Introduction to Biochemistry and Molecular Biology

Lecture {4}

pH & Buffers – Continued

"في ازديادِ العلمِ إرغامُ العِدى
وجمالُ العلمِ إصلاحُ العملْ"
-ابن الوردي

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APPROVED
0501113 – L4

Some notes regarding the previous lecture:

- Formic acid (pKa = 3.8) is more acidic than the lactic acids (pKa = 3.9).

- Buffer concentration does not affect the titration curve because we only care about equivalents, not molarity nor concentration.

- At the equivalence point, $[OH^-] = [H^+] \rightarrow$ complete neutralization.
  What is the concentration of the acetic acid needed to completely neutralize 50mM of (OH^-)?
  Answer: 50mM

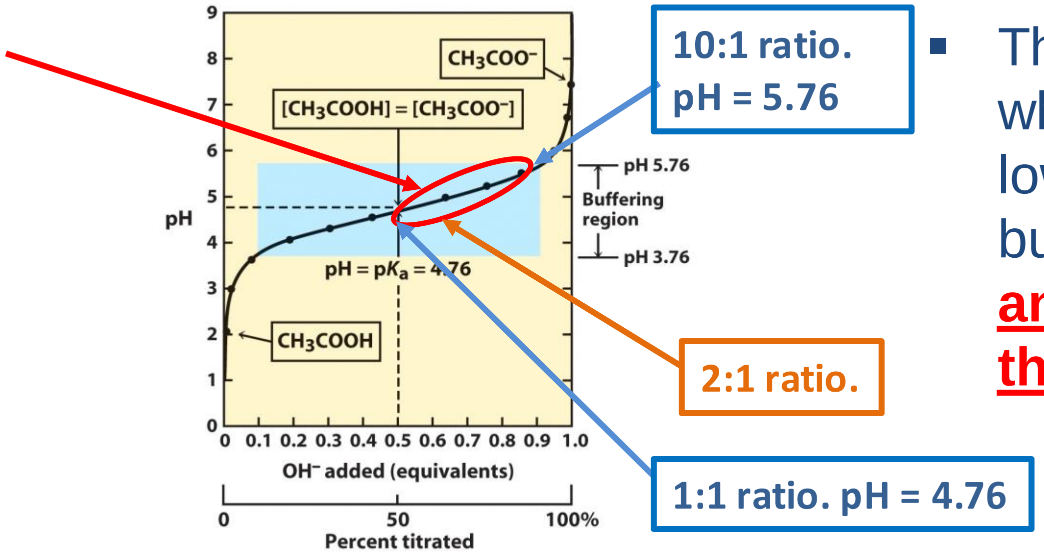
- Recall that buffers can only resist pH within the buffer range (pKa ± 1).

Some notes regarding the previous lecture:

- Regarding the following example from the previous lecture:

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

- You will not have access to a calculator to use Henderson-Hasselbalch's equation fully. However, you should use know that the ratio of acetate ions to acetic acid is 2:1, which is larger than 1:1 but smaller than 10:1. Therefore, the pH must lie in the region of the curve indicated by the red arrow:

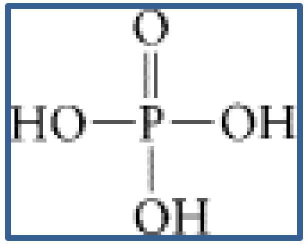


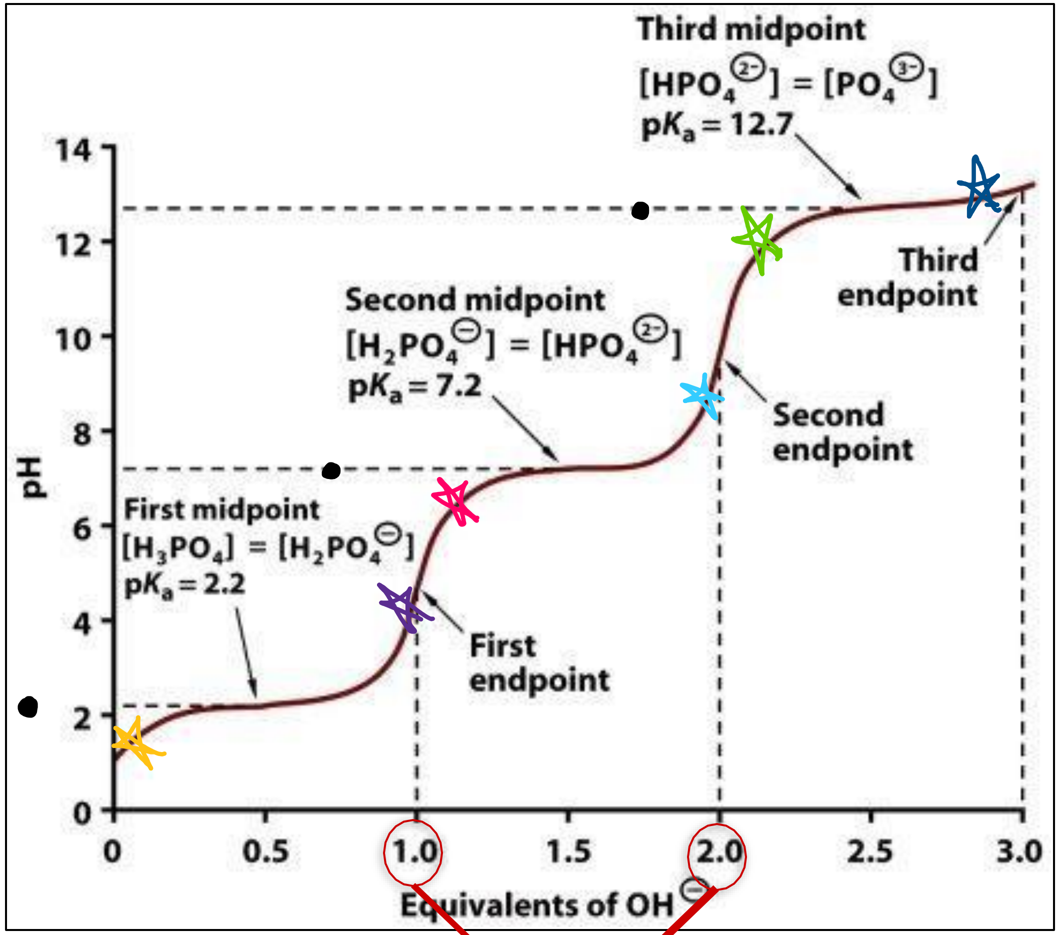
- Therefore, the pH must be higher than 4.76 (the midpoint, where $[CH_3COOH] = [CH_3COO^-]$ and the ratio is 1:1), but lower than 5.76 (which is pKa +1 - the upper limit of the buffering region, where the ratio is 10:1). **Only one answer option in the exam will contain a pH between these 4.76 and 5.76, so choose it.**

Titration curve of phosphate buffer

$$H_3PO_4 \underset{}{\overset{pK_{a(1)} = 2.14}{\rightleftharpoons}} \underset{+\ H^+}{H_2PO_4^-} \overset{pK_{a(2)} = 7.20}{\rightleftharpoons} \underset{+\ H^+}{HPO_4^{2-}} \overset{pK_{a(3)} = 12.4}{\rightleftharpoons} \underset{+\ H^+}{PO_4^{3-}}$$

We cannot deduce any information about buffer concentration from the curve.





What is the predominant form of buffer in:

- : H3PO4
- : H2PO4-
- : H2PO4-
- : HPO4-2
- : HPO4-2
- : PO4-3

Note values

Ease of removing a proton:

$H_3PO_4 > H_2PO_4^- > HPO_4^{2-} > PO_3^-$

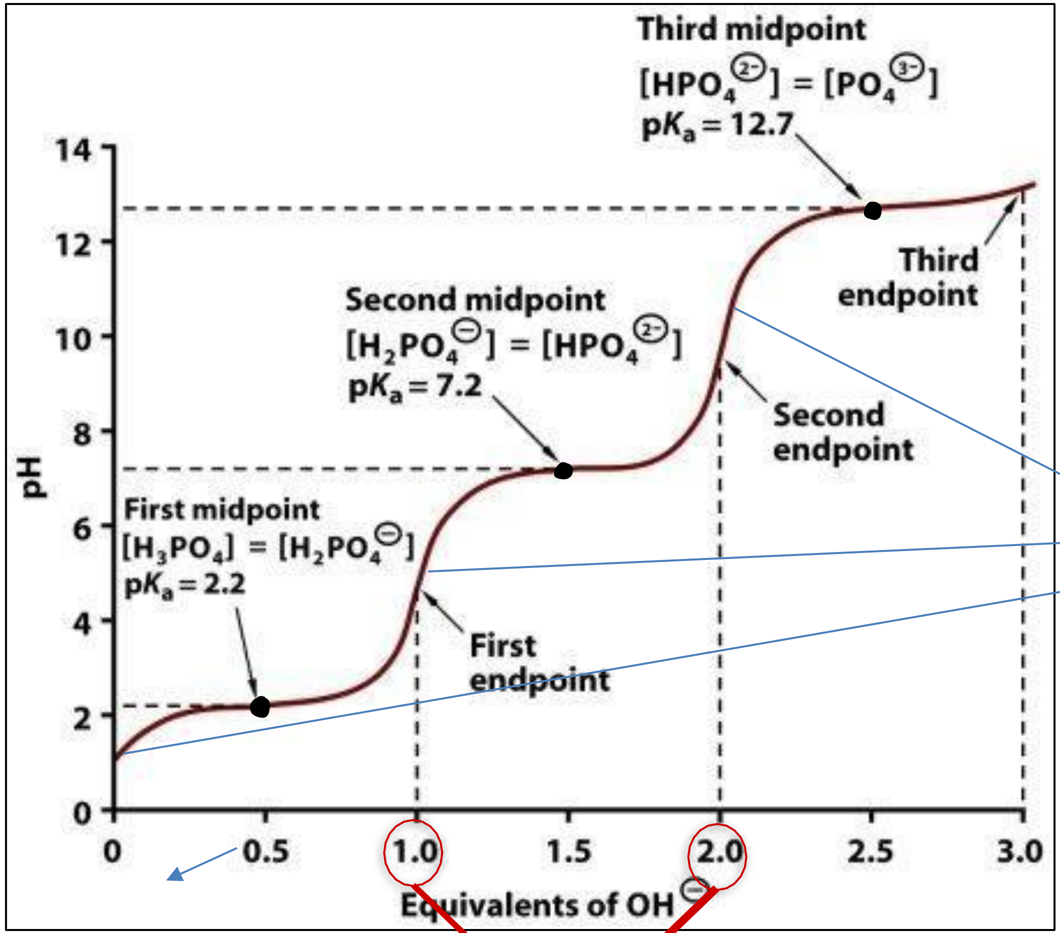
As the number of protons decreases as we go from H_3PO_4 to PO_3^-

Recall that, for each buffering region:

- The concentrations of the acid and the conjugate base are equal at the midpoint,
- The acid is the dominant form before the midpoint,
- The conjugate base is the dominant form after the midpoint
- We need 0.5 (additional) equivalents to reach the midpoint
- We need 1 (additional) equivalent to reach the equivalence point

We need 0.5 equivalents of OH^- to reach the first pKa, then an additional 0.5 equivalents **after the first equivalence point** to reach the second pKa, then another 0.5 equivalents **after the second equivalence point** to reach the third pKa.

$$H_3PO_4 \underset{}{\overset{pK_{a(1)} = 2.14}{\rightleftharpoons}} H_2PO_4^- + H^+ \overset{pK_{a(2)} = 7.20}{\rightleftharpoons} HPO_4^{2-} + H^+ \overset{pK_{a(3)} = 12.4}{\rightleftharpoons} PO_4^{3-} + H^+$$



There is no resistance to pH changes in these regions, as we are outside the buffering regions.

Note values

- Triprotic acids can dissociate into 3 protons, each proton has its own buffering region and pKa:

Proton	Midpoint	Buffering region (pH range)	Predominant buffer form
1st	2.2	1.2 – 3.2	H_3PO_4
2nd	7.2	6.2 – 8.2	$H_2PO_4^-$
3rd	12.4	11.4 – 13.4	HPO_4^{2-}

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?

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?
   - To this solution, 0.008 moles NaOH were added. What is the new pH? (ignore changes in volume).

Solutions are on the next page…

① $H_2PO_4^- \rightleftharpoons HPO_4^{-2} + H_3O^+$

0.1 M   0.3 M

- $V_{tot} = 100ml + 100 ml = 200ml = 0.2 L$
- moles of $(H_2PO_4^-) = 0.1 \times 0.1 = 0.01$ mol.
  $n = M \times V$
- moles of $(HPO_4^-) = 0.3 \times 0.1 = 0.03$ mol.
- $[H_2PO_4^-] = \frac{0.01}{0.2} = 0.05M$.
- $[HPO_4^-] = \frac{0.03}{0.2} = 0.15M$.

$pH = pKa + \log\left(\frac{HPO_4^{-2}}{H_2PO_4^-}\right)$

$7.2 = pKa + \log\left(\frac{0.15}{0.05}\right)$

$pka = 6.72$.

