Brønsted Acid Base Chemical Equilibrium. BUFFER SOLUTIONS

Aris Kaksis 2018. Riga Stradin`s University http://aris.gusc.lv/BioThermodynamics/BufferSolution.pdf

*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.**

Buffera 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** $\Leftrightarrow$ 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

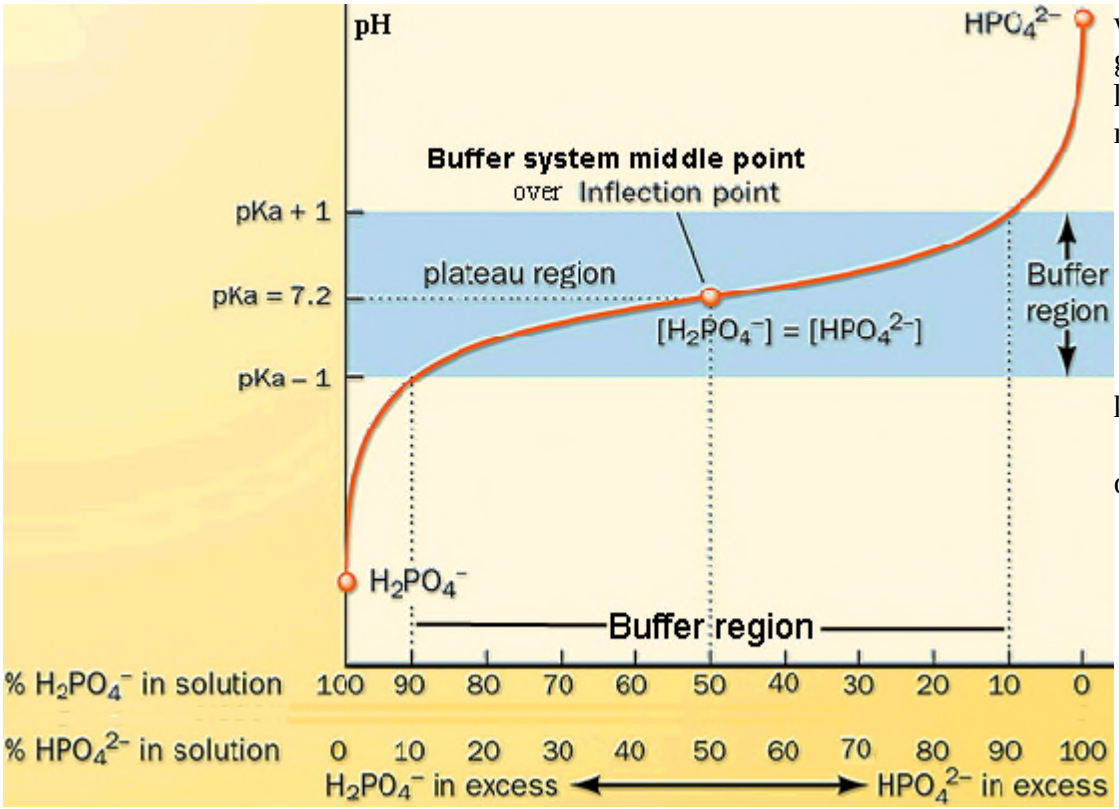
Having the general idea, that a buffer system consists of a protolytic acid and its conjugated base, several possible ways of practical composition of a buffer system can be used:

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 lowe 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 : $CH_3COOH \Leftrightarrow CH_3COO^- + H^+$
and sodium acetate as a salt dissociates completely : (strong electrolyte) :

$$CH_3COONa \Rightarrow CH_3COO^- + Na^+ \quad \alpha = 1$$

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) : $H_3O^+ + CH_3COO^- \Rightarrow CH_3COOH + H_2O$
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) : $OH^- + CH_3COOH \Rightarrow CH_3COO^- + H_2O$

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 NH_4OH/NH_4Cl**,** the same considerations should be referred to OH^- concentration.
The dissociation equilibrium of ammonium hydroxide is : $NH_4OH \Leftrightarrow NH_4^+ + OH^-$
Ammonium chloride, as every salt is a strong electrolyte and it is completely ionized in the solution :

$$NH_4Cl \Rightarrow NH_4^+ + Cl^- \quad \alpha = 1$$

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) :

$$OH^- + NH_4^+ \Rightarrow NH_4OH$$

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: $H_3O^+ + NH_4OH \Rightarrow NH^{4+} + 2H_2O$
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: $K_a = \frac{[H^+]\bullet[CH_3COO^-]}{[CH_3COOH]_{nondis}}$

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 :

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

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

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 $pK_a = -\log K_a$, the greater is acid K_a, the smaller is 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 an 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 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.

$pH=pK_a+\log\frac{C_{salt}}{C_{acid}}$

$pH=pK_a+\log\frac{n_{salt}}{n_{acid}}$ $pH=pK_a+\log\frac{n_{salt}/V}{n_{acid}/V}$

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.

$pH=pK_a+\log\frac{C'_{salt}\bullet V'_{salt}}{C'_{acid}\bullet V'_{acid}}$

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

$pH_{ac}=pK_a+\log\frac{n_{salt}-\Delta n_{ac}}{n_{acid}+\Delta n_{ac}}$

$pH_b=pK_a+\log\frac{n_{salt}+\Delta n_b}{n_{acid}-\Delta n_b}$

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 $\Delta pH= pH - pH_{ac}$ to decrease that. Adding the strong base, for example **NaOH**, change the buffer system **pH** value to increase that about $\Delta pH= pH_b - pH$.

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 **NH_4OH/NH_4Cl,** 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 : $K_b = \frac{[OH^-]\bullet[NH_4^+]}{[NH_4OH]_{nondis}}$.

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: $[NH_4^+] = C_{salt}$.

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 $[OH^-] = C_{base}$:

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

$pOH = pK_b + \log\frac{C_{salt}}{C_{base}}$ using water constant $pK_w = 14 = pH + pOH$ converting **pOH** to $pH = 14 - pK_b + \log\frac{C_{base}}{C_{salt}}$

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

$pOH = pK_b + \log\frac{n_{salt}}{n_{base}}$ $pOH = pK_b + \log\frac{n_{salt}/V}{n_{base}/V}$

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 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.

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

$pOH_{ac} = pK_b + \log\frac{n_{salt} + \Delta n_{ac}}{n_{base} - \Delta n_{ac}}$

$pOH_b = pK_b + \log\frac{n_{salt} + \Delta n_b}{n_{base} - \Delta n_b}$

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

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** ($pK_a = 4.74$ for acetic acid) can be calculated as follows: **pH** before addition of **HCl: pH = 4,74 + log(0.5/0.5) = 4.74 + log 1 = 4.74 + 0 = 4.74**

addition of **HCl** causes a reaction : $HCl + CH_3COONa \rightarrow CH_3COOH + NaCl$

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_{CH_3COONa} will decrease by **0.01** moles, therefore : **pH** after addition of **HCl**:

$pH_2 = 4.74 + \log((0.5 - 0.01) / (0.5 + 0.01)) = 4.74 + \log 0.996 = 4.74 - 0.002 = 4.738$

and the **pH** change is $\Delta pH = pH_1 - pH_2 = 0.002$.

At the same time, if this amount of **HCl** was added to **1** liter of pure water (the initial **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: $pH = -\log[H^+] = -\log 0.01 = -(-2) = 2$. Thus, the **pH** change in this case is $\Delta pH = 7 - 2 = 5$.

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

V. BUFFER CAPACITY β

The **pH** value of the buffer system is **Henderson Haselbalh** equation: $pH=pK_a+\log\frac{n_{salt}}{n_{acid}}$

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

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_{salt}' = C_{acid}'. The **C'** is proportional to buffer capacity **β**.

