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Synthesis of Carbonic Anhydrase CA indispensable Attractor.

Carbonic Anhydrase reactivity and generate Physiologic buffer solutions total equilibrium value pH=7.36 as Self-Organization Attractors. In reaction CO_2+2H_2O products (CO_{2aqua}) $H_3O^+ + HCO_3^-$ accumulate free energy content $G_{H3O++HCO3-}$=8.38 kJ/mol+60 kJ/mol is indispensable for functional activity of bicarbonate buffer system on the planet Earth for perfect reactions order in homeostasis complex processes.

CO_2 no reaction with water H_2O at absence of CA. CO_2 is small soluble and slow react with OH^-. Solubility $CO_{2gas}+H_2O+\Delta G \Leftrightarrow CO_{2aqua}+Q$ product constant: $K_{spCO2aqua}=[CO_{2aqua}]/[CO_{2gas}]/[H_2O]=0.034$ is unfavored but exothermic $\Delta H_{Hess}=\Delta H°_{CO2aq}-\Delta H°_{CO2gas}=-20.3$ kJ/mol.

$$\Delta G_{spCO2aqua}=-R\bullet T\bullet \ln(K_{spCO2aqua})=-8.3144*298.15*\ln(0.03397)/1000=8.3845 \text{ kJ/mol minimum.}$$

Air 0.04 % mol fraction $[CO_{2gas}]=0.0004$ dissolute concentration is:

$$[CO_{2aqua}]=K_{spCO2aqua}*[H_2O]*[CO_{2gas}]=0.03397*55.3457*0.0004=0.0007512 \text{ M.}$$

Carbon dioxide CO_{2aqua} react with OH^- times $10^{16.54}$ slower about neutralization reaction:

$H_3O^+ + HCO_3^- \Rightarrow CO_{2aqua}+2H_2O+\Delta G+Q$, jo neitralizācijas ātruma konstante ir $k_2=5{,}17*10^{18}\ M^{-2}s^{-1}$, bet reakcijā ar OH^- joniem: $CO_{2aqua}+OH^- \Rightarrow HCO_3^-$ ātruma konstante ir $k_{1OH}=1{,}5*10^2\ M^{-2}s^{-1}$. Eksotermiska reakcija Q:

$\Delta H_{Hess}=\Delta H°_{HCO3}-\Delta H°_{CO2}-\Delta H°_{OH}=-48{,}68$ kJ/mol. $K_{eqOH}=K_{eqCA}/K_{H2O}=2{,}906*10^{(-11)}/3{,}26/10^{(-18)}=9180981{,}6$

$v_{1OH}=k_{1OH}*[CO_{2aqua}]*[OH^-]=k_{1OH}*0{,}0076*10^{(-6{,}63)}=k_{1OH}*0{,}0000000178$;

$$v_{HCO3}=k_{HCO3}*[HCO_3^-]=k_{HCO3}*0{,}0154=$$

$$K_{eqOH}=K_{eqCA}/K_{H2O}=[HCO_3^-]/[CO_{2aqua}]/[OH^-]=2{,}993*10^{(-11)}/3{,}26/10^{(-18)}=k_{1OH}/k_{HCO3}=9180981{,}6.$$

$HCO_3^- \Rightarrow CO_{2aqua}+OH^-+\Delta G+Q$ decomposition reaction: $k_{HCO3}=1{,}5*10^{2}/9180981{,}6=0{,}000016338\ M^{-1}s^{-1}$;

$$K_{eqOH}=9180981{,}6 > K_{HomeostasisOH}=[HCO_3^-]/[CO_{2aqua}]/[OH^-]=0{,}0154/0{,}0076/10^{(-6{,}63)}=8643848$$

$$=\frac{[HCO_3^-].[H_3O^+]}{[CO_2]_{aqua}\cdot[OH^-].[H_3O^+]}=\frac{[HCO_3^-].[H_3O^+]}{[CO_2]_{aqua}\cdot K_{H2O}\cdot[H_2O]^2}=K_{eqOH}=K_{eqCA}/K_{H2O}=2{,}993*10^{(-11)}/3{,}26/10^{(-18)}=9180981{,}6;$$

Reaction with OH^- ions is tims 10^6 slower about CA carbonic anhydrase velocity constant.

