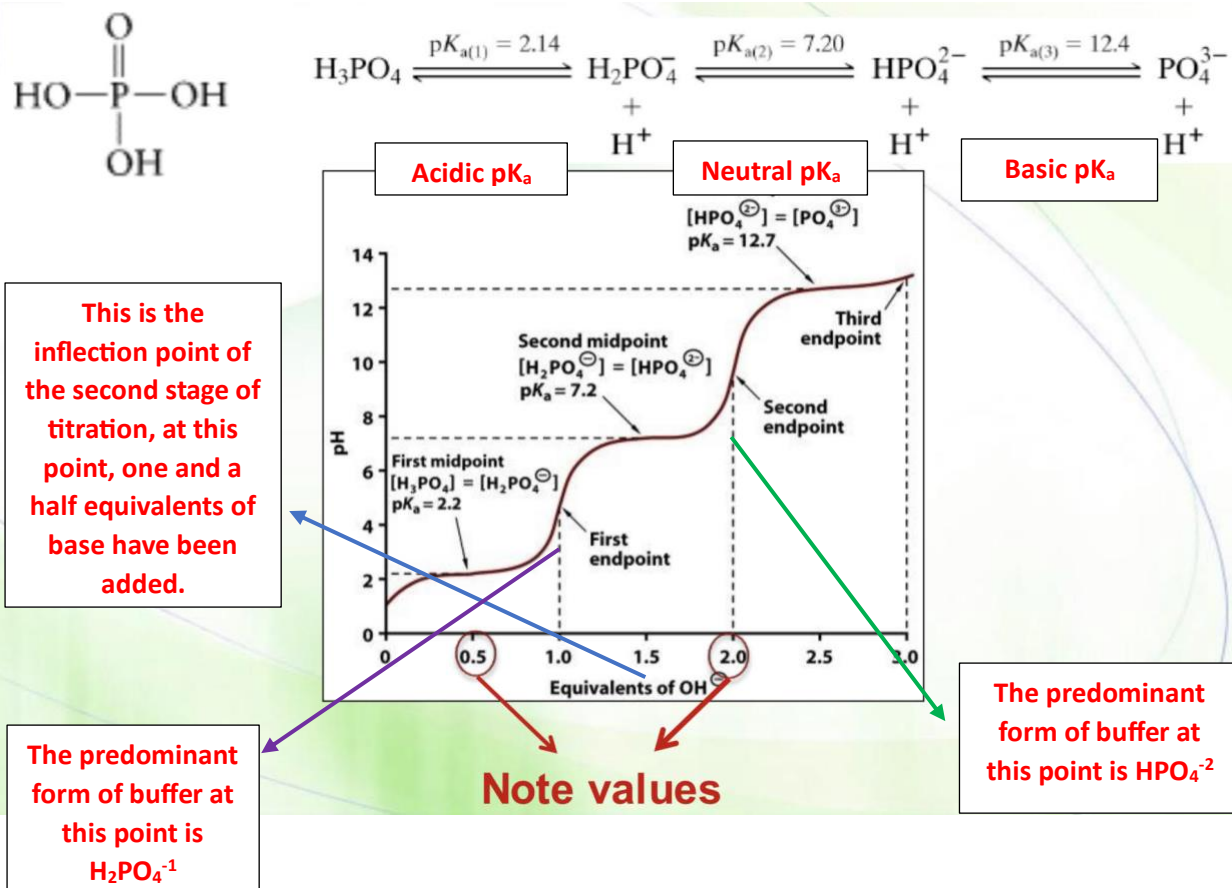
THE TITRATION CURVE OF PHOSPHATE BUFFER

Polyprotic acids are titrated through several stages depending on how many protons they contain. After the first proton is removed completely by completing the first stage of titration and it reaches the first endpoint, the second titration can begin in order to remove the second proton from the polyprotic acid.

If for example, the polyprotic acid contained three protons in each of its molecules such as in H_3PO_4 , then three equivalents of a strong base must be used in order to completely titrate the acid. One equivalent of the strong base is consumed by the end of each stage of titration.

