Protolytic equilibria in **water** BUFFER solutions. Brønsted Acid protolysis with water.

Buffer systems in the human organism tend to Prigogine attractor **pH** value **7.36** formed of two dominate phosphate and bicarbonate buffer systems with **over inflection point** on the middle pK_a=7,199 and pK_a=7,0512. That create protonate amines **-NH_3^+** and deprotonate carboxylates **-COO^-** for functional activity of enzymes in proteins, amino acids, carbonic acids and amines with broadband silencing interval from pH=6 to pH=7,36.

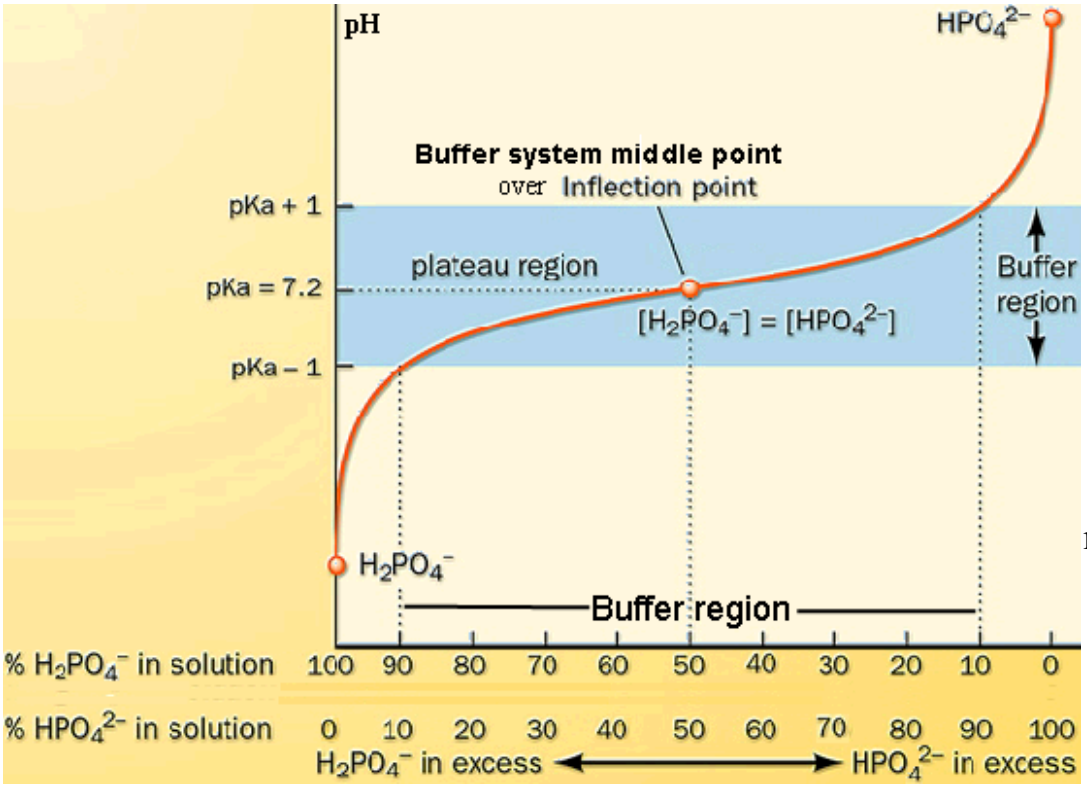
Dominate Phosphate $H_2PO_4^-+H_2O<=>HPO_4^{2-}+H_3O^+$ and Bicarbonate $CO_{2aqua}+2H_2O \xrightarrow{CA} H_3O^+ + HCO_3^-$.

Phosphate: $\mathbf{H_2PO_4^-}_{aq}+\mathbf{H_2O}+\Delta G+Q <=> \mathbf{HPO_4^{2-}}_{aq}+\mathbf{H_3O^+}$ CRC 2020 data I=0,25 M and pKa=7,199 at

equilibrium: $\frac{[HPO_4^{2-}]\cdot[H_3O^+]}{[H_2PO_4^-]\cdot[H_2O]}=K_{eq}=K_a/[H_2O]=10^{-7,199}/55,3=1,143*10^{-9}$; classic value $K_a=\frac{[HPO_4^{2-}]\cdot[H_3O^+]}{[H_2PO_4^-]}$;

Henderson Haselbalh $pH=pK_a+\log\frac{[HPO_4^{2-}]_{base}}{[H_2PO_4^-]_{acid}}$ homeostasis depends on components ratio $\frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$.

1. Dihydrogen phosphate *buffer system form phosphate, pyrophosphate, phosphate esters like ATP ect.* with *differing iby one deprotonated* H^+ *hydrogen ion less* $\mathbf{H_2PO_4^-}$/ $\mathbf{HPO_4^{2-}}$, where



the weak acid contains greater number of hydrogen ions plays the role of proton donor

$\mathbf{H_2PO_4^-}$

and deprotonated weak acid created base form contains one hydrogen atom less

$\mathbf{HPO_4^{2-}}$.

Buffer region ±1=pH one unite wide band region from middle point pK_a,

At over inflection point has gratest buffer capacity $\beta_{max}=0.55\cdot C_{buffer}$.

Carbonic acids, fatty acids, amino acids (proteins), protonate amines at Physiologic conditions pH=7,36 :

2a. $\mathbf{CH_3COOH+H_2O \Leftrightarrow H_3O^+ + CH_3COO^-}$; $K_a=\frac{[H^+]\cdot[CH_3COO^-]}{[CH_3COOH]_{nondis}}=10^{-4,76}$ $\quad K_a=1,74*10^{-5}$ M $=10^{-pKa}$

2b. $\mathbf{AA\text{-}COOH \Leftrightarrow AA\text{-}COO^- + H^+}$, $K_{aCOOH}=\frac{[AA\text{-}COO^-]\cdot[H^+]}{[AA\text{-}COOH]_{nondis}}=10^{-pKa}$ $\quad 2,0 < pK_{aAACOOH} < 4,9$;

2c. $\mathbf{AA\text{-}NH_3^+ \Leftrightarrow AA\text{-}NH_2 + H^+}$, $K_{aNH3+}=\frac{[AA\text{-}NH_2]\cdot[H^+]}{[AA\text{-}NH_3^+]_{protonate}}=10^{-pKa}$; $\quad pK_{aAANH3+} > 8,8$;

2d. $NH_4^+ + H_2O \Leftrightarrow H_3O^+ + NH_{3\,aqua}$; $K_a=\frac{[H^+]\cdot[NH_3]_{aqua}}{[NH_4^+]}=10^{-pKa}=10^{-9,25}$ $\quad K_a=\frac{10^{-14}}{1,78*10^{-5}}=10^{-9,25}$ M

3. ENZYME Carbonic Anhydrase **CA** as weak acid CO_{2aqua} reaction with water $\mathbf{CO_{2aqua}+H_2O}$ forms *Buffer system strong base* bicarbonate ion. *Weak acid to* $\mathbf{H_2O^{/CA/}CO_2/HCO_3^-}$ *base bicarbonate ion.*

$\mathbf{Q+CO_{2aqua} + 2H_2O \xrightarrow{CA} H_3O^+ + HCO_3^-}$ endothermic equilibrium $K_a=10^{-7,0512}$ M $=10^{-pKa}$;

Weak acid protolysis *Ostwald's* dilution law

The buffer system of weak acid protolytic equilibrium thermodynamic studies about pH value stability, if add water so dilute buffer solution and if add a strong acid or base.

1. CARBONIC ACID protolysis

Weak acid and classic dissociation form deprotonated conjugate base: $CH_3COOH \Leftrightarrow CH_3COO^- + H^+$.
Sodium acetate is the conjugate base strong electrolyte $\alpha = 1$: $CH_3COONa \Rightarrow CH_3COO^- + Na^+$.
As a great number of acetate ions salt do not let the dissociation of acetic acid as oppressed with acetate ions in products of dissociation equilibrium. According Le Chatelier's theorem acid dissociation is shifted to left. For this reason the dissociation degree of the acetic acid is close to zero $\alpha \Rightarrow 0$ but positive number.

If a strong acid is added to the buffer solution, the H_3O^+ ions react with base protonating CH_3COO^- acetate to form acetic acid : $H_3O^+ + CH_3COO^- \Leftrightarrow CH_3COOH + H_2O$

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

1) the strong acid (H_3O^+ ion) is transformed to a weak acid CH_3COOH.

2) the concentration of acetic acid **C** increases, therefore for strong acid **pH** is more acidic. In fact, a weak acid acetic acid dissociation degree **α** decreases depending on **C** according ***Ostwald's* dilution law**: $\alpha = \sqrt{\frac{K}{C}}$

For this reason, when the concentration of acetic acid grows, its dissociation degree is adjusted to be smaller and therefore the concentration of H_3O^+ ions and **pH** remains 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 buffer, the OH^- ions from the strong base react with the weak acid (acetic acid) :

$$OH^- + CH_3COOH \Rightarrow CH_3COO^- + H_2O$$

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

1) strong base OH^- ion deprotonates weak acid to form base form salt-acetate CH_3COO^- ion,
2) acetic acid was used, to do the concentration **C** of acetic acid decreases,
the dissociation degree **α** grows, hence, H_3O^+ concentration and **pH** remains constant. $\alpha = \sqrt{\frac{K}{C}}$

2. Protonate AMONIA weak acid NH_4^+ protolysis *Ostwald's* dilution law

Weak ammonium acid ions and deprotonated ammonia buffer solution: $NH_4^+ + H_2O \Leftrightarrow H_3O^+ + NH_{3aqua}$.

Ammonium chloride is a strong electrolyte $\alpha = 1$: : $NH_4Cl \Rightarrow NH_4^+ + Cl^-$

Base NH_{3aqua} protonation product NH_4^+ ions grate amount left side in buffer solution prevent protonation of ammonia as oppressed (as the presence of NH_4^+ shifts equilibrium to the right) and protonation degree for ammonia tends to zero but is asmall positive number $\alpha \Rightarrow 0$.

If a strong base is added to this solution OH^- ions react with weak acid NH_4^+ and form ammonia $NH_{3\ aqua}$:

$$OH^- + NH_4^+ \Rightarrow NH_{3\ aqua} + H_2O$$

Due to this reaction :

1) a very strong base OH^- ion is transformed into deprotonated weak acid form base $NH_{3\ aqua}$,

2) weak acid concentration **C** decreases deprotonation dissociation degree **α** is adjusted to be higher $\alpha = \sqrt{\frac{K}{C}}$.

Equilibrium : $NH_4^+ + H_2O \Leftrightarrow H_3O^+ + NH_{3\ aqua}$ shifts to right and H_3O^+ concentration **pH** remains constant.

When a strong acid is added, than H_3O^+ ions protonate ammonia $NH_{3\ aqua}$ and weak acid NH_4^+ concentration **C** increases but dissociation degree $\alpha = \sqrt{\frac{K}{C}}$ value decreases.

Strong base OH^- is transformed to buffer base $NH_{3\ aqua}$ but dissociation degree $\alpha = \sqrt{\frac{K}{C}}$ increases.

Henderson Haselbalh weak acid protolysis pH EQUATION

In discusion abovewe have prooved why **pH** of a buffer remains constant, but it is necessary to know, how particular value (**pK_a, n_{base} , n_{acid}**) will keep constant the **pH** by a given buffer solution.

1. Henderson Haselbalh pH expressions

The Henderson Haselbalh expression derives from weak acid deprotonation constant **K_a** expression.

In human body exist four type weak acids protolysis with water equilibria .

1. Phosphate, 2. carboxylate, 3. Ammonium ions, 4. Amino acids **AA** (carboxilate, protonate amines, tyrozine, cysteine).

1. Phosphate: $H_2PO_4^- + H_2O \Leftrightarrow H_3O^+ + HPO_4^{2-}$; $K_a = \frac{[H^+]\cdot[HPO_4^{2-}]}{[H_2PO_4^-]} = 10^{-7,199}$ $K_a = 6,3*10^{-8}$ M $= 10^{-pKa}$

2. $CH_3COOH + H_2O \Leftrightarrow H_3O^+ + CH_3COO^-$; $K_a = \frac{[H^+]\cdot[CH_3COO^-]}{[CH_3COOH]_{nondis}} = 10^{-4,76}$ $K_a = 1,74*10^{-5}$ M $= 10^{-pKa}$

3. $NH_4^+ + H_2O \Leftrightarrow H_3O^+ + NH_{3\ aqua}$; $K_a = \frac{[H^+]\cdot[NH_3]_{aqua}}{[NH_4^+]} = 10^{-pKa} = 10^{-9,25}$ $K_a = \frac{10^{-14}}{1,78*10^{-5}} = 5,618*10^{-10}$ M

4a. **AA-COOH** $\Leftrightarrow$ **AA-COO^-** + H^+ , $K_{aCOOH} = \frac{[AA\text{-}COO^-]\cdot[H^+]}{[AA\text{-}COOH]_{nondis}} = 10^{-pKa}$ $pK_{aAACOOH} < 4,9$;

4b. **AA-NH_3^+** $\Leftrightarrow$ **AA-NH_2** + H^+ , $K_{aNH3+} = \frac{[AA\text{-}NH_2]\cdot[H^+]}{[AA\text{-}NH_3^+]_{protonate}} = 10^{-pKa}$; $pK_{aAANH3+} > 8,8$;

4c. **Tyr**-phenol-**OH** $\Leftrightarrow$ **Tyr**-phenol-**O^-** + H^+, $K_{aTyr} = \frac{[Tyr\ O^-]\cdot[H^+]}{[Tyr\ OH]_{nedis}} = 10^{-10,07}$;

4d. **Cys-SH** $\Leftrightarrow$ **Cys-S^-** + H^+, $K_{aCys} = \frac{[Cys\ S^-]\cdot[H^+]}{[Cys\ SH]_{nedis}} = 10^{-8,18}$;

Tyrosine and cysteine at physiologic pH=7,36 are just not dissociate acids, which do not form buffer.

Ions origin in solution are two sources – weak acids and electrolytes. Deprotonated weak acid form base concentration in equilibrium constant **K_a** expression designated as **C_{base}**:

$[HPO_4^{2-}]$; $[CH_3COO^-]$; $[NH_{3\ aqua}]$; [**AA-COO^-**]; [**AA-NH_2**] ; **C_{base}** (base).

Weak acid concentration in constant **K_a** expression is **C_{acid}**:

$[H_2PO_4^-]$; $[CH_3COOH]_{nedis}$; $[NH_4^+]$; [**AA-COOH**] ; [**AA-NH_3^+**]; **C_{acid}** (weak acid)

Replacing in the equation of **K_a** the weak acid and deprotonated acid concentrations we have :

$K_a = \frac{[H^+]C_{base}}{C_{acid}}$. Calculate the $[H_3O^+] = \frac{K_a \bullet C_{acid}}{C_{base}}$. Taking a minus logarithm from both sides :

$\log[H^+] = -\log K_a - \log\frac{C_{acid}}{C_{base}}$ we got the Henderson Haselbalh equation $pH = -\log[H_3O^+] = pK_a + \log\frac{C_{base}}{C_{acid}}$

converting to **pH:** (note, logarithm mathematics rool **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) buffer system forming acid weakness pK_a exponent $K_a = 10^{-pKa}$;

2) deprotonated acid and weak acid ratio n_{base}/n_{acid} in 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 EXPRESSION

Henderson Haselbalh buffer solution **pH** form weak acids and deprotonated acid form base.