CA carbonic anhydrase protolysis reactivity functional activate bicarbonate buffer self-organizing with attraktor pH=7,36 generate concentration gradients H_3O^+, HCO_3^-, CO_{2aqua} for transport and osmosis. [9]

CA karbo anhidrāze liela ātruma protolīzē reaģēt CO_{2aqua} ar divām ūdens molekulām:

$CO_{2aqua}+2H_2O+\Delta G+Q=v1 \xrightarrow{CA} H_3O^+ + HCO_3^-$, kurā ātruma konstante ir: $k_{1CO2aqua}=1{,}5\times10^8\ M^{-1}s^{-1}$. [9]

Neutralization $H_3O^+ + HCO_3^- \xleftrightarrow{CA} CO_{2aqua}+2H_2O$ ātruma konstante ir reizes $10^{10{,}54}$ lielāka par karbo anhidrāzes ātruma konstanti: $k_2/k_{1CO2aqua}=5{,}17*10^{18}/1{,}5/10^8=10^{10{,}7}$. $K_{eqCA}=k_{1CO2aqua}/k_2=1{,}5*10^{8}/\mathbf{10^{18{,}7}}=2{,}993*10^{(-11)}$; $K_{eqOH}=k_{1OH}/k_2=1{,}5/10^2/5{,}17*10^{18}=10^{-16{,}7}$.

CA protolīzes līdzsvara konstanti aprēķina ātruma konstanšu attiecības izteiksmē:

$$K_{eqCAHCO3aqua}=k_{1CO2aqua}/k_2=\frac{[HCO_3^-]_{aqua}.[H_3O^+]}{[CO_2]_{aqua}\cdot[H_2O]^2}=K_{a\_CO2aqua}/[H_2O]^2=10^{-7{,}0512}/55{,}3^2=2{,}993*10^{-11}.$$

Bikarbonāta bufera sistēmas skābes protolīzes konstante $pK_{a\_CO2aqua}=7{,}0512$ ir draudzīga pH vērtībai 7,36:

$$K_{a\_CO2aqua}=K_{eqCAHCO3aqua}*[H_2O]^2=\frac{[HCO_3^-]_{aqua}.[H_3O^+]}{[CO_2]_{aqua}}=10^{-7{,}0512}=10^{-pKa\_CO2aqua}.$$

Oriģināla $pK_{a\_CO2aqua}=7{,}0512$ vērtība iegūta un aprēķināta BUFERA šķīdumam. [1]

Neutralization: $H_3O^+ + HCO_3^- \Leftrightarrow CO_{2aqua}+2H_2O$ ir inversa protolīzei un labvēlīga:

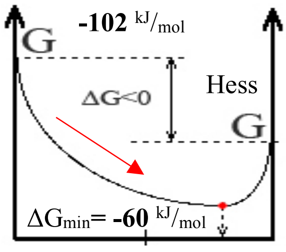
$$K_{eqNeitralizācijaHCO3aqua}=1/K_{eqCA}=1/2{,}993/10^{-11}=33412482242=\frac{[CO_2]_{aqua}\cdot[H_2O]^2}{[HCO_3^-]_{aqua}.[H_3O^+]}$$

Hesa brīvās enerģijas izmaiņa ir eksoerģiska: $\Delta G_{Hess}=2\Delta G°_{H2O}+\Delta G°_{CO2}-\Delta G°_{H3O}-\Delta G°_{HCO3}=\mathbf{-102}$ kJ/mol un entalpijas Hesa izmaiņa ir eksotermiska: $\Delta H_{Hess}=2\Delta H°_{H2O}+\Delta H°_{CO2}-\Delta H°_{H3O}-\Delta H°_{HCO3}=\mathbf{-7{,}2}$ kJ/mol .

Neutralization isr favored reaction. Brīvās enerģijas izmaiņas minimums izteiksmē ir negatīvs:

$$\Delta G_{eqNeitralizācijaHCO3aqua}=-R\bullet T\bullet \ln(K_{eqNeitralizācijaHCO3aqua})=-8{,}3144*298{,}15*\ln(33412482242)/1000=-60 \text{ kJ/mol.}$$

CO_{2aqua} protolīzes minimums ir pozitīvs: $\Delta G_{eqCAHCO3aqua}=-R\bullet T\bullet \ln(K_{eqCA\_HCO3aqua})=60$ kJ/mol.



