

Brønsted Acid Base Chemical Equilibrium. BUFFER SOLUTIONS

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Solutions, having ability to maintain a constant pH, despite addition of strong acid or strong base, are called buffer solutions.

Buffer systems play an important role in the human organism as they maintain pH at a level 7.36 despite the fact, that the metabolism products are very acidic - the daily production of acidic products is equivalent to 10 liters of 0.1 molar HCl.

Buffer solution biological action and role pH constant keeping in blood plasma pH=7,36, stomach pH=3, pancreas gland juice pH=8, mitochondria pH=7,36, skin pH=5,5 will be given in the following material.

A buffer system always consists of both forms of the same protolytic pair, or, in other words, of an acid a and its conjugated base b.

If both forms of a protolytic pair are present in solution at the same time, an equilibrium between them exists: $a \rightleftharpoons H^+ + b$, where a and b are acid and base of the protolytic pair respectively.

If an acid from outside will interfere into this equilibrium, the H^+ ions will react with the base of buffer system, more of the acid form of buffer system will be produced and the extra acidity H^+ will be absorbed in this way. Vice versa, if a base is added to the buffer system, it will react with the buffer acid and more of the buffer base will be formed and extra alkalinity OH^- will be absorbed.

1. POSSIBLE COMPOSITION of BUFFER SYSTEM REGION over INFLECTION POINT

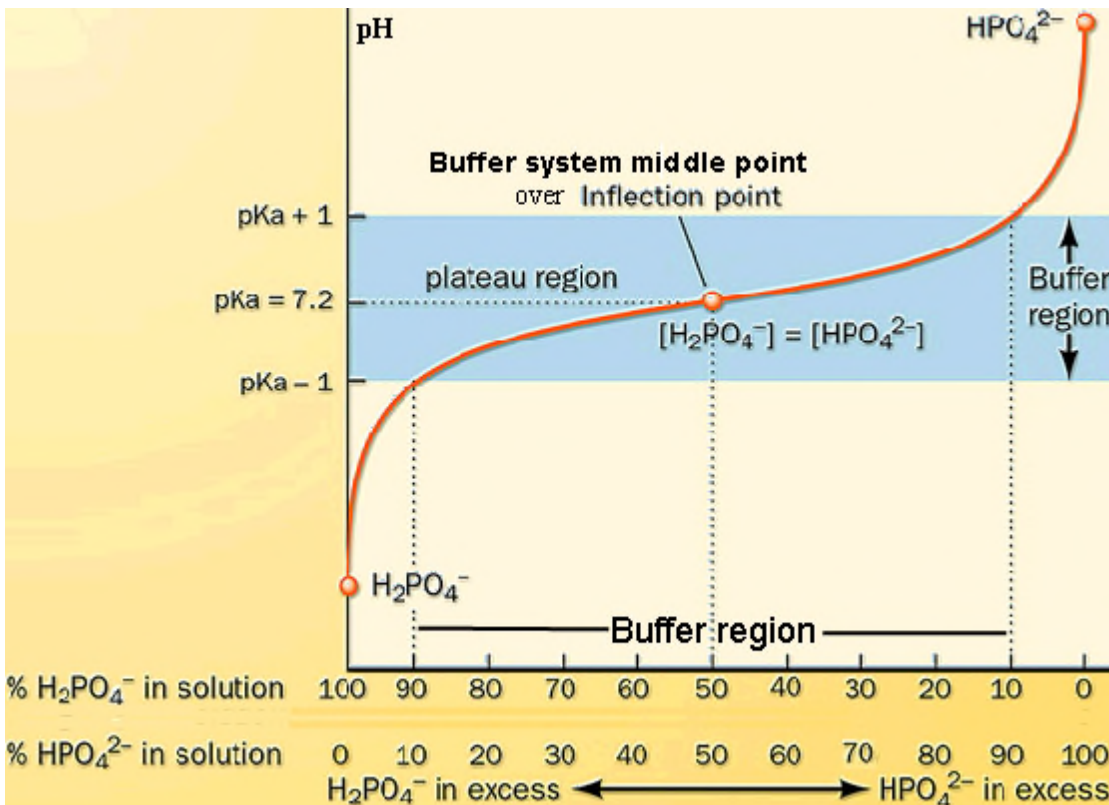
Having the general idea, that a buffer system consists of a protolytic acid and its conjugated base, several possible ways of practical composition of a buffer system can be used:

1. Buffer system can be composed of a weak acid and its salt with a strong base, for instance buffer system In this example the acid is acetic acid and the base of buffer is acetate ion CH_3COOH/CH_3COONa .

2. Buffer system can be composed of a weak base and its salt with a strong acid, for instance buffer system The base form of the buffer here is NH_4OH and the acid form is NH_4^+ ion NH_4OH/NH_4Cl .

3. Buffer system can be composed of a weak bivalent acid and its acidic salt, for instance, ENZYME Carbonic Anhydrase CA forms acid/base $H_2O^{CA}/CO_2/NaHCO_3$ equilibrium with hydro carbonate HCO_3^- anion.

4. Buffer system can be composed of two salts of the same polyvalent acid, differing in 1 hydrogen ion, as buffer systems $NaHCO_3/Na_2CO_3$ or NaH_2PO_4/Na_2HPO_4 ,



where the salt1, containing greater number of hydrogen ions plays the role

of the acid $H_2PO_4^-$.

and

the salt2, containing low number of hydrogen ion plays the role

of the base HPO_4^{2-} .

Buffer region middle point over Inflection point

MECHANISM OF BUFFER ACTION *Ostwald's dilution law*

To understand more precisely, why the pH value remains practically the same, when a strong acid or base is added to the buffer system, we have to write the dissociation reactions of both forms of the buffer.

II.1. ACETATE BUFFER SYSTEM

Let us first take acetate buffer as an example of a buffer, composed of acid and its salt.

Acetic acid dissociates according to equilibrium : $\text{CH}_3\text{COOH} \rightleftharpoons \text{CH}_3\text{COO}^- + \text{H}^+$

and sodium acetate as a salt dissociates completely : (strong electrolyte) :



As a great number of acetate ions is present in the solution due to presence of salt, the dissociation of acetic acid is oppressed, because acetate ions are products of dissociation equilibrium of acid, and if a great number of acetate ions already exists in the solution due to presence of salt, the dissociation equilibrium of acid is shifted to the left. For this reason the dissociation degree of the acetic acid is close to zero.

If a strong acid is added to the buffer solution in such a situation, the H_3O^+ ions of the strong acid will react with the base form of buffer (with the acetate ion) : $\text{H}_3\text{O}^+ + \text{CH}_3\text{COO}^- \Rightarrow \text{CH}_3\text{COOH} + \text{H}_2\text{O}$

Now there are 2 reasons, why the pH remains constant after this:

1) the strong acid (H_3O^+ ion is the existence form of all strong acids in water medium) is transformed to a weak acid CH_3COOH .

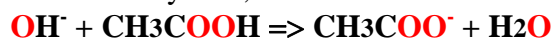
2) the concentration of acetic acid is increasing in this process, therefore it could be likely to say, that pH must become more acidic. In fact, according to *Ostwald's dilution law*, the dissociation degree α of a weak electrolyte

(acetic acid in this case) depends on the concentration: $\alpha = \sqrt{\frac{K}{C}}$

For this reason, when the concentration of acetic acid grows, its dissociation degree is adjusted to be smaller and therefore the concentration of H^+ ions and pH remain practically constant.

Assuming it all in a shorter way, the strong acid is transformed into a weak one and the dissociation degree of the weak acid is adjusted to be smaller, therefore pH remains constant.

If a strong base is added to acetate buffer system, the OH^- ions from the strong base react with the acid form of buffer (acetic acid) :



Now the same two reasons for practically constant pH can be seen :

1) a strong base (OH^- ion) is transformed into a strong base - CH_3COO^- ion,

2) acetic acid was used, to do this, but as the concentration C of acetic acid decreases, the dissociation degree α grows, hence, H^+ concentration and pH remains constant.

$$\alpha = \sqrt{\frac{K}{C}}$$

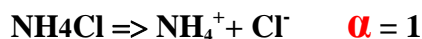
II.2. AMONIUM BUFFER SYSTEM

In another example, a buffer system, which is composed of a weak base and a salt, e.g. ammonium buffer $\text{NH}_4\text{OH}/\text{NH}_4\text{Cl}$, the same considerations should be referred to OH^- concentration.

The dissociation equilibrium of ammonium hydroxide is :

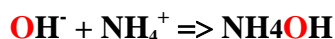


Ammonium chloride, as every salt is a strong electrolyte and it is completely ionized in the solution :



In the presence of NH_4^+ ions from the salt, the dissociation of ammonium hydroxide is oppressed (as the presence of reaction products shifts equilibrium to the left) and its $\alpha \rightarrow 0$.

If a strong base is added to this solution, it will react with the acid form of the buffer (with NH_4^+ ion) :

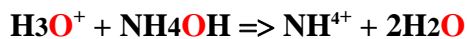


Due to this reaction :

1) a very strong base OH^- ion is transformed into a weak base NH_4OH ,

2) concentration of this weak base grows in this process, but as the dissociation degree α is adjusted to be lower, OH^- concentration and pOH and, consequently, pH remain practically constant.

When a strong acid is added to buffer system, its H_3O^+ ions react with the base form of buffer- the ammonium hydroxide:



1) a strong acid (H_3O^+) is transformed to a strong acid (NH_4^+),

2) buffer base NH_4OH is used for this, but, as the concentration of base decreases,

the dissociation degree α increases and OH^- concentration, pOH and pH remain practically constant.

III. Henderson Haselbalh pH VALUE OF THE BUFFER SYSTEM EQUATION

Using the understandings, discussed above, one can understand, why **pH** of a buffer remains constant, but it is necessary to know, what particular value of **pH** will be kept constant by a given buffer.

III.1. Henderson Haselbalh pH COMPOSED FROM A WEAK ACID AND ITS SALT BUFFER

To derive the **pH** equation, we have to start with the considerations, already discussed above. First, we have to

write the equation for a dissociation constant of the buffer acid: $K_a = \frac{[H^+][CH_3COO^-]}{[CH_3COOH]_{nondis}}$

Knowing, as the acetate ions in the solutions come from two sources - from acid and from the salt, we have to decide, how to replace the concentration of acetate ion in the equation of equilibrium constant. As the salt is completely dissociated and the acid's dissociation is completely oppressed by the presence of acetate ions from the salt, acetate ion concentration in solution is practically equal to the concentration of salt $[CH_3COO^-] = C_{salt}$.

At the same time, as the dissociation of acid is completely oppressed by presence of salt, the concentration of nondissociated molecules of acid is practically equal to the initial concentration of acid: $[CH_3COOH]_{nondis} = C_{acid}$.

Replacing the concentrations of acetate ion and nondissociated acid in the equation of **K** we have :

$$K_a = \frac{[H^+]C_{salt}}{C_{acid}} \text{ Solving this for } [H^+] : [H^+] = \frac{K_a \cdot C_{acid}}{C_{salt}}. \text{ Taking a minus logarithm from both sides :}$$

$$\log[H^+] = -\log K_a - \log \frac{C_{acid}}{C_{salt}} \text{ we got the } \mathbf{Henderson Haselbalh} \text{ equation } \mathbf{pH} = -\log[H^+] = \mathbf{pK}_a + \log \frac{C_{salt}}{C_{acid}}$$

converting to **pH**: (note, that $\log a/b = -\log b/a$)

Factors, that affect the pH value of a buffer system The **pH** value, that is kept **constant** by a buffer.

- 1) on the strength of the acid, included in buffer system (K_a is the measure of acid strength) and
- 2) on the ratio between salt and acid amount n_{salt}/n_{acid} of buffer solution volume **V**.
- 3) not **pH** depends on dilution of buffer solution. Drinking the water leave safe the blood **pH=7.36** constant.
- 4) Fourth factor, that affects **pH** of a buffer system, is temperature - increases of temperature increase the value of K_a and this shifts **pH** to lower values (as $\mathbf{pK}_a = -\log K_a$, the greater is acid K_a , the smaller is \mathbf{pK}_a).

DIFFERENT FORMS OF pH Henderson Haselbalh EQUATION

The form of **pH** equation, which we have derived for a buffer, consisting of weak acid and its salt contains the final concentrations of buffer components in a **ready buffer solution**. Let us change the form of **pH** equation for this reason. First, considering, that the concentration (molarity) of a buffer component can be expressed as $C_M = n/V$, where **n** is number of moles and buffer system volume **V** is common. (**V** can be canceled, because **V** here is the

$$\mathbf{pH} = \mathbf{pK}_a + \log \frac{C_{salt}}{C_{acid}}$$

$$\mathbf{pH} = \mathbf{pK}_a + \log \frac{n_{salt}}{n_{acid}}$$

$$\mathbf{pH} = \mathbf{pK}_a + \log \frac{n_{salt} / V}{n_{acid} / V}$$

same total volume of buffer solution in both cases). It is very often necessary to express the **pH** of a buffer through the concentrations of the **two initial solutions of acid and salt**, that are practically mixed together to obtain the buffer solution.