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

Components amount ratio logarithm forms **pH** value. **pH** expression of C_{base}/C_{acid} converting to number of moles ratio n_{base}/n_{acid} as buffer system volume **V** is common and can to scratch.

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

It is very often necessary to express the **pH** of a buffer through the concentrations of the two initial solutions of weak acid and deprotonated acid base form. So practical mix together solutions.

If the buffer solution is prepared from two solutions than numbers of moles calculate **n = C'V'**, where **C'** and **V'** are the concentration and the volume of the initial solutions. Mixing total buffer solution volume is $V_{buf}=V'_{base}+V'_{acid}$. The **Henderson Haselbalh** equation is used for practical calculations for **pH** .

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

Δn_{ac} is a strong acid moles, for example **HCl**, added to buffer solution, which decreases Brensted base amount $n_{base}-\Delta n_{ac}$ and increases the buffer weak 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 $\Delta pH= pH_b - pH$.

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

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

EXAMPLE OF BUFFER ACTION studies

Now, when the equation for buffer pH is derived, we can study 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**

Strong acid 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 **ΔpH = 5 = 7 - 2**.

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**.

BUFFER CAPACITY β

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

$$\mathbf{pH=pK_a+\log\frac{n_{base}}{n_{acid}}}$$

where $\mathbf{n_{base}}$ and $\mathbf{n_{acid}}$ are the numbers of equivalents of salt and acid respectively.

If an acid is added to buffer solution, it will react with the base $\mathbf{n_{base}}$ and will decrease (at the same time, as more weak acid will be formed $\mathbf{n_{acid}}$ will increase).

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 $\mathbf{n_{base}}$ of the base, present in buffer system, all base 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 weak 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 $\mathbf{n_{acid}}$ of weak 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 $\mathbf{\beta = \frac{\Delta n}{\Delta pH \bullet V_{buffer}} = \left(\frac{mol}{Liter}\right)}$

where $\Delta\mathbf{n}$ is the number of equivalentmols of the strong acid or base, that is added to the buffer,

$\Delta\mathbf{pH}$ is the **pH** change, caused by the addition of strong acid $\Delta\mathbf{n_{ac}}$ or strong base $\Delta\mathbf{n_b}$,

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

Buffer capacity units are equivalent mol/Liter. The definition of buffer capacity in words is as follows :

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

On middle point buffer capacity is affected by four reasons :

***1**.the total summary concentration of buffer solution* $\mathbf{C_{base}' + C_{acid}' = C'}$

Buffer capacity is proportional to summary total concentration $\mathbf{C' = C_{base}' + C_{acid}'}$.

***2**. the ratio between buffer components* on **middle point** is $\frac{\mathbf{n_{base}}}{\mathbf{n_{acid}}}=1$ with reaching

2. <u>maximal value</u> $\mathbf{\beta_{acid}=\beta_{base}=0.55\cdot C'}$. **Henderson Haselbalh** buffer equation on middle point $\mathbf{pH=pK_a+\log\frac{n_{base}}{n_{acid}}}$ is equal to weak acid constant $\mathbf{pH=pK_a}$ value. because $\mathbf{\log\frac{n_{baze}}{n_{skab}}=\log1=0}$.

3. deviated from the ratio one $\mathbf{n_{base}/n_{acid}=1}$ „middle point" both buffer capacities against strong acid $\mathbf{\beta_{ac}}$ and buffer capacity against strong base $\mathbf{\beta_b}$ fast becomes smaller.

Single weak acid buffer system action broad $\mathbf{pH=pK_a\pm1}$ is in two units of **pH**.

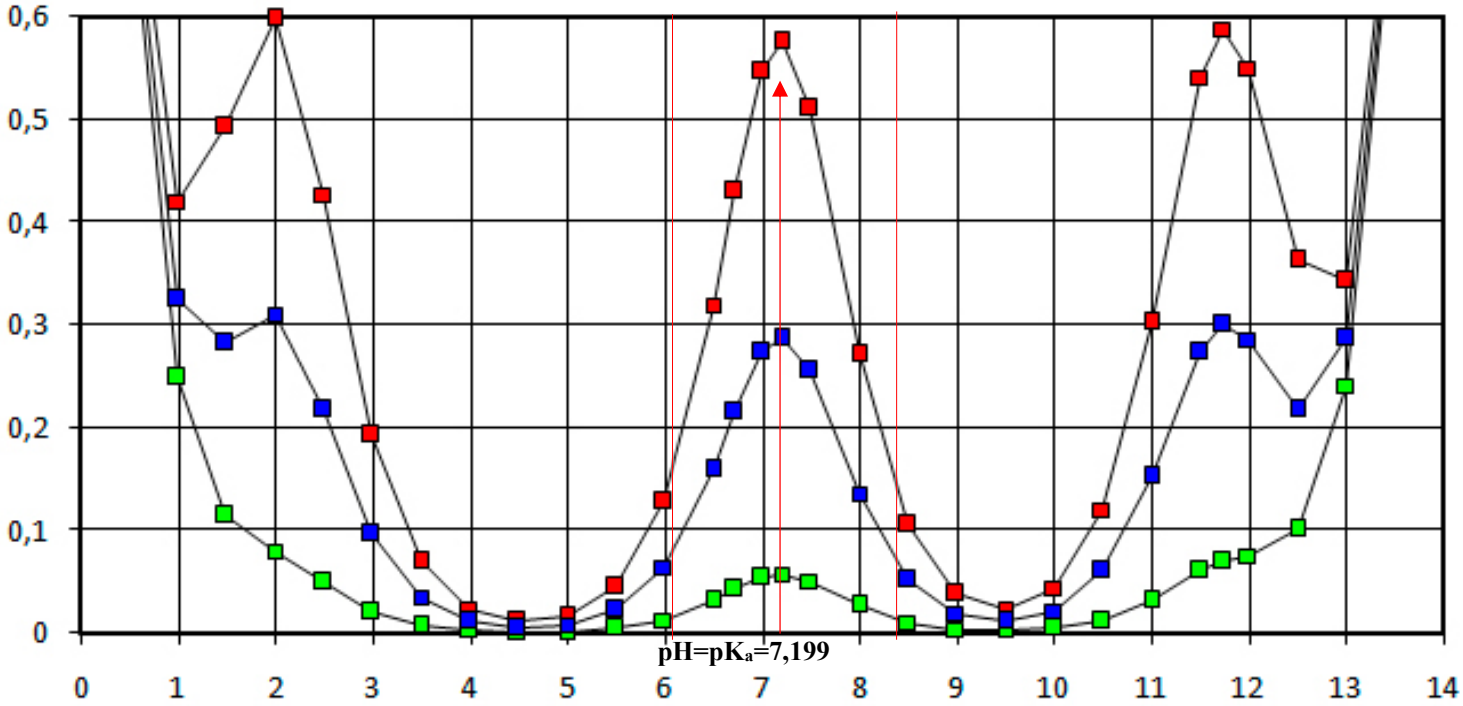
4. Buffer capacities on „middle point" are *symmetrically* equal $\mathbf{\beta_{ac}=\beta_b}$. Added strong acid pH decreases about $\Delta\mathbf{pH=-1}$, but added strong base pH increases about $\Delta\mathbf{pH=+1}$.

5. Amino acids and proteins using 47 pK_a constants create broadband buffer systems with inactive buffer capacity silencing zone pH 6 to 7,36. On this zone dominate phosphate pK_a=7,199 and bicarbonate pK_a=7,0512 buffer systems maintaining 7,36 pH.

Phosphate buffer system $H_2PO_4^-/HPO_4^{2-}$; $pH=pK_a+\log\frac{[HPO_4^{2-}]}{[H_2PO_4^-]}=7{,}199+\log\frac{1{,}45}{1}=7{,}36$

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

β , eq.mol/L **$pK_a=7.199$, $H_2PO_4^-/HPO_4^{2-}$**



Buffer system **middle point pH=pK_a=7,199** over inflection point maximum of buffer capacity β=0.55

pH

Concentration of Buffer solution C_{buffer}= 1 M — red

Concentration of Buffer solution C_{buffer}=0.5 M — blue

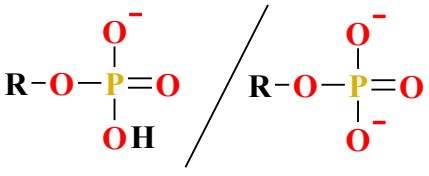
Concentration of Buffer solution C_{buffer}=0.1 M — green

$H_2PO_4^-$ weak acid, contains one number hydrogen more and **$H_2PO_4^-$** is weak acid.

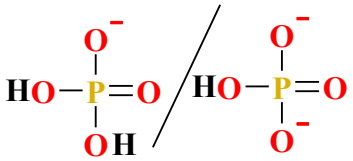
HPO_4^{2-} deprotonated weak acid form of base, contains one hydrogen les and **HPO_4^{2-}** is protolytic base

1) Biological important phosphate buffer system **$H_2PO_4^-$ / HPO_4^{2-}** with pK=7,199 value.

1a) Biological ubiquities exist phosphate buffer system of the organic esters of phosphoric acid so as ATP (adenosine tri phosphate), ADP (adenosine diphosphate), 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 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. Total concentration 0,115 M=[$H_2PO_4^-$]+[HPO_4^{2-}] in muscle cells cytosole.



2) Inactive silencing interval ΔpH from 6 to 7,36 indispensible for proteins - amino acids charged negative **–COO⁻** and positive **–NH₃⁺** make enzymes functional activity.

Like to hemoglobin proteins as long chain polypeptides and free amino acids with four type weak acid groups constitute 47 values of weak acid cknstants: $pK_{a\text{-}COOH}$, $pK_{a\text{-}NH3+}$, $pK_{aRgroup}$.

Amino Acid	pK_{aCOOH}	pK_{aNH3+}	$pK_{aRgroup}$
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 $pK_{a\text{-}COOH}^-$ value smaller about 7,36:
$pK_{a\text{-}COOH}^-$=4.25<4,9 (fatty acids) <7,36 and
given smallest $pK_{a\text{-}NH3+}$ value grater about 7,36 < 9,04 = $pK_{a\text{-}NH3+}$
20 amino acids have four protolytic pK_a equilibria in 47 groups:

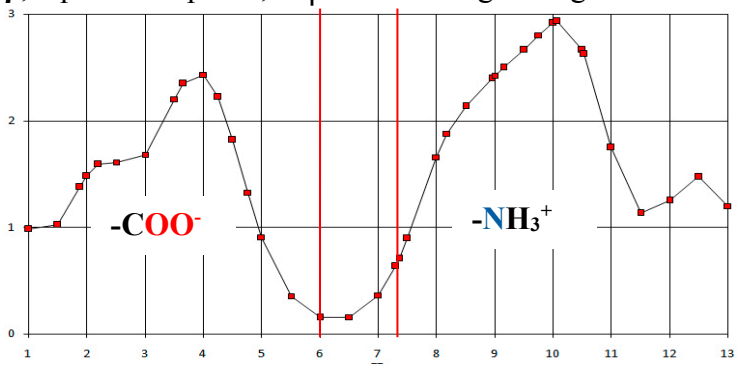
1. **R-COOH** ⇔**R-COO⁻** +**H⁺**, 22 groups of 47
2. **R-NH₃⁺** ⇔**R-NH₂** + **H⁺** 22+1 group of 47
3. **Tyrosine**-phenol-**OH**⇔**Tyrosine**-phenolate-**O⁻** +**H⁺** one group,
4. **Cysteine-SH** ⇔**Cysteine-S⁻** + **H⁺** one group .

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

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

Human Physiologic pH=7,36 dominate Bicarbonate, Phosphate buffers perform proteins - amino acids charged negative **–COO⁻** and positive **–NH₃⁺** make enzymes functional activity.

β, eq.mmol/L pH=7,36



-COOH pK_a values are on interval from 2 to 4,9 and **–NH₃⁺** pK_a values are on interval from 8 to 10 .
Proteins buffer have silence region from **pH=6** to 7,36 .

23 thousand protein molecules total buffer solution concentration C_{buffer}=2,3 mM . Buffer capacity at physiologic pH=7,36 is **β** =0,7 mM. Indispensible inactive silencing interval ΔpH from 6 to 7,36 providing attractor pH=7,36 with two dominate buffer systems pH Bicarbonate and Phosphates

Shuttle hemoglobin-based bicarbonate $4HCO_3^-$, proton H^+ to oxygen O_{2aqua} concentration sensitive exchange:
$4O_{2aqua}+(H^+His63,58)_4Hb_T$..salt bridges..$(HCO_3^-)_4+4\mathbf{H_2O}<=>Hb_R(O_2)_4+4\mathbf{H_3O^+}+4HCO_3^-$:
Arterial $[\mathbf{O_2}]=6\cdot10^{-5}$ M $[Hb_R(O_2)]=0,96$, venous $[\mathbf{O_2}]= 0,486\cdot10^{-5}$ M $[Hb_R(O_2)]=0,66$ homeostasis
$[(H^+)_4Hb_T$..salt bridges..$(HCO_3^-)_4]=0,04$, venous $[(H^+)_4Hb_T$..salt bridges..$(HCO_3^-)_4]=0,34$
$K=[Hb_R(O_2)]*[\mathbf{H_3O^+}]^4*[HCO_3^-]^4/[(H^+)_4Hb_T$salt bridges$(HCO_3^-)_4]/[H_2O]^4/[\mathbf{O_2}]^4=400000*2,23*10^{-44}$;
arterial K=0,96*(10^(-7,36))^4*(0,0154)^4/0,04/55^4/6/10^(-5)<>0,96/0,04/6/10^(-5)=400000*
=(10^(-7,36))^4*(0,0154)^4/55^4=2,23*10^(-44)
venous K=0,66*(10^(-7,36))^4*(0,0154)^4/0,34/55^4/0,486/10^(-5)<>0,66/0,34/0,486/10^(-5)=399419
Circulation cycle generate $[\mathbf{H^+}]=459*6\cdot10^{-5}$ M =0,0275 M=$[\mathbf{HCO_3^-}]=[H^+]$;
Normal $[HCO_3^-]$=0,0154 M, $[CO_{2aqua}]$=0,0076 M and pH=7,36
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 $(\mathbf{H^+}His63,58)_4\mathbf{Hb_T}$ (Tense state), oxy hemoglobin $(\mathbf{O_2}His63,58)_4\mathbf{Hb_R}$ (**Relax** state) and with carbonic anhydrase **CA** driven bicarbonate buffer systems a joint physiological mechanism of action carries out the inhaled $\mathbf{O_2}$ and exhaled $\mathbf{CO_2}$ between AIR in ***lungs*** and tissues on interface human body / environment**.**

3) Third bicarbonate buffer system in human organism creates oxidation reactions.

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 $Q + CO_{2aqua} + 2H_2O <^{CA}> H_3O^+ + HCO_3^-$ 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_3O^+ concentration increase as oxidation product CO_{2aqua} forms two H_3O^+ and HCO_3^-.

Absence of carbonic anhydrase **CA** reaction drives to left as CO_2 evaporated out consuming H_3O^+ and HCO_3^- in **lungs** and acid concentration $[H_3O^+]$ remains stabilized at homeostasis level **pH**=7.36. If concentration H_3O^+ decreases, so increases **pH>7.36** in **kidneys**, carbonic anhydrase equilibrium is shifted to the right and the extra amount of HCO_3^- passes into urine and is transported out and pH stabilizes to homeostasis **pH=7.36** level according Le Chatelier's principle.