Reactant $H_3O^+ + HCO_3^-$
products $CO_{2aqua} + 2H_2O$.

Figure 2. Exothermic and exoergic neutralization Hess Free energy change $\Delta G_{eqNeutralization}$ negative **-102** $^{kJ}/_{mol}$, but minimizes $\Delta \mathbf{G_{min}} = \Delta G_{eqNeutralization} = \mathbf{-60}$ $^{kJ}/_{mol}$ reaching equilibrium mixture: $K_{eqNeutralization}=34459000000$ at presence of CA Carbonic Anhydrase. Carbon dioxide reaction with hydroxide anions is slow because of small factorials: velocity and concentrations $k_{1OH}=1.5*10^2$ $M^{-2}s^{-1}$, $[CO_{2aqua}]=0.0007512$ M, $[OH^-]=10^{-6.64}$ M. Carbonic Anhydrase synthesis solve for bioenergetic perfect order homeostasis as Self-Organization Attractor. [3,4]

pH=7.36 multipurpose Self-Organization Attractor creates positive and negative **charged groups R-COO$^-$, R-NH$_3^+$, HPO$_4^{2-}$, R-PO$_4^{2-}$, HCO$_3^-$** as free and linked in **R** molecules: amino acids, proteins, nucleic acids, carbohydrates, coenzymes.

Buffer systems in the Life organism trend to Self-Organization Attractor **pH** value **7.36**. Each of dominate buffer system have 7.36 friendly maximum (Figure 3. and 4.) of the buffer capacity: dihydrogen phosphate pK_{a_H2PO4}=7.199 [1] and Carbonic Anhydrase create protolysis calculate constant: $pK_{a\_CO2aqua}$=7.0512. [1]

Table 2. [6] Proteins as long chain polypeptides and free amino acids with four type acid groups constitute 47 values for classic acid constants. In three forms $pK_{a\text{-}COOH}$, $pK_{a\text{-}NH3+}$, $pK_{aRgroup}$:
for deprotonate carboxylate negative anion **R-COO$^-$**,
for protonate positive charged ammonium cation **R-NH$_3^+$**,
neutral phenolic acid Tyr–**OH** and Cys-**SH** neutral sulfhydryl groups.

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

20 amino acids four type protolysis groups classic 47 acids constant pK_a values:

1. **R-COOH<=>R-COO$^-$+H$^+$**, 22 values of groups 47;
2. **R-NH$_3^+$<=>R-NH$_2$+ H$^+$** 22+1=23 values of groups 47;
3. **Tyr-**phenol-**OH<=>Tyr-**phenolate-**O$^-$** +**H$^+$**;
4. **Cysteine-SH<=>Cysteine-S$^-$+H$^+$** one group.

Biochemical environment Self-Organization Attractor pH=7.36 creates molecules functional activity as charged groups: carboxylate **R-COO$^-$** and ammonium **R-NH$_3^+$**.

Maximal carboxylate $pK_{a\text{-}COOH^-}$ value smaller about 7.36=pH: $pK_{a\text{-}COOH}$=4.25< including fatty acids too 4.9<7.36 and smallest ammonium $pK_{a\text{-}NH3+}$ value grater about 7.36<9.04=$pK_{a\text{-}NH3+}$.

NpK_a number of parallel protolytic equilibria average mean pK_{a_mean} value is calculated as:
$pK_{a\_mean}=(\Sigma pK_{aRgroup}+\Sigma pK_{aNH3}+\Sigma pK_{aCOOH})/NpK_a$.
Ostwald's dilution law calculates the pH of solution at concentration C logarithm:

$$pH=\frac{pK_{a\_mean}-\log C}{2}.$$

Shuttle hemoglobin stabilized **multipurpose Self-Organization Attractor pH=7.36**.