② • 0.02mol of acetic acid, $V = 1L$, $pKa = 4.8$

$[CH_3COOH] = \frac{0.02}{1} = 0.02M$

$pKa = -\log(ka)$

$Ka = 10^{-pka} \rightarrow Ka = 10^{-4.8} = 1.58 \times 10^{-5}$

$Ka = \frac{[H^+]^2}{[HA]} \rightarrow 1.58 \times 10^{-5} = \frac{[H^+]^2}{0.02}$

$[H^+] = \sqrt{0.02 \times 1.58 \times 10^{-5}}$

$[H^+] = 5.62 \times 10^{-4} M$

$pH = -\log[H^+]$

$pH \approx 3.25$

- $n_{(NaOH)} = 0.008\,mol$

$$CH_3COOH \rightleftharpoons CH_3COO^- + H_3O^+$$

before $0.02\ mol$ $\quad$ 0 $\quad \longrightarrow$ it is very small amount in the beginning of the rxn.

after $0.02 - 0.008$ $\quad$ $+0.008$

$= 0.012\ mol$

$$[CH_3COOH] = \frac{0.012}{1L} = 0.012\,M$$

$$[CH_3COO^-] = \frac{0.008}{1L} = 0.008\,M$$

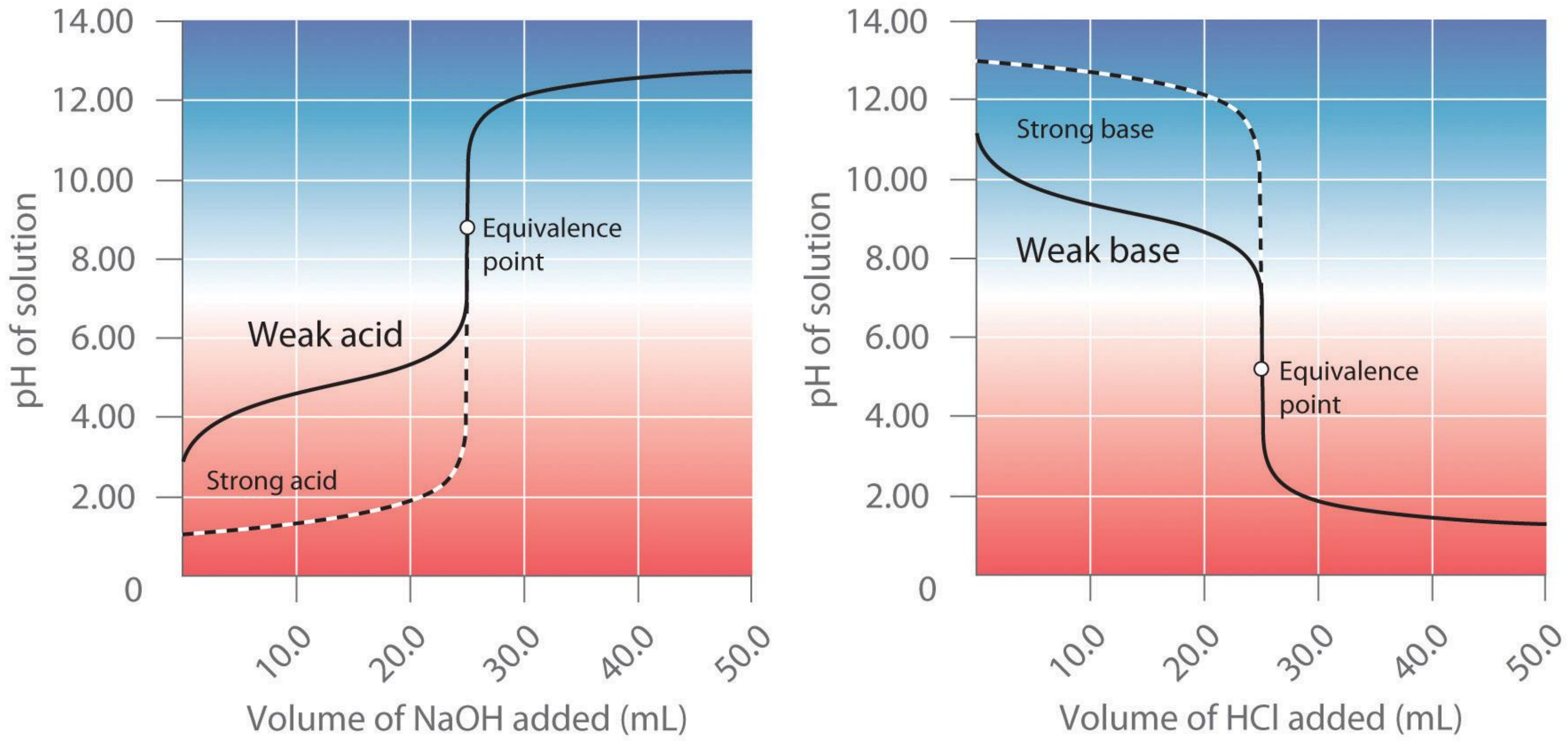
$$pH = pKa + \log\left(\frac{[CH_3COO^-]}{[CH_3COOH]}\right)$$