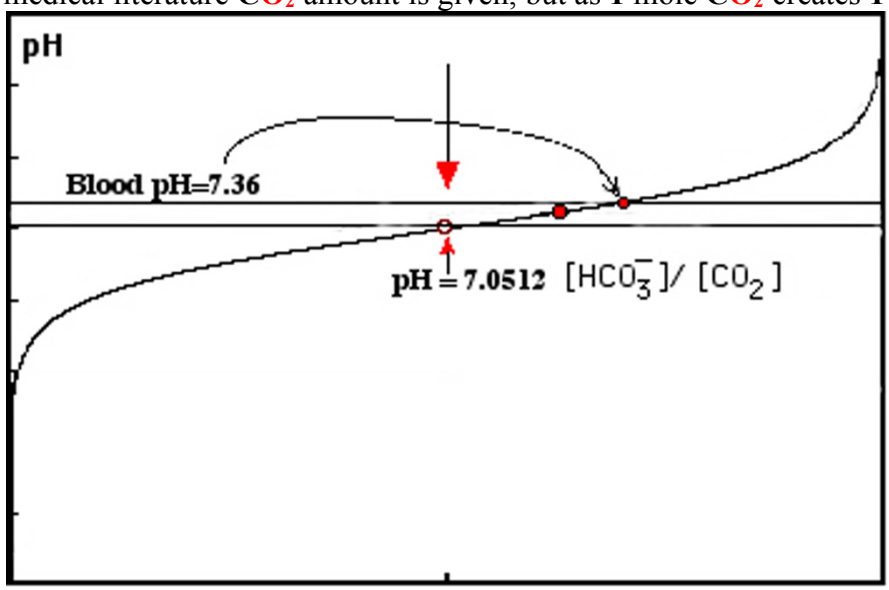
The Brønsted acid is blood-water solution CO_{2aqua}**,** which in. The dissolved into water H_2O (into blood) carbonic dioxide CO_{2aqua} occurring in cell converted with carbonic anhydrase **CA** to $H_3O^+ + HCO_3^-$**.** The water H_2O and carbonic dioxide CO_{2aqua}, finally, is acid in direct equilibrium 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_3O^+ are involved into blood **pH** formation according buffer solution

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

$\frac{[HCO_3^-]}{[CO_{2\,aqua}]} = 10^{(pH-pK)} = 10^{(7.36-7.0512)} = 10^{0.3088} = \frac{2.0361}{1}$ 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 $[CO_{2aqua}]$.

Alkaline reserve at **7.36** = **pH** is **normal** as $\frac{[HCO_3^-]}{[CO_{2\,aqua}]} = \frac{2.0361}{1}$.

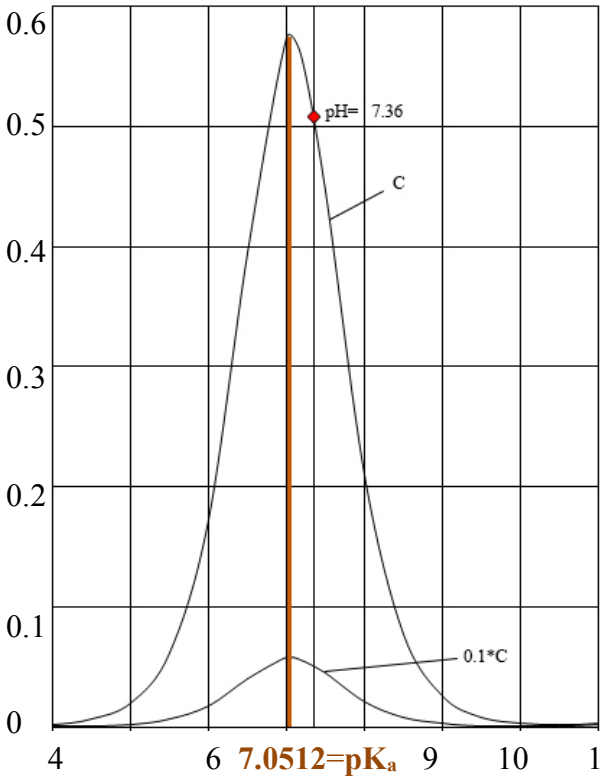
HCO_3^-0% **50%** 100% **salt** – buffer system **base**

CO_2+ $2H_2O$ 100% **50%** 0% **weak acid** buffer component

As soon as H_3O^+ concentration grows for some reason, Carbonic anhydrase **CA** equilibrium is shifted to left and channeling H_3O^+ and HCO_3^- transported CO_2 out by respiration in **lungs** so acid concentration [H_3O^+] stabilizes. If concentration H_3O^+ 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_3O^+ 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 **7.36** is Prigogine attractor for organisms.



*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•10^{-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_3O^+ are included into equation for blood **pH**.

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

I) $O_{2AIR}+H_2O \overset{membrane}{\rightleftharpoons} H_2O+O_{2aqua}$; $4O_{2aqua}+$ **deoxy**$(H^+His63,58)_4Hb_T <=[O_2]=6\cdot10^{-5}$ M$=>oxyHb_R(O_2)_4+4H^+$,

Oxidation products $C_6H_{12}O_6+6O_{2aqua}+6H_2O \leftarrow \underline{Krebs\_Cycle} \rightarrow 6CO_{2aqua}+12H_2O \leftarrow \underline{CA} \rightarrow 6H_3O^+ + 6HCO_3^-$

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

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

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

Enthalpy heat consumed ΔH_H for reaction endothermic: $\Delta H_H=\Delta H°_{H3O}-\Delta H°_{HCO3}-2\Delta H°_{H2O}-\Delta H°_{CO2}=9{,}7576$ $^{kJ}/_{mol}$

$= -285{,}81-689{,}93-(2*-285{,}85-413{,}7076) = -975{,}74+985{,}3276=9{,}7576$ $^{kJ}/_{mol}$ endothermic

Endothermic as needed heat supply to drive reaction forwards. Hess free energy change endoergic positive:

$\Delta G_{Hess}=\Delta G°_{H3O}+\Delta G°_{HCO3}-2\Delta G°_{H2O}-\Delta G°_{CO2}=-213{,}2746$**-544,9688**$-(2*-237{,}191-385{,}98)=102$ $^{kJ}/_{mol}$.

Enzyme **CA** make classic acid constant weak $K_a = 10^{-7.0512}$ or exponent $pK_a = 7{,}0512$ so very close to **pH** value 7,36. Water concentration $[H_2O]$=55.3 M is constant so included in value K_{eq}.

Enzyme **CA** drive reaction with two water molecules endoergic:

$CO_{2aqua}+2H_2O \leftarrow \underline{CA} \rightarrow H_3O^+ + HCO_3^-$; $10^{-7.0512}=K_a=[H_2O]^2K_{eq}=[H_3O^+][HCO_3^-]/[CO_{2aqua}]$ as unfavored

$\frac{[HCO_3^-]_{aqua}\cdot[H_3O^+]}{[CO_2]_{aqua}\cdot[H_2O]^2} = K_{eq}=K_a/[H_2O]^2=10^{-7{,}0512}/55{,}3457339^2=2{,}906*10^{-11}=10^{-10.54}$. Free energy change is

$\Delta G_{eq}=-RTln(K_{eq})=-8.3144\bullet298.15\bullet\ln(10^{-10.224})=60.145$ $^{kJ}/_{mol}$ smaller as Hess value 102 $^{kJ}/_{mo}$ according Prigogine energy change minimum for equilibrium, where R=8,3144 $^{J}/_{mol/K}$ and T=298,15 K (25° C).

II) process second gradual reaction concentration gradient and electrochemical membrane potential bicarbonate ion HCO_3^- and proton H^+ 1. $E_H=P\bullet lg([10^{-pH}{}_{extraMit}/10^{-pH}{}_{Mitochon})=0{,}06154*lg(10\text{^}2{,}36)=0{,}14523$ **V**;

2. $E_{HCO3-Mitochon}= -P\bullet log([HCO_3^-{}_{cytosol}]/[HCO_3^-{}_{Mitochon}])= -0{,}06154*log(0.0154/0.0338919)= 0{,}0210821$ **V;**

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

3. $\Delta G_{HCO3-}=RTln([HCO_{3cytosol}]/[HCO_{3Mitoch}])=8{,}3144*310{,}15*log(0{,}0154/0{,}0338919)= -2{,}0341094$ $^{kJ}/_{mol}$;

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

Total $\Delta G_{total}=\Delta G_F+(\Delta G_{HCO3-}+\Delta G_{H+})=-16{,}0471+(-2{,}0341094)+(-23{,}3943)= -41{,}4755$ $^{kJ}/_{mol}$ exoergic transfer.

exoergic reaction is driven by concentration gradients through **proton** and **bicarbonate channels** of **membrane**.

II) process third gradual neutralisation:

$H_3O^+ + HCO_3^- \rightarrow 2H_2O+CO_{2aqua}+Q=$**7,1928** $^{kJ}/_{mol}$ exothermic$+\Delta G=$**-102** $^{kJ}/_{mol}$ exoergic.

$\Delta G_{Hess}=2\Delta G°_{H2O}+\Delta G°_{CO2}-\Delta G°_{H3O}-\Delta G°_{HCO3}=2*-237{,}191-385{,}98-(-213{,}2746$**-544,9688**$)=$**-102** $^{kJ}/_{mol}$;

$\Delta H_{Hess}=2\Delta H°_{H2O}+\Delta H°_{CO2}-\Delta H°_{H3O}-\Delta H°_{HCO3}=2*-285{,}85-413{,}7976-(-285{,}81$**-692,4948**$)=$**-7,1928** $^{kJ}/_{mol}$;

$v_2=k_2\bullet[H_3O^+][HCO_3^-]=$**1,6958*10^15**$*10^{(-5)}*0{,}0154=261153200$ M^2s^{-1}; Neutralisation velocity;

Extra Mitochondrial pH=5 at presence of CA and Alveolar epithelia cell surface pH=5 at absence CA.

II) process fourth gradual reaction is non-enzymatic evaporation: $[CO_{2aqua}]=0{,}0004*1{,}878=0{,}00075125$ M;

Evaporation at absence Carbonic Anhydrase CA $CO_{2aqua}+$**Q**$(20{,}3$ $^{kJ}/_{mol})$ endothermic $<=>CO_2\uparrow_{gas}+$**ΔG**$(-8{,}379$ $^{kJ}/_{mol})$;

Substance	$\Delta H°_{Hess}$, $^{kJ}/_{mol}$	$\Delta S°_{Hess}$, $^{J}/_{mol/K}$	$\Delta G°_{Hess}$, $^{kJ}/_{mol}$
H_3O^+	-285,81	-3,854	-213,274599
$-OH^-$	-230,015	-10,9	-157,2
HCO_3^-	-689,93	98,324	-586,93988
HCO_3^-	**-692,4948**	**-494,768**	**-544,9688**
H_2O	-285,85	69,9565	-237,191
H_2O	**-286,65**	**-453,188**	**-151,549**
CO_{2aqua}	-413,7976	117,5704	-385,98
$CO_2\uparrow_{gas}$	-393,509	213,74	-394,359

Evaporation $\Delta H_{Hess}=\Delta H°_{CO2gas}-\Delta H°_{CO2aq}=20{,}3$ $^{kJ}/_{mol}$ $=-393{,}509+413.7976=20{,}3$ $^{kJ}/_{mol}$; **endothermic**.....

Evaporation $\Delta G_{Hess}=\Delta G°_{CO2gas}-\Delta G°_{CO2aq}=-8{,}379$ $^{kJ}/_{mol}$ $=-394{,}359+385{,}98= -8{,}379$ $^{kJ}/_{mol}$ **exoergic**.....

Solubility $\Delta G_{Hess}=\Delta G°_{CO2aq}-\Delta G°_{CO2gas}=8{,}379$ $^{kJ}/_{mol}$

$K_{sp}=K_{eq}=EXP(-\Delta G_{eq}/R/T)=0{,}034045=1/29{,}375$

$\frac{[CO_2\ aqua]}{[CO_2\ gas]\cdot[H_2O]} = K_{sp}=0{,}034045=1/29{,}375$

$\frac{[CO_2\ gas]\cdot[H_2O]}{[CO_2\ aqua]} = K_{evaporation}=29{,}375$; Evaporation equilibrium at absence Carbonic Anhydrase CA.

$[CO_2\uparrow_{gas}]=29{,}375*[CO_{2aqua}]/[H_2O]=29{,}375*0{,}0076/55{,}3457339=0{,}00403$ mol fraction; pH=7,36 .

$[HCO_3^-]=0{,}0154$ M and $[CO_{2aqua}]=0{,}0076$ M if pH=7,36; At pH=5=7,0512+log(0,001/$[CO_{2aqua}]$);

10^(5-7,0512)= 0,001/$[CO_{2aqua}]$; $[CO_{2aqua}]=0{,}001/10\text{^}(5-7{,}0512)=0{,}1125$; pH=5 ;

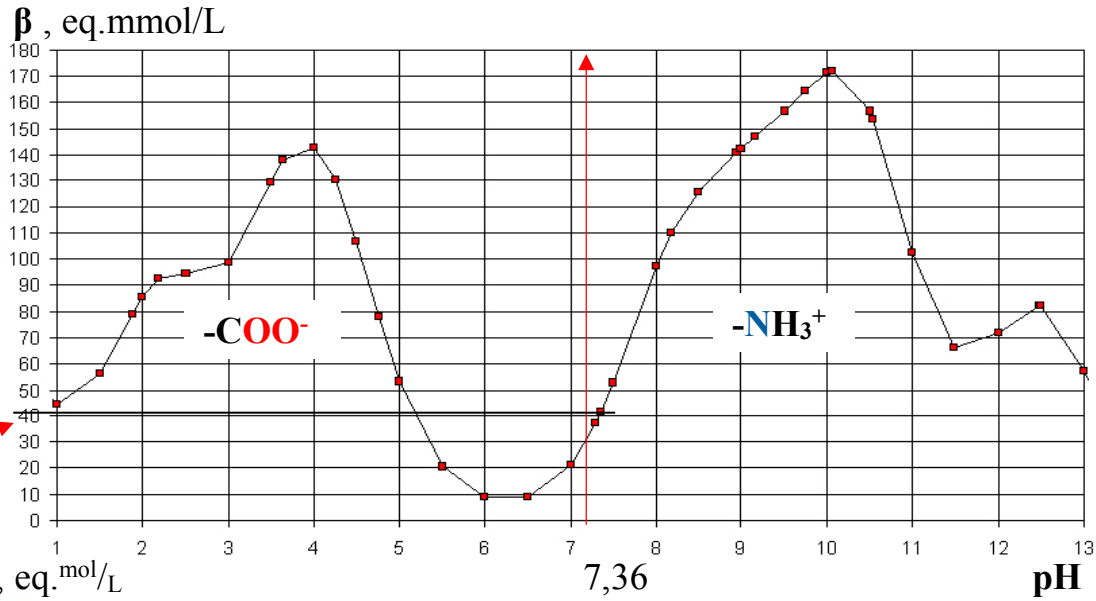
$[CO_2\uparrow_{gas}]=29{,}375*[CO_{2aqua}]/[H_2O]=29{,}375*0{,}1125/55{,}3457339=0{,}05971$ mol fraction; Atmospheric 0,0004.