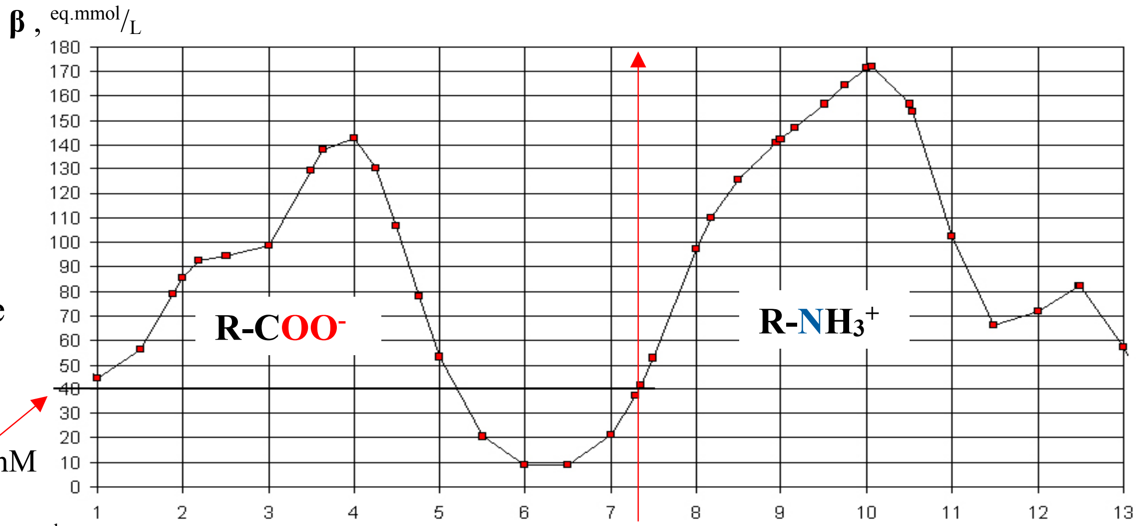
Hemoglobin in tissue desorbs oxygen **O_{2aqua}** for exchange to HCO_3^- and H^+ but in lungs releases HCO_3^- and H^+ due to adsorption of oxygen **O_{2aqua}**. [6] Exchange equilibrium depends on oxygen concentration in arterial $6\cdot10^{-5}$ M and venous $0.426\cdot10^{-5}$ M according actual hemoglobin sensitive equilibrium to oxygen concentration in blood: [6] O_{2aqua}+(H$^+$His63,58)Hb_T...salt bridges...(HCO_3^-)+**H_2O**<=>$Hb_R(O_2)$+**H_3O^+**+HCO_3^-:
arterial concentration [**O_{2aqua}**]=$6\cdot10^{-5}$ M ,
fraction [(H$^+$)Hb_T...salt bridges...(HCO_3^-)]=0.04 , fraction [$Hb_R(O_2)$]=0.96, [6]
venous concentration [**O_{2aqua}**]=$0.426\cdot10^{-5}$ M ,
fraction [(H$^+$)Hb_T...salt bridges...(HCO_3^-)]=0.37, fraction [$Hb_R(O_2)$]=0.63. [6]
In one blood circulation organism consume 0.96-0.63=0.33 fraction of oxygen from arterial saturated fraction 0.96=[$Hb_R(O_2)$]. [6] Stabilized Norma concentrations [HCO_3^-]=0.0154 M, [CO_{2aqua}]=0.0076 M sustain Self-Organization Attractor pH=7.36.

Henderson Haselbalh expression for Brensted protolysis calculates Attractor value 7.36:

$$pH=pK_a+\log\frac{[HCO_3^-]}{[CO_2]_{aqua}}=7.0512+\log(0.0154\ M/0.0076\ M)=7.36.$$