Now, if the buffer solution is prepared from a solution of salt and a solution of acid, the numbers of moles can be replaced by $n = C'V'$, where **C'** and **V'** are the concentration and the volume of the initial solutions.

$$\mathbf{pH} = \mathbf{pK}_a + \log \frac{C'_{salt} \cdot V'_{salt}}{C'_{acid} \cdot V'_{acid}}$$

Thus, the final form of equation is obtained: where **C'** and **V'** are the concentration and the volume of the initial salt and acid solutions in the bottles of salt C'_{salt} and acid C'_{acid} . This final form is the most commonly used

Henderson Haselbalh equation for **pH** calculation

$$\mathbf{pH}_{ac} = \mathbf{pK}_a + \log \frac{n_{salt} - \Delta n_{ac}}{n_{acid} + \Delta n_{ac}}$$

The Δn_{ac} is number of strong acid moles, for example **HCl**, added to buffer solution, which decreases the buffer system salt as Brensted base amount $n_{salt} - \Delta n_{ac}$ and increases the buffer system acid amount $n_{acid} + \Delta n_{ac}$, thus change the buffer system **pH** value about $\Delta \mathbf{pH} = \mathbf{pH} - \mathbf{pH}_{ac}$ to decrease that. Adding the strong base, for example **NaOH**, change the buffer system **pH** value to increase that about $\Delta \mathbf{pH} = \mathbf{pH}_b - \mathbf{pH}$.

$$\mathbf{pH}_b = \mathbf{pK}_a + \log \frac{n_{salt} + \Delta n_b}{n_{acid} - \Delta n_b}$$

III.2. Henderson Haselbalh pH IN A BUFFER, COMPOSED FROM WEAK BASE AND SALT

In a buffer, composed from a weak base and a salt, for example, ammonium buffer $\text{NH}_4\text{OH}/\text{NH}_4\text{Cl}$, dissociation reactions of base and salt have to be written prior to deriving **Henderson Haselbalh** equation of pOH and equation of equilibrium constant for base dissociation has to be written : $K_b = \frac{[\text{NH}_4^+][\text{OH}^-]}{[\text{NH}_4\text{OH}]_{\text{nondis}}}$.

As the presence of completely dissociated salt creates a great number of NH_4^+ ions, the dissociation of base is completely oppressed and for this reason all NH_4^+ ions can be considered to come only from salt. Thus, the NH_4^+ concentration in this case can be replaced by the initial concentration of the salt: $[\text{NH}_4^+] = C_{\text{salt}}$.

At the same time, as the dissociation of base is completely oppressed, the concentration of nondissociated molecules of the base can be replaced by the initial concentration of base and solving this for $[\text{OH}^-] = C_{\text{base}}$:

$$K_b = \frac{[\text{OH}^-] \cdot C_{\text{salt}}}{C_{\text{base}}} ; [\text{OH}^-] = K_b \cdot \frac{C_{\text{base}}}{C_{\text{salt}}} \text{ after taking } -\log: -\log[\text{OH}^-] = \text{pOH} = -\log K_b - \log \frac{C_{\text{base}}}{C_{\text{salt}}}$$

$$\text{pOH} = \text{p}K_b + \log \frac{C_{\text{salt}}}{C_{\text{base}}} \quad \text{using water constant } \text{p}K_w = 14 = \text{pH} + \text{pOH} \text{ converting } \text{pOH} \text{ to } \text{pH} = 14 - \text{p}K_b + \log \frac{C_{\text{base}}}{C_{\text{salt}}}$$

For simplicity don not change **Henderson Haselbalh** pOH equation to pH

$$\text{pOH} = \text{p}K_b + \log \frac{n_{\text{salt}}}{n_{\text{base}}} \quad \text{pOH} = \text{p}K_b + \log \frac{n_{\text{salt}} / V}{n_{\text{base}} / V} \quad \left| \begin{array}{l} \text{First, considering, that the concentration (molarity) of a} \\ \text{buffer component can be expressed as } C_M = n/V, \text{ where } n \text{ is} \\ \text{number of moles and buffer system volume } V \text{ is common.} \end{array} \right.$$

(V can be canceled, because V here is the same total volume of buffer solution in both cases).

Now, if the buffer solution is prepared from a solution of salt and a solution of base, the numbers of moles can be replaced by $n = C'V'$, where C' and V' are the concentration C'_{salt} , C'_{base} and the volume V'_{salt} , V'_{base} of the initial solutions.

$$\text{pOH} = \text{p}K_b + \log \frac{C'_{\text{salt}} \cdot V'_{\text{salt}}}{C'_{\text{base}} \cdot V'_{\text{base}}} \quad \text{Thus, the final form of equation is obtained: where } C' \text{ and } V' \text{ are the concentration and the volume of the initial salt and base solutions in the bottles of salt } C'_{\text{salt}} \text{ un base } C'_{\text{base}}. \text{ This final form is the most commonly used one.}$$

$$\text{pOH}_{\text{ac}} = \text{p}K_b + \log \frac{n_{\text{salt}} + \Delta n_{\text{ac}}}{n_{\text{base}} - \Delta n_{\text{ac}}} \quad \text{The } \Delta n_{\text{ac}} \text{ is number of strong acid moles, for example } \text{HCl}, \text{ added to buffer solution, which increases the buffer system salt amount } n_{\text{salt}} + \Delta n_{\text{ac}} \text{ as Brensted acid and decreases the buffer system base amount } n_{\text{base}} - \Delta n_{\text{ac}}, \text{ thus change the buffer system } \text{pOH} \text{ value } \Delta \text{pOH} = \text{pOH}_{\text{ac}} - \text{pOH} \text{ to increase that. Adding the strong base, for example } \text{NaOH}, \text{ change the buffer system } \text{pOH} \text{ value to decrease that } \Delta \text{pOH} = \text{pOH} - \text{pOH}_b.$$

$$\text{pOH}_b = \text{p}K_b + \log \frac{n_{\text{salt}} + \Delta n_b}{n_{\text{base}} - \Delta n_b}$$

III.4. EXAMPLE OF BUFFER ACTION

Now, when the equation for buffer pH is derived, we can illustrate the buffer action.

Let us imagine, that **0.01** mole of **HCl** is added to a buffer system, containing **0.5** moles of acetic acid and **0.5** moles of sodium acetate. pH values before and after addition of **HCl** ($\text{p}K_a = 4.74$ for acetic acid) can be calculated as follows: pH before addition of **HCl**: $\text{pH} = 4.74 + \log(0.5/0.5) = 4.74 + \log 1 = 4.74 + 0 = 4.74$

addition of **HCl** causes a reaction : $\text{HCl} + \text{CH}_3\text{COONa} \rightarrow \text{CH}_3\text{COOH} + \text{NaCl}$

As the number of moles of **HCl** is **0.01**, the number of moles of acetic acid will increase by **0.01** moles and $n_{\text{CH}_3\text{COONa}}$ will decrease by **0.01** moles, therefore : pH after addition of **HCl**:

$$\text{pH}_2 = 4.74 + \log((0.5 - 0.01) / (0.5 + 0.01)) = 4.74 + \log 0.996 = 4.74 - 0.002 = 4.738$$

and the pH change is $\Delta \text{pH} = \text{pH}_1 - \text{pH}_2 = 0.002$.

At the same time, if this amount of **HCl** was added to **1** liter of pure water (the initial $\text{pH} = 7$ in pure water), after addition of **HCl**, concentration of H^+ ions would be **0.01** mole/l (as **HCl** is added to **1** l of H_2O), making pH of solution: $\text{pH} = -\log [\text{H}^+] = -\log 0.01 = -(-2) = 2$. Thus, the pH change in this case is $\Delta \text{pH} = 7 - 2 = 5$.

As one can see, the pH change, caused by **HCl** in a buffer solution is negligible when compared to the pH change, caused by the same amount of acid in pure water, where the change from $\text{pH} = 7$ to $\text{pH} = 2$ (from neutral

to strongly acidic) is drastic for hydrogen ion $[\text{H}^+]$ concentration $\frac{[\text{H}^+]_{\text{HCl}}}{[\text{H}^+]} = \frac{10^{-2}}{10^{-7}} = 10^5 = 100000 \text{ times.}$

V. BUFFER CAPACITY β

The **pH** value of the buffer system is **Henderson Haselbalh** equation:

$$\text{pH} = \text{pK}_a + \log \frac{n_{\text{salt}}}{n_{\text{acid}}}$$

where n_{salt} and n_{acid} are the numbers of equivalents of salt and acid

respectively.

If an acid is added to buffer solution, it will react with the salt of buffer system therefore

n_{salt} will decrease (n_{acid} will increase at the same time, as more buffer acid will be formed).

This means, that the buffer system cannot stand against just any amount of added acid. If the number of equivalents of the added strong acid reaches the number of equivalents n_{salt} of the salt, present in buffer system, all salt will be used up and the resistant **pH** constant buffer system doesn't exist anymore.

As well, if a strong base is added to the buffer system, it will use the acid of buffer system and the buffer system can stand against addition of base only until the number of equivalents of the added base is equal to the number of equivalents n_{acid} of the buffer acid.

From the discussion above one has to make a conclusion, that a value, that characterizes the ability of buffer system to stand against addition of strong acid or strong base, is necessary. Such a value is buffer capacity, which

is expressed as

$$\beta = \frac{\Delta n}{\Delta \text{pH} \cdot V_{\text{buffer}}} = \left(\frac{\text{mol}}{\text{Liter}} \right)$$

where Δn is the number of equivalents **of the strong acid or base**, that is added to the buffer,

ΔpH is the **pH** change, caused by the addition of strong acid Δn_{ac} or base Δn_{b} ,

V_{buffer} is the volume of the buffer solution, to which the strong acid or base is added.

Buffer capacity units are equivalent mol/Liter, the same as for C_N , because **pH** has no unit. The definition of buffer capacity in words is as follows :

*Buffer capacity β shows, what strong acid mol numbers Δn_{ac} or a strong base Δn_{b} can be added to 1 liter V_{buffer} of buffer solution to shift its **pH** value for 1 **pH** unit.*

Buffer capacity can be both determined experimentally or calculated. To determine the buffer capacity experimentally, a known number of equivalents of a strong acid or base is added to a known volume of the buffer solution and **pH** is measured before and after addition of it.

In general, the **buffer capacity** is affected by four reasons :

1. the total concentration of buffer solution $C_{\text{salt}}' = C_{\text{acid}}'$. The C' is proportional to buffer capacity β .

2. the ratio between buffer components $\frac{n_{\text{salt}}}{n_{\text{acid}}} = 1$ on **middle point** of buffer solution:

2. a) maximal value $\beta_{\text{acid}} = \beta_{\text{base}} = 0.55 \cdot C'$ buffer capacity have on **middle - over inflection point**. Buffer solution have *symmetric* equal values $\beta_{\text{acid}} = \beta_{\text{base}} = 0.55 \cdot C'$;

if the ratio between buffer components $\frac{n_{\text{salt}}}{n_{\text{acid}}} = 1$ is one.

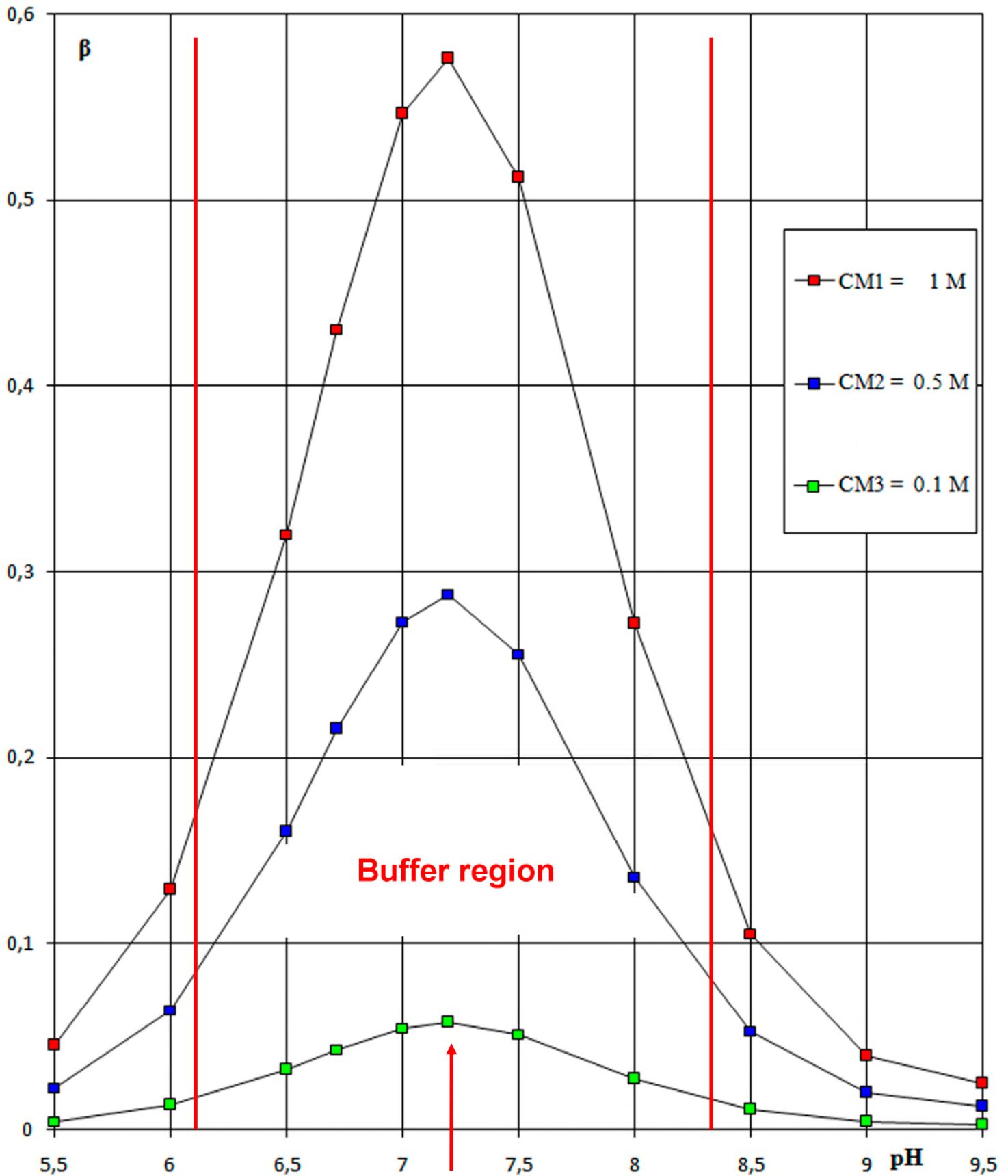
2. b) as soon as the ratio between buffer components deviates from **middle point** $n_{\text{salt}}/n_{\text{acid}} = 1$, both buffer capacities β_{acid} and β_{base} becomes fast smaller as $0.55 \cdot C'$

2. c) as soon as the salt/acid ratio in buffer solution deviates from **middle point** $n_{\text{salt}}/n_{\text{acid}} = 1$, buffer capacity becomes *asymmetric* (β_{acid} and β_{base} differ from each other).