Three buffer systems of human organism: proteins + phosphates + bicarbonate
Total buffer capacity: proteins +([$H_2PO_4^-$]+[HPO_4^{2-}])+([CO_{2aqua}]+[HCO_3^-])
Total buffer capacity: 100% = **4,7%** + **93,9%** + **1,4%**;

Cytosol muscle cells

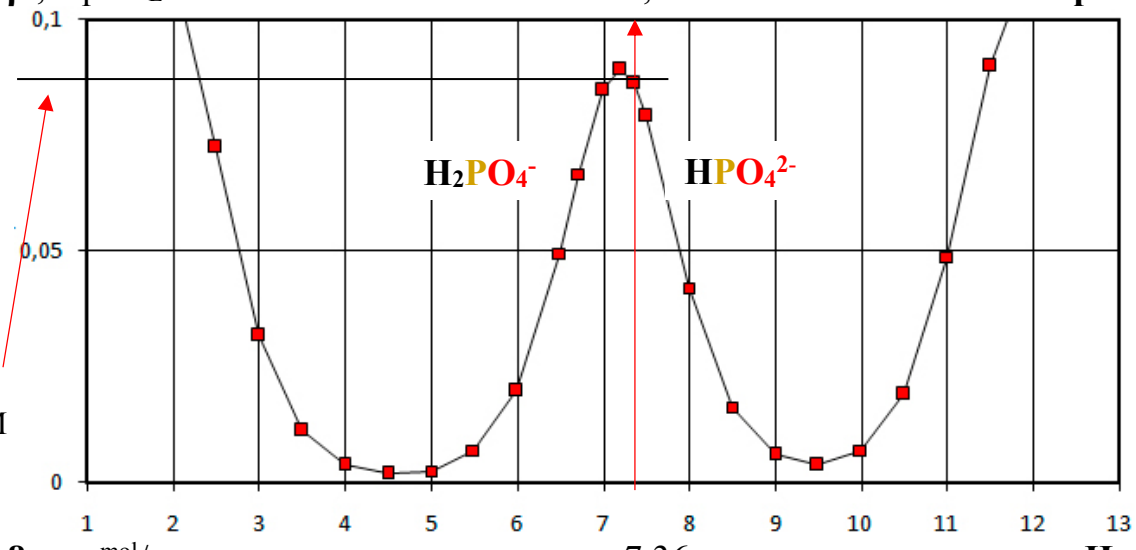
Buffer capacity is strong acid Δn_{sk} or base Δn_b equivalent·mol / in one Liter of buffer solution **ΔpH=±1**

Proteins buffer have silence region from **pH=6** to 7,36 . 23 thousand protein molecules total buffer solution concentration C_{buffer}=135 mM groups 3 mM pmuscle roteins
Buffer capacity at physiologic pH=7,36 is
β =40 mM
4,7 %=40/852*100%

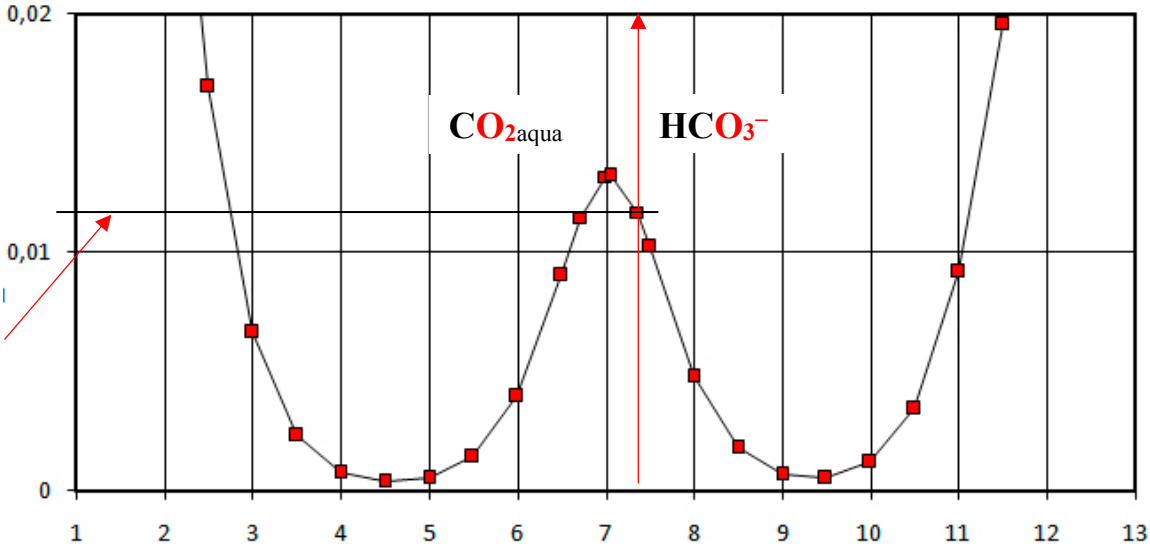


Total phosphate buffer systems concentration [$H_2PO_4^-$]+[HPO_4^{2-}] in muscle cells cytosole is C_{buffer}=0,155 M

Buffer capacity at physiologic pH=7,36 is
β =800 mM
93,9 %=800/852*100%



Total bicarbonate buffer system concentration [CO_{2aqua}]+[HCO_3^-] in blood plasma is C_{buffer}=0,023 M
Buffer capacity at physiologic pH=7,36 is
β =12 mM
1,4%=12/852*100%



Total

Buffer capacity β_{sum}=40+800+12=852 eq.$^{mmol}/_{L}$ 7,36 **pH**

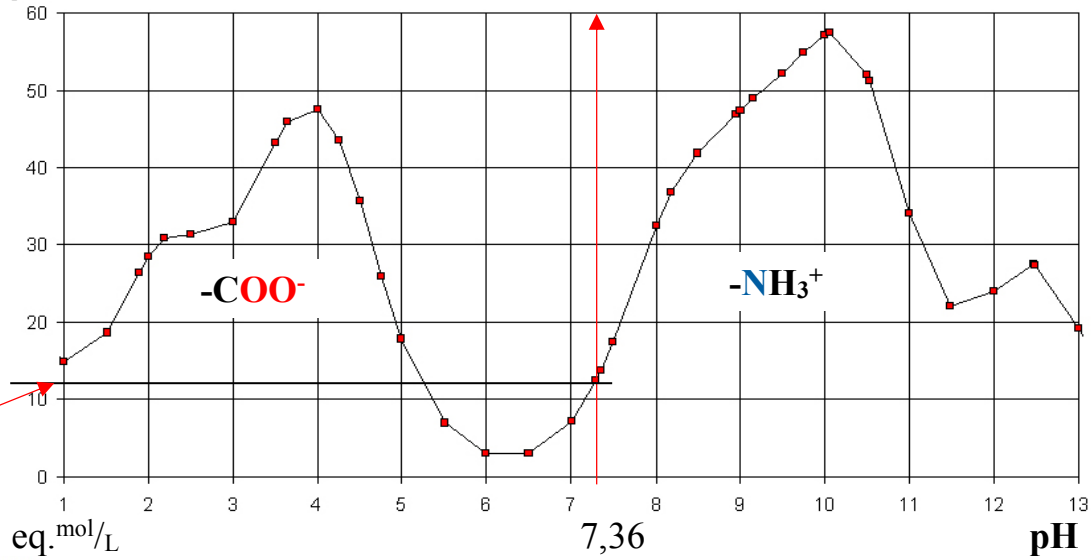
Three buffer systems in human organism 1) proteins, 2) phosphates and 3) bicarbonate sum

Three buffer systems of human organism: proteins + phosphates + bicarbonate
Total buffer capacity: proteins +([$H_2PO_4^-$]+[HPO_4^{2-}])+([CO_{2aqua}]+[HCO_3^-])
Total buffer capacity: 100% = **46,15%** + **7,7%** + **46,15%**;

Extra Cellular space Blood plasma

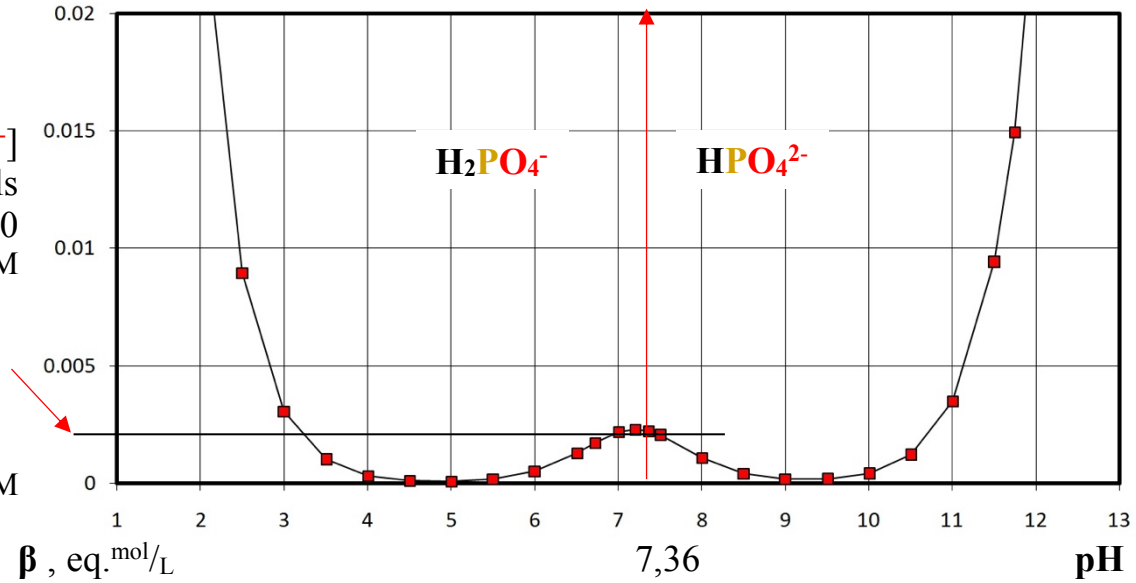
Buffer capacity is strong acid Δn_{sk} or base Δn_b equivalent·mol / in one Liter of buffer solution **ΔpH=±1**

Proteins buffer have silence region from **pH=6** to 7,36 . 23 thousand protein molecules total buffer solution concentration C_{buffer}=45 mM groups 1 mM albumin
Buffer capacity at physiologic pH=7,36 is
β =12 mM
46,15%=12/26*100%



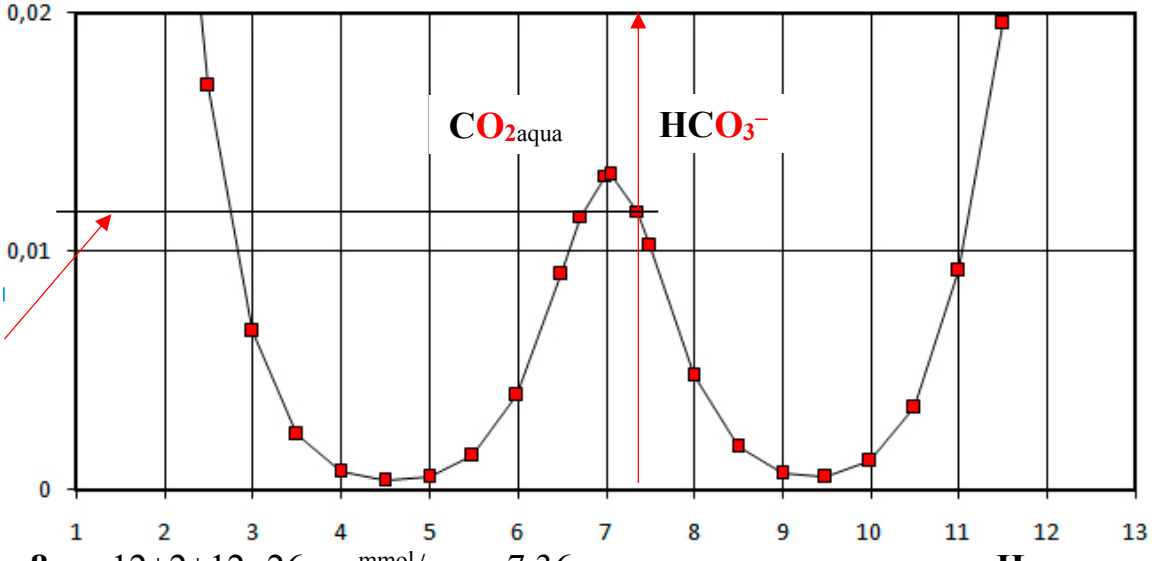
Total phosphate buffer systems concentration [$H_2PO_4^-$]+[HPO_4^{2-}] in muscle cells cytosole is C_{buffer}=0,0
β =2 mM
7,7 %==2/26*100%
M

Buffer capacity at physiologic pH=7,36 is
β =2 mM
7,7 %==2/26*100%



Total bicarbonate buffer system concentration [CO_{2aqua}]+[HCO_3^-] in blood plasma is C_{buffer}=0,023 M
Buffer capacity at physiologic pH=7,36 is

β =12 mM
45,45%=12/26,4*100%



Total
Buffer capacity β_{sum}=12+2+12=26 eq.$^{mmol}/_{L}$

Three buffer systems in human organism 1) proteins, 2) phosphates and 3) bicarbonate sum

1) **Hepta peptide Ser-Cys-Arg-Tyr-Asp-Lys-Glu** eight protolytic equilibria constants:

pK_a : 9,15, 8,18, 12,48, 10,07, 3,65, 10,53, 4,25, 2,19

$$K_{aCOOH}=\frac{[Glu\text{-}COO^-]\cdot[H^+]}{[Glu\text{-}COOH]_{nedis}};\ K_{aGluCOOH}=\frac{[Glu\text{-}COO^-]\cdot[H^+]}{[Glu\text{-}COOH]_{nedis}};\ K_{aLysNH3+}=\frac{[Lys\,NH_2]\cdot[H^+]}{[Lys\,NH_3^+]_{protonēts}};$$

$$K_{aAspCOOH}=\frac{[Asp\,OO^-]\cdot[H^+]}{[Asp\,OOH]_{nedis}};\ K_{aTyrFenolsOH}=\frac{[Rfenol\,O^-]\cdot[H^+]}{[Rfenol\,OH]_{nedis}};\ K_{aRArgNH+}=\frac{[Arg\,NH]\cdot[H^+]}{[Arg\,NH_2^+]_{protonēts}};$$

$$K_{aCysSH}=\frac{[R\text{-}CS^-]\cdot[H^+]}{[R\text{-}CSH]_{nedis}};\ K_{aNH3+}=\frac{[Ser\,NH_2]\cdot[H^+]}{[Ser\,NH_3^+]_{protonēts}};$$