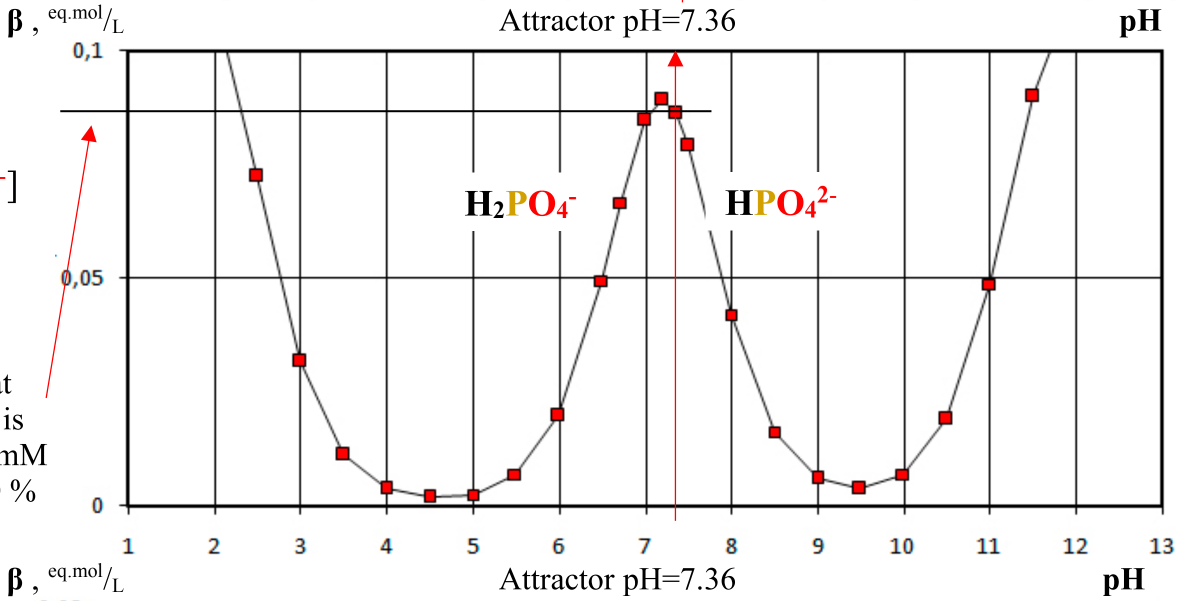
Self-Organization Attractor 7.36 creates functional activity of molecules with charged groups negative , positive: **HPO$_4^{2-}$, HCO$_3^-$, R-COO$^-$, R-NH$_3^+$, R-PO$_4^{2-}$** as free and linked in molecules **R**: amino acids, proteins, nucleic acids, carbohydrates, coenzymes. 11th and 12th pages: BUFFER solution. [1]

Proteins buffer have silence region from **pH=6** to 7.36 . 23 thousand protein total buffer solution concentration is C_{buffer}=3 mM. Muscle cytosol proteins the Buffer capacity at physiologic pH=7.36 is **β** =40 mM
30.3 %=40/132*100 %