Phosphate buffer system $\text{NaH}_2\text{PO}_4 / \text{Na}_2\text{HPO}_4$

$$\text{pH} = \text{pK}_a + \log \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}$$

Buffer capacity strong acid Δn_{ac} or strong base Δn_b equivalent mole/ into one Liter buffer solution $\Delta \text{pH}=1$



Buffer system **middle point $\text{pH}=\text{pK}_a=7,199$ over inflection point** Maximum Buffer Capacity $\beta=0.55$

Na_2HPO_4 salt, containing lower number of hydrogen ions plays the role of the **base HPO_4^{2-}**

NaH_2PO_4 salt, containing greater number of hydrogen ions, plays the role of the **acid H_2PO_4^-**

IV. CALCULATUION EXAMPLES ABOUT pH OF BUFFER SOLUTIONS

1. Calculate **pH** of a formiate buffer (**HCOOH/HCOONa**), if the buffer is composed from **300 mL** of **0.15 M HCOOH** and **200 mL** of **0.09 M HCOONa** solutions, $K_{\text{HCOOH}}=2 \cdot 10^{-4}$

$$\text{pH} = \text{p}K_a + \log \frac{C_{\text{salt}} \cdot V_{\text{salt}}}{C_{\text{acid}} \cdot V_{\text{acid}}} = -\log 2 \cdot 10^{-4} + \log \frac{0.09 \cdot 200}{0.15 \cdot 300} = 3.7 - 0.398 = 3.3$$

2. Calculate **pH** of a buffer, composed from **80 mL 0.1 M NH₄OH** and **120 mL of 0.17 M NH₄Cl** solutions, $K_{\text{NH}_4\text{OH}}=1.8 \cdot 10^{-5}$.

$$\text{pH} = 14 - \text{p}K_b + \log \frac{C_b \cdot V_b}{C_s \cdot V_s} = 14 - (-\log 1.8 \cdot 10^{-5}) + \log \frac{0.1 \cdot 80}{0.17 \cdot 120} = 14 - 4.74 + \log 0.39 = 9.26 - 0.41 = 8.85$$

3. Calculate, how many milliliters of **0.1 M HCOOH** and of **0.2 M HCOONa** have to be taken to obtain a buffer, having **pH = 3.0** and total volume **1 liter**, $K_{\text{HCOOH}} = 2 \cdot 10^{-4}$.

When writing **pH** equation for this case, volume of salt can be named **x** and then the volume of acid in this

case is **(1000-x) mL**: $3.0 = -\log 2 \cdot 10^{-4} + \log \left(\frac{0.2x}{0.1(1000-x)} \right) \rightarrow 3.0 = 3.7 + \log \left(\frac{0.2x}{0.1(1000-x)} \right)$

$$\log \left(\frac{0.2x}{0.1(1000-x)} \right) = -0.7 \rightarrow \left(\frac{0.2x}{0.1(1000-x)} \right) = 10^{-0.7} = 0.199$$

$$0.2x = 0.199(1000 - 0.1x);$$

$$0.2x + 19.9 = 0.0199x$$

$$0.2199x = 19.9;$$

$$x = 90,5 \text{ mL}$$

$$V_{\text{salt}} = x = 90.5 \text{ mL};$$

$$V_{\text{acid}} = 1000 - x = 909.5 \text{ mL}$$

4. Calculations of buffer capacity (see theory in the next chapter).

Calculate the **pH** change and buffer capacity, observed when **10 mL** of **0.5 M NaOH** are added to a buffer system, composed of **100 mL** of **0.2 M NaHCO₃** and **200 mL** of **0.3 M Na₂CO₃**, $K_{\text{HCO}_3^-} = 4.69 \cdot 10^{-11}$

a) **pH** before addition of **NaOH** is: $\text{pH}_1 = -\log 4.69 \cdot 10^{-11} + \log \frac{0.3 \cdot 200}{0.2 \cdot 100} = 10.32 + \log 3 = 10.8$

b) addition of **NaOH** causes a reaction : $\text{NaOH} + \text{NaHCO}_3 \rightarrow \text{Na}_2\text{CO}_3 + \text{H}_2\text{O}$

as the number of moles of **NaOH** is **n = 0.01 • 0.5 = 0.005**, the number of moles of **Na₂CO₃** increases by **0.005** moles and the number of moles of **NaHCO₃** decreases by **0.005** moles. The number of moles of salt (**Na₂CO₃**) in the initial buffer was

$$n_{\text{salt}} = 0.2 \cdot 0.3 = 0.06 \text{ moles}$$

The number of moles of acid in initial buffer was (acid is **NaHCO₃** here) : **n_{acid} = 0.1 • 0.2 = 0.02 moles** thus, after the addition of **NaOH** **pH** becomes:

$$\text{pH}_2 = -\log 4.69 \cdot 10^{-11} + \log \frac{0.06 + 0.005}{0.02 - 0.005} = 10.32 + \log 4.33 = 10.97$$

c) buffer capacity of the solution is found as:

$$\beta = \frac{n_{\text{NaOH}}}{\Delta \text{pH} \cdot V_{\text{buffer}}} = \frac{0.05}{(10.97 - 10.8) \cdot (0.1 + 0.2)} = 0.98 \text{ mol/L}$$

Buffer System Research by numerical Experiment 1.

Let us prove, that buffer capacity depends on the concentration of buffer solution.

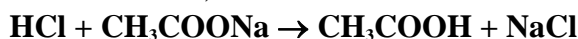
To do this, let us compare the buffer capacities of two solutions, having the same acid/salt ratio, but different total concentrations.

If we add the same amount of **HCl** 1 milli equivalents (meq) of **HCl** to two different buffer solutions, one having **200** meq of acetic acid and **200** meq of sodium acetate, other having **20** meq acetic acid and **20** meq of sodium acetate in **1** liter of the buffer, the buffer capacities will be as follows.

a) The initial pH of both buffer solutions will be the same: $\text{pH} = \text{pK}_{\text{CH}_3\text{COOH}}$:

$$\text{pH}_1 = \text{pK}_{\text{CH}_3\text{COOH}} + \log \frac{200}{200} = \text{pK}_{\text{CH}_3\text{COOH}} + \log \frac{20}{20} = 4,74 - 0 = 4,74 \quad (\text{as } \log 1 = 0)$$

In both of these buffer solutions, if HCl is added, it will react with the salt :



b) as **10** meq of **HCl** are added, n_{salt} decreases for **1** meq and n_{acid} increases for **1** meq. The **pH** values after the addition of **HCl** will be:

In the more concentrated buffer system :

$$\text{pH}_2 = \text{pK}_{\text{CH}_3\text{COOH}} + \log \frac{200-1}{200+1} = 4,74 + \log \frac{199}{201} = 4,74 + \log 0,9900 = 4,74 + (-0,00434) = 4,73566$$

In the more diluted buffer system :

$$\text{pH}_2 = \text{pK}_{\text{CH}_3\text{COOH}} + \log \frac{20-1}{20+1} = 4,74 + \log \frac{19}{21} = 4,74 + \log 0,90476 = 4,74 + (-0,0434) = 4,69653$$

c) The **pH** change will be $\Delta\text{pH} = \text{pH}_1 - \text{pH}_2$:

in ten times more concentrated system:

$$\Delta\text{pH} = 4.74 - 4.73566 = 0.00434$$

in ten times more diluted system:

$$\Delta\text{pH} = 4.74 - 4.69653 = 0.0434$$

d) The buffer capacities against acid will be : $\beta_{\text{ac}} = \frac{\Delta n_{\text{HCl}}}{\Delta\text{pH} \cdot V_{\text{buffer}}}$

in ten times more concentrated solution: $\beta_{\text{ac}} = \frac{1 \text{ekv} \cdot \text{mmol}}{0.00434 \cdot 1000 \text{mL}} = 0.23 \text{ eq} \cdot \text{mol} / \text{L}$

in ten times more diluted solution : $\beta_{\text{ac}} = \frac{1 \text{ekv} \cdot \text{mmol}}{0.0434 \cdot 1000 \text{mL}} = 0.023 \text{ eq} \cdot \text{mol} / \text{L}$

As we could see from the results of calculation, the buffer capacity of buffer system is proportional to concentration at $C' = 200 \text{ meq/L}$ $\beta_{\text{ac}} = 0.23 \text{ eq} \cdot \text{mol} / \text{L}$ and for ten times diluted concentration $C' = 20 \text{ meq/L}$ $\beta_{\text{ac}} = 0.023 \text{ eq} \cdot \text{mol} / \text{L}$.

Research the "middle point" of buffer system 2

Let us prove, that, if the salt/acid ratio in a buffer solution is **1:1**, β_{acid} and β_{base} are equal. The more concentrated solution of the previous example will be used for this, therefore the initial **pH** value is the same **4.74** and after addition of **1** meq **HCl** :

$$\text{pH}_1 = \text{pK}_{\text{CH}_3\text{COOH}} + \log \frac{200-1}{200+1} = 4,74 + \log \frac{199}{201} = 4,74 + \log 0,9900 = 4,74 + (-0,00434) = 4,73566$$

If a strong base, for example, **KOH** is added to the buffer system, it will react with the acid of buffer system and more salt will be produced : $\text{KOH} + \text{CH}_3\text{COOH} \rightarrow \text{CH}_3\text{COOK} + \text{H}_2\text{O}$

If **1** meq of **KOH** are added, n_{acid} will decrease for **1** meq and n_{salt} will increase for **1** meq, hence, the **pH** after addition of **KOH** will be:

$$\text{pH}_2 = \text{pK}_{\text{CH}_3\text{COOH}} + \log \frac{200+1}{200-1} = 4,74 + \log \frac{201}{199} = 4,74 + \log 1,01 = 4,74 + 0.00434 = 4.74434$$

now the **pH** change against the acid **HCl** is $\Delta\text{pH}_1 = 4.74 - 4.73566 = 0.00434$

now the **pH** change against the base **KOH** is $\Delta\text{pH}_2 = 4.74434 - 4.74 = 0.00434$

and $\beta_{\text{base}} = (1 \text{ meq}) / (0.00434 \cdot 1000 \text{mL}) = 0.23 \text{ eq} \cdot \text{mol} / \text{L}$,

which is the same value, that was previously calculated for $\beta_{\text{acid}} = 0.23 \text{ eq} \cdot \text{mol} / \text{L}$.

Experimental Research of Buffer System with Alkaline Reserve 3.

Let us prove, that in a buffer solution, containing the same total number of equivalents of acid and base, but having the salt/acid ratio other than 1:1, β_{acid} and β_{base} are not any more equal to each other and that both of them are smaller, than in a solution, having acid/salt ratio, equal to 1:1.

For example, let us choose a buffer solution, containing 200 meq of CH_3COOK and 20 meq CH_3COOH . The summary number of equivalents is $200 + 20 = 220$, approximately the same, than in the more concentrated buffer from example 1 (where 200 meq CH_3COOK and 200 meq CH_3COOH).