$$= 4.8 + \log\left(\frac{0.008}{0.012}\right) = 4.62.$$

Extra information:

It is good to know this idea. 💡

When we add a strong acid to the buffer, the PH will decrease, so the curve will drop from a high pH instead of moving up from a low pH.



(a) Weak acid titrated with strong base **(b) Weak base titrated with strong acid**

Biological buffers in human body

- 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

Bicarbonate buffer

$$CO_2\,(d) + H_2O \xrightleftharpoons{K_h} H_2CO_3$$

$$H_2CO_3 \xrightleftharpoons{K_a} H^+ + HCO_3^-$$

This is an enzymatic reaction (it occurs through the action of an enzyme), though it may sometimes take place spontaneously (without the presence of an enzyme). It occurs in the lungs and in red blood cells

Blood (instantaneously)

This reaction is controlled by the renal system (the kidneys)

$$\mathbf{CO_2 + H_20 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-}$$

Lungs

(within minutes)

H_2CO_3 (carbonic acid) is highly unstable and immediately dissociates in two possible ways: either into the ions H^+ and HCO_3^-, or into CO_2 and H_2O. So you will not find undissociated H_2CO_3 in the blood.

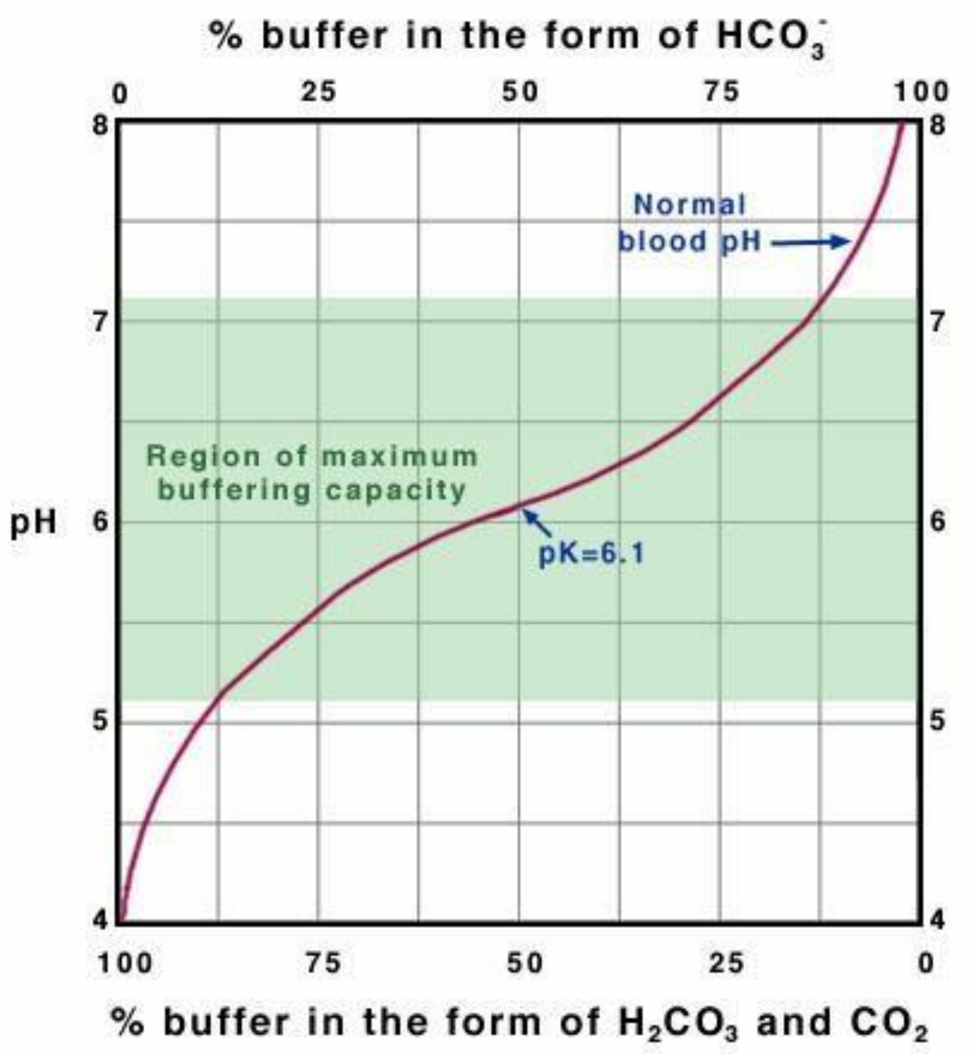
Excretion via kidneys (hours to days)