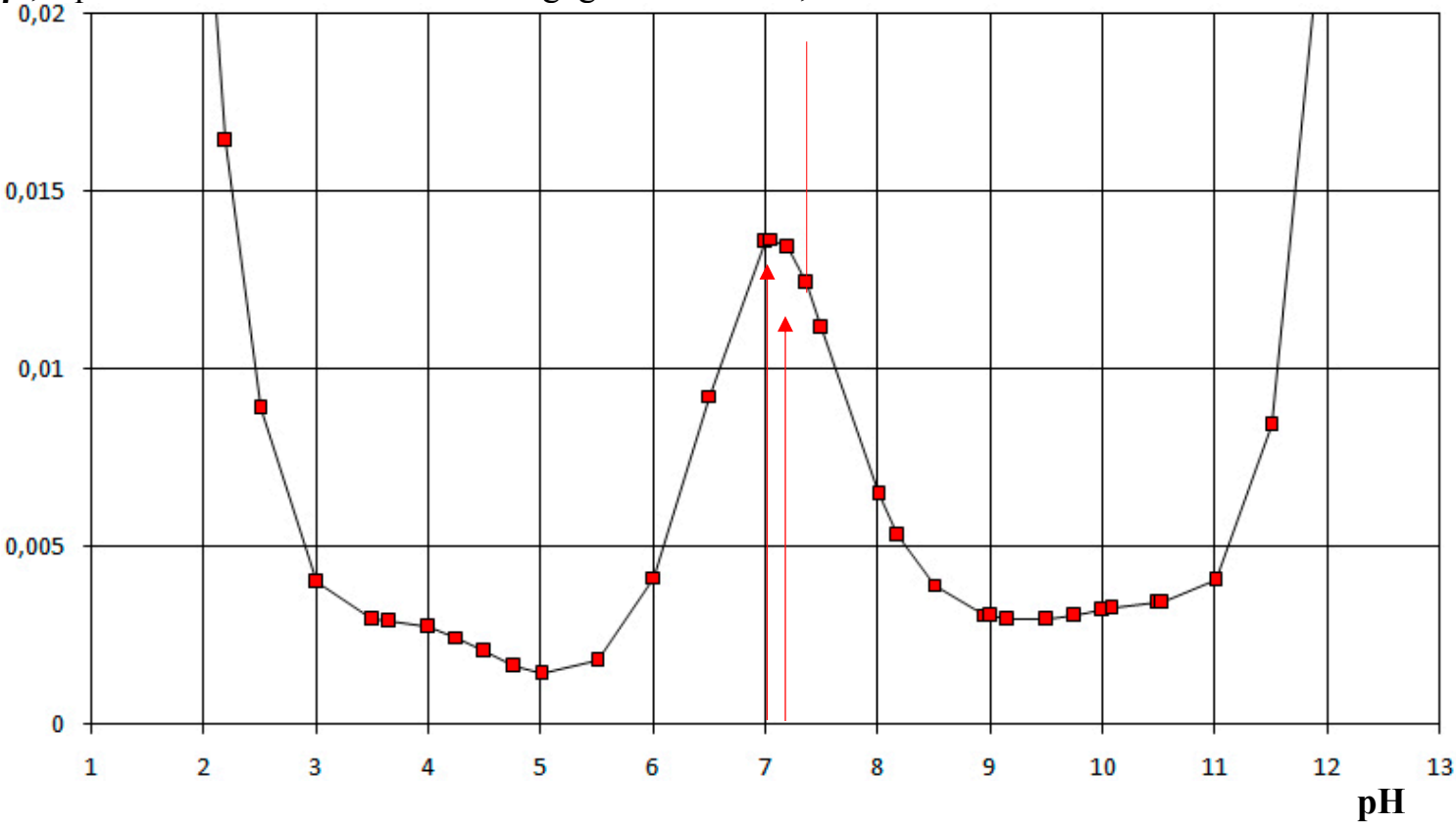
Prigogine attractor 7,36 value locates on Δ**pH**=1,36 broad silence interval from 6 to 7,36.

2) **Phosphate buffer $H_2PO_4^-$/ HPO_4^{2-}**; $7.36=pH=pK_a+\log\frac{[HPO_4^{2-}]}{[H_2PO_4^-]}=7{,}199+\log\frac{1{,}45}{1}=7{,}36$

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

$$7.36=pH=pK+\log\frac{[HCO_3^-]}{[CO_{2\,aqua}]}=7.0512+\log\frac{2{,}0361}{1}=7{,}36;$$

β , eq.mol/L Prigogine attractor 7,36 value



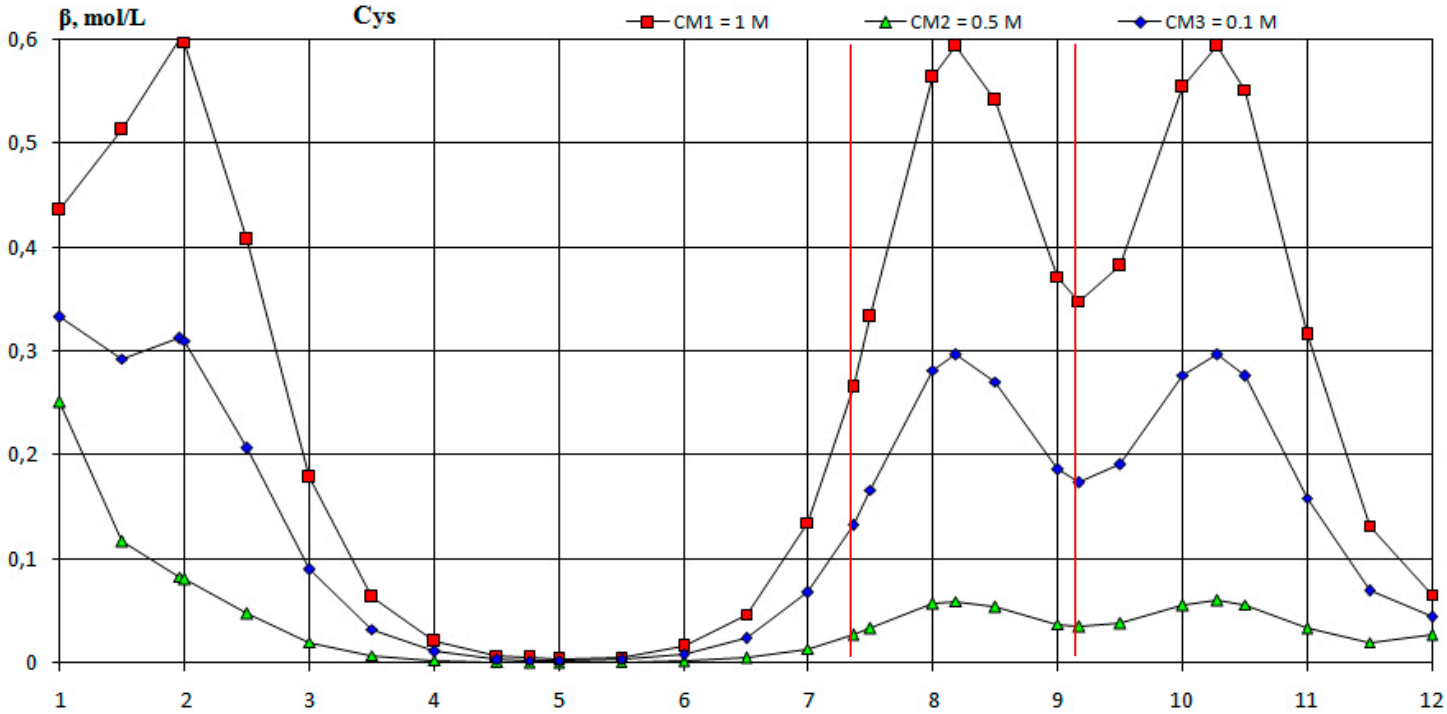
Three buffer systems sum in living organisms forms broadband buffer capacity maximum at bicarbonate protolytic constant value pK_a=7,0512 in range from pH=5 to pH=9.

Amino acids (proteins) broadband buffer system Cysteine Cys

$$K_{aCOOH} = \frac{[AA\text{-}COO^-]\cdot[H^+]}{[AA\text{-}COOH]_{nedis}};\ K_{aNH3+} = \frac{[AA\text{-}NH_2]\cdot[H^+]}{[AA\text{-}NH_3^+]_{protonēts}};\ K_{aRSH} = \frac{[R\text{-}CS^-]\cdot[H^+]}{[R\text{-}CSH]_{nedis}};$$

Buffer capacity strong acid Δn_{ac} or strong base Δn_b mol /Liter

Prigogine attractor 7,36 value included in five Δ**pH**=5,86 units interval from 6,36 to 12,1



Concentration of Buffer solution C_{buffer}= 1 M red

Concentration of Buffer solution C_{buffer}=0.5 M blue

Concentration of Buffer solution C_{buffer}=0.1 M green

1 Buffer solution dilution dose no change value pH is constant as $\mathbf{n_{salt}/n_{acid}}$= constant.

the same for ten times diluted buffer $\mathbf{n_{salt}/n_{acid}}$=const. amount ratio logarithm is log(1)=0!

1.a Water drinking in human organism pH=7,36 value do not change and not intact!

2. Buffer capacity is proportional to concentration **β~C** !

3. Broadband buffer system capacity has tree maximal values β_{max} . Mark on graph !

pK_{aCOOH} =1,96, K_{aNH3+} =10,28, pK_{aRSH} =8,18 .

4. Tree maximal values are β_{max} = 0,6•**C** jo **β=0,6•1** $^{mol}/_{L}$ **= 0.6** $^{ekv.mol}/_{L}$!

5. Buffer solution middle point **pH**=9,17 capacity against acid and base

are symmetric equal β_{ac}**=0,35** $^{ekv.mol}/_{L}$ **=**β_b, ,

adding strong acid **0,35** $^{ekv.mol}/_{L}$ Δ**pH**=-1 decreases about one unit,

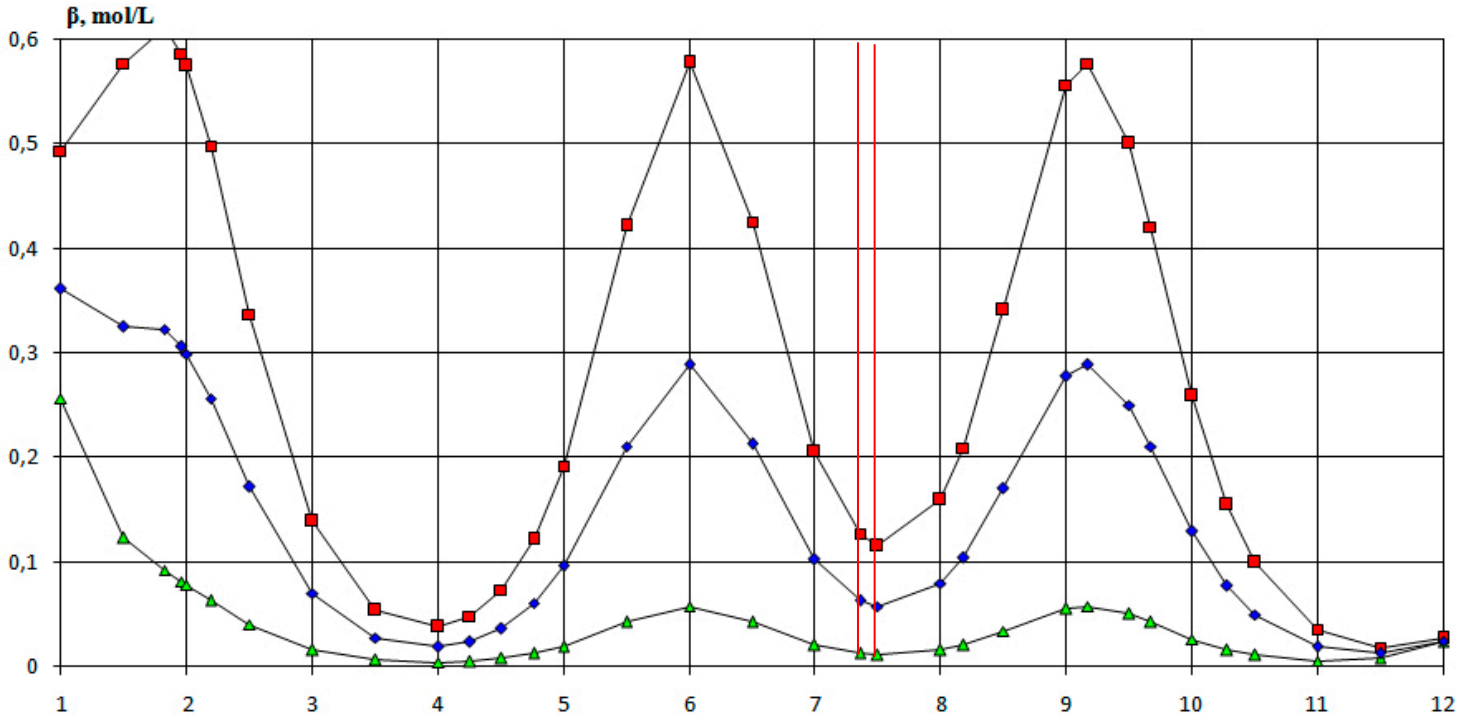
adding strong base **0,35** $^{ekv.mol}/_{L}$ Δ**pH**=+1 increases about one unit!

Amino acids (proteins) broadband buffer system Histidine His

$$K_{aCOOH} = \frac{[AA\text{-}COO^-]\cdot[H^+]}{[AA\text{-}COOH]_{nedis}};\ K_{aNH3+} = \frac{[AA\text{-}NH_2]\cdot[H^+]}{[AA\text{-}NH_3^+]_{protonēts}};\ K_{aNH+} = \frac{[RN]\cdot[H^+]}{[RNH^+]_{protonēts}};$$

Buffer capacity strong acid Δn_{ac} or strong base Δn_b mol /Liter

Prigogine attractor 7,36 value included in seven Δ**pH**=7 units interval from 4 līdz 11



Concentration of Buffer solution C_{buffer}= 1 M red

Concentration of Buffer solution C_{buffer}=0.5 M blue

Concentration of Buffer solution C_{buffer}=0.1 M green

1 Buffer solution dilution dose no change value pH is constant as $\mathbf{n_{salt}/n_{acid}}$= constant.

the same for ten times diluted buffer $\mathbf{n_{salt}/n_{acid}}$=const. amount ratio logarithm is log(1)=0!

1.a Water drinking in human organism pH=7,36 value do not change and not intact!

2. Buffer capacity is proportional to concentration **β~C** !

3. Broadband buffer system capacity has tree maximal values β_{max} . Mark on graph !

pK_{aCOOH}=1,82, K_{aNH3+}=9,17, p K_{aNH+}=6 .