Initial pH value of this chosen buffers solution is:

$$\text{pH} = \text{pK}_{\text{CH}_3\text{COOH}} + \log \frac{200}{20} = 4,74 + \log 10 = 4,74 + 1 = 5,74$$

If 1 meq HCl are added, n_{salt} decreases for 1 meq and n_{acid} increases for 1 meq, therefore after acid addition:

$$\text{pH}_1 = \text{pK}_{\text{CH}_3\text{COOH}} + \log \frac{200-1}{20+1} = 4,74 + \log \frac{199}{21} = 4,74 + \log 9,4765 = 4,74 + 0,9766 = 5,7166$$

$$\Delta\text{pH}_1 = \text{pH}_1 - \text{pH} = 5,7166 - 5,74 = 0,023 ; \text{ and } \beta_{\text{ac}} = \frac{\Delta n_{\text{HCl}}}{\Delta\text{pH} \cdot V_{\text{buffer}}} = \frac{1 \text{ eq} \cdot \text{mmol}}{0,023 \cdot 1000 \text{ mL}} = 0,0430 \text{ eq} \cdot \text{mol} / \text{L}$$

If 1 meq of KOH are added to the same buffer solution, KOH reacts with acetic acid, n_{acid} decreases for 1 meq and n_{salt} increases for 1 meq. After the addition of KOH the pH value will be :

$$\text{pH}_2 = \text{pK}_{\text{CH}_3\text{COOH}} + \log \frac{200+1}{20-1} = 4,74 + \log \frac{201}{19} = 4,74 + \log 10,5789 = 4,74 + 1,02444 = 5,76444$$

$$\Delta\text{pH}_2 = \text{pH}_2 - \text{pH} = 5,76444 - 5,74 = 0,02444 \text{ and } \beta_{\text{b}} = \frac{\Delta n_{\text{KOH}}}{\Delta\text{pH} \cdot V_{\text{buffer}}} = \frac{1 \text{ eq} \cdot \text{mmol}}{0,02444 \cdot 1000 \text{ mL}} = 0,0409 \text{ eq} \cdot \text{mol} / \text{L}$$

Comparing $\beta_{\text{acid}} = 0,0430 \text{ eq} \cdot \text{mol} / \text{L}$ and $\beta_{\text{base}} = 0,0409 \text{ eq} \cdot \text{mol} / \text{L}$ one can see, that the buffer capacity of this buffer system against acid is greater, than against base. This is a logical result, because the reserve of salt (salt reacts with added acid) is much greater, than the reserve of the buffer acid (buffer acid reacts with added base).

Comparing the buffer capacities of this solution to the buffer capacities of a solution, containing 200 meq salt and 200 meq acid (from example 1 $\beta_{\text{ac}} = 0,23 \text{ eq} \cdot \text{mol}$; $\beta_{\text{b}} = 0,23 \text{ eq} \cdot \text{mol}$), one can see, that both values are much smaller for the buffer system, in which salt/acid ratio differs from “middle point” of buffer system 1:1.

Research of Buffer System with low Alkaline Reserve 4

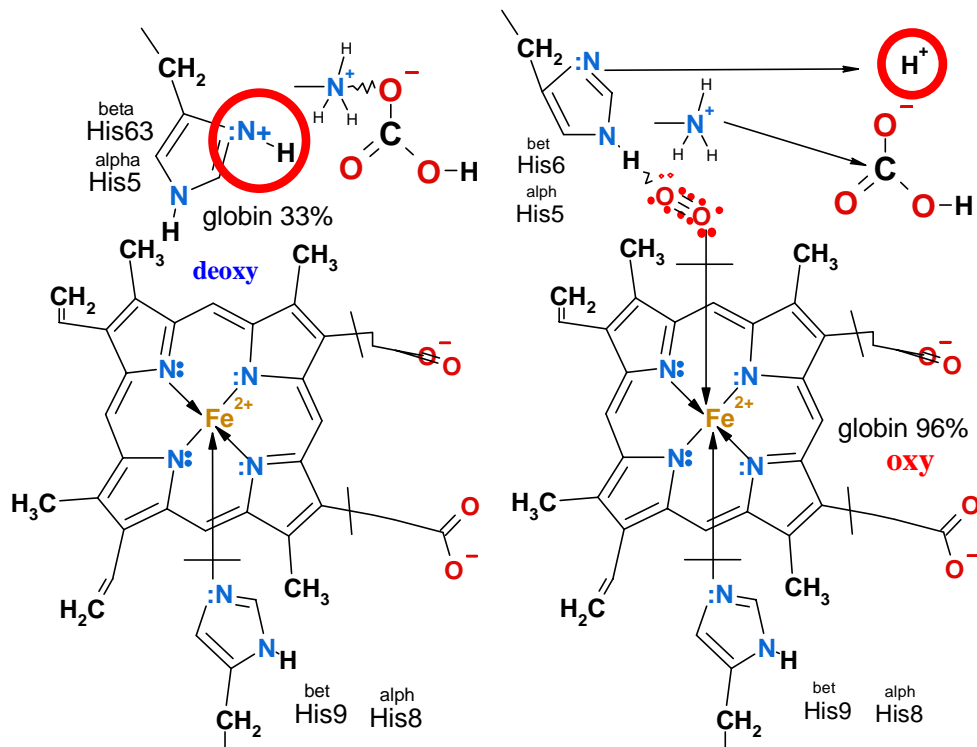
If we calculated buffer capacities of a solution, containing 20 meq salt and 200 meq acid, we would find out, that the values are the same, than in previous example, but they are replaced by each other: now

$\beta_{\text{acid}} = 0,0409 \text{ eq} \cdot \text{mol} / \text{L}$ and $\beta_{\text{base}} = 0,0430 \text{ eq} \cdot \text{mol} / \text{L}$. This also easy to understand, because in this case the alkaline reserve of salt is small and therefore the capacity against acid is lower, but the reserve of acetic acid is great and therefore the capacity against base is high.

In the biological bodies buffer solutions **alkaline reserve** always exceeds the acids, that has the sense to work against the metabolic production of acids (acetic acid, formic acid, pyruvic acid, lactic acid, glycerin acids, malic acid, succinic acid, citric acid and wastes of fatty acids like as palmitic acid, butyric acid, stearic acid and so on more other).

Shuttle deoxy - oxy hemoglobin with Carbonic Anhydrase enzyme in **O₂** , **CO₂** metabolism stabilize physiologic pH=7.36 and oxygen arterial concentration [**O₂blood**]=6·10⁻⁵ M

I) Oxygen **O₂** from AIR 20.95% **O₂**↑gas assimilation reaction dissolution in water to form **O₂aqua** exothermic $\Delta H_r = -55,7 \text{ kJ/mol}$ and exoergic $\Delta G_r = -27,7 \text{ kJ/mol}$ as water soluble 1) $\text{O}_{2\text{AIR}} + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{O} + \text{O}_{2\text{aqua}} + \text{Q} + \Delta G$. Penetrate in Human body through aquaporins osmosis by concentration gradient [**O₂**]=9,768·10⁻⁵ M to **venous** blood [**O₂**]=1,85·10⁻⁵ M $\Delta G_{\text{O}_2} = RT \ln([\text{O}_{2\text{Blood}}]/[\text{O}_{2\text{aqua}}]) = -4,29 \text{ kJ/mol}$ exoergic movement: 2) $\text{O}_{2\text{aqua}} + \text{H}_2\text{O} \xrightarrow{\text{Aquaporins}} \text{H}_2\text{O} + \text{O}_{2\text{aqua}} + \Delta G$ $\Delta G_{\text{H}_2\text{O}} = RT \ln([\text{H}_2\text{O}]_{\text{right}}/[\text{H}_2\text{O}]_{\text{left}}) = -8,3144 \cdot 310,15 \cdot \ln(0,305/0,2) = -1,088 \text{ kJ/mol}$ exoergic $\Delta G_{\text{O}_2+} = -5,379 \text{ kJ/mol}$. 4**O₂aqua** from blood plasma adsorbs **deoxy** hemoglobin **Hb_T** of inspired fresh AIR releases four protons 4**H⁺**, 4**HCO₃⁻**: $4\text{O}_{2\text{aqua}} + (\text{H}^+\text{His63,58})_4\text{Hb}_T \cdot \text{salt bridges}(\text{HCO}_3^-)_4 \leftrightarrow \text{Hb}_R(\text{O}_2)_4 + 4\text{H}^+ + 4\text{HCO}_3^-$ stabilizing arterial concentration [**O₂**]=6·10⁻⁵ M. [**O₂blood**]=6·10⁻⁵ M concentration sensitive equilibrium $(\text{H}^+\text{His63,58})_4\text{Hb}_T \leftrightarrow \text{Hb}_R(\text{O}_2)_4$ shift to right regulates erythrocytes glycolysis metabolite **BPG⁵⁻** as two **phosphate 2,3-esters** **G⁻** $\text{H}_2\text{COPO}_3^{2-} - \text{HCOPO}_3^{2-} - \text{COO}^-$ glycerate dihydroxy acid salt with homeostasis concentration [**BPG⁵⁻**]=5 mM, so **BPG⁵⁻** pushed out of **cavity** to stabilize and store reserves 459 times higher as arterial blood concentration [**O₂blood**]=6·10⁻⁵ M amount [**O₂amount**]=459·6·10⁻⁵ M=0,02754 M. [O2Solutions.pdf](#). Oxygen adsorbs by donor-acceptor bond on iron(II) **Fe²⁺** in coordination center of heme and releases four protons **H⁺** **Hb_R(O₂)₄+4H⁺**. Proton water sticks $\text{H}^+ + \text{H}_2\text{O} \rightarrow \text{H}_3\text{O}^+$ forms hydroxonium ion. *In tissues* desorbed oxygen [**O₂desorbed**] restore oxygen concentration [**O₂**]=6·10⁻⁵ M in blood plasma 459 times and **deoxy**-hemoglobin capture four protons **H⁺** $(\text{H}^+\text{His63,58})_4\text{Hb}_T$ so keeps continuously pH=7,36±0,01. Oxygen desorbed Krebs cycle converts to mitochondrial oxidative phosphorylation product **CO₂aqua** **II** pathway with carbonic anhydrase (CA) shift to right concentration gradient **CO₂** produces amount 0,0339 M **HCO₃⁻**. Shuttle **deoxy** hemoglobin **Hb_T** capture [**H⁺**]=0,0275 M. So is stabilized constant pH=7,36±0,01 value.



II) $\text{Q}_{\text{aqua}} + \text{CO}_{2\text{aqua}} + 2\text{H}_2\text{O} \xleftarrow{\text{CA}} \text{H}_3\text{O}^+ + \text{HCO}_3^- \xleftarrow{\text{Membrane}} \text{H}_3\text{O}^+ + \text{HCO}_3^- \leftrightarrow \text{H}_2\text{O} + \text{H}_2\text{CO}_3 + \text{Q}_{\text{gas}} \leftrightarrow \text{H}_2\text{O} + \text{CO}_2\uparrow_{\text{gas}} + \text{H}_2\text{O}$.
 endothermic $\Delta H_r = +9,75 \text{ kJ/mol}$; athermic $\Delta H_r = 0 \text{ kJ/mol}$; exothermic $\Delta H_r = -9,76 \text{ kJ/mol}$; endothermic $\Delta H_r = +20,3 \text{ kJ/mol}$;
 endoergic $\Delta G_r = +58,4 \text{ kJ/mol}$; exoergic $\Delta G_r = -22,5 - 1,96 \text{ kJ/mol}$; exoergic $\Delta G_r = -58,2 \text{ kJ/mol}$; exoergic $\Delta G_r = -8,54 \text{ kJ/mol}$;
 II) $\text{Q}_{\text{aqua}} + \text{CO}_{2\text{aqua}} + 2\text{H}_2\text{O} \xleftarrow{\text{CA}} \text{H}_3\text{O}^+ + \text{HCO}_3^- + \text{Q} \xleftarrow{\text{Membrane}} \text{H}_2\text{O} + \text{CO}_2\uparrow_{\text{gas}} + \text{H}_2\text{O}\uparrow_{\text{gas}}$.
 endothermic $\Delta H_r = +9,75 \text{ kJ/mol}$; endothermic $\Delta H_r = +54,5 \text{ kJ/mol}$; summary endothermic $\Delta H_r = +64,25 \text{ kJ/mol}$;
 endoergic $\Delta G_r = +58,4 \text{ kJ/mol}$; exoergic $\Delta G_r = -82,1 \text{ kJ/mol}$; summary exoergic $\Delta G_r = -23,7 \text{ kJ/mol}$;

Shuttle is venous **deoxy Hb_T**, adsorbs four molecules 4**O₂** from fresh AIR, acidify water medium with 4**H⁺**, promoting **CO₂** breathe out: Each **H⁺** and **HCO₃⁻** ion amount [**H⁺**]=459·6·10⁻⁵ M =0,0275 M=[**HCO₃⁻**] shifts equilibrium to right $\text{H}^+ + \text{HCO}_3^- + \text{Q} \leftrightarrow \text{H}_2\text{O} + \text{CO}_2\uparrow_{\text{gas}}$ via membrane channels. So pH=7,36 remains constant, as one bicarbonate ion and one hydrogen ion produce one **CO₂** right side.