The first stage of titration of a polyprotic acid occurs at the lowest pH compared to the rest of the stages, this is because the more protons the polyprotic acid has, the more acidic it, and thus the lower the value of pK_a of the acid at that stage is.

The conjugate base produced as a result of the first stage of titration ($\text{H}_2\text{PO}_4^{2-}$), is going to be the weak acid of the second stage of titration. Each stage of titration has its own midpoint/inflection point with a pH value equal to the value of pK_a for the acid in that certain stage.



MORE EXERCISES:

- What is the pK_a of a dihydrogen phosphate buffer when pH of 7.2 is obtained when 100 ml of 0.1 M NaH_2PO_4 is mixed with 100 ml of 0.1 M Na_2HPO_4 ?

It is very important to notice here in this question that when both solutions are mixed together, the concentrations of the weak acid and its conjugate base will both change because of the increase in volume, and that when substituting the values in the Henderson-Hasselbalch equation; we must substitute the concentrations after the mixing not before.

Another thing worthy of mentioning is that even though the weak acid and its conjugate base in this case have a different number of protons, the number of equivalents for each mole is equal for both compounds. This is because while $\text{H}_2\text{PO}_4^{1-}$ acts as acid that can release 2 protons, HPO_4^{2-} acts as a base that can accept 2 protons.

After substituting in the Henderson-Hasselbalch equation,
the final answer is: $pK_a = 7.2$

- A solution was prepared by dissolving 0.02 moles of acetic acid ($pK_a = 4.8$) in water to give 1 liter of solution. What is the pH?
 - What is the pH?
 $pK_a = -\log K_a \rightarrow K_a = 10^{-pK_a} = 10^{-4.8} = 1.585 \cdot 10^{-5}$
 $1.585 \cdot 10^{-5} = x^2/0.02 \rightarrow x = 5.63 \cdot 10^{-4} = [\text{H}^+] = [\text{CH}_3\text{COO}^-]$
 $pH = -\log 5.63 \cdot 10^{-4} = 3.25$
 - To this solution was then added 0.008 moles of concentrated sodium hydroxide (NaOH). What is the new pH? (In this problem, you may ignore changes in volume due to the addition of NaOH). NaOH is a strong base that will react completely with acetic acid, this would decrease the moles of acetic acid to become 0.012 moles while adding $80 \cdot 10^{-4}$ moles of acetate (the conjugate base) to the solution as a result of this reaction.

This would result in a pH of 4.62.

BUFFERS IN THE HUMAN BODY:

We are going to talk about biological buffers in our bodies, how our bodies maintain pH, all of which is very important as a small or simple increase or

decrease in the body's pH may lead to death because it directly affects our CNS.

From the 2nd semester -physiology-neurophysiology: how changes in pH affect CNS:

(Doctor didn't mention it, don't memorize it, Just for further understanding)
Acidosis depresses neuronal activity, fall in pH may cause a comatose state.
Alkalosis however, increases neuronal excitability and may cause cerebral epileptic seizures.

Small differences in pH represent a huge change in H^+ concentration (as we mentioned before, when we compared between orange and lemon juice) 2 units change in pH = hundredfold change in H^+ concentration.
Our bodies can't tolerate this change.

Although Biological Buffers differ a lot than Chemical Buffers, they do share some common aspects.

pH must be maintained in the ECF as well as the ICF, So we need buffers that work on the ECF and others that work on the ICF and others might work on both.

Some of the Biological Buffers in the human body:

1-Carbonic acid-bicarbonate system (BLOOD)

Considered to be the buffering system that has the highest capacity in the ECF specifically (Strongest one).

2-Dihydrogen phosphate-monohydrogen phosphate system (intracellular) ATP, glucose-6-phosphate, bisphosphoglycerate (RBC)

Also called the phosphate buffer system

***This is at the Second stage ($\text{H}_2\text{PO}_4^- \longrightarrow \text{HPO}_4^{2-}$) of H_3PO_4 titration only, WHY?

Because pK_a is 7.2 which is close to the blood's pH 7.4 (in cells 6.96.7). Therefore we can't take a buffer with a pK_a of 2 or 12 (will kill us) .We need a buffer with a pK_a close to the blood.