4. Tree maximal values are β_{max} = 0,58•**C** jo **β=0,58•1** $^{mol}/_{L}$ **= 0.058** $^{ekv.mol}/_{L}$!

5. Buffer solution middle point **pH**=7,5 capacity against acid and base

are symmetric equal β_{ac}**=0,12** $^{ekv.mol}/_{L}$ =β_b, ,

adding strong acid **0,12** $^{ekv.mol}/_{L}$ Δ**pH**=-1 decreases about one unit,

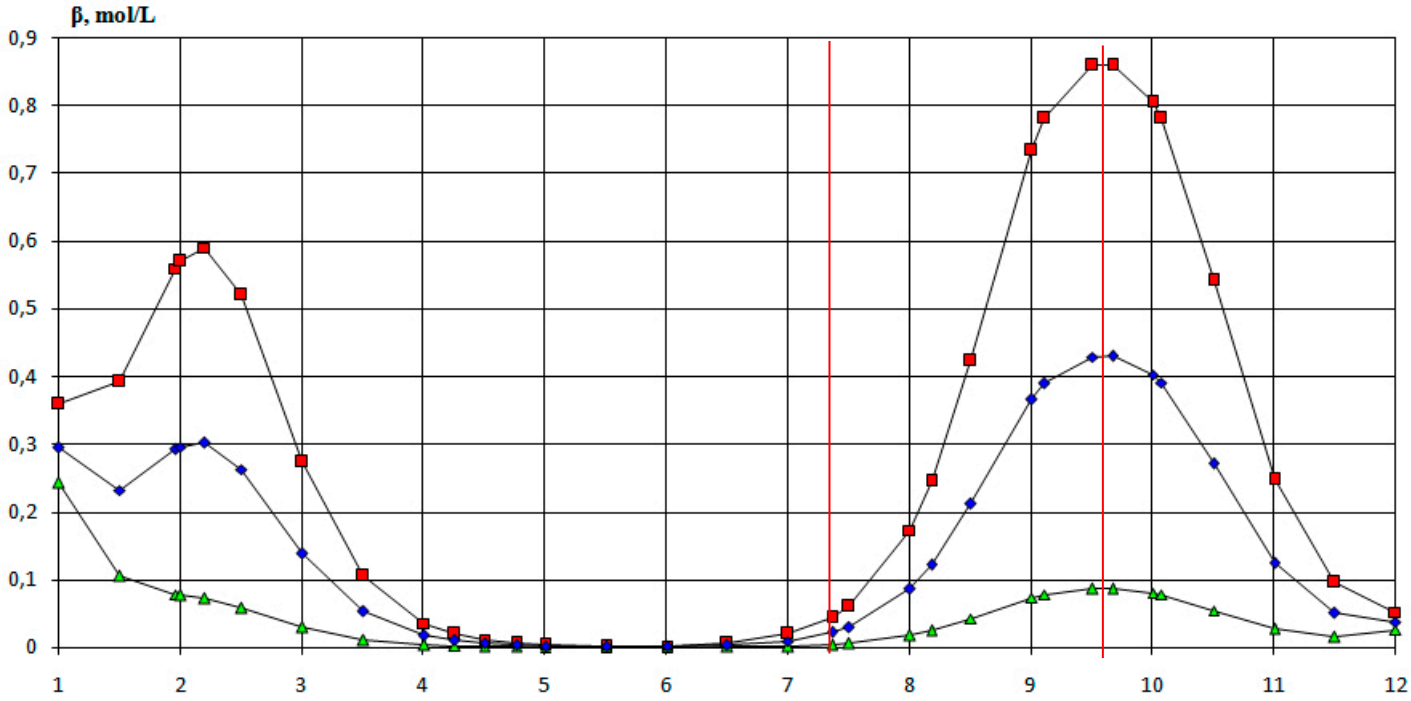
adding strong base **0,12** $^{ekv.mol}/_{L}$ Δ**pH**=+1 increases about one unit!

Amino acids (proteins) broadband buffer system Tyrosine Tyr

$$K_{aCOOH} = \frac{[AA\text{-}COO^-]\cdot[H^+]}{[AA\text{-}COOH]_{nedis}};\ K_{aNH3+} = \frac{[AA\text{-}NH_2]\cdot[H^+]}{[AA\text{-}NH_3^+]_{protonēts}};\ K_{aRphenolOH} = \frac{[RfenolO^-]\cdot[H^+]}{[RfenolOH]_{nedis}};$$

Buffer capacity strong acid Δn_{ac} or strong base Δn_b mol /Liter

Prigogine attractor 7,36 value included in piecu Δ**pH**=4,5 vienību intervālā no 7,5 maksimums 9,6 līdz 12



Concentration of Buffer solution C_{buffer}= 1 M red

Concentration of Buffer solution C_{buffer}=0.5 M blue

Concentration of Buffer solution C_{buffer}=0.1 M green

1 Buffer solution dilution dose no change value pH is constant as $\mathbf{n_{salt}/n_{acid}}$= constant.

the same for ten times diluted buffer $\mathbf{n_{salt}/n_{acid}}$=const. amount ratio logarithm is log(1)=0!

1.a Water drinking in human organism pH=7,36 value do not change and not intact!

2. Buffer capacity is proportional to concentration **β~C** !

3. Broadband buffer system capacity has tree maximal values β_{max} . Mark on graph !

pK_{aCOOH} =2,2, K_{aNH3+} =9,11, $K_{aRphenolOH}$ =10,07 .

4. Maximum pH=9,6 value β_{max}=0,86•**C** , jo **β=0,86•1** $^{mol}/_{L}$**=0.86** $^{ekv.mol}/_{L}$ maximal!

5. Buffer solution maximum **pH**=9,6 capacity against acid and base

are symmetric equal β_{ac}**=0,86** $^{ekv.mol}/_{L}$ **=**β_b, ,

adding strong acid **0,86** $^{ekv.mol}/_{L}$ Δ**pH**=-1 decreases about one unit,

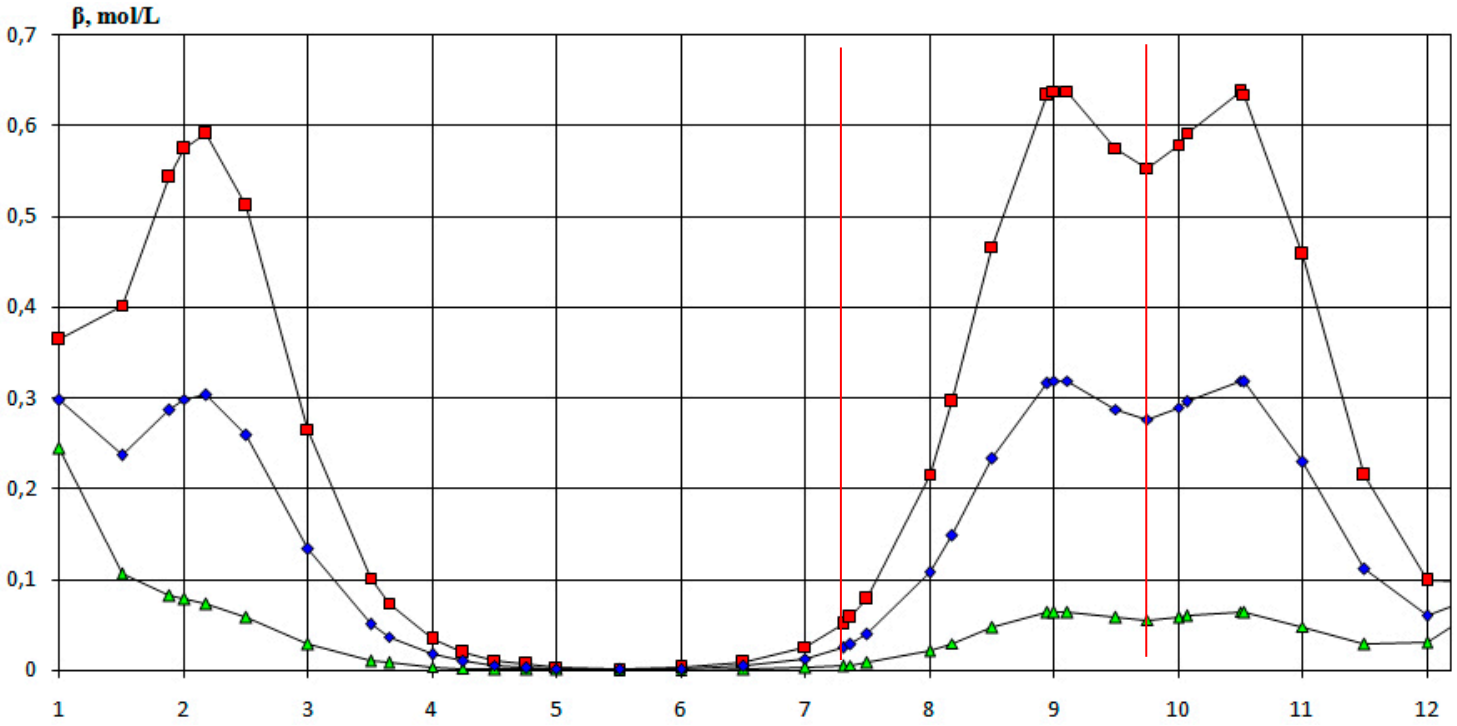
adding strong base **0,86** $^{ekv.mol}/_{L}$ Δ**pH**=+1 increases about one unit!

Amino acids (proteins) broadband buffer system Lysine Lys

$$K_{aCOOH} = \frac{[AA\text{-}COO^-]\cdot[H^+]}{[AA\text{-}COOH]_{nedis}};\ K_{aNH3+} = \frac{[AA\text{-}NH_2]\cdot[H^+]}{[AA\text{-}NH_3^+]_{protonēts}};\ K_{aRNH3+} = \frac{[RNH_2]\cdot[H^+]}{[RNH_3^+]_{protonēts}};$$

Buffer capacity strong acid Δn_{ac} or strong base Δn_b mol /Liter

Prigogine attractor 7,36 value included in septiņu Δ**pH**=4,9 vienību intervālā no 7,3 līdz 12,2



Concentration of Buffer solution C_{buffer}= 1 M red

Concentration of Buffer solution C_{buffer}=0.5 M blue

Concentration of Buffer solution C_{buffer}=0.1 M green

1 Buffer solution dilution dose no change value pH is constant as $\mathbf{n_{salt}/n_{acid}}$= constant.

the same for ten times diluted buffer $\mathbf{n_{salt}/n_{acid}}$=const. amount ratio logarithm is log(1)=0!

1.a Water drinking in human organism pH=7,36 value do not change and not intact!

2. Buffer capacity is proportional to concentration **β~C** !

3. Broadband buffer system capacity has tree maximal values β_{max} . Mark on graph !

pK_{aCOOH}=2,18, K_{aNH3+}=8,95, pK_{aRNH3+}=10,53 .

4. Middle point pH=9,75 value is β_{max}=0,55•**C** , jo **β=0,55•1** $^{mol}/_{L}$**=0.55** $^{ekv.mol}/_{L}$!

5. Buffer solution middle point **pH**=9,75 capacity against acid and base

are symmetric equal β_{ac}**=0,12** $^{ekv.mol}/_{L}$ **=**β_b, ,

adding strong acid **0,55** $^{ekv.mol}/_{L}$ Δ**pH**=-1 decreases about one unit,

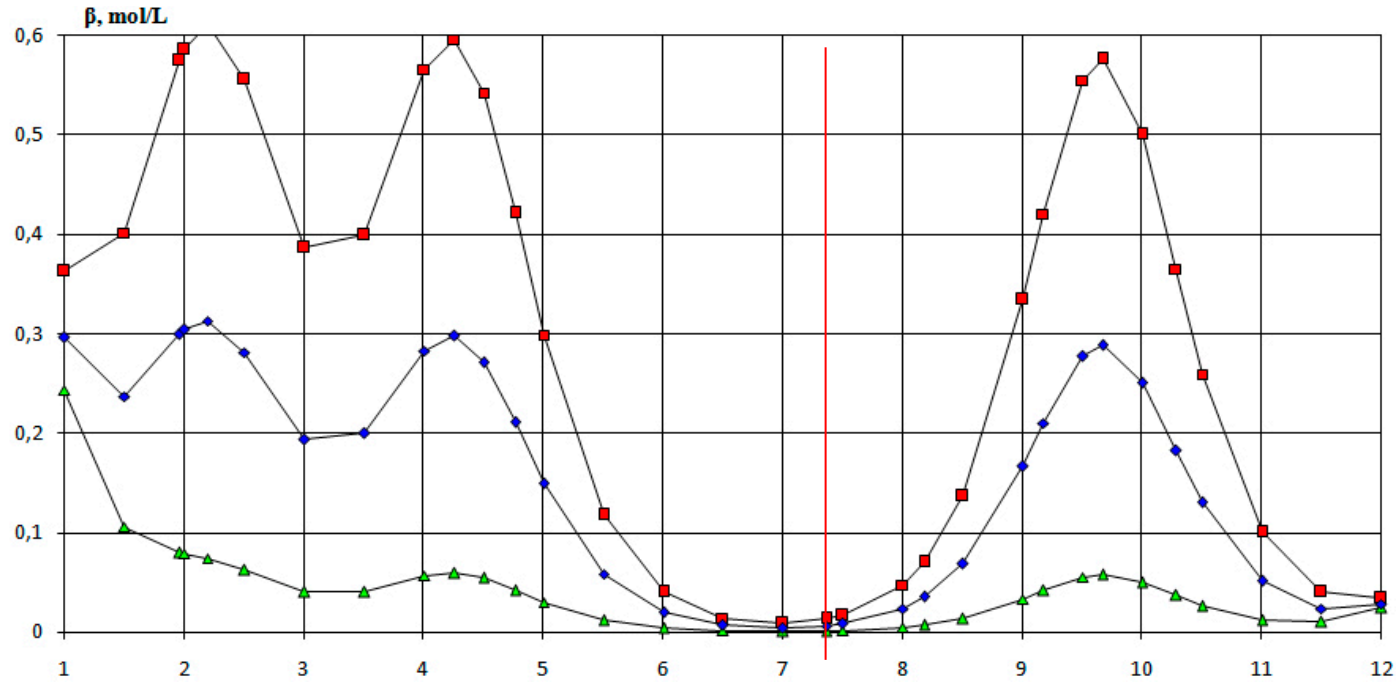
adding strong base **0,55** $^{ekv.mol}/_{L}$ Δ**pH**=+1 increases about one unit!

Amino acids (proteins) broadband buffer system Aspartate Asp

$$K_{aCOOH} = \frac{[AA\text{-}COO^-]\cdot[H^+]}{[AA\text{-}COOH]_{nedis}};\ K_{aNH3+} = \frac{[AA\text{-}NH_2]\cdot[H^+]}{[AA\text{-}NH_3^+]_{protonēts}};\ K_{aRCOOH} = \frac{[R\text{-}COO^-]\cdot[H^+]}{[R\text{-}COOH]_{nedis}};$$

Buffer capacity strong acid Δn_{ac} or strong base Δn_b mol /Liter

Prigogine attractor 7,36 value not included in aspartate buffer systems



Concentration of Buffer solution C_{buffer}= 1 M red

Concentration of Buffer solution C_{buffer}=0.5 M blue

Concentration of Buffer solution C_{buffer}=0.1 M green

1 Buffer solution dilution dose no change value pH is constant as $\mathbf{n_{salt}/n_{acid}}$= constant.

the same for ten times diluted buffer $\mathbf{n_{salt}/n_{acid}}$=const. amount ratio logarithm is log(1)=0!

1.a Water drinking in human organism pH=7,36 value do not change and not intact!

2. Buffer capacity is proportional to concentration **β~C** !

3. Broadband buffer system capacity has tree maximal values $\mathbf{\beta_{max}}$ outside of Prigogine attractor 7,36. Mark on graph ! pK_{aCOOH} =1,88, K_{aNH3+} =9,6, K_{aRCOOH} =3,65 .