The epithelial cell surface of **lungs** has the specific building surface as square area is: S=950 nm x 950 nm= 0.9 μm² on super thin 0.6 nm layer within water small volume: 0.5415·10⁻³ μm³ = 0.5415·10⁻¹⁸ L. Created acidity in thin water layer volume increases up to pH=5.5 if one proton **H⁺** crosses the membrane channels reaching the surface so hydrogen ion concentration is: [**H₃O⁺**]=10^{-pH}=10^{-5.5} M. Respiration of fresh AIR in lungs Hemoglobin released protons **H⁺** during oxygen adsorbition for total amount concentration:

[**O₂adsorbed**]=[**H₃O⁺**]=459·6·10⁻⁵ M = 0,02754 M forms hydrogen ion [**H₃O⁺**]_{right}/[**H₃O⁺**]_{left}=10^{-5.5}/0,0275 concentration gradient, which drives exoergic $\Delta G = -22,5 \text{ kJ/mol}$ proton movement through epithelial cell membrane proton channels: $\text{H}_3\text{O}^+_{\text{left}} \xleftarrow{\text{proton channel}} \text{H}_3\text{O}^+_{\text{right}} + \Delta G$. General process $\text{H}_2\text{O} + \text{CO}_2\uparrow_{\text{gas}} + \text{H}_2\text{O}\uparrow_{\text{gas}}$ require heat supply endothermic $\Delta H = 54,5 \text{ kJ/mol}$ to drive spontaneous $\Delta G = -82,0679 \text{ kJ/mol}$ products evaporation $\text{CO}_2\uparrow_{\text{gas}}$ and $\text{H}_2\text{O}\uparrow_{\text{gas}}$ keeping moisture **H₂O** on surface of membrane. Hydrogen ions water acidity shift endothermic $\Delta H_r = +54,5 \text{ kJ/mol}$ and exoergic $\Delta G_r = -82,1 \text{ kJ/mol}$ decomposition $\text{H}_3\text{O}^+ + \text{HCO}_3^-$ breath out to AIR $\text{CO}_2\uparrow_{\text{gas}}$ with $\text{H}_2\text{O}\uparrow_{\text{gas}}$:
 endothermic $\Delta H_r = +54,5 \text{ kJ/mol}$; $\text{H}_3\text{O}^+ + \text{HCO}_3^- + \text{Q} \xleftarrow{\text{Membrane}} \text{H}_2\text{O} + \text{CO}_2\uparrow_{\text{gas}} + \text{H}_2\text{O}\uparrow_{\text{gas}} + \Delta G_r = -82,1 \text{ kJ/mol}$. exoergic .

Brønsted Acid/Base CA and hemoglobin shuttle enzymes of O_2 , $CO_{2\text{aqua}}/HCO_3^- + H^+$

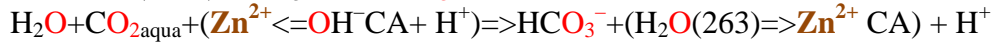
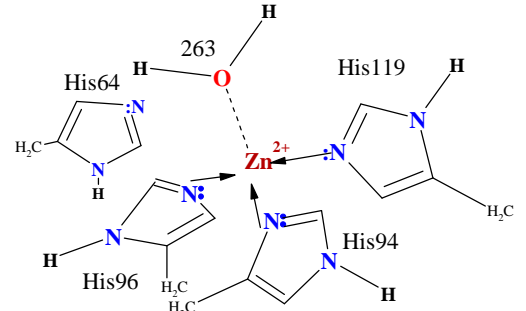
Enzyme **Carbonic anhydrase (CA)** made acid/base equilibrium $H_2O-CA-CO_2/HCO_3^- + H_3O^+$

There are **shuttle** buffer systems, that act in the human organism and allow **pH** of the organism to be stabilized constant in narrow interval allowed changes ($pH = 7.36_{-0,01}^{+0,02}$) despite the fact, that organism

produces great amount of metabolic $[CO_{2\text{Krebs}}] = 0,0275$ M. The CA made amount of acidic products is $[H_3O^+] = [HCO_3^-] = 0,01695$ M compensated by buffer solution. CA buffer of blood are connected to **shuttle** hemoglobin captured proton H^+ by oxygen $O_{2\text{aqua}}$ desorbition due to Krebs product $CO_{2\text{aqua}}$ target cells *in tissues*:

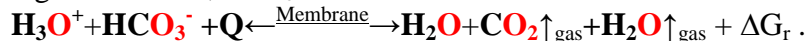
Hydrogen carbonate buffer system carbonic anhydrase equilibrium keeps weak acid $CO_{2\text{aqua}}$ and bicarbonate ions at homeostasis normal amounts $[HCO_3^-] = 0.0154$ M, $[CO_{2\text{aqua}}] = 0.0076$ M, referring to 56,23 mL (50-60 mL) released volume CO_2 from 100 mL blood as *alkaline reserve* 2,036 / 1 in clinic evaluation.

Carbon dioxide forms by oxidation of carbohydrates, of fats and of proteins. Bicarbonate is created as product in hydration $2H_2O$ of $CO_{2\text{aqua}}$ by CA enzyme Zn^{2+} ion active coordination center. It's location in enzyme carbonic anhydrase Zn^{2+} ion coordination pocket:

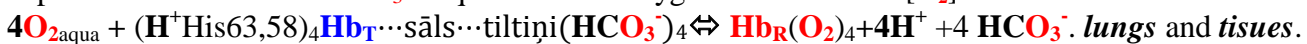


$Hb_R(O_2)_4 + 4H^+ \rightleftharpoons 4O_{2\text{aqua}} + (H^+His63,58)_4Hb_T$ stabilizing arterial concentration $[O_2] = 6 \cdot 10^{-5}$ M in blood. **Deoxy** hemoglobin ($H^+His63,58$) $_4Hb_T$ capture four protons $4H^+$ at histidine residues and $4HCO_3^-$ in venous hemoglobin form of erythrocytes **deoxy** ($H^+His63,58$) $_4Hb_T$ (**Tense** state). In **lungs shuttle** absorbs oxygen in arterial **oxy** hemoglobin ($O_2His63,58$) $_4Hb_R$ (**Relax** state) releasing $4H^+$ and $4HCO_3^-$.

1) First of four human buffer systems is enzyme CA made Brønsted Acid/Base endothermic equilibrium: $Q + CO_{2\text{aqua}} + 2H_2O \xleftarrow{CA} H_3O^+ + HCO_3^-$ which consume heat Q of Krebs cycle complexes exothermic reactions. Shift to right supported by high water $2H_2O$ concentration $[H_2O]^2 = (993,36/18,0153)^2 = 55,139^2 = 3040,4$ and by low stabilized $pH = 7,36 \pm 0,01$ of hydrogen ions H_3O^+ concentration $[H_3O^+] = 10^{-7,36}$ M in products. $CO_{2\text{Krebs}}$ as bicarbonate salt bridge linked $HCO_3^- \dots H_3^+N$ — and equal produced protons $[H^+] = [CO_{2\text{Krebs}}] = 0,0275 = [HCO_3^-]$ captures **deoxy** ($H^+His63,58$) $_4Hb_T$ **shuttle** and brings to **lungs**. **Lungs** evaporates $CO_2 \uparrow_{\text{gas}} + H_2O \uparrow_{\text{gas}}$ endothermic $\Delta H_r = +54,5$ kJ/mol, but exoergic $\Delta G_r = -82,1$ kJ/mol:



Symbol ($H^+His63,58$) $_4Hb_T$ to a **Shuttle** molecule of **deoxy** hemoglobin is inconvenient to write every time the complicated structure of hemoglobin. **Deoxy** hemoglobin is capturing and **oxy** hemoglobin completely deprotonated $4H^+$ and $4HCO_3^-$. Equilibrium is oxygen concentration $[O_2] = 6 \cdot 10^{-5}$ M sensitive:



Lungs venous blood hemoglobin saturation with oxygen 459 times restore circulated arterial blood $[O_2] = 6 \cdot 10^{-5}$ M amount in one liter [O2Solutions.pdf](#) Adsorbed four $4O_{2\text{aqua}}$ ($O_2His63,58$) $_4Hb_R + 4H^+ + 4HCO_3^-$ in products release four protons $4H^+$ and bicarbonate ions $4HCO_3^-$, promoting evaporation $CO_2 \uparrow_{\text{gas}} + H_2O \uparrow_{\text{gas}}$ on **lungs** epithelia surface, and removing out of organism $[H^+] = 459 \cdot 6 \cdot 10^{-5} = 0,0275$ M amount $H^+ + H_2O \Rightarrow H_3O^+$, that is equal to total by respiration evaporated $[CO_2 \uparrow_{\text{gas}}] = 0,0275$ M amount.

Shift to the left ($O_2His63,58$) $_4Hb_R + 4H^+ + 4HCO_3^-$ from **deoxy** captured **shuttle** ($H^+His63,58$) $_4Hb_T$ oxygen depending concentration $[O_2] = 6 \cdot 10^{-5}$ M adsorbition-desorbition equilibrium explain pH stabilization at 7.36.

That explain, why pH is not changed, despite Krebs cycle acid $CO_{2\text{aqua}}$ product which involved in CA equilibrium. Henderson-Haselbalh homeostasis pH value expression leave the ratio $[HCO_3^-]/[CO_{2\text{aqua}}] = 2,0263$ practically unchanged as intact both concentrations bicarbonate $[HCO_3^-]$ and carbon dioxide $[CO_{2\text{aqua}}]$:

$7.36 = pH = pK + \log([HCO_3^-]/[CO_{2\text{aqua}}]) = 7.0512 + \log([HCO_3^-]/[CO_2])$ and anti logarithm is being alkaline reserve $[HCO_3^-]/[CO_{2\text{aqua}}] = 10^{(pH-pK)} = 10^{(7.36-7.0512)} = 10^{0.3088} = 2,0361/1$. **Lungs** when in venous blood erythrocytes **deoxy** ($H^+His63,58$) $_4Hb_T$ (**Tense**) **Shuttle** hemoglobin by oxygen $O_{2\text{aqua}}$ adsorbition release of protons H^+ and HCO_3^- so in **Lungs** evaporates carbon dioxide $CO_2 \uparrow_{\text{gas}}$ as breathed out in AIR.

In such a way two equilibria stabilize arterial oxygen concentration $[O_{2\text{aqua}}] = 6 \cdot 10^{-5}$ M with **shuttle** hemoglobin by oxygen adsorbition-desorbition and CA buffer system made value $pH = 7,36$ with Krebs cycle drive the exchange metabolism of O_2 and CO_2 respiration to interface human body / environment.

2) Second buffer system, that is present in blood, is the protein buffer system. This one has to be explained a little more, as it differs from the usual buffer systems that are composed from weak acid/salt or weak base/salt. Like hemoglobin **Hb** proteins are long chain polypeptides of amino acids with four type protolytic acid groups:

Amino Acid	pK _{aCOOH}	pK _{aNH3+}	pK _{aRgrupa}
Isoleucine	2.36	9.68	
Valine	2.32	9.62	
Leucine	2.36	9.60	
Phenylalanine	1.83	9.13	
Cysteine	1.96	10.28	8.18
Methionine	2.28	9.21	
Alanine	2.34	9.69	
Proline	1.99	10.96	
Glycine	2.34	9.60	
Threonine	2.11	9.62	
Serine	2.21	9.15	
Tryptophan	2.38	9.39	
Tyrosine	2.20	9.11	10.07
Histidine	1.82	9.17	6.00
Aspartate	1.88	9.60	3.65
Glutamate	2.19	9.67	4.25
Asparagine	2.02	8.80	
Glutamine	2.17	9.13	
Lysine	2.18	8.95	10.53
Arginine	2.17	9.04	12.48

-COO⁻ deprotonated carboxyl negative anion salt groups,
 protonated positive charged ammonium groups **-NH₃⁺**,
 neutral phenolic acid **-OH** and **-SH** neutral sulfhydryl groups.

In physiologic medium pH=7,36 ±0.01

Carbonic acid groups deprotonated negative charged **-COO⁻** and amino groups **R-NH₃⁺** protonated positive charged.

Table given maximal pK_{a-COOH⁻} value smaller about 7,36:

$$pK_{a-COOH^-} = 4.25 < 7,36 \text{ and}$$

given smallest pK_{a-NH3+} value greater about 7,36 < 9,04 = pK_{a-NH3+}
 20 amino acids have four protolytic pK_a equilibria in 47 groups:

1. **R-COOH** ⇌ **R-COO⁻** + **H⁺**, 22 groups of 47
2. **R-NH₃⁺** ⇌ **R-NH₂** + **H⁺** 22+1 group of 47
3. **Tyrosine-phenol-OH** ⇌ **Tyrosine-phenolate-O⁻** + **H⁺** one group,
4. **Cysteine-SH** ⇌ **Cysteine-S⁻** + **H⁺** one group .