Titration curve of bicarbonate buffer

Note pKa

Although H_2CO_3 contains two protons, only one of them can dissociate. Therefore, the titration curve of H_2CO_3 has only one buffering region instead of two.

Normal blood pH range: 7.35 to 7.45



Normal blood pH is much higher than the pKa, making it outside the buffering range. However, the carbonic acid-bicarbonate system can still act as a buffer. We will see why in the next slide.

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 CO_2 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.

Due to the metabolic reactions occurring in muscles and other tissue, large amounts of CO_2 are produced in the body. This CO_2 exits the cells and enters red blood cells, where it is reacted with water to form large amounts of carbonic acid (which then dissociates into its ions that may leave the red cells and enter the blood plasma as they are hydrophilic). These large amounts of carbonic acid are important in ensuring that the buffer is effective under blood pH conditions.

The lungs and kidneys monitor and regulate the levels of carbon dioxide and bicarbonate in the blood. If we were to add carbonic acid and bicarbonate to a closed container, mix them and record their pH, it will be the same as the pKa. However, in an open system like our bodies that continuously interact with their environment, this is not the case.

Metabolic → kidneys → control the levels of bicarbonate ions

Respiratory → lungs → control the levels of carbon dioxide

Acidosis and alkalosis

- Both pathological conditions can be either metabolic or respiratory.

- Acidosis (pH< 7.35)
  - Metabolic: production of ketone bodies (starvation and diabetes)
  - Respiratory: pulmonary (asthma; emphysema)

- Alkalosis (pH > 7.45)
  - Metabolic: excessive administration of salts
  - Respiratory: hyperventilation (anxiety)

Respiratory conditions

Respiratory Acidosis

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow \mathbf{CO_2} + H_2O$$

Carbon dioxide levels increase → the reactions moves towards producing more carbonic acid and hydrogen bicarbonate ions (in the direction of the thick red arrow). As a result of the increase in the number of protons, the pH decreases below 7.35. However, it does not decrease drastically (e.g. from 7.35 to 7.25).

Respiratory Alkalosis

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow {\scriptstyle CO_2} + H_2O$$

Carbon dioxide levels decrease → the reactions moves towards producing more carbon dioxide (in the direction of the thick red arrow). As a result of the decrease in the number of protons, the pH increases.

Metabolic conditions

See slide 19 for the causes of each. Both metabolic conditions result from the kidney not being able to regulate proton concentrations in the blood fast enough to counteract the change.