4. Tree maximal values of $\mathbf{\beta_{max}}$ = 0,55•C jo **β=0,55•1** $^{mol}/_{L}$ **= 0.55** $^{ekv.mol}/_{L}$!

5. Physiologic pH=7,36 not depends on aspartate buffer solution

are symmetrical equal $\mathbf{\beta_{ac}=0,0}$ $^{ekv.mol}/_{L}$ $\mathbf{=\beta_b}$, ,

adding strong acid **0,0** $^{ekv.mol}/_{L}$ **ΔpH**=0 does not have resistance as buffer capacity,

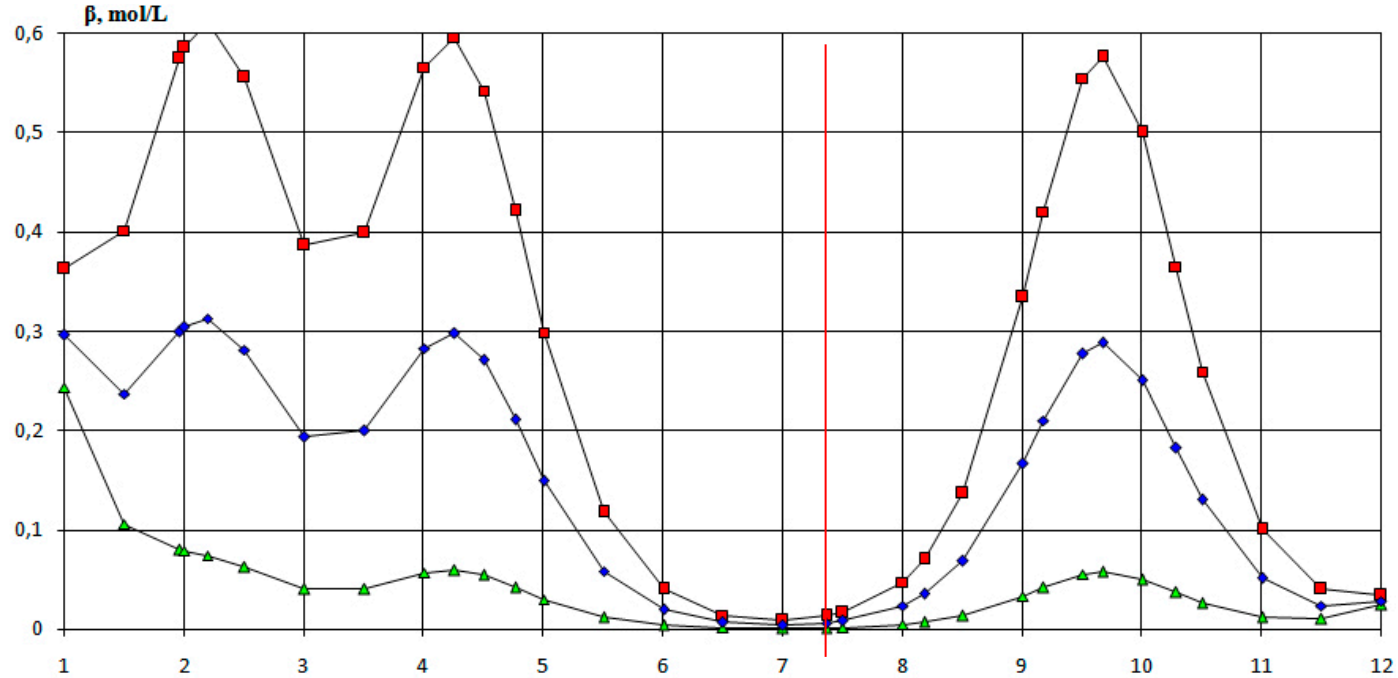
adding strong base **0,0** $^{ekv.mol}/_{L}$ **ΔpH**=0 does not have resistance as buffer capacity!

Amino acids (proteins) broadband buffer system Glutamate Glu

$$K_{aCOOH} = \frac{[AA\text{-}COO^-]\cdot[H^+]}{[AA\text{-}COOH]_{nedis}};\ K_{aNH3+} = \frac{[AA\text{-}NH_2]\cdot[H^+]}{[AA\text{-}NH_3^+]_{protonēts}};\ K_{aRCOOH} = \frac{[R\text{-}COO^-]\cdot[H^+]}{[R\text{-}COOH]_{nedis}};$$

Buffer capacity strong acid Δn_{ac} or strong base Δn_b mol /Liter

Prigogine attractor 7,36 value not included in glutamate buffer systems



Concentration of Buffer solution C_{buffer}= 1 M red

Concentration of Buffer solution C_{buffer}=0.5 M blue

Concentration of Buffer solution C_{buffer}=0.1 M green

1 Buffer solution dilution dose no change value pH is constant as $\mathbf{n_{salt}/n_{acid}}$= constant.

the same for ten times diluted buffer $\mathbf{n_{salt}/n_{acid}}$=const. amount ratio logarithm is log(1)=0!

1.a Water drinking in human organism pH=7,36 value do not change and not intact!

2. Buffer capacity is proportional to concentration **β~C** !

3. Broadband buffer system capacity has tree maximal values β_{max} outside of Prigogine attraktor 7,36. Mark on graph ! pK_{aCOOH} =2,19, K_{aNH3+} =9,67, K_{aRCOOH} =4,25 .

4. Tree maximal values of β_{max} = 0,55•**C** jo **β=0,55•1** $^{mol}/_{L}$ = **0.55** $^{ekv.mol}/_{L}$!

5. Physiologic pH=7,36 not depends on glutamate buffer solution

are symmetrical equal β_{ac}**=0,0** $^{ekv.mol}/_{L}$ =β_b, ,

adding strong acid **0,0** $^{ekv.mol}/_{L}$ **ΔpH**=0 does not have resistance as buffer capacity,

adding strong base **0,0** $^{ekv.mol}/_{L}$ **ΔpH**=0 does not have resistance as buffer capacity!

Proteins have broadband buffer systems with activity minimum in range from 6 to 7,36. Human organism dominate bicarbonate and phosphate buffer systems. In organisms processes leed Prigogine attractor in direction pH=7,36. Carbonic anhydrase CA enzyme drive protolysis reaction by pK_a=7,0512 value and phosphate protolysis reaction by constant pK_a=7,199 value determnes physiologic pH value 7,36 with alkaline reserve : 2/1 un 1,45/1 respectively.

Hepta peptide Ser-Cys-Arg-Tyr-Asp-Lys-Glu eight protolytic equilibria

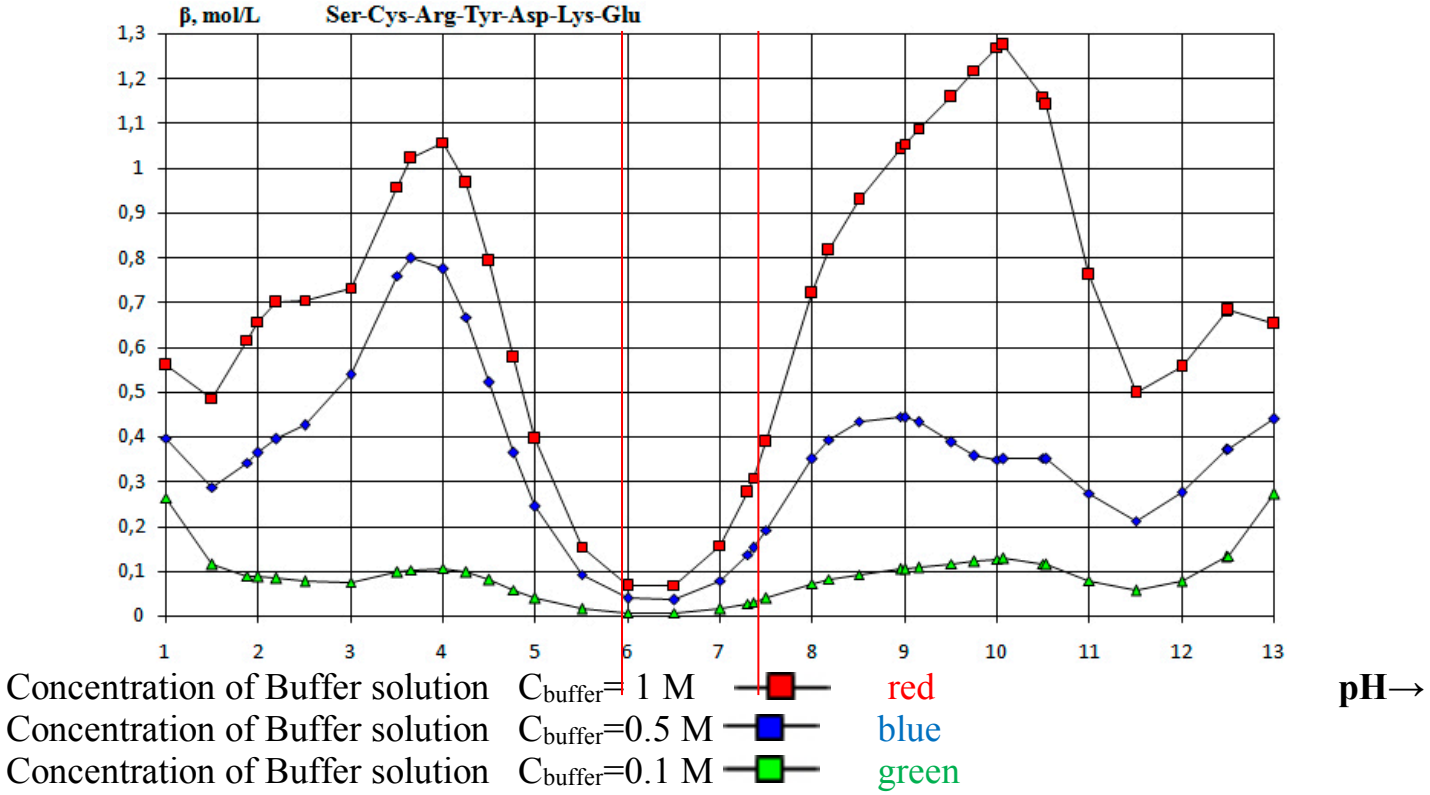
pK_a : 9,15, 8,18, 12,48, 10,07, 3,65, 10,53, 4,25, 2,19

$$K_{aCOOH}=\frac{[\text{Glu-COO}^-]\cdot[H^+]}{[\text{Glu-COOH}]_{nedis}};\ K_{aGluCOOH}=\frac{[\text{Glu-COO}^-]\cdot[H^+]}{[\text{Glu-COOH}]_{nedis}};\ K_{aLysNH3+}=\frac{[\text{Lys}NH_2]\cdot[H^+]}{[\text{Lys}NH_3^+]_{protonēts}};$$

$$K_{aAspCOOH}=\frac{[\text{Asp}COO^-]\cdot[H^+]}{[\text{Asp}COOH]_{nedis}};\ K_{aTyrFenolsOH}=\frac{[\text{Rfenol}O^-]\cdot[H^+]}{[\text{Rfenol}OH]_{nedis}};\ K_{aRArgNH+}=\frac{[\text{Arg}NH]\cdot[H^+]}{[\text{Arg}NH_2^+]_{protonēts}};$$

$$K_{aCysSH}=\frac{[\text{R-C}S^-]\cdot[H^+]}{[\text{R-C}SH]_{nedis}};\ K_{aNH3+}=\frac{[\text{Ser}NH_2]\cdot[H^+]}{[\text{Ser}NH_3^+]_{protonēts}};$$

Prigogine attractor 7,36 value included in Δ**pH**=1,36 units interval from 6 to 7,36.



Concentration of Buffer solution C_{buffer}= 1 M red

Concentration of Buffer solution C_{buffer}=0.5 M blue

Concentration of Buffer solution C_{buffer}=0.1 M green

Proteins show buffer activity minimum in interval from pH=6 to pH=7,36 values. It depends on twenty proteinogenic amino acids 47 protolytic constants, which are shown in table on

7th page: http://aris.gusc.lv/BioThermodynamics/BufferSolution.pdf.

Small rise at 7,36 from 7 to 7,36enhance resistence against adding acid.

Carbonic anhydrase CA enzyme drive protolysis reaction by pK_a=7,0512 value and phosphate protolysis reaction by constant pK_a=7,199 value determnes physiologic pH value 7,36 with alkaline reserve : 2/1 un 1,45/1 respectively.

Hepta peptide His-Cys-Arg-Tyr-Asp-Lys-Glu nine protolytic equilibria

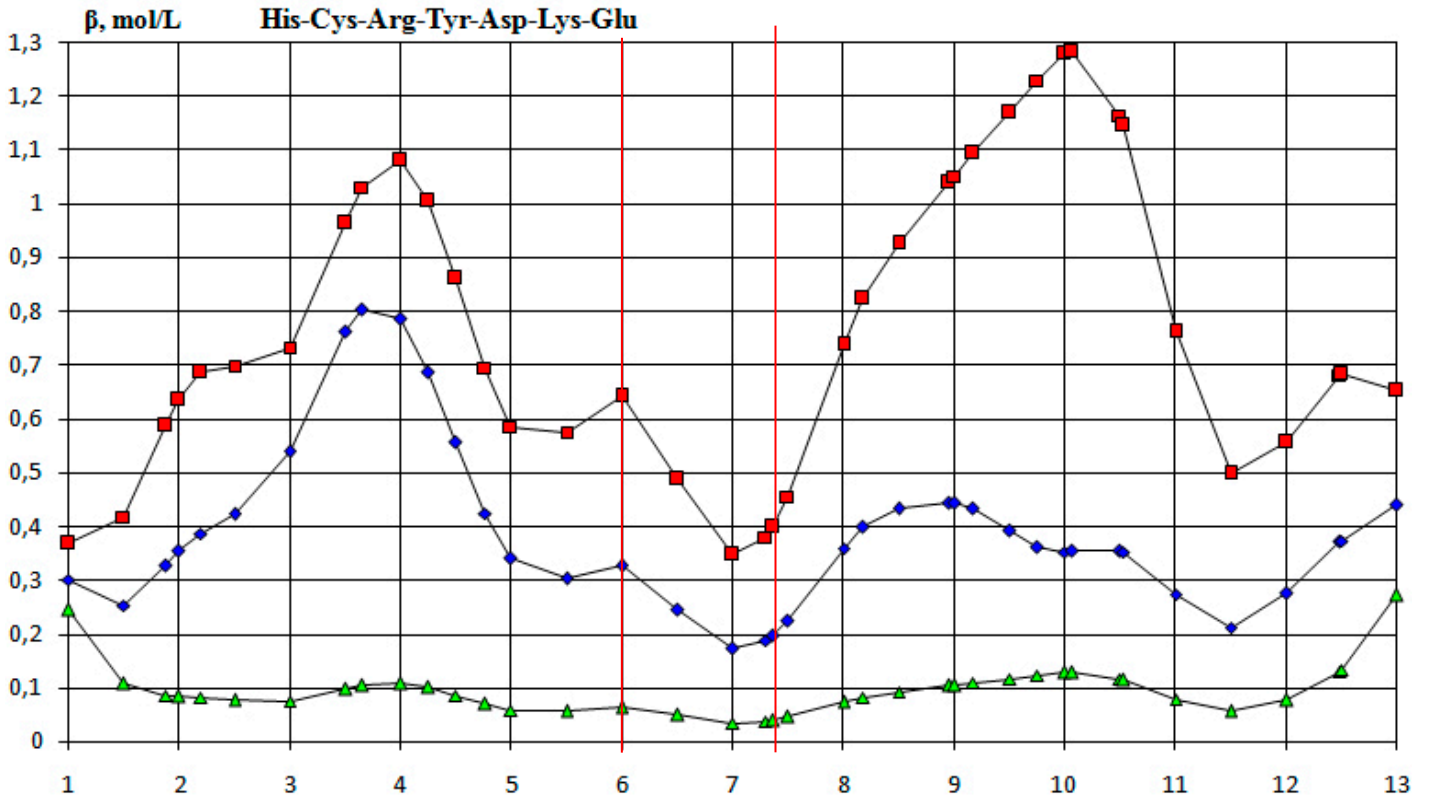
pK_a : 9,17, 6,00, 8,18, 12,48, 10,07, 3,65, 10,53, 4,25, 2,19

$$K_{aCOOH}=\frac{[Glu\text{-}COO^-]\cdot[H^+]}{[Glu\text{-}COOH]_{nedis}};\ K_{aGluCOOH}=\frac{[Glu\text{-}COO^-]\cdot[H^+]}{[Glu\text{-}COOH]_{nedis}};\ K_{aLysNH3+}=\frac{[Lys\,NH_2]\cdot[H^+]}{[Lys\,NH_3^+]_{protonēts}};$$

$$K_{aAspCOOH}=\frac{[Asp\,OO^-]\cdot[H^+]}{[Asp\,OOH]_{nedis}};\ K_{aTyrFenolsOH}=\frac{[Rfenol\,O^-]\cdot[H^+]}{[Rfenol\,OH]_{nedis}};\ K_{aRArgNH+}=\frac{[Arg\,NH]\cdot[H^+]}{[Arg\,NH_2^+]_{protonēts}};$$

$$K_{aCysSH}=\frac{[R\text{-}CS^-]\cdot[H^+]}{[R\text{-}CSH]_{nedis}};\ K_{aRHisNH+}=\frac{[His\,N]\cdot[H^+]}{[His\,NH^+]_{protonēts}};\ K_{aNH3+}=\frac{[His\,NH_2]\cdot[H^+]}{[His\,NH_3^+]_{protonēts}};$$

Prigogine attractor 7,36 value included in Δ**pH**=1,36 units interval from 6 to 7,36.