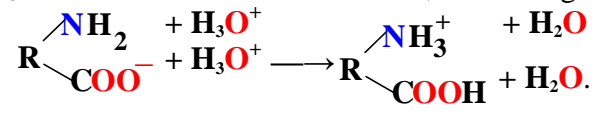
NpK_a number of parallel protolytic equilibria average pK_a value is calculated as pK_a=(Σ pK_{a R group}+ pK_{a-NH3+}+ pK_{a-COOH})/NpK_a

In *Ostwald's dilution law* calculates one the pH of solution at

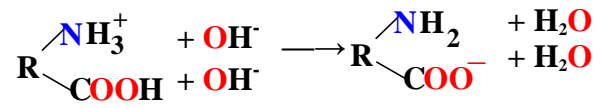
$$\text{concentration } C \text{ logarithm: } \text{pH} = \frac{\text{pK}_a - \log C}{2} = \dots$$

14th page: http://aris.gusc.lv/BioThermodynamics/Data_bookSpring2015CT.pdf

properties and acidic properties protonated ammonium groups **-NH₃⁺**. If an acid is added to solution, containing protein like hemoglobin Hb, the **H₃O⁺** ions will react with basic amino group and basic carboxylate group. The strong acid **H₃O⁺** will be transformed into water the weak base **H₂O**.

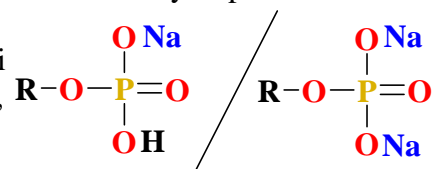


If a strong base is added to protein-containing solution, the **OH⁻** ions react with the carboxylic groups and the strong base **OH⁻** will be transformed into water the weak acid **H₂O**.

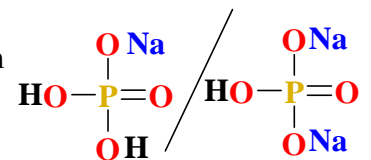


3) Biological important phosphate buffer system **NaH₂PO₄/Na₂HPO₄** pK=7,199 we will study as practical work.

4) Biological ubiquities exist besides the inorganic phosphate buffer system, buffer systems of the organic esters of phosphoric acid so as ATP (adenosine triphosphate), ADP (adenosine di phosphate), CTP, CDP, GTP, GDP, TTP, TDP, UTP, UDP, NADH B₃ vitamin, FADH₂ B₂ vitamin, phospho proteins, glucose phosphate, fructose phosphate, etc. :



If there are any difficulties to understand the structure of last two groups of compounds, remember, that phosphoric acid can be shown in structure as in the ester of phosphoric acid one of the hydrogen atoms is replaced by an organic radical. Practically the buffer system consists of a mono substituted and bi substituted salts of the ester. Likely as for phosphates **NaH₂PO₄/Na₂HPO₄**.



Not all of these 4 buffer systems act in the same organism body water solutions.

In *erythrocytes* main are bicarbonate buffer with **shuttle** hemoglobin-based proton oxygen **O₂aq** sensitive exchange: **(O₂His63,58)₄Hb_R + 4H⁺ ← [O₂aq] = 6 · 10⁻⁵ M → 4O₂aq + (H⁺His63,58)₄Hb_T**. Krebs cycle product **CO₂aq** exchanged to bicarbonate buffer solution: **Q + CO₂aq + 2H₂O ←^{CA}→ H₃O⁺ + HCO₃⁻**.

In blood *plasma* dominate enzyme **CA** bicarbonate **pH=7.36±0,01**, protein and phosphate buffer solutions.

In sweat, urine and digestive apparatus dominates bicarbonate system and phosphate system is too present.

Besides the normal "chemical" mechanisms of buffer action in maintaining constant **pH=7.36±0,01**, with **deoxy** hemoglobin **(H⁺His63,58)₄Hb_T (Tense state)**, **oxy** hemoglobin **(O₂His63,58)₄Hb_R (Relax state)** and with carbonic anhydrase **CA** driven bicarbonate buffer systems are a joint physiological mechanism of action, which carries out the exchange of breathed in **O₂** and breathed out **CO₂** between AIR in *lungs* and tissues on interface human body / environment.

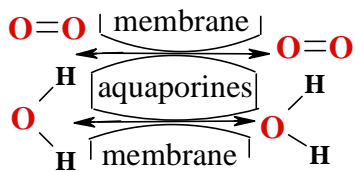
Human **shuttle hemoglobin-bicarbonate** buffer system and Krebs cycle driven respiration from AIR **O₂** and breathed out **CO₂** action **physiologic** mechanism

Before we have to order three molecules involved in the buffer systems. The **shuttle oxy** hemoglobin, second is **carbonic anhydrase CA** with constant value $pK=7.0512$ and **shuttle deoxy** hemoglobin:

oxy Hb_R(O₂)₄ + 4H⁺ <=> deoxy (H⁺_{His63,58})₄Hb_T + 4O_{2aqua}, where completely deprotonated 4 H⁺ **oxy Hb_R** but **deoxy** hemoglobin **Hb_T** capturing four protons 4 H⁺ and 4 HCO₃⁻ as desorbing four oxygen 4O_{2aqua} molecules.

Shuttle and **carbonic anhydrase CA** stabilize exchange process from AIR **O₂** to breathed out in to AIR **CO₂**.

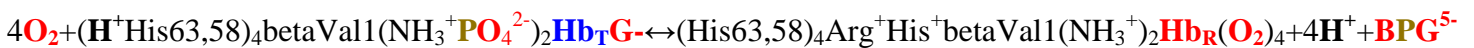
Two **I** and **II** pathways are happen of gradual reactions: **I) O_{2AIR} + H₂O $\xrightleftharpoons{\text{aquaporin}}$ H₂O + O_{2aqua}**



Process in lungs **I) Pathway first reaction** on cell wall membrane aquaporins penetrating water **H₂O** with oxygen **O_{2aqua}** by rate 10⁹ sec⁻¹ reach erythrocyte cells and oxygen concentration in blood plasma significant changes from **venous** blood [O₂]=1,85•10⁻⁵ M to arterial blood plasma in water becomes [O₂]=6•10⁻⁵ M.

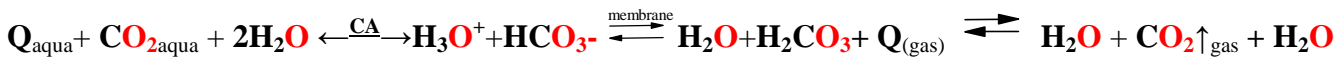
Bisphospho glycerate **BPG⁵⁻** drive hemoglobin **O₂** adsorbition <=> desorbition equilibrium sensitive to concentration.

It saturates arterial **shuttle oxy** hemoglobin with oxygen 459 times over [O₂]=6•10⁻⁵ M stored reserve 0,0275 M and pushed out of **shuttle deoxy** hemoglobin bisphospho glycerate **BPG⁵⁻** releases 4H⁺ and 4 HCO₃⁻.

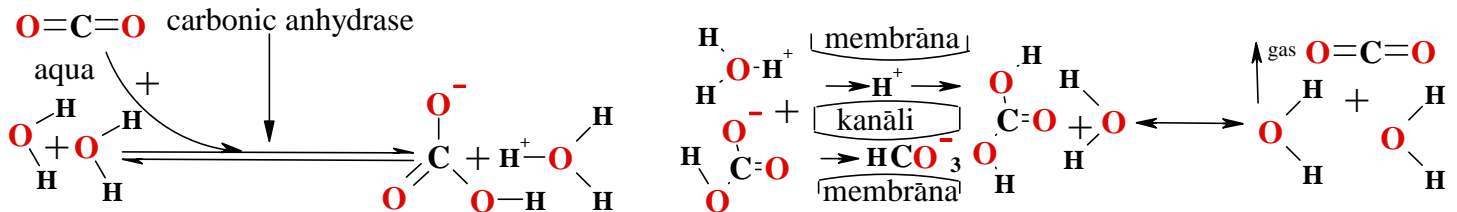


Each adsorbed oxygen molecules **O_{2aqua}** on hemoglobin releases proton **H⁺** which increases acidity on epithelial cell surface of **lungs**. The epithelial cell surface of **lungs** has the specific building: super thin 0.6 nm water layer on surface $S=950 \text{ nm} \times 950 \text{ nm} = 0.9 \mu\text{m}^2$ as square within small volume $0.5415 \cdot 10^{-3} \mu\text{m}^3 = 0.5415 \cdot 10^{-18} \text{ L}$ in liters created acidity increases up to $pH=5.5$ if one proton crosses the membrane channel reaching the surface and that cause fast decomposition of carbonic acid **H₂CO₃** to evolving **CO₂↑** gas is breathed out to AIR.

II) pathway start from metabolic Krebs cycle oxidation with oxygen **O_{2aqua}** produces **CO_{2aqua}** *in tissues* cells:



Enzyme Carbonic Anhydrase (CA) drive to right equilibrium mixture in three gradual reactions first is endothermic: $Q + 2H_2O + CO_{2\text{aqua}} \xleftarrow{CA} H_3O^{+} + HCO_3^{-}$.



Second gradual exothermic reaction forms Carbonic acid $H^{+} + HCO_3^{-} \xrightarrow{\text{membrane}} H_2CO_3 + Q$. Proton **H⁺** and bicarbonate **HCO₃⁻** through channels drive concentration gradients for $[H_3O^{+}]_{\text{right}}/[H_3O^{+}]_{\text{left}} = 10^{-7,36}_{\text{right}}/0,0339$ and for bicarbonate ions $[HCO_3^{-}]_{\text{right}}/[HCO_3^{-}]_{\text{left}} = 0,0154 M_{\text{right}}/0,0339 M_{\text{left}}$ breathing out of organism to AIR gas **CO₂↑_{gas}**.

Third gradual reaction on **lung** epithelial cell surface (outside organism) with absence CA decomposes carbonic acid **H₂CO₃** to gas **CO₂↑_{gas}** in endothermic reaction: $H_2CO_3 + Q_{\text{(gas)}} \rightarrow H_2O + CO_2\uparrow_{\text{gas}}$. Heat supply is important for support the breathing out of organism.

Processes in tissues. As soon as the *arterial* blood reaches *tissues*, the following reactions occur.

Metabolic $\text{CO}_{2\text{aqua}}$ product enzyme Carbonic Anhydrase (CA) converts to HCO_3^- bicarbonate and hydroxonium H_3O^+ ions according $\text{pH}=7.36$ *alkaline reserve* $2.036/1=[\text{HCO}_3^-]/[\text{CO}_2]=0,0339\text{ M}/0,01665\text{ M}$.
 1) Tissues blood oxygen concentration little decreases below $[\text{O}_{2\text{aqua}}]=6\cdot 10^{-5}\text{ M}$ arterial concentration. Oxygen concentration sensitive **shuttle** equilibrium $(\text{O}_2\text{His63,58})_4\text{Hb}_R+4\text{H}^+\rightleftharpoons 4\text{O}_{2\text{aqua}}+(\text{H}^+\text{His63,58})_4\text{Hb}_T$ shifts right restoring 459 times arterial concentration $[\text{O}_{2\text{aqua}}]=6\cdot 10^{-5}\text{ M}$ level amount from reserves of **oxy** hemoglobin $(\text{O}_2\text{His63,58})_4\text{Hb}_R$. Hemoglobin desorbing oxygen reach decreased **venous** blood level $[\text{O}_2]=1,85\cdot 10^{-5}\text{ M}$ *in lungs*. Each desorbed oxygen replaces proton H^+ at distal histidine His63,58 in hemoglobin $(\text{H}^+\text{His63,58})_4\text{Hb}_T$ (**Tense** state) and bind produced metabolic product HCO_3^- prevent acidity effect stabilizing $\text{pH}=7.36$ constant.

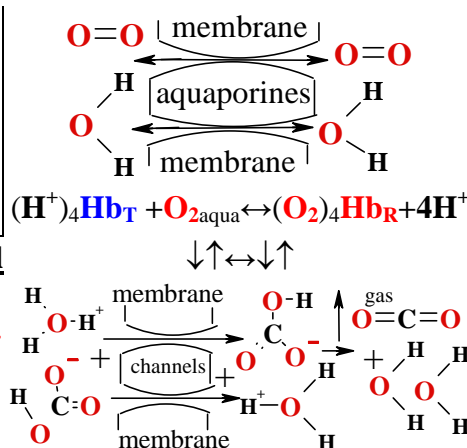
2) Krebs cycle metabolite $\text{CO}_{2\text{aqua}}$ endothermic reaction with water in *tissues* drive carbonic anhydrase shift equilibrium to right $\text{Q} + \text{CO}_{2\text{aqua}} + 2\text{H}_2\text{O} \xleftarrow{\text{CA}} \text{H}_3\text{O}^+ + \text{HCO}_3^-$ forming ratio $1/2,0361 = [\text{CO}_{2\text{aqua}}]/[\text{HCO}_3^-]$. Enzyme Carbonic Anhydrase (CA) equilibrium shifts reaction towards bicarbonate anion to prevent of carbonic dioxide accumulation, according Le Chatelier's due to high water $[\text{H}_2\text{O}]$ concentration 55.3 M, low hydrogen cation concentration $[\text{H}_3\text{O}^+]=10^{-7.36}\text{ M}$, enzyme CA constant $\text{pK}=7.0512$ value as friendly for physiologic $\text{pH}=7,36$ value. CA absence out side human organism as isolated with cell membranes shifts to some fold more acidic as enough at $\text{pH}=5,5$ on the surface for spontaneous carbonic acid bubbling $\text{Q} + \text{H}_2\text{CO}_3 \rightarrow \text{H}_2\text{O} + \text{CO}_2\uparrow_{\text{gas}}$.