Metabolic Acidosis

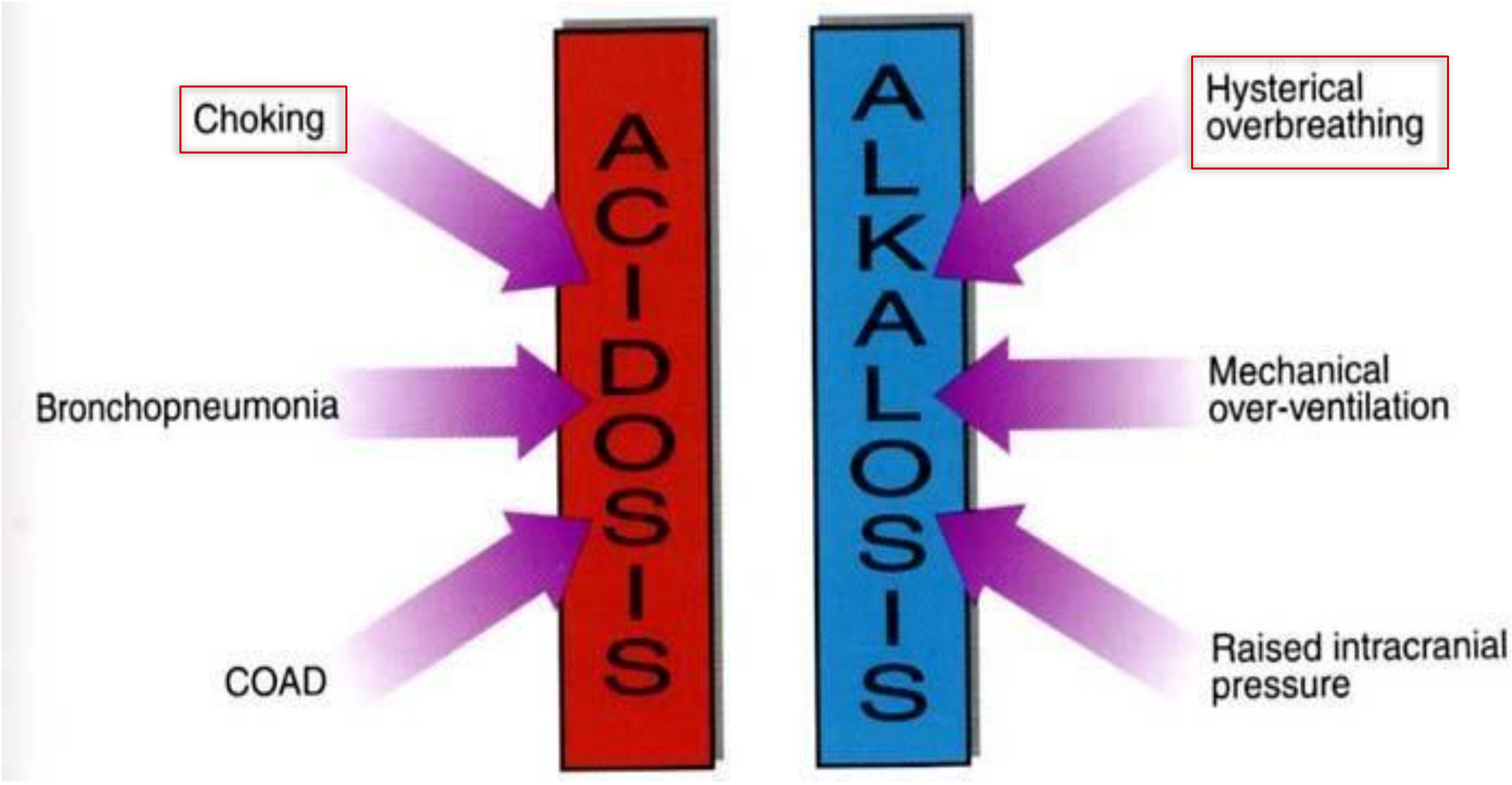
$$\mathbf{H^+} + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow CO_2 + H_2O$$

Metabolic Alkalosis

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

When there is a problem with lung function, the kidneys come to the rescue to keep the proton concentrations (and therefore blood pH levels) within the normal range.
The opposite is also true: When there is a problem with kidney function, the lungs come to the rescue.