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

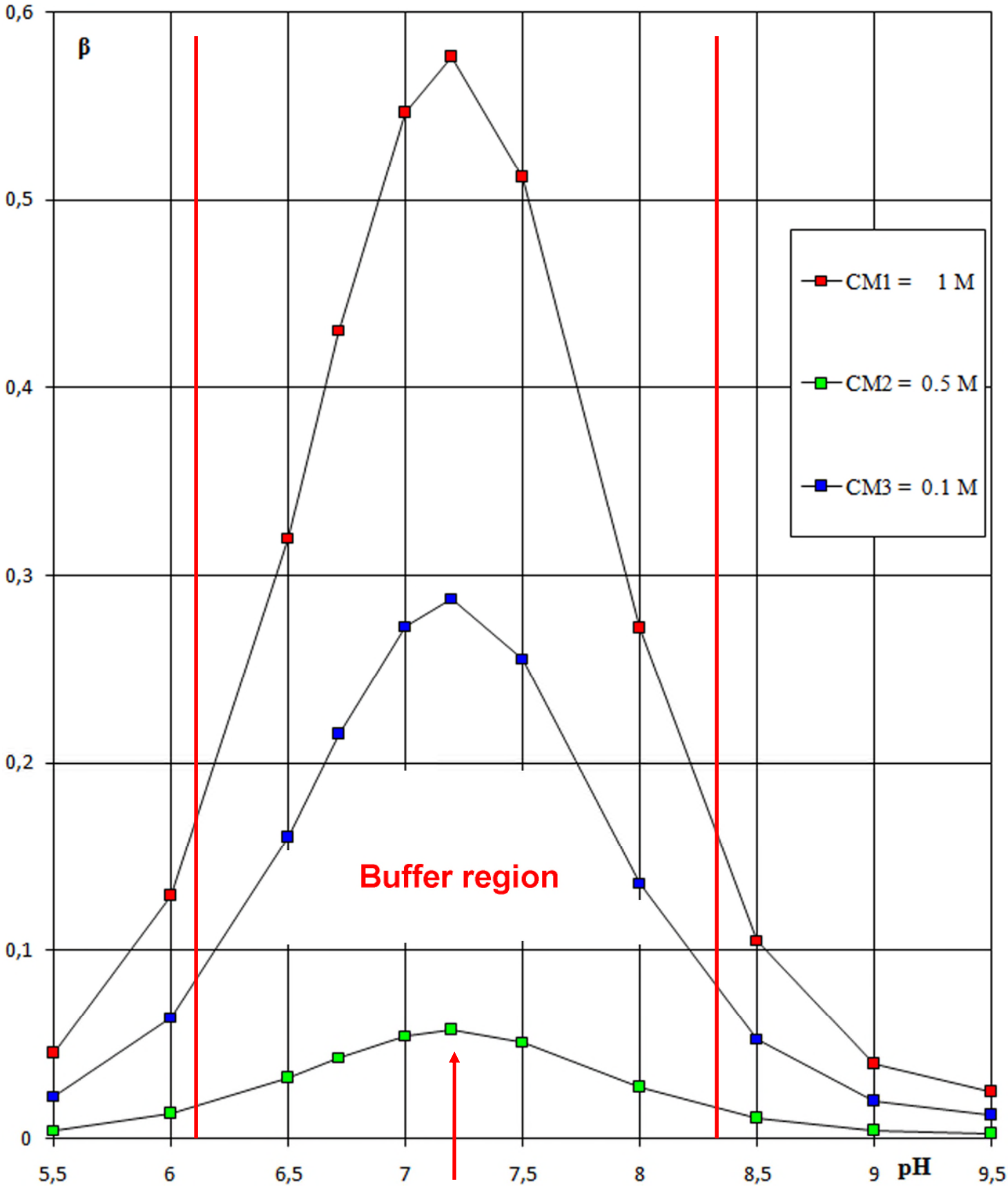
2. a) maximal value $\beta_{acid}=\beta_{base}=0.55\cdot C'$ buffer capacity have on **middle - over inflection point**. Buffer solution have ***symmetric*** equal values $\beta_{acid}=\beta_{base}=0.55\cdot C'$;
if the ratio between buffer components $\frac{n_{salt}}{n_{acid}}$=**1** is one.

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

Phosphate buffer system NaH_2PO_4 / Na_2HPO_4 $pH = pK_a + \log\frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$

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



Buffer system **middle point $pH=pK_a=7,199$ over inflection point** Maximum Buffer Capacity β=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 **$H_2PO_4^-$** ions, plays the role of the **acid**

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, $K_{HCOOH}=2\bullet10^{-4}$

$$pH=pK_a+\log\frac{C_{salt}\bullet V_{salt}}{C_{acid}\bullet V_{acid}}=-\log 2\bullet10^{-4}+\log\frac{0.09\bullet 200}{0.15\bullet 300}=3.7-0.398=3.3$$

2. Calculate **pH** of a buffer, composed from **80** mL **0.1** M NH_4OH and **120** mL of **0.17** M NH_4Cl solutions, $K_{NH_4OH}=1.8\bullet10^{-5}$.

$$pH=14-pK_b+\log\frac{C_b\bullet V_b}{C_s\bullet V_s}=14-(-\log 1.8\bullet10^{-5})+\log\frac{0.1\bullet 80}{0.17\bullet 120}=14-4.74+\log0.39=9.26-0.41=8.85$$

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, $K_{HCOOH}=2\bullet10^{-4}$.

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: $3.0=-\log 2\bullet10^{-4}+\log\left(\frac{0.2x}{0.1(1000-x)}\right)\rightarrow \quad 3.0=3.7+\log\left(\frac{0.2x}{0.1(1000-x)}\right)$

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

$0.2x = 0.199(100 - 0.1x);$
$0.2x + 19.9 = 0.0199x$
$0.2199\ x = 19.9\ ;$
$x = 90{,}5\ mL$
$V_{salt} = x = 90.5\ mL\ ;$
$V_{acid} = 1000 - x = 909.5\ mL$

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 $NaHCO_3$ and **200** mL of **0.3** M Na_2CO_3, $K_{HCO_3^-}=4.69\bullet10^{-11}$

a) **pH** before addition of **NaOH** is: $pH_1=-\log 4.69\bullet10^{-11}+\log\frac{0.3\bullet 200}{0.2\bullet 100}=10.32+\log3=10.8$

b) addition of **NaOH** causes a reaction : $NaOH + NaHCO_3 \rightarrow Na_2CO_3 + H_2O$

as the number of moles of **NaOH** is **n** = **0.01•0.5** = **0.005**, the number of moles of Na_2CO_3 increases by **0.005** moles and the number of moles of $NaHCO_3$ decreases by **0.005** moles. The number of moles of salt (Na_2CO_3) in the initial buffer was $n_{salt}=0.2\bullet0.3=0.06$ moles

The number of moles of acid in initial buffer was (acid is $NaHCO_3$ here) : $n_{acid}=0.1\bullet0.2=0.02$ moles

thus, after the addition of **NaOH pH** becomes:

$$pH_2=-\log 4.69\bullet10^{-11}+\log\frac{0.06+0.005}{0.02-0.005}=10.32+\log4.33=10.97$$

c) buffer capacity of the solution is found as:

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

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: $\mathbf{pH = pK_{CH_3COOH}}$:

$$\mathbf{pH_1 = pK_{CH_3COOH} + \log\frac{200}{200} = pK_{CH_3COOH} + \log\frac{20}{20} = 4{,}74 - 0 = 4{,}74}$$ (as **log 1 = 0**)

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

$$\mathbf{HCl + CH_3COONa \rightarrow CH_3COOH + NaCl}$$

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

In the more concentrated buffer system :

$$\mathbf{pH_2 = pK_{CH_3COOH} + \log\frac{200-1}{200+1} = 4{,}74 + \log\frac{199}{201} = 4{,}74 + \log 0{,}9900 = 4{,}74 + (-0{,}00434) = 4{,}73566}$$

In the more diluted buffer system :

$$\mathbf{pH_2 = pK_{CH_3COOH} + \log\frac{20-1}{20+1} = 4{,}74 + \log\frac{19}{21} = 4{,}74 + \log 0{,}90476 = 4{,}74 + (-0{,}0434) = 4{,}69653}$$

c) The **pH** change will be $\mathbf{\Delta pH = pH_1 - pH_2}$:

in ten times more concentrated system: $\mathbf{\Delta pH = 4.74 - 4.73566 = 0.00434}$

in ten times more diluted system: $\mathbf{\Delta pH = 4.74 - 4.69653 = 0.0434}$

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

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

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

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

$$\mathbf{pH_1 = pK_{CH_3COOH} + \log\frac{200-1}{200+1} = 4{,}74 + \log\frac{199}{201} = 4{,}74 + \log 0{,}9900 = 4{,}74 + (-0{,}00434) = 4{,}73566}$$