We follow full cycle of the process, going back the content of **venous** blood , that to know what mechanism of enzymes: carbonic anhydrase (CA) and **shuttle** molecules hemoglobin work in living organisms.

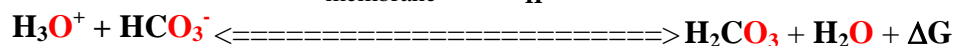
First, hemoglobin are **shuttles** molecules of oxygen $[\text{O}_{2\text{aqua}}]=6\cdot 10^{-5}\text{ M}$ concentration sensitive equilibrium *in lungs* $(\text{O}_2\text{His63,58})_4\text{Hb}_R+4\text{H}^+\rightarrow 4\text{O}_{2\text{aqua}} + (\text{H}^+\text{His63,58})_4\text{Hb}_T$ stabilize arterial blood concentration to prevent deficiency (hypoxia) and avoid oxidative stress limiting oxygen concentration. **Shuttle in tissues** desorbs oxygen by proton replaces prevent acidose and stabilize $\text{pH}=7,36$. **Shuttle in lungs** adsorbs oxygen releasing protons on epithelial cell surface so keeping acidity $\text{pH}=5,5$ promote decomposition of carbonic acid out in AIR.

Second, enzyme CA equilibrium $\text{H}_2\text{O}/\text{CA}/\text{CO}_{2\text{aqua}}$ stabilize at $\text{pH}=7,36$ so prevent acidose. Evaporation: endothermic $\Delta H_f = +54,5\text{ kJ/mol}$; $\text{H}_3\text{O}^+ + \text{HCO}_3^- + \text{Q} \xleftarrow{\text{Membrane}} \text{H}_2\text{O} + \text{CO}_2\uparrow_{\text{gas}} + \text{H}_2\text{O}\uparrow_{\text{gas}} + \Delta G_f = -82,1\text{ kJ/mol}$. exoergic. Equilibrium keep surface moisture H_2O be side breath out to AIR carbon dioxide $\text{CO}_2\uparrow_{\text{gas}}$ and water vapor $\text{H}_2\text{O}\uparrow_{\text{gas}}$. For moisture membrane proton channels are permeable H^+ , unless **proton H^+** impermeable for dray **channels**. Therefore membrane is equipped by aquaporins, which are water and solute oxygen $\text{O}=\text{O}$ permeable in both directions: $\text{O}=\text{O} + \text{H}_2\text{O}$ aquaporin channels $\rightleftharpoons \text{H}_2\text{O} + \text{O}=\text{O}$. AQP1 transfer rate is $3\cdot 10^9$ per second.

For protons crossing the membrane through proton channels, necessary water molecules locate both side of the membrane and aquaporins are supplier of water H_2O molecules to moisture **alveolar lungs** surface.



Free energy change $\Delta G = -60\text{ kJ/mol}$ for Reaction of H_2CO_3 formation is **exoergic** $\Delta G < 0$ negative therefore promotes spontaneous neutralization reaction $\text{H}_3\text{O}^+ + \text{HCO}_3^- \rightleftharpoons \text{H}_2\text{CO}_3 + \text{H}_2\text{O} + \Delta G$ **alveolar surface** in lungs consuming $+Q$ heat and evolving water $+ \text{H}_2\text{O}$ supporting surface moisture $\text{H}_2\text{CO}_3 + \text{Q} \rightleftharpoons \text{CO}_2\uparrow_{\text{gas}} + \text{H}_2\text{O}$ endothermic reaction



Human pH=7,36 of blood Henderson Haselbalh CA equation homeostasis

Main buffer system CA using hemoglobin shuttle stabilizes pH=7,36 and arterial level $[O_{2\text{aqua}}] = 6 \cdot 10^{-5} \text{ M}$:
deoxy hemoglobin $(H^+ \text{His63,58})_4 \text{Hb}_T$ (Tense state) \rightleftharpoons **oxy hemoglobin** $(O_2 \text{His63,58})_4 \text{Hb}_R$ (Relax state) $+ 4H^+$

Carbonic Anhydrase (CA) driven – bicarbonate $2H_2O \xrightarrow{CA} CO_{2\text{aqua}} / H_3O^+ + HCO_3^-$ buffer system

Organism store H^+ and HCO_3^- as Krebs cycle metabolic product carbonic dioxide, if CA produced buffer system acidic form $CO_{2\text{aqua}}$ and H_3O^+ . For this reason, the acid form have to be transported out of organism in two metabolites through proton channels H^+ across membranes and through bicarbonate channels HCO_3^- with **deoxy hemoglobin shuttle** $4O_{2\text{aqua}} + (H^+ \text{His63,58})_4 \text{Hb}_T \rightleftharpoons (O_2 \text{His63,58})_4 \text{Hb}_R + 4H^+$ capturing proton in distal histidine and salt bridge linked $HCO_3^- \dots H_3^+ N$ - bicarbonate. Effective of controlled acid form's is breathing out $CO_2 \uparrow_{\text{gas}}$, that stabilize pH of blood pH=7.36 by metabolites exchange via AIR with oxygen O_2 respiration in and carbon dioxide CO_2 breathing out.

Carbonic anhydrase CA make conversion of $CO_{2\text{aqua}}$ to bicarbonate anion HCO_3^- in to water medium fast and establish acid-base $Q + CO_{2\text{aqua}} + 2H_2O \xleftarrow{CA} H_3O^+ + HCO_3^-$ **endothermic** equilibrium at pH=7,36 as producing right side reaction products $H_3O^+ + HCO_3^-$ demanding to heat. So Heating +Q shifts equilibrium right side and as soon as H^+ concentration increase as three Krebs cycle product $CO_{2\text{aqua}}$ forms two H_3O^+ and HCO_3^- . Instantly carbonic anhydrase CA equilibrium is by respiration shifted to left as CO_2 evaporated out consuming H^+ and HCO_3^- in **lungs** and acid concentration $[H^+]$ **remains** stabilized at homeostasis level pH=7.36. If concentration H^+ decreases, so increases **pH>7.36**, carbonic anhydrase equilibrium is shifted to the right and the extra amount of HCO_3^- through **kidneys** passes into urine and is transported out and pH stabilizes to homeostasis pH=7.36 level according Le Chatelier's theorem.

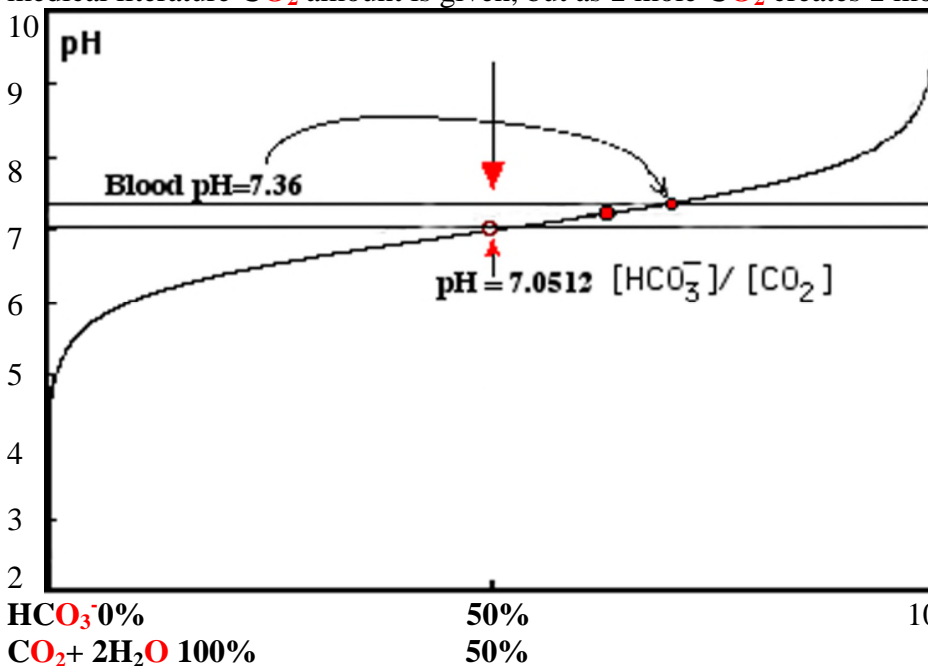
The Brønsted acid is blood-water solution $CO_{2\text{aqua}}$, which in. The dissolved into water H_2O (into blood) carbonic dioxide $CO_{2\text{aqua}}$ occurring in cell converted with carbonic anhydrase CA to $H^+ + HCO_3^-$. The water H_2O and carbonic dioxide $CO_{2\text{aqua}}$, finally, is acid in direct equilibrium with HCO_3^- base plus ions H^+ .

Carbonic anhydrase equilibrium constant $pK=7.0512$ decreases concentration acid form $CO_{2\text{aqua}}$ into water avoid accumulation therefore hydrogen carbonate HCO_3^- and hydrogen ions H^+ are involved into blood pH formation according buffer solution

Henderson-Haselbalh equation: $7.36 = \text{pH} = \text{pK} + \log \left(\frac{[HCO_3^-]}{[CO_{2\text{aqua}}]} \right) = 7.0512 + \log \left(\frac{[HCO_3^-]}{[CO_{2\text{aqua}}]} \right);$

$\frac{[HCO_3^-]}{[CO_{2\text{aqua}}]} = 10^{(\text{pH}-\text{pK})} = 10^{(7.36-7.0512)} = 10^{0.3088} = \frac{2.0361}{1}$ the ratio $[HCO_3^-]/[CO_{2\text{aqua}}]$ being approximately 2/1. In

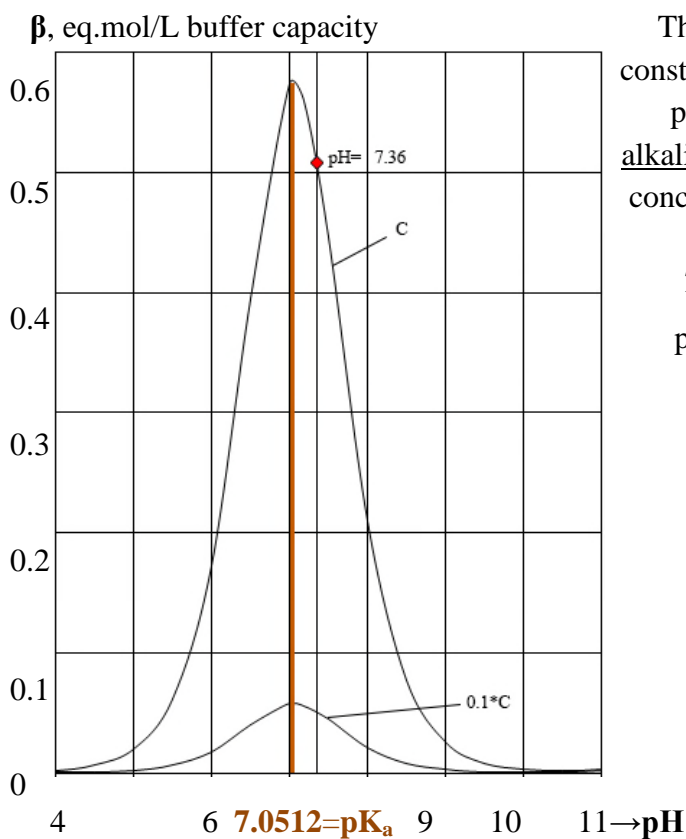
medical literature CO_2 amount is given, but as 1 mole CO_2 creates 1 mole $H_2O \xrightarrow{CA} CO_{2\text{aqua}}$, it is the same.



Buffer region middle point is the over inflection point in graph \circ :
 $\text{pH} = \text{pK}_a = 7.0512; [HCO_3^-]/[CO_2] = 1$
 is one as well buffer component concentrations are equal $[HCO_3^-] = [CO_2]$ as well as bicarbonate salt $[HCO_3^-]$ concentration is equal to Brønsted **weak acid** dissolved in blood CO_2 concentration $[CO_2]$.
 Alkaline reserve at $7.36 = \text{pH}$ is **normal** as $\frac{[HCO_3^-]}{[CO_{2\text{aqua}}]} = \frac{2.0361}{1}$.

As soon as H^+ concentration grows for some reason, Carbonic anhydrase CA equilibrium is shifted to left and channeling H^+ and HCO_3^- transported CO_2 out by respiration in **lungs** so acid concentration $[H^+]$ stabilizes. If concentration H^+ decreases, carbonic anhydrase CA equilibrium is shifted to the right and the extra amount of HCO_3^- through **kidneys** passes into urine. Bicarbonate channels in **kidney** cells are open at higher values of $pH > 7.36$ from side of blood circulation, but **lungs** channel transport are opened for H^+ and HCO_3^- at lower values $pH < 7.36$.

$$\text{assuming } C=1M = [HCO_3^-] + [CO_{2\text{aqua}}]$$



This value $pK=7.0512$ is carbonic anhydrase made equilibrium constant very friendly to blood $pH=7.36$. As most of metabolism products are acidic, the organism has to have a reserve of alkalinity. For this reason the ratio between HCO_3^- and $CO_{2\text{aqua}}$ concentrations is **2/1**. The pH value of physiological conditions blood homeostasis is **7.36**.