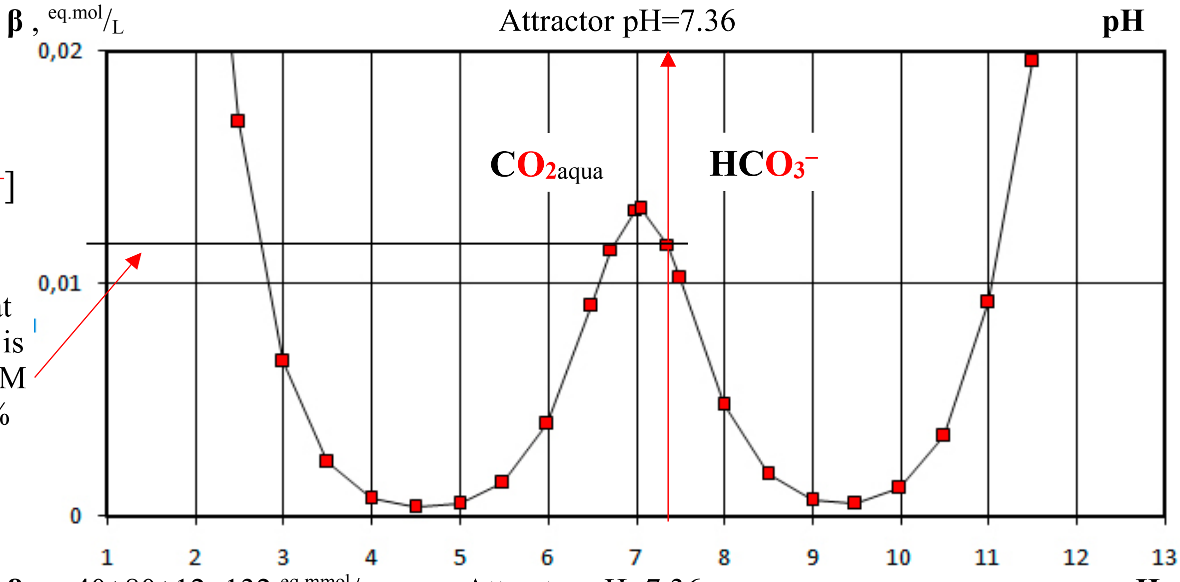


Total phosphate buffer systems concentration [$\mathbf{H_2PO_4^-}$]+[$\mathbf{HPO_4^{2-}}$] in muscle cells cytosol is C_{buffer}=0.155 M

The Buffer capacity at physiologic pH=7.36 is **β** =80 mM
66.6 %=80/132*100 %



Total bicarbonate buffer system concentration [$\mathbf{CO_{2aqua}}$]+[$\mathbf{HCO_3^-}$] is
C_{buffer}=0.023 M.
The Buffer capacity at physiologic pH=7.36 is **β** =12 mM
9.1 %=12/132*100 %



Total Buffer capacity β_{sum}=40+80+12=132 eq.mmol/L ; Attractor pH=7.36 **pH**

Figure 3. Cytosol muscle cells. Buffer capacities versus pH values from 1 to 13. Actual buffer capacity at Attractor pH=7.36 for two dominate phosphate , bicarbonate and total protein made buffer capacity sum.

proteins + phosphate + bicarbonate ,
at pH=7.36: proteins +([$\mathbf{H_2PO_4^-}$]+[$\mathbf{HPO_4^{2-}}$])+([$\mathbf{CO_{2aqua}}$]+[$\mathbf{HCO_3^-}$]),
total buffer capacity: 100 % = **30.3 %** + **66.6 %** + **9.1** %;

Buffer capacity is acid $\Delta \mathbf{n_{ac}}$ or base $\Delta \mathbf{n_b}$ equivalent_mols/ in one Liter changing pH per one unit **ΔpH=±1**.

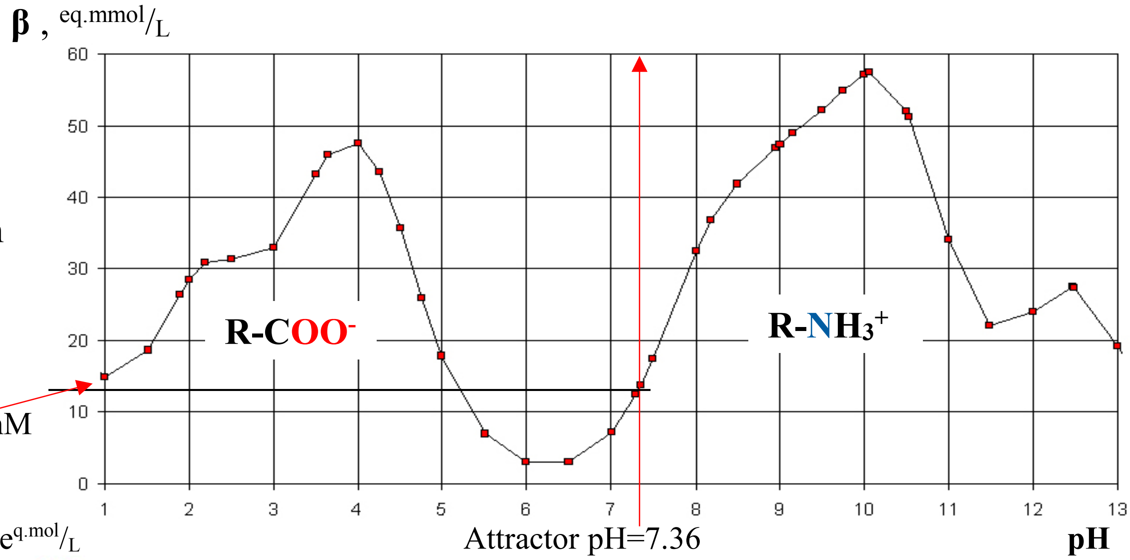
Three type buffer systems create multipurpose Self-Organized Attractor pH=7.36 for perfect homeostasis order with charged groups as free and linked in molecules R.