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 : $\mathbf{KOH + CH_3COOH \rightarrow CH_3COOK + H_2O}$

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

$$\mathbf{pH_2 = pK_{CH_3COOH} + \log\frac{200+1}{200-1} = 4.74 + \log\frac{201}{199} = 4.74 + \log 1{,}01 = 4.74 + 0.00434 = 4.74434}$$

now the **pH** change against the acid **HCl** is $\mathbf{\Delta pH_1 = 4.74 - 4.73566 = 0.00434}$

now the **pH** change against the base **KOH** is $\mathbf{\Delta pH_2 = 4.74434 - 4.74 = 0.00434}$

and β_{base} **= (1 meq) / (0.00434• 1000mL) = 0.23 eq•mol/L,**

which is the same value, that was previously calculated for β_{acid} **= 0.23 eq•mol/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:

$$\mathbf{pH = pK_{CH_3COOH} + \log\frac{200}{20} = 4{,}74 + \log 10 = 4{,}74 + 1 = 5{,}74}$$

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

$$\mathbf{pH_1 = pK_{CH_3COOH} + \log\frac{200-1}{20+1} = 4{,}74 + \log\frac{199}{21} = 4{,}74 + \log 9{,}4765 = 4{,}74 + 0{,}9766 = 5{,}7166}$$

$\mathbf{\Delta pH_1 = pH_1 - pH = 5.7166 - 5.74 = 0.023}$ **;** and $\beta_{ac} = \frac{\Delta n_{HCl}}{\Delta pH \bullet V_{bufer}} = \frac{1eq \bullet mmol}{0{,}023 \bullet 1000mL} = 0{,}0430$ **eq•mol** /L

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

$$\mathbf{pH_2 = pK_{CH_3COOH} + \log\frac{200+1}{20-1} = 4{,}74 + \log\frac{201}{19} = 4{,}74 + \log 10{,}5789 = 4{,}74 + 1{,}02444 = 5{,}76444}$$

$\mathbf{\Delta pH_2 = pH_2 - pH = 5{,}76444 - 5.74 = 0.02444}$ and $\beta_b = \frac{\Delta n_{KOH}}{\Delta pH \bullet V_{bufer}} = \frac{1eq \bullet mmol}{0{,}02444 \bullet 1000mL} = 0{,}0409$ **eq•mol** /L

Comparing β_{acid} = **0**,**0430 eq•mol** /L and β_{base}**,** = **0**,**0409 eq•mol** /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** β_{ac} = **0**,**23 eq•mol;** β_b = **0**,**23 eq•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

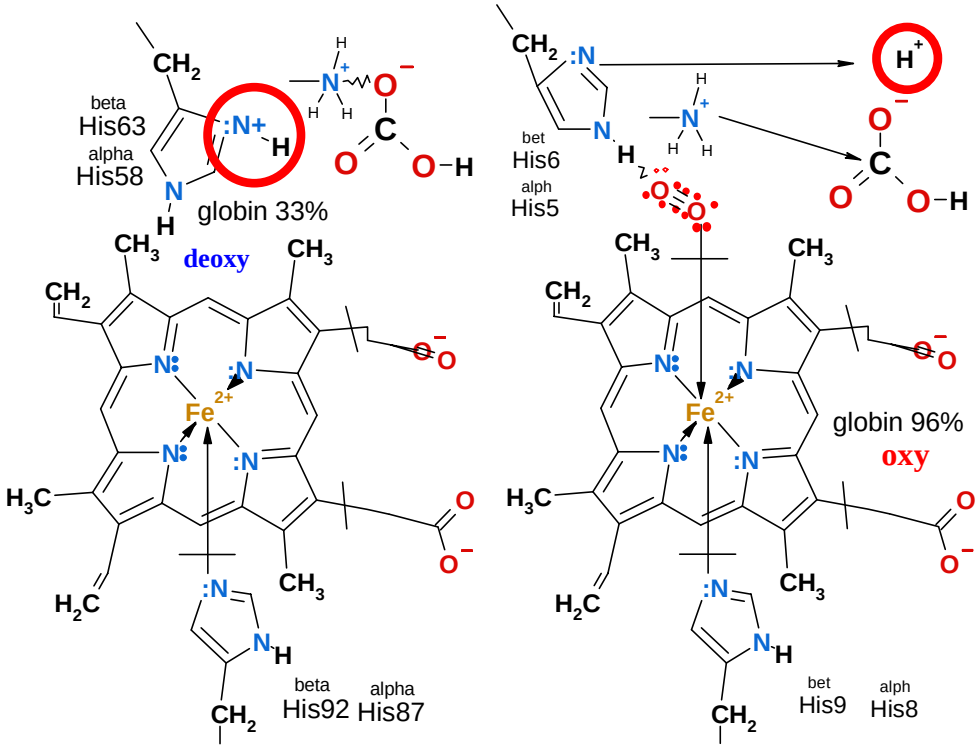
If we calculated buffer capacities of a solution, containing **20** meq salt and **200** meq acid, we would find out, that the values are the same, than in previous example, but they are replaced by each other: now β_{acid} = **0**,**0409**eq•mol/L and β_{base} = **0**,**0430** eq•mol/L. This also easy 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 buffera 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, succsinic 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 [O_{2blood}]=6·10^{-5} M

I) Oxygen O_2 from **AIR** 20.95% O_2↑gas assimilation reaction dissolution in water to form O_{2aqua} exothermic ΔH_r=-55,7 $^{kJ}/_{mol}$ and exoergic ΔG_r= -27,7 $^{kJ}/_{mol}$ as water soluble **1)** $O_{2AIR}+H_2O \Leftrightarrow H_2O+O_{2aqua}+Q+\Delta G$. Penetrate in Human body through aquaporins osmosis by concentration gradient [O_2]=9,768·10^{-5} M to venous blood [O_2]=1,85•10^{-5} M ΔG_{O2}= RT**ln**([O_{2Blood}]/[O_{2aqua}])= - 4,29 $^{kJ}/_{mol}$ exoergic movement: 2) $O_{2aqua}+H_2O \xrightarrow{Aquaporins} H_2O+O_{2aqua}+\Delta G$ ΔG_{H2O} =RT**ln**([H_2O]$_{right}$/[H_2O]$_{left}$)= -8,3144*310,15***ln**(0,305/0,2)= -1.088 $^{kJ}/_{mol}$ exoergic ΔG_{O2+}= -5,379$^{kJ}/_{mol}$. $4O_{2aqua}$ from blood plasma adsorbs **deoxy** hemoglobin **Hb_T** of inspired fresh AIR releases four protons $4H^+$, $4HCO_3^-$: $4O_{2aqua}+(H^+His63,58)_4Hb_T$ ·salt bridges$(HCO_3^-)_4 \Leftrightarrow Hb_R(O_2)_4+4H^++4HCO_3^-$ stabilizing arterial concentration [O_2]=6·10^{-5} M.

[O_{2Blood}]=6•10^{-5} M concentration sensitive equilibrium $(H^+His63,58)_4Hb_T \leftrightarrow Hb_R(O_2)_4$ shift to right regulates erythrocytes glycolysis metabolite **BPG^{5-}** as two **phosphate 2,3-esters** G^- $H_2COPO_3^{2-}$-$HCOPO_3^{2-}$-COO^- glycerate dihydroxy acid salt with homeostasis concentration [BPG^{5-}]=5 mM, so BPG^{5-} pushed out of **cavity** to stabilize and store reserves 459 times higher as arterial blood concentration **[**O_{2Blood}]=6•10^{-5}M amount [$O_{2amount}$]=459*6•10^{-5} M=0,02754 M.