3- Proteins (Protein Buffers System)

- Hemoglobin in blood (in RBCs)
- Other proteins in blood and cells

They can act as buffers in both extracellular and intracellular fluid compartments, any protein in our body can act as a buffer. We will talk more about it later.

THE BICARBONATE BUFFER SYSTEM:

As we all know, when we breath (inhalation) oxygen enters our body to the lungs where gas exchange occurs in the alveoli → RBC → hemoglobin.
(Both oxygen or CO₂ are gases that dissolve in water as all of the medians in our body are composed of water)

Oxygen will reach capillaries where another gas exchange occurs, oxygen then diffuses to cells and CO₂ is diffused into the blood to reach the lungs.

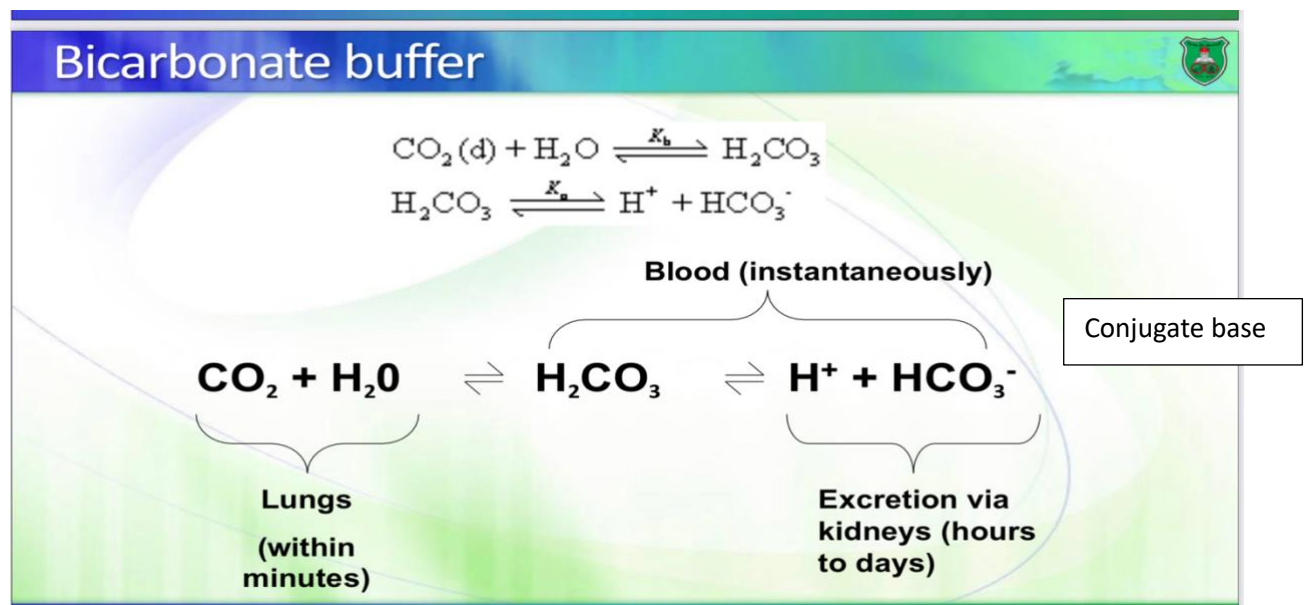
Before exhalation, a part of CO₂ may interact with water producing carbonic acid H₂CO₃ (a weak acid in its two stages).

H₂CO₃ disassociates producing H⁺ and HCO₃⁻.

HCO₃⁻ will not disassociate further inside our body. WHY??

Because its pK_a is outside the range of pH in the surrounding area.

(As we mentioned earlier in the Henderson-Hasselbalch equation, the acid disassociates further when the surrounding pH is larger than its pK_a).



This (carbonic acid + bicarbonate ion) is a biological buffer that shares some principles of chemical buffers and differs in others.