The *alkaline reserve* $2.036/1 = [HCO_3^-]/[CO_{2\text{aqua}}]$ at blood $pH=7.36$ can be controlled by adding H_2SO_4 to a sample of

100 mL blood reacts with included in salt HCO_3^- and the $CO_{2\text{aqua}}$ is liberated. If **56.23 mL (50-60 mL)**

of gaseous CO_2 are liberated from **100 mL** of blood, the controlled *alkaline reserve* in homeostasis is normal and

total *alkaline reserve* amount concentration

0.023M = $[HCO_3^-] + [CO_{2\text{aqua}}]$ is in homeostasis normal as sum of $[HCO_3^-] = 0.0154 M$ and $[CO_{2\text{aqua}}] = 0.0076M$.

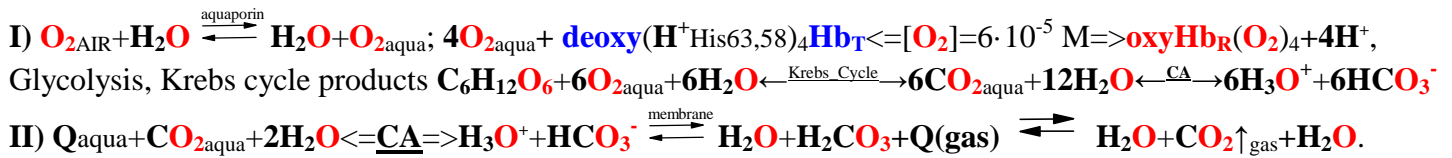
Two types of diseases occur, if the acid-base balance is distorted in the organism alkalosis and acidosis.

1) *Respiratory alkalosis* occurs, if **lungs** are hyperventilated, for example, during anesthesia. If $CO_{2\text{aqua}}$ concentration decreases $pH > 7.36$ alkalosis due to hyperventilation, the blood vessels are broadened and their tonus is lowered as a result of it, therefore O_2 supply to brain is shortened.

For this reason it is necessary to use AIR mixtures of O_2 and CO_2 during anesthesia instead of pure oxygen. If respiratory alkalosis occurs for other reasons than hyperventilation of **lungs**, the ratio **2/1** of the buffer components can be re-established in a longer period of breathing normal, CO_2 -containing AIR 350 ppm.

2) *Respiratory acidosis* occurs in the cases, when the concentration of CO_2 in the AIR is increased. The result of this is that the action of breathing muscles becomes more difficult. Again, this can be canceled, if the patient starts breathing normal AIR. However, if increased CO_2 content in the AIR lasts long, metabolic acidosis occurs $pH < 7.36$. Metabolic acidosis hemoglobin reserves depleted oxygen concentration below venous $[O_2] = 1,85 \cdot 10^{-5} M$. For this reason only the concentrations of carbonic dioxide $CO_{2\text{aqua}}$ into water H_2O (avoid carbonic acid H_2CO_3 formation) and bicarbonate HCO_3^- and hydrogen ions H^+ are included into equation for blood pH .

There are two sequences, which drive enzymes **CA** and **shuttle** hemoglobin governed gradual reactions



II) process first gradual reaction enzyme Carbonic anhydrase **CA** made equilibrium:



Enthalpy heat consumed ΔH_r for reaction endothermic: $\Delta H_r = \Delta H^\circ_{H_3O} - \Delta H^\circ_{HCO_3} - 2\Delta H^\circ_{H_2O} - \Delta H^\circ_{CO_2} = 9,7576 \text{ kJ/mol}$
 $= -285,81 - 689,93 - (2 \cdot -285,85 - 413,7076) = -975,74 + 985,3276 = 9,7576 \text{ kJ/mol}$ endothermic

Endothermic as needed heat supply to drive reaction forwards.

Entropy decrease $\Delta S_r < 0$ negative as enzyme Carbonic Anhydrase **CA** governed reaction:

$\Delta S_r = \Delta S^\circ_{H_3O} + \Delta S^\circ_{HCO_3} - 2\Delta S^\circ_{H_2O} - \Delta S^\circ_{CO_2} = -3.854 + 98.324 - (2 \cdot 69.956 + 117.57) = 94.47 - 257.482 = -163.0134 \text{ J/mol/K}$

$\Delta G_r = \Delta H_r - T \cdot \Delta S_r = 9,7576 - (298,15 \cdot -0,1630134) = 58,36 \text{ kJ/mol}$ endoergic **free energy** accumulated in products

by **CA** governed reaction. $\Delta S_{\text{dispersed}} = -\Delta H_r / T = -9.5876 / 298.15 = -32,727 \text{ J/K/mol}$.

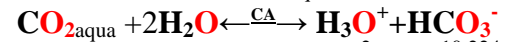
$\Delta S_{\text{total}} = \Delta S_r + \Delta S_{\text{dispersed}} = -163,0134 - 32,7271 = -195,7405 \text{ J/K/mol}$. $T \cdot \Delta S_{\text{total}} = -0,1957405 \cdot 298,15 = -58,36 \text{ kJ/mol}$

$\Delta G_r = \Delta G^\circ_{H_3O} + \Delta G^\circ_{HCO_3} - 2\Delta G^\circ_{H_2O} - \Delta G^\circ_{CO_2} = -213,275 - 586,94 - (2 \cdot -237,19 - 385,98) = +60,145 \text{ kJ/mol}$ endoergic.

Carbonic anhydrase make the equilibrium constant $K_{eq} = 10^{-7.0512}$ or exponent $pK_{eq} = 7,0512$ is constant and very close to **pH** value 7,36. Water concentration $[H_2O] = 55.3 \text{ M}$ is constant so included in value

$$K_{eq} = K \cdot [H_2O]^2 = 10^{-10.224} \cdot (997,07/18,0153)^2 = 10^{-7.0512} \text{ M} = 10^{-pK_{eq}} \text{ exponent value } pK_{eq} = 7.0512.$$

II) process first gradual reaction driven by enzyme **CA**



$$10^{-7.0512} = K_{eq} = [H_2O]^2 \cdot K = [H_3O^+][HCO_3^-] / [CO_{2aqua}] \text{ as } K = [H_3O^+][HCO_3^-] / ([CO_{2aqua}] \cdot [H_2O]^2) = 10^{-10.224}$$

$\Delta G = -RT \ln(K) = -8.3144 \cdot 298.15 \cdot \ln(1 \cdot 10^{-10.224}) = 60.145 \text{ kJ/mol}$ where $R = 8,3144 \text{ J/mol/K}$ and $T = 310,15 \text{ K}$ (25°C).

$K_{eq} = \text{EXP}(-\Delta G_r / RT) \cdot [H_2O]^2 = (10^{-10.5372}) \cdot [H_2O]^2 = (10^{-10.5372}) \cdot (997,07/18,0153)^2 = 10^{-7.0512} = 10^{-pK_{eq}}$

II) process second gradual reaction concentration gradient and electrochemical membrane potential

bicarbonate ion HCO_3^- and proton H^+ 1. $E_H = P \cdot \lg([10^{-pH_{\text{extraMit}}}] / [10^{-pH_{\text{Mitochon}}}] = 0,06154 \cdot \lg(10^{2,36}) = 0,14523 \text{ V}$

2. $E_{HCO_3^- \text{ Mitochon}} = -P \cdot \lg([HCO_3^-]_{\text{cytosol}} / [HCO_3^-]_{\text{Mitochon}}) = -0,06154 \cdot \lg(0,0154 / 0,0338919) = 0,0210821 \text{ V}$

$E_{\text{sum}} = 0,14523 + 0,0210821 = 0,1663168 \text{ V} = E_{\text{membrane}}$; $\Delta G_F = nFE = -1 \cdot 96485 \cdot 0,1663168 = -16,0471 \text{ kJ/mol}$

3. $\Delta G_{HCO_3^-} = RT \ln([HCO_3^-]_{\text{cytosol}} / [HCO_3^-]_{\text{Mitochon}}) = 8,3144 \cdot 310,15 \cdot \lg(0,0154 / 0,0338919) = -2,0341094 \text{ kJ/mol}$

4. $\Delta G_{H^+} = -RT \ln([H_3O^+]_{\text{extraMit}} / [H_3O^+]_{\text{Mitochon}}) = -RT \ln(10^{-7,36} / 10^{-5}) = -8,3144 \cdot 310,15 \cdot \ln(10^{2,36}) = -23,3943 \text{ kJ/mol}$

Total $\Delta G_{\text{total}} = \Delta G_F + (\Delta G_{HCO_3^-} + \Delta G_{H^+}) = -16,0471 + (-2,0341094) + (-23,3943) = -41,4755 \text{ kJ/mol}$ exoergic transfer.

II) process third gradual Carbonic acid formation: $H_3O^+ + HCO_3^- \rightarrow H_2O + H_2CO_3 + Q$ exothermic.

$\Delta H_r = \Delta H^\circ_{H_2O} + \Delta H^\circ_{H_2CO_3} - \Delta H^\circ_{H_3O} - \Delta H^\circ_{HCO_3} = -285.85 - 699,65 - (-285.81 - 689.93) = -985.5 + 975.74 = -9.76 \text{ kJ/mol}$

$\Delta S_r = \Delta S^\circ_{H_2O} + \Delta S^\circ_{H_2CO_3} - \Delta S^\circ_{H_3O} - \Delta S^\circ_{HCO_3} = 69.956 + 187 - (-3.854 + 98.324) = 256.956 - 94.47 = 162,486 \text{ J/mol/K} \dots \dots$

$\Delta S_{\text{dispersed}} = -\Delta H_r / T = 9.76 / 298.15 = +32.735 \text{ J/K/mol} \dots$

$\Delta G_r = \Delta H_r - T \cdot \Delta S_r = -9.76 - 298.15 \cdot 0.129751 = -38.695 \text{ kJ/mol}$ exoergic reaction is driven by concentration

gradients through **proton** and **bicarbonate channels** of **membrane**.

$\Delta G = \Delta G^\circ_{H_2O} + \Delta G^\circ_{H_2CO_3} - \Delta G^\circ_{H_3O} - \Delta G^\circ_{HCO_3} = -237.19 - 623.17 - (-213.275 - 586.94) = -860.36 + 800.215 = -60.145 \text{ kJ/mol} \dots$

$\Delta S_{\text{total}} = \Delta S_r + \Delta S_{\text{dispersed}} = 32.735 + 162,486 = 129.751 \text{ J/K/mol} \dots$

II) process fourth gradual reaction is non-enzymatic decomposition $H_2CO_3 \rightleftharpoons CO_2 \uparrow_{\text{gas}} + H_2O$;

$\Delta G = \Delta G^\circ_{H_2O} + \Delta G^\circ_{CO_2} - \Delta G^\circ_{H_2CO_3} = -237.19 - 385.98 - 623.17 = -623.17 + 623.17 = 0.0 \text{ kJ/mol}$ is **anenergetic** or **neutral**.

Enthalpy change decomposition reaction of carbonic acid $Q + H_2CO_3 \rightleftharpoons CO_2 \uparrow_{\text{gas}} + H_2O$ endothermic

Substance	$\Delta H^\circ_r, \text{ kJ/mol}$	$\Delta S^\circ_r, \text{ J/mol/K}$	$\Delta G^\circ_r, \text{ kJ/mol}$	$\Delta H = \Delta H^\circ_{H_2O} + \Delta H^\circ_{CO_2} - \Delta H^\circ_{H_2CO_3} = +20.291 \text{ kJ/mol}$
H_3O^+	-285.81	-3.854	-213,275	$= -286 - 393.509 - (-699.65) = -679.509 + 699.65 = +20.291 \text{ kJ/mol}$
HCO_3^-	-689.93	98.324	-586,94	is endothermic exactly with the cooling effects....
$H_2O \uparrow_{\text{gas}}$	-241,8352	188,7402		$\Delta S_{\text{dispersed}} = -\Delta H_r / T = -20.291 / 298.15 = -68.056 \text{ J/K/mol} \dots$
H_2O	-285.85	69.9565	-237,191	$\Delta S_r = \Delta S^\circ_{H_2O} + \Delta S^\circ_{CO_2} - \Delta S^\circ_{H_2CO_3} = +96,696 \text{ J/mol/K} \dots$
$CO_2 \uparrow_{\text{gas}}$	-393.509	213.74	-394,359	$= 69.956 + 213,74 - (187.) = 257.482 - 94.47 = +96,696 \text{ J/mol/K} \dots$
CO_{2aqua}	-413.7976	117.5704	-385,98	$T \cdot \Delta S_{\text{total}} = 28,64 \text{ J/K/mol} \cdot 298,15 \text{ K} = +8,539 \text{ kJ/mol}$
H_2CO_3	-699,65	187.00	-623,17	bound $T \Delta S_n \leftarrow$ lost free energy $\Delta G_{\text{reverse reaction}} \leftarrow \dots$

endothemic $\Delta H^\circ_{\text{reaction}} = +20,291 \text{ kJ/mol}$; cooling $Q = -20,291 \text{ kJ/mol}$ spontaneous $\Delta G^\circ_{\text{reaction}} = -8,539 \text{ kJ/mol}$.