O2Solutions.pdf. Oxygen adsorbs by donor-acceptor bond on iron(II) Fe^{2+} in coordination center of heme and releases four protons H^+ $Hb_R(O_2)_4+4H^+$. Proton water sticks $H^++H_2O \rightarrow H_3O^+$ forms hydroxonium ion. ***In tissues*** desorbed oxygen **[**$O_{2desobed}$**]** restore oxygen concentration [O_2]=6·10^{-5} M in blood plasma 459 times and deoxy–hemoglobin capture four protons H^+ $(H^+His63,58)_4Hb_T$ so keeps continuously pH=7,36±0,01.

Oxygen desorbed Krebs cycle converts to mitochondrial oxidative phosphorylation product CO_{2aqua}. **II)** pathway with carbonic anhydrase (**CA**) shift to right concentration gradient CO_2 produces amount 0,0339 M HCO_3^-. **Shuttle deoxy** hemoglobin Hb_T capture [H^+]=0,0275 M. So is stabilized constant pH=7,36±0,01 value.

II) $Q_{aqua}+CO_{2aqua}+2H_2O \xleftarrow{CA} H_3O^++HCO_3^- \xleftarrow{Membrane} H_3O^++HCO_3^- \Leftrightarrow H_2O+H_2CO_3+Q_{gas} \leftrightarrow H_2O+CO_2\uparrow_{gas}+H_2O$.
endothermicΔH_r= +9.75 $^{kJ}/_{mol}$; athermic ΔH_r= 0 $^{kJ}/_{mol}$; exothermic ΔHr=-9.76 $^{kJ}/_{mol}$; endothermic ΔH_r= +20.3 $^{kJ}/_{mol}$;
endoergic ΔG_r= +58.4 $^{kJ}/_{mol}$; exoergic ΔG_r=-22.5-1,96 $^{kJ}/_{mol}$;; exoergic ΔGr=-58.2 $^{kJ}/_{mol}$; exoergic ΔG_r= -8,54 $^{kJ}/_{mol}$;
II) $Q_{aqua}+CO_{2aqua}+2H_2O \xleftarrow{CA} H_3O^++HCO_3^- +Q \xleftarrow{Membrane} H_2O+CO_2\uparrow_{gas}+H_2O\uparrow_{gas}$.
endothermicΔH_r= +9.75 $^{kJ}/_{mol}$; endothermic ΔH_r= +54,5 $^{kJ}/_{mol}$; summary endothermic ΔH_r= +64,25 $^{kJ}/_{mol}$;
endoergic ΔG_r= +58.4 $^{kJ}/_{mol}$; exoergic ΔG_r= -82,1 $^{kJ}/_{mol}$; summary exoergic ΔG_r= -23,7 $^{kJ}/_{mol}$;

Shuttle is venous **deoxy Hb_T** , adsorbs four molecules $4O_2$ from fresh AIR, acidify water medium with $4H^+$, promoting CO_2 breathe out: Each H^+ and HCO_3^- ion amount [H^+]=459*6•10^{-5} M =0,0275 M=[HCO_3^-] shifts equilibrium to right $H^+ +HCO_3^-+ Q \leftrightarrow H_2O +CO_2\uparrow_{gas}$ via membrane channels. So pH=7,36 remains constant, as one bicarbonate ion and one hydrogen ion produce one CO_2 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 μm^2 on super thin 0.6 nm layer within water small volume: 0.5415•10^{-3} μm^3 = 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: [H_3O^+]=10^{-pH}=10$^{-5.5}$ M. Respiration of fresh AIR in lungs Hemoglobin released protons H^+ during oxygen adsorbtion for total amount concentration:

[$O_{2adsorbed}$**]**=[H_3O^+]=459*6•10^{-5} M= 0,02754 M forms hydrogen ion $[H_3O^+]_{right}/[H_3O^+]_{left}$=10$^{-5,5}$/0,0275 concentration gradient, which drives exoergic ΔG = -22,5 $^{kJ}/_{mol}$ proton movement through epithelial cell membrane proton channels: $H_3O^+_{left} \xleftarrow{proton\_channel} H_3O^+_{right} +\Delta G$. General process $H_2O+CO_2\uparrow_{gas}+H_2O\uparrow_{gas}$ require heat supply endothermic ΔH=54,5 $^{kJ}/_{mol}$ to drive spontaneous ΔG= -82,0679 kJ/mol products evaporation $CO_2\uparrow_{gas}$ and $H_2O\uparrow_{gas}$ keeping moisture H_2O on surface of membrane. Hydrogen ions water acidity shift endothermic ΔH_r= +54,5 $^{kJ}/_{mol}$ and exoergic ΔG_r= -82,1 $^{kJ}/_{mol}$ decomposition $H_3O^++HCO_3^-$ breath out to AIR $CO_2\uparrow_{gas}$ with $H_2O\uparrow_{gas}$:

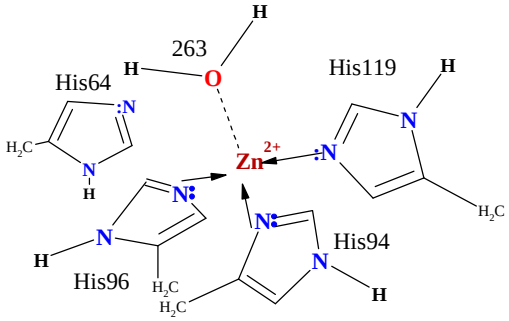
endothermic ΔH_r= +54,5 $^{kJ}/_{mol}$; $H_3O^++HCO_3^- +Q \xleftarrow{Membrane} H_2O+CO_2\uparrow_{gas}+H_2O\uparrow_{gas}$ + ΔG_r= -82,1 $^{kJ}/_{mol}$. exoergic .

Brønsted Acid/Base **CA** and hemoglobin **shuttle** enzymes of O_2 , CO_{2aqua} /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** = $7.36^{+0,02}_{-0,01}$) despite the fact, that organism produces great amount of metabolic [CO_{2Krebs}]= 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_{2aqua} desorbtion due to Krebs product CO_{2aqua} target cells ***in tissues***:

Hydrogen carbonate buffer system carbonic anhydrase equilibrium keeps weak acid CO_{2aqua} and bicarbonate ions at homeostasis normal amounts [HCO_3^-]=0.0154 M, [CO_{2aqua}]=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_{2aqua} by CA enzyme Zn^{2+} ion active coordination center. It's location in enzyme carbonic anhydrase Zn^{2+} ion coordination pocket:

$$CO_{2aqua} + 2H_2O \rightleftharpoons CA(Zn^{2+}) \rightleftharpoons H_3O^+ + HCO_3^-$$

$$H_2O+CO_{2aqua}+(Zn^{2+}<=OH^-CA+H^+)=>HCO_3^-+(H_2O(263)=>Zn^{2+}\ CA) + H^+$$

$Hb_R(O_2)_4$+$4H^+$ ⇔ $4O_{2aqua}$ + $(H^+His63,58)_4Hb_T$ stabilizing arterial concentration [O_2]=$6\cdot10^{-5}$ M in blood. Deoxy hemoglobin $(H^+His63,58)_4Hb_T$ capture four protons 4 H^+ at histidine residues and 4 HCO_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 4 H^+ and 4 HCO_3^- .

1) First of four human buffer systems is enzyme **CA** made Brønsted Acid/Base endothermic equilibrium: $Q+CO_{2aqua}+2H_2O \xleftarrow{CA}\rightarrow H_3O^+ + HCO_3^-$ 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±0,01 of hydrogen ions H_3O^+ concentration $[H_3O^+] = 10^{-7,36}$ M in products. CO_{2Krebs} as bicarbonate salt bridge linked $HCO_3^-\ldots H_3{}^+N$— and equal produced protons $[H^+]=[CO_{2Krebs}]=0,0275=[HCO_3^-]$ captures deoxy $(H^+His63,58)_4Hb_T$ **shuttle** and brings to ***lungs***. ***Lungs*** evaporates $CO_2\uparrow_{gas}$+ $H_2O\uparrow_{gas}$ endothermic ΔH_r= +54,5 $^{kJ}/_{mol}$, but exoergic ΔG_r= -82,1 $^{kJ}/_{mol}$:

$$H_3O^+ + HCO_3^- + Q \xleftarrow{Membrane}\rightarrow H_2O + CO_2\uparrow_{gas} + H_2O\uparrow_{gas} + \Delta G_r\ .$$

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 4 H^+ and 4 HCO_3^-. Equilibrium is oxygen concentration [O_2]=$6\cdot10^{-5}$ M sensitive:

$4O_{2aqua}$ + $(H^+His63,58)_4Hb_T\cdots sāls\cdots tiltiņi(HCO_3^-)_4$⇔ $Hb_R(O_2)_4$+$4H^+$ +4 HCO_3^-. ***lungs*** and ***tisues***.