Proteins buffer have silence region from **pH=6** to 7.36 . Protein total buffer solution concentration C_{buffer}=1 mM for albumin.

The Buffer capacity at physiologic pH=7.36 is

β =12 mM

46.15 %=12/26*100 %

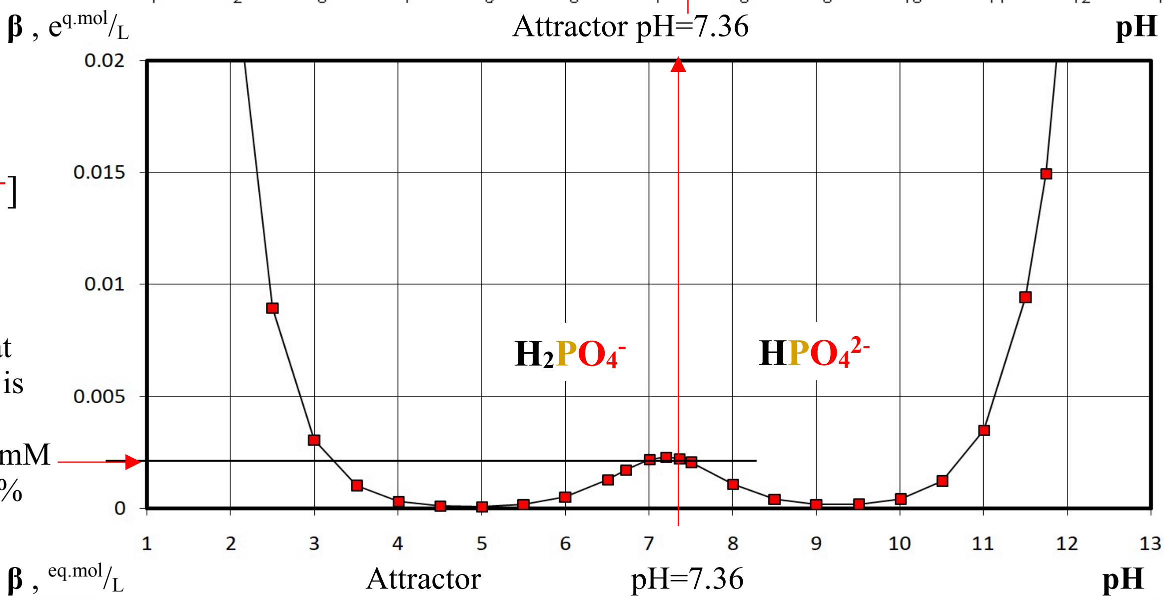


Total phosphate buffer systems concentration [$H_2PO_4^-$]+[HPO_4^{2-}] in blood plasma C_{buffer}=0.004 M.