Concentration of Buffer solution C_{buffer}= 1 M red

Concentration of Buffer solution C_{buffer}=0.5 M blue

Concentration of Buffer solution C_{buffer}=0.1 M green

Histidine do not have influence to phosphate and bicarbonate buffer systems , becouse joined in coordinative compounds with electron pair acceptor ions Zn^{2+}, Fe^{2+}, Fe^{3+} and at physiologic pH=7,36 histidine is deprotonated du to pK_a=6 value protonation preseeds only for smal values as pH< 6 in acidic medium.

Proteins have buffer activity minimum in range from pH=7 to pH=7,36 values.

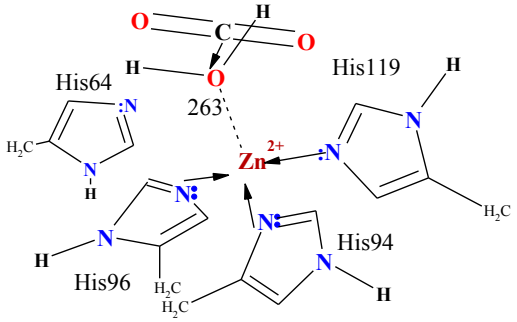
That determine twenty proteinogenic amino acids with 47 protolytic constants values, that given in table ob 7th page: http://aris.gusc.lv/BioThermodynamics/BufferSolution.pdf.

Brønsted Acid/Base **CA** and hemoglobin **shuttle** enzymes of O_2 ⇔ 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} + $2H_2O$ ⇄ CA(Zn^{2+}) ⇄ H_3O^+ + HCO_3^-

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

$Hb_R(O_2)_4$+**4**H^+ ⇔ **4**O_{2aqua} + (H^+His63,58)$_4Hb_T$ stabilizing arterial concentration [O_2]=6·10^{-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}+$2H_2O$ ←^{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±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^-…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←^{Membrane}→H_2O+$CO_2\uparrow_{gas}$+$H_2O\uparrow_{gas}$ + ΔG_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]=6·10^{-5} M sensitive:
4O_{2aqua} + (H^+His63,58)$_4Hb_T$···sāls···tiltiņi(HCO_3^-)$_4$⇔ $Hb_R(O_2)_4$+**4**H^+ +4 HCO_3^-. ***lungs*** and ***tisues***.

Lungs venous blood hemoglobin saturation with oxygen 459 times restore circulated arterial blood [O_2]=6·10^{-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]=6·10^{-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.

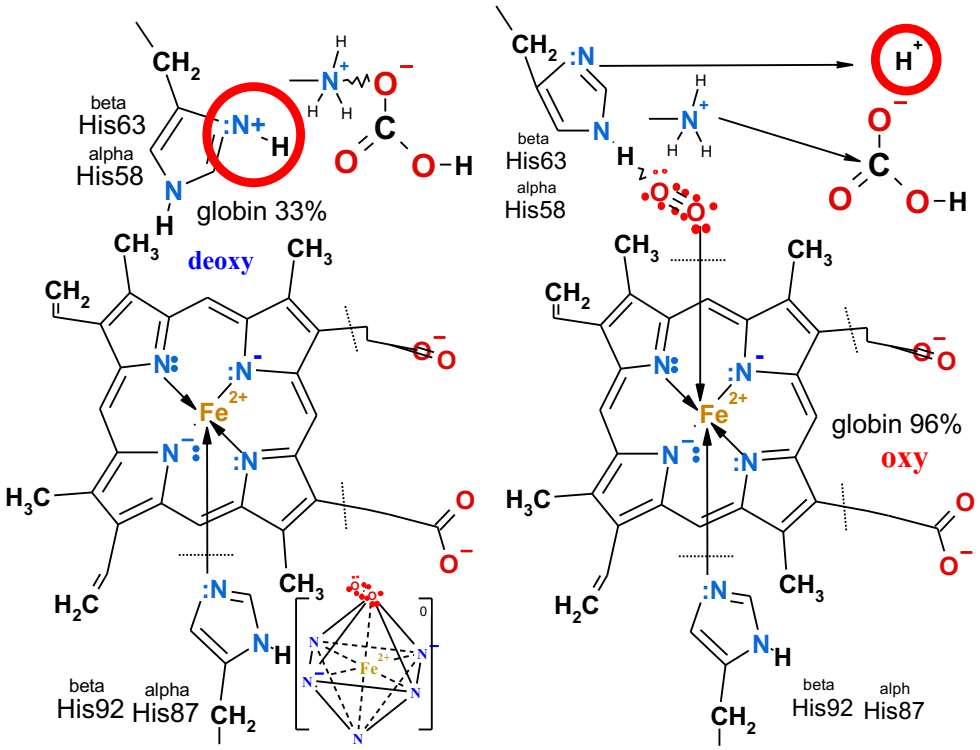
In such a way two equilibria stabilize arterial oxygen concentration [O_{2aqua}]=6·10^{-5} M with **shuttle** hemoglobin by oxygen adsorbtion-desorbtion and **CA** buffer system made value pH=7,36 with Krebs cycle drive the exchange metabolism of O_2 and CO_2 respiration to interface human body / environment.

Shuttle deoxy - oxy **hemoglobin** with Carbonic Anhydrase enzyme in O_2 , CO_2 metabolism stabilize physiologic pH=7.36 and oxygen arterial concentration $[O_{2blood}]=6\cdot10^{-5}$ M

I) Oxygen O_2 from **AIR** 20.95% $O_2\uparrow$gas assimilation reaction dissolution in water to form O_{2aqua} exothermic ΔH_r=-11,7 $^{kJ}/_{mol}$ and endoergic ΔG_{sum}= 12,11 $^{kJ}/_{mol}$ as water soluble **1)** $O_{2AIR}+H_2O \Leftrightarrow H_2O+O_{2aqua}+Q+\Delta G$. Concentration gradient $[O_2]=9{,}768\cdot10^{-5}$ M to venous blood $[O_2]=1{,}85\cdot10^{-5}$ M exoergic ΔG_{O2}= RT**ln**($[O_{2Blood}]/[O_{2aqua}]$)= - 4,29 $^{kJ}/_{mol}$ osmosis: 2) $O_{2aqua}+H_2O \xrightarrow{Aquaporins} H_2O+O_{2aqua}+\Delta G_{H2O}$ =RTln($[H_2O]_{right}/[H_2O]_{left}$)= -1.088 $^{kJ}/_{mol}$ exoergic.

Sum is $\Delta G_{sum}=\Delta G_{O2}+\Delta G_{H2O}+\Delta G_r$= +12,11 $^{kJ}/_{mol}$ endoergic at inspiration of fresh AIR but exothermic. $4O_{2aqua}$ from blood plasma adsorbs **deoxy** hemoglobin Hb_T releases four protons $4H^+$, $4HCO_3^-$ stabilizing arterial concentration $4O_{2aqua}+(H^+His63,58)_4Hb_T\cdot$salt bridges$(HCO_3^-)_4 \Leftrightarrow Hb_R(O_2)_4+4H^++4HCO_3^-$: $[O_2]=6\cdot10^{-5}$ M and pH=7,36..

$[O_{2Blood}]=6\bullet10^{-5}$ 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 $[O_{2Blood}]=6\bullet10^{-5}$M amount $[O_{2amount}]=459*6\bullet10^{-5}$ 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 $[O_2]=6\cdot10^{-5}$ 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 $[H^+]=459*6\bullet10^{-5}$ 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: $0.5415\bullet10^{-3}$ μm^3 = $0.5415\bullet10^{-18}$ 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:

$[O_{2adsorbed}]=[H_3O^+]=459*6\bullet10^{-5}$ 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 $H_2O\uparrow_{gas}$:

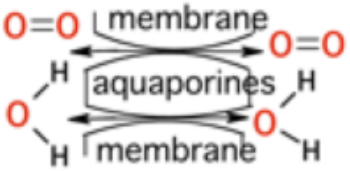
endothermic ΔH_r= +54,5 $^{kJ}/_{mol}$; $H_3O^++HCO_3^- +Q \xleftarrow{Membrane} H_2O+CO_2\uparrow_{gas}+H_2O\uparrow_{gas}$ + ΔG_r= -82,1 $^{kJ}/_{mol}$. exoergic .

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)** $O_{2AIR} + H_2O \overset{aquaporin}{\rightleftarrows} H_2O + O_{2aqua}$



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^-.

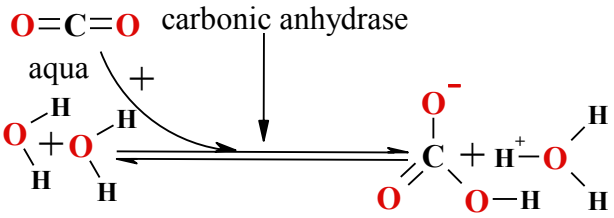
$4O_2$+$(H^+His63,58)_4betaVal1(NH_3^+PO_4^{2-})_2Hb_TG$-↔$(His63,58)_4Arg^+His^+betaVal1(NH_3^+)_2Hb_R(O_2)_4$+$4H^+$+$BPG^{5-}$

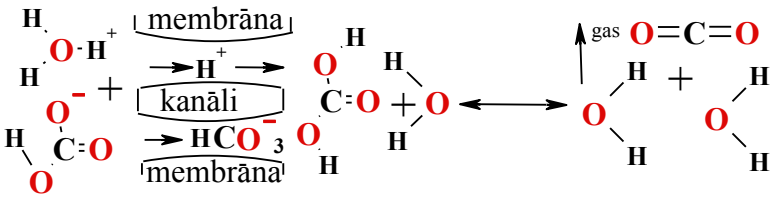
Each adsorbed oxygen molecules O_{2aqua} on hemoglobin releases proton H^+ which increases acidy on epithelial cell surface of ***lungs***. The epithelial cell surface of ***lungs*** has the specific building: super thin 0.6 nm water layer on surface S=950 nm x 950 nm= 0.9 μm^2 as square within small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

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

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 $H^+ + HCO_3^- \rightarrow \overset{membrane}{\rightarrow} H_2CO_3 + Q$. 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.

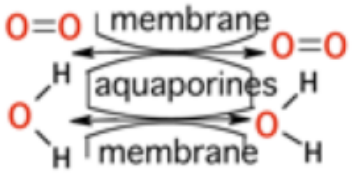
2) Krebs cycle metabolite CO_{2aqua} endothermic reaction with water in ***tissues*** drive carbonic anhydrase shift equilibrium to right $Q + CO_{2aqua} + 2H_2O \xleftarrow{CA} H_3O^+ + HCO_3^-$ forming ratio 1/2,0361 = [CO_{2aqua}]/[HCO_3^-]. Enzyme Carbonic Anhydrase (**CA**) equilibrium shifts reaction 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

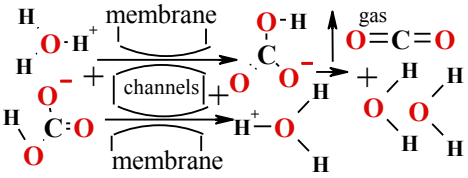
$H_3O^+ + HCO_3^- <=> H_2CO_3 + H_2O + \Delta G$

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

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

IV. BUFFER SOLUTIONS pH studies

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, $K_{HCOOH}=2\cdot10^{-4}$

$$pH=pK_a+\log\frac{C_{salt}\bullet V_{salt}}{C_{acid}\bullet V_{acid}}= -\log 2\bullet10^{-4}+\log\frac{0.09\bullet200}{0.15\bullet300}=3.7-0.398=3.3$$

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, $K_{NH_4OH}=1.8\cdot10^{-5}$.

$$pH=14-pK_b+\log\frac{C_b\bullet V_b}{C_s\bullet V_s}=14-(-\log 1.8\bullet10^{-5})+\log\frac{0.1\bullet80}{0.17\bullet120}=14-4.74+\log0.39=9.26-0.41=8.85$$

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, $K_{HCOOH}=2\cdot10^{-4}$.

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, $K_{HCO_3^-}=4.69\cdot10^{-11}$

a) **pH** before addition of **NaOH** is: $pH_1=-\log 4.69\bullet10^{-11}+\log\frac{0.3\bullet200}{0.2\bullet100}=10.32+\log3=10.8$

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

as the number of moles of **NaOH** is $n = 0.01\cdot0.5 = 0.005$, 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 $n_{salt}= 0.2\cdot0.3 = 0.06$ moles

The number of moles of acid in initial buffer was (acid is $NaHCO_3$ here) : $n_{acid}= 0.1\cdot0.2 = 0.02$ 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:

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

Buffer System studies by numerical Experiment

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: $\beta_{ac} = \dfrac{1ekv \bullet mmol}{0.00434 \bullet 1000mL} = 0.23$ **eq•mol** /L

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

As we could see from the results of calculation, the buffer capacity of buffer system is proportional to concentration at $C^{'}$ = **200** meq/L β_{ac} = **0.23 eq•mol** /L and for ten times diluted concentration $C^{'}$ = **20** meq/L β_{ac} = **0.023 eq•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 $\mathbf{\beta_{base} = (1\ meq) / (0.00434 \bullet 1000mL) = 0.23\ eq \bullet mol/L,}$

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

Experimental Study of Buffer System with Alkaline Reserve

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:

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

$\Delta\mathbf{pH_1 = pH_1 - pH = 5.7166 - 5.74 = 0.023}$ **;** 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 :

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

$\Delta\mathbf{pH_2 = pH_2 - pH = 5{,}76444 - 5.74 = 0.02444}$ 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).