Lungs venous blood hemoglobin saturation with oxygen 459 times restore circulated arterial blood [O_2]=$6\cdot10^{-5}$ M amount in one liter O2Solutions.pdf Adsorbed four $4O_{2aqua}$ $(O_2His63,58)_4Hb_R$+$4H^+$+4HCO_3^- in products release four protons 4 H^+ and bicarbonate ions 4 HCO_3^- , promoting evaporation $CO_2\uparrow_{gas}$+$H_2O\uparrow_{gas}$ on ***lungs*** epithelia surface, and removing out of organism $[H^+]=459*6*10^{-5}$ =0,0275 M amount $H^+ + H_2O => H_3O^+$, that is equal to total by respiration evaporated [$CO_2\uparrow_{gas}$] =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]=$6\cdot10^{-5}$ M adsorbtion-desorbtion equilibrium explain pH stabilization at 7.36.

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

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

In such a way two equilibria stabilize arterial oxygen concentration [O_{2aqua}]=$6\cdot10^{-5}$ M with **shuttle** hemoglobin by oxygen adsorbtion-desorbtion 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. A protein like hemoglobin **Hb** is a long chain of amino acid remainders, but this long chain still as amino acid and protein molecules have four type acidic functional groups: **-COOH** neutral, **-NH_3^+** positive charged, phenol **-OH** neutral, **-SH** neutral.

Amino Acid	$pK_{a\text{-}COOH}$	$pK_{a\text{-}NH3+}$	$pK_{a\ R\ group}$
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

At physiologic pH=7, 36 ±0.01 carboxylic groups **R-COO^-** negative charged and amino groups **R-NH_3^+** positive charged.

For example, glutamic acid pK_a reference to physiologic pH value smaller $pK_{aR\text{-}COO^-}$=4.25<7,36, pK_{aCOO^-}=2,19<7,36 and for amine is greater: 9,67=$pK_{a\text{-}NH3+}$>7,36 .

In table shown constants pK_a of four parallel protolytic equilibria:

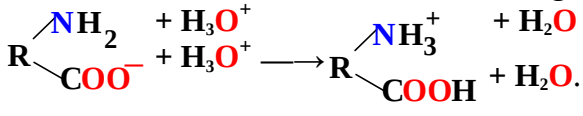
Acid ⇔ base + H^+

1. **R-COOH** ⇔**R-COO^-** +H^+,
2. **R-NH_3^+** ⇔**R-NH_2** + H^+
3. **Tyrosine**-phenol-**OH**⇔**Tyrosine**-phenol-**O^-** +H^+,
4. **Cysteine-SH** ⇔**Cysteine-S^-** + H^+

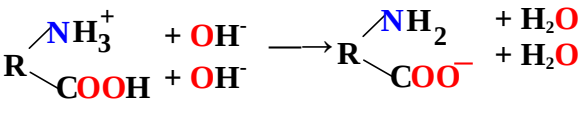
NpK_a number of parallel protolytic equilibria average pK_a value is calculated as pK_a=(Σ $pK_{a\ R\ group}$+ $pK_{a\text{-}NH3+}$+ $pK_{a\text{-}COOH}$)/NpK_a

In *Ostwald's dilution law* calculates one the pH of solution at concentration C logarithm: $pH=\frac{pK_a - \log C}{2}$=.....

Molecule like hemoglobin at blood plasma pH=7.36 has deprotonated carboxylate groups **-COO^-** with basic properties and acidic properties protonated ammonium groups –**NH_3^+**. If an acid is added to solution, containing protein like hemoglobin Hb, the **H_3O^+** ions will react with basic amino group and basic carboxylate group The strong acid **H_3O^+** will be transformed into water the weak base **H_2O** .

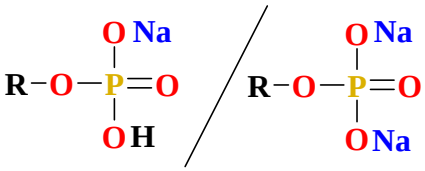


If a strong base is added to protein-containing solution, the **OH^-** ions react with the carboxylic groups and the strong base **OH^-** will be transformed into water the weak acid **H_2O**.

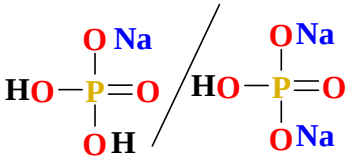


3) Biological important phosphate buffer system **NaH_2PO_4/Na_2HPO_4** pK=7,199 we will study as practical work.

4) Biological ubiquities exist besides the inorganic phosphate buffer system, buffer systems of the organic esters of phosphoric acid so as ATP (adenosine tri phosphate), ADP (adenosine di phosphate), CTP, CDP, GTP, GDP, TTP, TDP, UTP, UDP, NADH B_3 vitamin, $FADH_2$ B_2 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 **NaH_2PO_4/Na_2HPO_4**.



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 O_{2aqua} sensitive exchange: $(O_2His63,58)_4Hb_R$ **+4H^+**←[O_{2aqua}]=6·10^{-5} M → **4O_{2aqua}** + $(H^+His63,58)_4Hb_T$. Krebs cycle product **CO_{2aqua}** exchanged to bicarbonate buffer solution: **Q+CO_{2aqua}+2H_2O** $\leftarrow\overset{CA}{\rightarrow}$**$H_3O^+$+$HCO_3^-$**.

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.

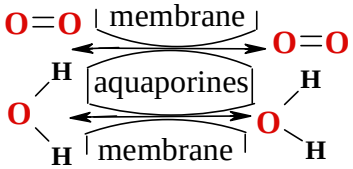
Besides the normal "chemical" mechanisms of buffer action in maintaining constant **pH=7.36±**0,01, with deoxy hemoglobin $(H^+His63,58)_4Hb_T$ (Tense state), oxy hemoglobin $(O_2His63,58)_4Hb_R$ (**Relax** state) and with carbonic anhydrase **CA** driven bicarbonate buffer systems are a joint physiological mechanism of action, which carries out the exchange of breathed in **O_2** and breathed out **CO_2** between AIR in ***lungs*** and tissues on interface human body / environment**.**

Human **shuttle hemoglobin-bicarbonate** buffer system and Krebs cycle driven respiration from AIR O_2 and breathed out CO_2 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 $Hb_R(O_2)_4$ + $4H^+$ <=> **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 HCO_3^- as desorbing four oxygen $4O_{2aqua}$ molecules. **Shuttle** and **carbonic anhydrase CA** stabilize exchange process from AIR O_2 to breathed out in to AIR CO_2.

Two **I** and **II pathways** are happen of gradual reactions: **I)** $O_{2AIR} + H_2O \overset{aquaporin}{\rightleftharpoons} H_2O + O_{2aqua}$



Process ***in lungs*** **I) Pathway** first reaction on cell wall membrane aquaporins penetrating water H_2O with oxygen O_{2aqua} by rate 10^9 sec^{-1} reach erythrocyte cells and oxygen concentration in blood plasma significant changes from **venous** blood $[O_2]=1{,}85\cdot10^{-5}$ M to arterial blood plasma in water becomes $[O_2]=6\cdot10^{-5}$ M.

Bisphospho glycerate BPG^{5-} drive hemoglobin O_2 adsorbtion⇔desorbtion equilibrium sensitive to concentration. It saturates arterial **shuttle oxy** hemoglobin with oxygen 459 times over $[O_2]=6\cdot10^{-5}$ M stored reserve 0,0275 M and pushed out of **shuttle deoxy** hemoglobin bisphospho glycerate BPG^{5-} releases $4H^+$ and 4 HCO_3^-.