The difference is that biological buffers are open systems so they are open to other body systems that contribute to control pH, while chemical buffers act in closed controlled systems + **when preparing chemical buffers we use titration (unless we add substances by titration we have no other source for the chemical buffer).**

Buffer systems act as the first line of defense that deal with a large change in the pH (a quick transient solution) then other systems come to act permanently (final solution).

Second line of defense is the respiratory system (*lungs*) third one is the renal system

(The respiratory system is considered the second defense line and renal system is the third because breathing is a fast process (18 times / minute in adults) while renal functions take much longer)

الدكتورة شبهت ال buffer بالدفاع المدني هدفه الحفاظ على حياة المريض و عملياته الحيوية مؤقتا لحين الوصول الى المستشفى، ثم يأتي دور الأطباء و الممرضين بإصلاح المشاكل بصورة دائمة تقريبا.

Respiratory Response:

High amounts of CO_2 ———> High exhalation (increases breathing rate).

Low amounts of CO_2 ———> Preserves CO_2 levels in the blood and prevents further loss (decreases breathing rate).

Renal Response:

High amounts of HCO_3^- ———> Excretion.

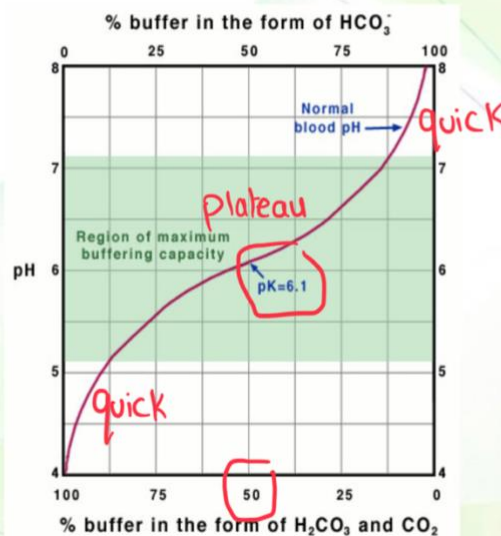
Low amounts of HCO_3^- ———> Reabsorption.

Titration curve of bicarbonate buffer



Note pKa

Text



Notice that the pK_a is 6.1, and as we know it has to work from $pH = 5.1 - 7.1$

Blood's pH however, is 7.4!! So why is this buffer considered to be the strongest in our blood and why does it have the highest capacity??

Remember that it's a biological buffer which means that it's open to other systems, and by applying the Henderson-Hasselbalch equation:

$$pH = 7.4 \quad pK_a = 6.1$$

$$7.4 = 6.1 + \log [HCO_3^-] / [CO_2]$$

$$\log (HCO_3^- / CO_2) = 1.3$$

$$[HCO_3^-] / [CO_2] = 20$$

Note that the acid is H₂CO₃ but we express it as CO₂ because CO₂ is its source. For example, in blood tests we measure CO₂ concentration (or pressure pCO₂ because it's a gas that can be converted to molarity).

The ratio between the acid and its conjugate base is 20, which means that the conjugate base is 20 times larger than the acid.

In chemical buffers they were relatively close in concentration, but in biological buffers the conjugate base concentration a lot is larger than that of the acid, WHY?

Now we have to answer two questions:

Why is the pH higher than the value of the weak acid's (H_2CO_3) pK_a , and why is the conjugate base's (HCO_3^-) concentration larger?

If an acid entered the body, the acid will react with HCO_3^- .

If a base entered the body, it will react with H_2CO_3 .

ركزوا معي منيح

- Now, let's consider **the first case** ———> **an acid enters the body:**

As we mentioned it needs to react with a base (HCO_3^-)

In the biological system, the only source of the base is the disassociation of the acid H_2CO_3 which is a **weak** acid, therefore it disassociates in very small amounts (unlike chemical buffers where we can use titration to increase the salt/conjugate base concentrations).

Our bodies however, require high capacity (higher concentration of conjugate base/ HCO_3^- molecules) to deal with high acid concentrations that enter the body, and the small dissociation of H_2CO_3 isn't enough to provide a high buffering capacity.

Unlike chemical buffers, we can't use titration processes in our bodies to increase the concentration of HCO_3^- , so how do we increase the acid dissociation rate?