The Buffer capacity at physiologic pH=7.36 is

β =2 mM

7.7 %==2/26*100 %

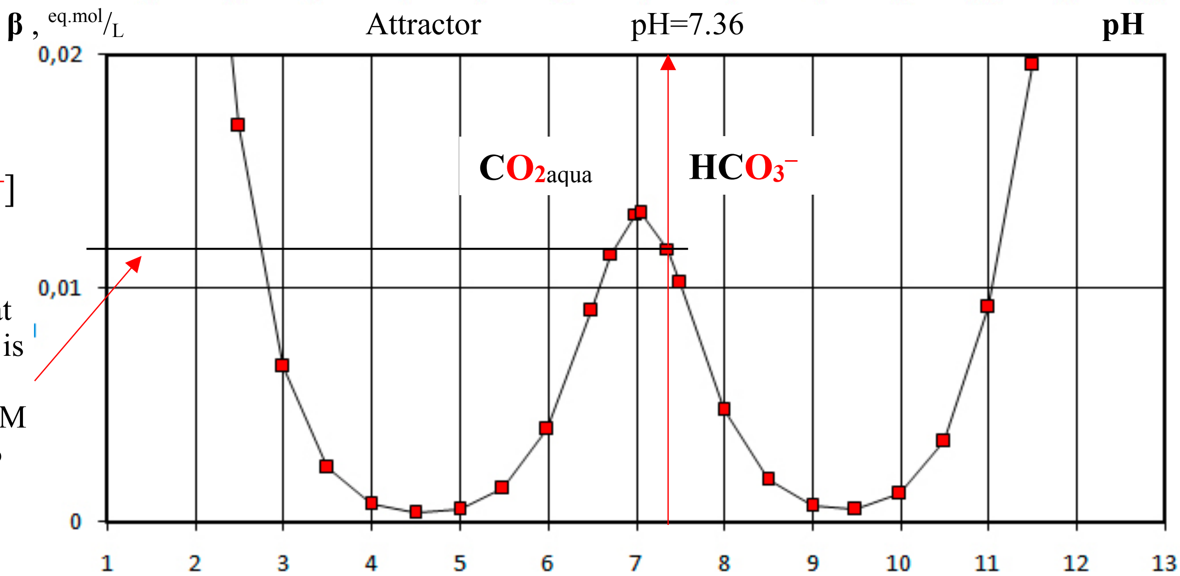


Total bicarbonate buffer system concentration [CO_{2aqua}]+[HCO_3^-] in blood plasma is C_{buffer}=0.023 M.

The Buffer capacity at physiologic pH=7.36 is

β =12 mM

46.15 %=12/26*100 %



Total Buffer capacity β_{sum}=12+2+12=26 eq.mmol/L Attractor pH=7.36 **pH**

Figure 4. Extra Cellular space Blood plasma. Buffer capacities versus pH values from 1 to 13. Actual buffer capacity at Attractor pH=7.36 for two dominate phosphate , bicarbonate and total protein made buffer capacity sum. proteins + phosphate + bicarbonate ,

at pH=7.36: proteins +([$H_2PO_4^-$]+[HPO_4^{2-}])+([CO_{2aqua}]+[HCO_3^-]),

total buffer capacity: 100 % = **46.15 %** + **7.7 %** + **46.15** %;

Buffer capacity is acid Δn_{ac} or base Δn_b equivalent_mols/ in one Liter changing pH per one unit **ΔpH=**±1. Three type buffer systems create multipurpose Self-Organized Attractor pH=7.36 for perfect homeostasis order with charged groups as free and linked in molecules R .

11th and 12th pages: BUFFER solution. [1]

References.

1. David R. Lide. CRC Handbook of Chemistry and Physics .90th ed. Taylor and Francis Group LLC; 2010 .
2. Prigogine I, Defey R. Chemical Thermodynamics. Longmans Green & co ©; 1954.
3. Prigogine I, Nicolis G. Self-Organization in Non-Equilibrium Systems. Wiley, 1977.
4. Prigogine I. Time, Structure and Fluctuations. Lecture, The Nobel Praise in Chemistry; 1977.
5. Kuman M. New light on the attractors creating order out of the chaos. *Int J Complement Alt Med.* **11**(6), 337, (2018) ;
6. Nelson DL, Cox MM. Lehninger Principles of Biochemistry. 5th ed. New York: W.H. Freman and company; 2008.
7. Xing W, Yin G, Zhang J. Rotating Electrode Method and Oxygen Reduction Electrocatalysts. *Elsevier*; 6 (2014) .
8. Alberty RA. Biochemical Thermodynamic's : Applications of Mathematics. John Wiley & Sons, Inc. 1-463, (2006).
9. Pinard MA, Mahon B, McKenna R. Probing the Surface of Human Carbonic Anhydrase for Clues towards the Design of Isoform Specific Inhibitors. *BioMed Research International*; **2015**, 3 (2015).
10. Kotz JC, Purcell KF. Chemistry and chemical reactivity. Saunders College Publishing; 1991.
11. White VM. THE CARBON CYCLE, ISOTOPES, AND CLIMATE I and II. Lectures 37, 38; 2003 .
12. Hanania J, Pomerantz C, Stenhouse K, Toor J, Donev J. Carbon cycle. University of Calgary's 2020 .
13. Der wohltemperierte Planet. *Der Spiegel*. 2007 Nr.19:148-154. German .
14. Kaksis A. The Biosphere Self-Organization Attractors drive perfect order homeostasis reactions to link bioenergetic with functionally activate oxygen and carbon dioxide molecules. 7th International Conference on New Trends in Chemistry September 25-26, 2021.27-32.