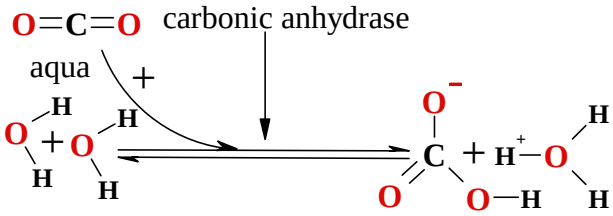
$4O_2+(H^+His63,58)_4betaVal1(NH_3^+PO_4^{2-})_2Hb_TG- \leftrightarrow (His63,58)_4Arg^+His^+betaVal1(NH_3^+)_2Hb_R(O_2)_4+4H^++BPG^{5-}$

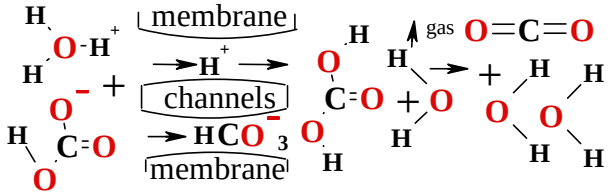
Each adsorbed oxygen molecules O_{2aqua} on hemoglobin releases proton H^+ which increases acidy on epithelial cell surface of ***lungs***. The epithelial cell surface of ***lungs*** has the specific building: super thin 0.6 nm water layer on surface S=950 nm x 950 nm= 0.9 μm^2 as square within small volume $0.5415\cdot10^{-3}$ $\mu m^3=0.5415\cdot10^{-18}$ 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 H_2CO_3 to evolving $CO_2\uparrow$ gas is breathed out to AIR.

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

$$Q_{aqua} + CO_{2aqua} + 2H_2O \overset{CA}{\longleftrightarrow} H_3O^+ + HCO_3^- \overset{membrane}{\rightleftharpoons} H_2O + H_2CO_3 + Q_{(gas)} \rightleftharpoons H_2O + CO_2\uparrow_{gas} + H_2O$$

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





Second gradual **exothermic** reaction forms Carbonic acid $H^+ + HCO_3^- \overset{membrane}{\longrightarrow} H_2CO_3 + Q$. Proton H^+ and bicarbonate HCO_3^- through channels drive concentration gradients for $[H_3O^+]_{right}/[H_3O^+]_{left}=10^{-7{,}36}{}_{right}/0{,}0339$ and for bicarbonate ions $[HCO_3^-]_{right}/[HCO_3^-]_{left}=0{,}0154\ M_{right}/0{,}0339\ M_{left}$ breathing out of organism to AIR gas $CO_2\uparrow_{gas}$.

Third gradual reaction on ***lung*** epithelial cell surface (outside organism) with absence **CA** decomposes carbonic acid H_2CO_3 to gas $CO_2\uparrow_{gas}$ in endothermic reaction: $H_2CO_3 + Q(gas) \rightarrow H_2O + CO_2\uparrow_{gas}$. 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 CO_{2aqua} product enzyme Carbonic Anhydrase (**CA**) converts to HCO_3^- bicarbonate and hydroxonium H_3O^+ ions according pH=7.36 ***alkaline reserve*** 2.036/1=$[HCO_3^-]/[CO_2]$= 0,0339 M/0,01665 M .

1) Tissues blood oxygen concentration little decreases below $[O_{2aqua}]=6\cdot10^{-5}$ M arterial concentration. Oxygen concentration sensitive **shuttle** equilibrium $(O_2His63,58)_4Hb_R+4H^+ => 4O_{2aqua}+(H^+His63,58)_4Hb_T$ shifts right restoring 459 times arterial concentration $[O_{2aqua}]=6\cdot10^{-5}$ M level amount from reserves of **oxy** hemoglobin $(O_2His63,58)_4Hb_R$. Hemoglobin desorbing oxygen reach decreased venous blood level $[O_2]=1{,}85\bullet10^{-5}$ M ***in lungs***. Each desorbed oxygen replaces proton H^+ at distal histidine His63,58 in hemoglobin $(H^+His63,58)_4Hb_T$ (Tense state) and bind produced metabolic product HCO_3^- prevent acidity effect stabilizing pH=7.36 constant.

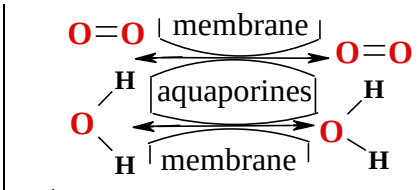
2**)** Krebs cycle metabolite CO_{2aqua} endothermic reaction with water in ***tissues*** drive carbonic anhydrase shift equilibrium to right $Q + CO_{2aqua} + 2H_2O \xleftarrow{CA} H_3O^+ + HCO_3^-$ forming ratio 1/2,0361 = $[CO_{2aqua}]/[HCO_3^-]$. Enzyme Carbonic Anhydrase (**CA**) equilibrium shifts reaction towards bicarbonate anion to prevent of carbonic dioxide accumulation, according Le Chatelier's due to high water $[H_2O]$ concentration 55.3 M, low hydrogen cat ion concentration $[H_3O^+]=10^{-7.36}$ M, enzyme **CA** constant pK=7.0512 value as friendly for physiologic pH=7,36 value. **CA** absence out side human organism as isolated with cell membranes shifts to some fold more acidic as enough at pH=5,5 on the surface for spontaneous carbonic acid bubbling $Q + H_2CO_3 \rightarrow H_2O + CO_2\uparrow_{gas}$.

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 $[O_{2aqua}]=6\cdot10^{-5}$ M concentration sensitive equilibrium ***in lungs*** $(O_2His63,58)_4Hb_R + 4H^+ \rightarrow 4O_{2aqua} + (H^+His63,58)_4Hb_T$ 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 pH=7,36. **Shuttle** in ***lungs*** adsorbs oxygen releasing protons on epithelial cell surface so keeping acidity pH=5,5 promote decomposition of carbonic acid out in AIR.

Second, enzyme **CA** equilibrium $H_2O/CA/CO_{2aqua}$ stabilize at pH=7,36 so prevent acidose. Evaporation: endothermic ΔH_r= +54,5 $^{kJ}/_{mol}$; $H_3O^+ + HCO_3^- + Q \xleftarrow{Membrane} H_2O + CO_2\uparrow_{gas} + H_2O\uparrow_{gas}$ + ΔG_r= -82,1 $^{kJ}/_{mol}$. exoergic. Equilibrium keep surface moisture H_2O be side breath out to AIR carbon dioxide $CO_2\uparrow_{gas}$ and water vapor $H_2O\uparrow_{gas}$. 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 $O=O$ permeable in both directions: $O=O + H_2O$ aquaporin channels $\Leftrightarrow H_2O + O=O$. **AQP1** transfer rate is $3\cdot10^9$ 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.



$(H^+)_4Hb_T + O_{2aqua} \leftrightarrow (O_2)_4Hb_R + 4H^+$

$\downarrow\uparrow \leftrightarrow \downarrow\uparrow$

Free energy change **ΔG**= -60 $^{kJ}/_{mol}$ for Reaction of H_2CO_3 formation is **exoergic ΔG**<0 negative therefore promotes spontaneous neutralization reaction

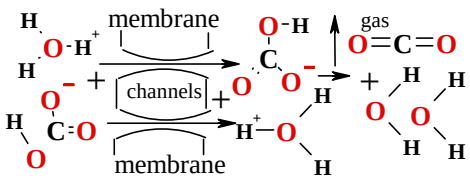
$H_3O^+ + HCO_3^- <=> H_2CO_3 + H_2O + \Delta G$

Inside the cell–cytosol

CA with water consumes heat **+Q**

$CO_2 + 2H_2O + Q \xleftarrow{CA} H_3O^+ + HCO_3^-$

aqua exothermic



alveolar surface in **lungs** consuming **+Q** heat and evolving water + H_2O supporting surface moisture

$H_2CO_3 + Q \rightleftharpoons CO_2\uparrow_{gas} + H_2O$

endothermic reaction

$H_3O^+ + HCO_3^- <==========> H_2CO_3 + H_2O + \Delta G$

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 $[O_{2aqua}] = 6 \cdot 10^{-5}$ M:
deoxy **hemoglobin**$(H^+His63,58)_4Hb_T$(Tense state)<=>**oxy hemoglobin**$(O_2His63,58)_4Hb_R$(**Relax** state)**+4H**$^+$

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

Organism store H^+ and HCO_3^- as Krebs cycle metabolic product carbonic dioxide, if **CA** produced buffer system acidic form CO_{2aqua} 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** $4O_{2aqua} + (H^+His63,58)_4Hb_T <=> (O_2His63,58)_4Hb_R + 4H^+$ capturing proton in distal histidine and salt bridge linked $HCO_3^- \ldots H_3^+N$- bicarbonate. Effective of controlled acid form's is breathing out $CO_2\uparrow_{gas}$, 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.