When we discussed the Henderson-Hasselbalch equation, we said that the acid disassociation increases when the pH increases, therefore we need the pH to be out of the buffer range (7.4) to drive the acid to disassociate more (acid disassociation increases due to the surrounding environment conditions ———> difference in pH).

IN OTHER WORDS, the environment (pH) needs to be suitable to supply enough amount of the base (HCO_3^-) which is why the major component of the buffer system is the HCO_3^- .

- **The second case** ———> **a base enters the body:**

Now we need a high acid concentration in order to neutralize the added base, and this is EASY to accomplish, because the body can react CO_2 with water to give H_2CO_3 (problem solved).

Why is this buffer effective?



- Even though the normal blood pH of 7.4 is outside the optimal buffering range of the bicarbonate buffer, which is 6.1, this buffer pair is important due to two properties:
 - bicarbonate is present in a relatively high concentration in the ECF (24mmol/L).
 - the components of the buffer system are effectively under physiological control: the CO₂ by the lungs, and the bicarbonate by the kidneys.
 - It is an open system (not a closed system like in laboratory).
 - An open system is a system that continuously interacts with its environment.

PATHOLOGICAL CONDITIONS

Normal blood pH ranges between 7.36 – 7.44 (averages at 7.4)

pH<7.36 is called Acidosis

pH>7.44 is called Alkalosis

The suffix “Osis” means that the whole body is affected (intracellular and extracellular fluid compartments) by the change in pH.

If the change occurs in the blood only, the suffix is “Emia” (as in acidemia).

If pH reaches 7 the person will die, and a pH of 8 is impossible to achieve in human fluid compartments (ECF and ICF, not including certain areas in the body such as in the GI tract).

ACIDOSIS AND ALKALOSIS CAN OCCUR DUE TO METABOLIC REASONS OR RESPIRATORY REASONS.

ACIDOSIS:

An example of Metabolic Acidosis:

Starvation: when a person eats the body secretes insulin to deal with high blood sugar, within 2 hours insulin will decrease the sugar rate to FASTING BLOOD SUGAR.

This rate may lead to fainting because the brain isn't supplied with enough sugar, so we either eat more or our body breaks down glycogen.

Glycogen will be depleted in about 12-13 hours .

After glycogen stores are depleted energy can be obtained from lipids.

Glucagon hormone attaches to adipose tissue or fatty acid and signal lipid (fatty acid) breakdown.

Fatty acids are long chains of carboxylic acids that are broken down to produce large amounts of acetyl CoA that enter Krebs cycle to produce energy.

The fatty acids produce large amounts of acetyl CoA that can't be all used in Krebs cycle, so they are used to form ketone bodies (contains a Ketone functional group and carboxyl groups) which are considered to be acids.

Large amounts of ketone bodies therefore lead to acidosis (Ketoacidosis)

An example of that is a diabetic patient that doesn't know about his condition or a patient that neglects it.

What happens in this case is that the blood sugar is always high and doesn't go down to fasting blood sugar because insulin isn't working or we have resistance. The person's cells won't receive enough sugar and will be in starvation, which will activate fatty acid breakdown —-> high amounts of acetyl CoA —-> Ketogenesis —-> Ketoacidosis/

One example of Ketone bodies is Acetone, so the patient may smell like Acetone as the body keeps excreting Acetone. People who follow Keto diet may have this as well.

اللّٰهُمَّ عَلِّمْنَا مَا يَنْفَعُنَا وَانْفَعْنَا بِمَا عَلَّمْتَنَا وَزِدْنَا عِلْمًا

This is a challenge problem for those of you who would like to solve extra questions:

A buffer is made by mixing a 100ml solution of 0.56M Na_2HPO_4 with another 200ml solution of 0.14M of Na_3PO_4 . What is the pH before and after 100ml of 0.04M $\text{Ca}(\text{OH})_2$ is added to the buffer, knowing that the pK_a value of HPO_4^{2-} is 12.4.

The answer: the pH before = 12.09, the pH after is 12.275

The End

V2