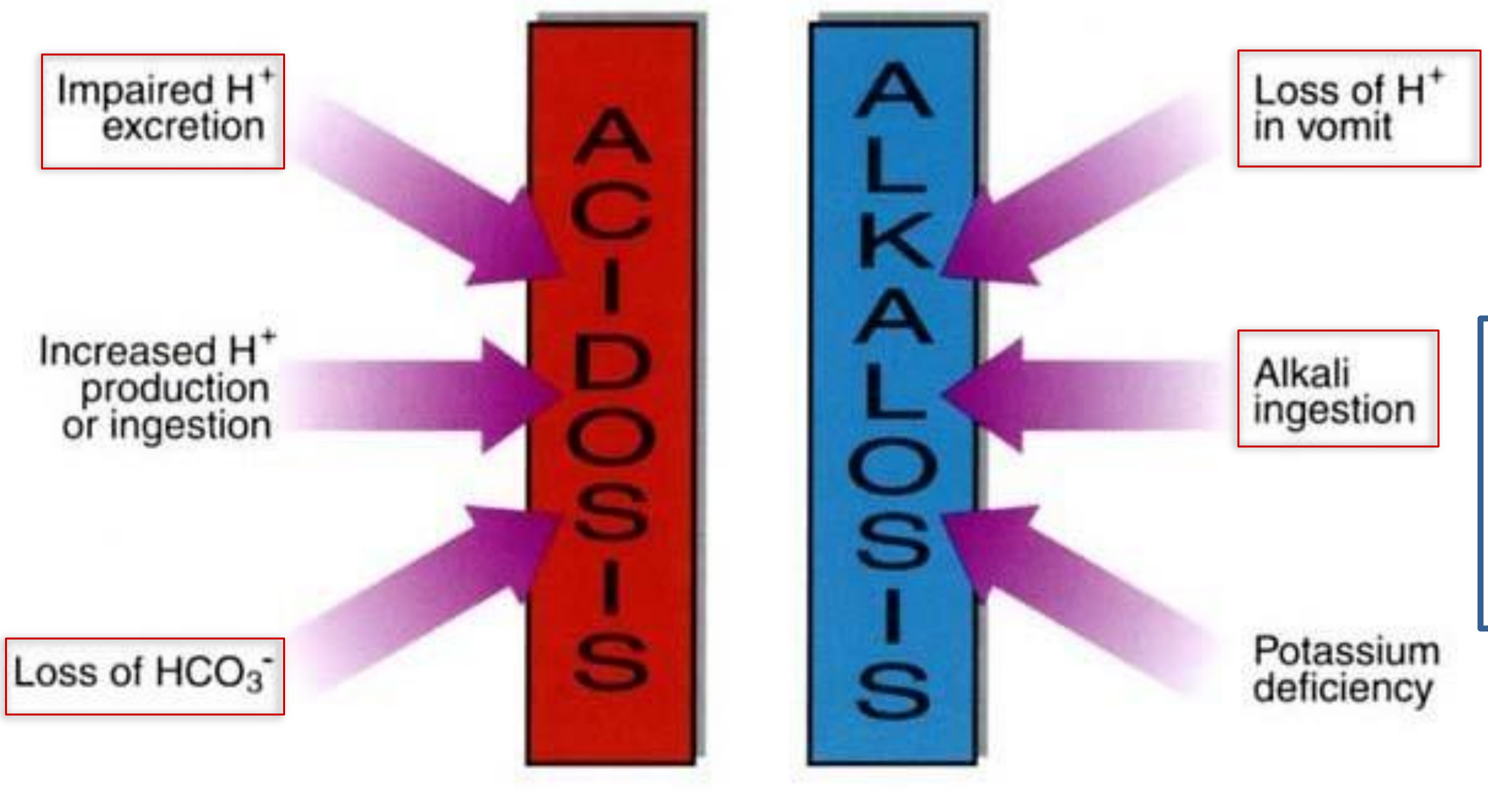
Causes of respiratory acid-base disorders



Hysterical overbreathing (hyperventilation) can happen, for example, in a panic attack. Hyperventilation results in dizziness as a mechanism used by the brain to return the breathing rate (and therefore the blood pH) back to normal. A temporary solution we can use is breathing in a bag. This causes the person to re-inhale their own carbon dioxide and therefore prevent its levels from dropping in the blood

Causes of metabolic acid-base disorders

May be a result of impaired kidney function



By taking alkaline to increase the pH of the stomach in the case of a condition called ”heartburn”

Compensation

- Compensation: A change in HCO_3^- or pCO_2 as a result of the primary event in order to return the pH to normal levels.
- If the underlying problem is metabolic, hyperventilation or hypoventilation alters pCO_2; it is called respiratory compensation.
- If the problem is respiratory, renal mechanisms drives metabolic compensation via changing $[HCO_3^-]$.
- Complete compensation if brought back within normal limits
- Partial compensation if the pH is still outside norms.

However, the levels of bicarbonate ions and carbon dioxide are not normal despite the pH being within the normal range

However, it is not too far from the normal range (7.35 – 7.45).
The levels of bicarbonate ions would also be outside the normal range.

As previously mentioned, when there is a problem with lung function, the kidneys compensate for this by regulating the concentrations of bicarbonate ions and protons (and therefore blood pH levels) within the normal range.
When there is a problem with kidney function, the lungs come to regulate the levels of carbon dioxide.

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

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V1 → V2	• Slide number 19 next to “Alkali ingestion”.	• To decrease the PH of stomach.	• To increase the PH.
V2 → V3			
V3 → V4			

Additional Resources:

رسالة من الفريق العلمي:

1. https://youtu.be/jW2-Xqg8lhw?si=T5VU_D8ZCLRUPLRA
2. Campbell/Farrell's Biochemistry – 7th edition:

- Titration curves (chapter 2 – sec. 2.4).
- Buffers (sec. 2.5).
- Biochemical connection (pages 55 +56).

3. Mark's Basic Medical Biochemistry: chapter 4 – sec. 4.3 + 4.4).