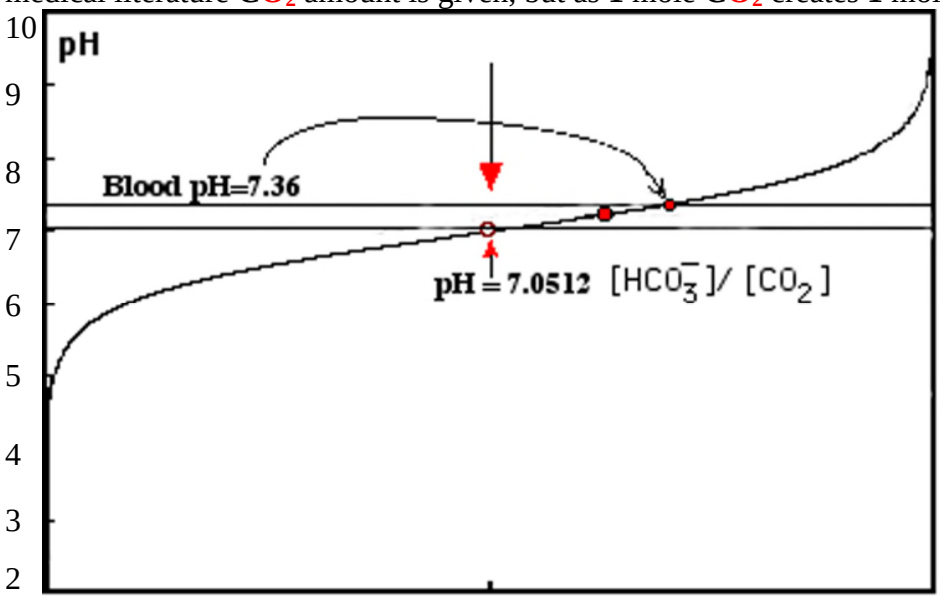
Carbonic anhydrase **CA** make conversion of CO_{2aqua} to bicarbonate anion HCO_3^- in to water medium fast and establish acid-base **Q** $+CO_{2aqua} + 2H_2O \xleftarrow{CA} H_3O^+ + HCO_3^-$ **endothermic** equilibrium at pH=7,36 as producing right side reaction products $H_3O^+ + HCO_3^-$ demanding to heat. So Heating **+Q** shifts equilibrium right side and as soon as H^+ concentration increase as three Krebs cycle product CO_{2aqua} 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_{2aqua}**,** which in. The dissolved into water H_2O (into blood) carbonic dioxide CO_{2aqua} occurring in cell converted with carbonic anhydrase **CA** to $H^+ + HCO_3^-$**.** The water H_2O and carbonic dioxide CO_{2aqua}, 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_{2aqua} 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: $7.36 = pH = pK + \log\left(\frac{[HCO_3^-]}{[CO_{2aqua}]}\right) = 7.0512 + \log\left(\frac{[HCO_3^-]}{[CO_{2aqua}]}\right)$;

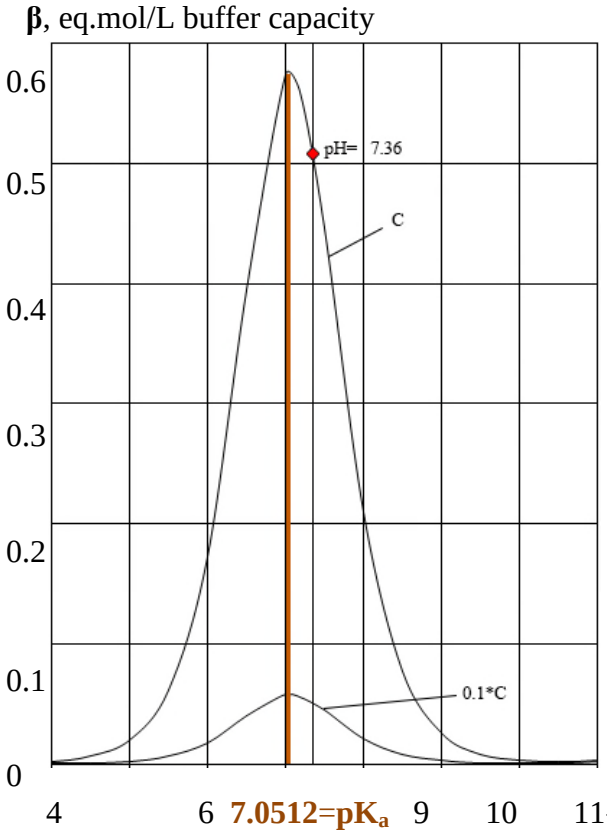
$\frac{[HCO_3^-]}{[CO_{2aqua}]} = 10^{(pH-pK)} = 10^{(7.36-7.0512)} = 10^{0.3088} = \frac{2.0361}{1}$ the ratio $[HCO_3^-]/[CO_{2aqua}]$ being approximately **2/1**. In medical literature CO_2 amount is given, but as **1** mole CO_2 creates **1** mole $H_2O^{/CA/}CO_{2aqua}$**,** it is the same.



Buffer region middle point is the over inflection point in graph ○: $pH = pK_a = 7.0512$; $[HCO_3^-]/[CO_2] = 1$ 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** = **pH** is **normal** as $\frac{[HCO_3^-]}{[CO_{2aqua}]} = \frac{2.0361}{1}$.

HCO_3^- 0%	**50%**	100% **salt –** buffer system **base**
CO_2 + $2H_2O$ 100%	**50%**	0% **weak acid** buffer component

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



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_{2aqua} concentrations is **2/1**. The **pH** value of physiological conditions blood homeostasis is **7.36.**

*The **alkaline reserve*** 2.036/1=$[HCO_3^-]/[CO_{2aqua}]$ 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_{2aqua} 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** = $[HCO_3^-]+[CO_{2aqua}]$ is in homeostasis **normal** as sum of $[HCO_3^-]$ = **0.0154 M** and $[CO_{2aqua}]$=**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_{2aqua} concentration decreases **pH>7.36 alkalosys** 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. Hoverer, 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 $[O_2]=1{,}85\cdot10^{-5}$ M.

For this reason only the concentrations of carbonic dioxide CO_{2aqua} 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

I) $O_{2AIR}+H_2O \overset{aquaporin}{\rightleftarrows} H_2O+O_{2aqua}$; $4O_{2aqua}+$ **deoxy**$(H^+His63,58)_4Hb_T <=[O_2]=6\cdot10^{-5}$ M=>**oxyHb**$_R(O_2)_4+4H^+$,
Glycolysis, Krebs cycle products $C_6H_{12}O_6+6O_{2aqua}+6H_2O \leftarrow \overset{Krebs\_Cycle}{\longrightarrow} 6CO_{2aqua}+12H_2O \leftarrow \overset{CA}{\longrightarrow} 6H_3O^+ +6HCO_3^-$

II) $Q_{aqua}+CO_{2aqua}+2H_2O <= \underline{CA} => H_3O^+ +HCO_3^- \overset{membrane}{\rightleftarrows} H_2O+H_2CO_3+Q(gas) \rightleftarrows H_2O+CO_2\uparrow_{gas}+H_2O$.

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

Free energy consumes ΔG_r for reaction endoergic: $CO_{2aqua}+2H_2O+\Delta G+Q <= \overset{CA}{=>} H_3O^+ +HCO_3^-$

Enthalpy heat consumed ΔH_r for reaction endothermic: $\Delta H_r=\Delta H°_{H3O}-\Delta H°_{HCO3}-2\Delta H°_{H2O}-\Delta H°_{CO2}$=9,7576 $^{kJ}/_{mol}$
= -285,81-689,93-(2*-285,85-413,7076) = -975,74+985,3276=9,7576 $^{kJ}/_{mol}$ endothermic
Endothermic as needed heat supply to drive reaction forwards.

Entropy decrease ΔS_r **<0** negative as enzyme Carbonic Anhydrase **CA** governed reaction**:**
$\Delta S_r=\Delta S°_{H3O}+\Delta S°_{HCO3}-2\Delta S°_{H2O}-\Delta S°_{CO2}$=-3.854+98.324-(2*69.956+117.57)=94.47-257.482= -163.0134 $^{J}/_{mol/K}$.
$\Delta G_r=\Delta H_r-T*\Delta S_r$=9,7576-(298,15*-0,1630134)=58,36 kJ/mol endoergic **free** energy accumulated in products by **CA** governed reaction. $\Delta S_{dispersed}$= - ΔH_r/ T = -9.5876 /298.15= -32,727 J/K/mol.
$\Delta S_{total}=\Delta S_r+\Delta S_{dispersed}$=-163,0134-32,7271= -195,7405 J/K/mol. $T\bullet\Delta S_{total}$=-0,1957405*298,15= **-58,36** kJ/mol
$\Delta G_r=\Delta G°_{H3O}+\Delta G°_{HCO3}-2\Delta G°_{H2O}-\Delta G°_{CO2}$=-213,275-586,94-(2*-237,19-385,98)= +60,145 $^{kJ}/_{mol}$ 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 M is constant so included in value
$K_{eq}=K\bullet[H_2O]^2=10^{-10.224}\bullet(997,07/18,0153)^2=10^{-7.0512}$ M$=10^{-pKeq}$ exponent value pK_{eq}=7.0512.

II) process first gradual reaction driven by enzyme **CA** $CO_{2aqua}+2H_2O \leftarrow \overset{CA}{\longrightarrow} H_3O^+ +HCO_3^-$
$10^{-7.0512}=K_{eq}=[H_2O]^2\cdot K=[H_3O^+][HCO_3^-]/[CO_{2aqua}]$ as $K=[H_3O^+][HCO_3^-]/([CO_{2aqua}]\cdot[H_2O]^2)=10^{-10.224}$
$\Delta G=-RTln(K)$=-8.3144•298.15•ln(1•$10^{-10.224}$)= 60.145 $^{kJ}/_{mol}$ where R=8,3144 J/mol/K and T=310,15 K (25°C).
$K_{eq}=EXP(-\Delta G_r/R/T)*[H_2O]^2=(10^{-10.5372})*[H_2O]^2=(10^{-10.5372})*(997,07/18,0153)^2=10^{-7.0512}=10^{-pKeq}$

II) process second gradual reaction concentration gradient and electrochemical membrane potential bicarbonate ion HCO_3^- and proton H^+ 1. $E_H=P\bullet lg([10^{-pH}{}_{extraMit}/10^{-pH}{}_{Mitochon})$=0,06154***lg**(10^2,36)=0,14523 **V**
2. $E_{HCO3\text{-}Mitochon}$= **-P•log**($[HCO_3^-{}_{cytosol}]/[HCO_3^-{}_{Mitochon}]$)= -0,06154***log(**0.0154/0,0338919)= 0,0210821 **V**
E_{sum}=0,14523+0,0210821=0,1663168 V =$E_{membrane}$; ΔG_F=**nFE**=-1*96485***0,1663168= -16,0471 kJ/mol
3. $\Delta G_{HCO3\text{-}}$=**RTln([HCO**$_{3cytosol}$**]/[HCO**$_{3Mitoch}$**])**=8,3144*310,15***log**(0,0154/0,0338919)= -2,0341094 kJ/mol
4. ΔG_{H+}=**-RTln**($[H_3O^+]_{extraMit}/[H_3O^+]_{Mitochon}$)=**-RTln**($10^{-7,36}/10^{-5}$)=-8,3144*310,15***ln**(10^**2,36**)= -23,3943 $^{kJ}/_{mol}$
Total $\Delta G_{total}=\Delta G_F+(\Delta G_{HCO3\text{-}}+\Delta G_{H+})$=-16,0471 +(-2,0341094)+(-23,3943)= -41,4755 $^{kJ}/_{mol}$ exoergic transfer.

II) process third gradual Carbonic acid formation: $H_3O^+ +HCO_3^- \rightarrow H_2O+H_2CO_3+Q$ exothermic.
$\Delta H_r=\Delta H°_{H2O}+\Delta H°_{H2CO3}-\Delta H°_{H3O}-\Delta H°_{HCO3}$= -285.85-699,65-(-285.81-689.93)= =-985.5+975.74= -9.76 $^{kJ}/_{mol}$
$\Delta S_r=\Delta S°_{H2O}+\Delta S°_{H2CO3}-\Delta S°_{H3O}-\Delta S°_{HCO3}$=69.956+187-(-3.854+98.324)=256.956-94.47= 162,486 $^{J}/_{mol/K}$.....
$\Delta S_{dispersed}$ = - $\Delta H_r/T$ = 9.76 /298.15= +32.735 J/K/mol....
$\Delta G_r=\Delta H_r-T*\Delta S_r$= -9.76 -298.15*0.129751=-38.695 kJ/mol **exoergic** reaction is driven by concentration gradients through **proton** and **bicarbonate channels** of **membrane**.
$\Delta G=\Delta G°_{H2O}+\Delta G°_{H2CO3}-\Delta G°_{H3O}-\Delta G°_{HCO3}$=-237.19-623.17-(-213.275-586.94)=-860.36+800.215= -60.145 $^{kJ}/_{mol}$.
$\Delta S_{total}=\Delta S_r+\Delta S_{dispersed}$=32.735 +162,486 =129.751 J/K/mol....

II) process fourth gradual reaction is non-enzymatic decomposition $H_2CO_3 <=> CO_2\uparrow_{gas}+H_2O$;
$\Delta G=\Delta G°_{H2O}+\Delta G°_{CO2}-\Delta G°_{H2CO3}$= -237.19-385.98-623.17)= -623.17+623.17=0.0 $^{kJ}/_{mol}$ is **anenergic** or neutral.

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

Substance	$\Delta H°_r$, $^{kJ}/_{mol}$	$\Delta S°_r$, $^{J}/_{mol/K}$	$\Delta G°_r$, $^{kJ}/_{mol}$
H_3O^+	-285.81	-3.854	-213,275
HCO_3^-	-689.93	98.324	-586,94
$H_2O\uparrow_{gas}$	-241,8352	188,7402	
H_2O	-285.85	69.9565	-237,191
$CO_2\uparrow_{gas}$	-393.509	213.74	-394,359
CO_{2aqua}	-413.7976	117.5704	-385,98
H_2CO_3	-699,65	187.00	-623,17

$\Delta H=\Delta H°_{H2O}+\Delta H°_{CO2}-\Delta H°_{H2CO3}$= +20.291 $^{kJ}/_{mol}$
=-286-393.509-(-699.65)= -679.509+699.65= +20.291 $^{kJ}/_{mol}$
is endothermic exactly with the **cooling** effects.....
$\Delta S_{dispersed}$ =-$\Delta H_r/T$ = -20.291/298.15= -68.056 J/K/mol....
$\Delta S_r=\Delta S°_{H2O}+\Delta S°_{CO2}-\Delta S°_{H2CO3}$= +96,696 $^{J}/_{mol/K}$....
= 69.956+213,74-(187.)= 257.482-94.47=+96,696 $^{J}/_{mol/K}$....
$T\bullet\Delta S_{total}$=28,64 $^{J}/_{mol}$•298,15 K=**+8.539** kJ/mol
bound TΔSn← lost free energy ΔGreverse$_{reaction}$←....
endothermic $\Delta H°_{reaction}$ =+20,291 kJ/mol; cooling Q= -20,291 kJ/mol spontaneous $\Delta G°_{reaction}$ =-8,539 kJ/mol .