THEORETICAL SUPPORT FOR STRUCTURAL BIOCHEMISTRY COURSE (DESCRIPTIVE)

Topics:

- Water structure.
- Physical properties of water.
- Water ionization. Ionic result of water.
- The pH measure.
- Buffer systems.

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The human body is comprised of approximately 65% water, which acts as a solvent for the substances which we need. Being a good nucleophilic, often times water is a direct participant in hydrolysis and condensation reactions.

Water is an ideal solvent for our organism, having the role of transportation of carbohydrates, water soluble vitamins, as well as proteins from the place of absorption to various tissues.

Water intervenes in the purification of the organism from final producers in metabolism and also participates in maintaining the body temperature. The unique properties of water are derived from its structure.

STRUCTURE OF WATER:

Water is an oxygen hydride, formed from two atoms of hydrogen and one of oxygen, bound by covalent bonds. The angle between the two covalent bonds is 104.5°, and the distance O-H is 0,965 Å.
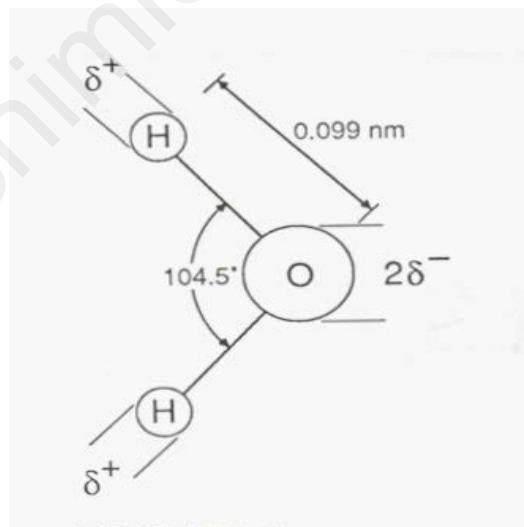

Within the water molecule, the oxygen atom, which is a powerful electronegative, attracts the two hydrogen electrons. This leads to polar **H-**

O connections in which the hydrogen atoms are positive and the oxygen atom is negative.

This forms a dipolar molecule with an uneven distribution of electrons between the hydrogen and oxygen atoms, which allows to act as a solvent. As such, water is a dipole molecule.

The neighboring molecules of liquid water interact between themselves. The intermolecular bonding of water results from the attraction of the partially negative property of the oxygen atom with the partially positive hydrogen atoms of the neighbouring water molecule. This results in electrostatic bonds called **hydrogen bonds**.
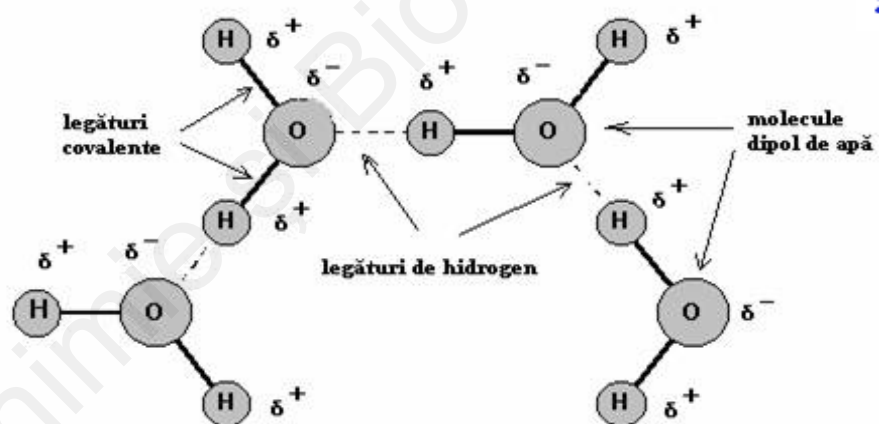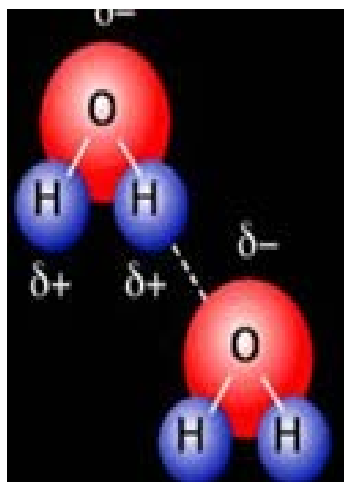
Due to the electrons' tetrahedral arrangement around the oxygen atoms, each molecule of water is potentially capable of forming four hydrogen bonds with neighboring water molecules. However, these bonds are weaker than covalent bonds. One water molecule sets hydrogen bonds with four other molecules only at very low temperatures (-183°C). Parts of the hydrogen bonds break at high temperature due to thermal agitation.
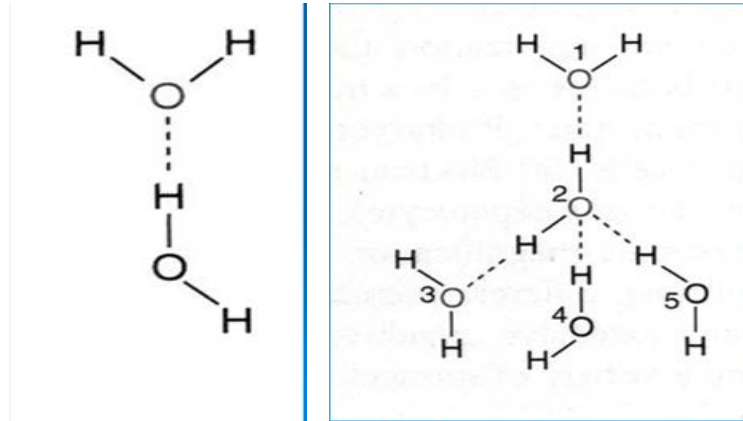
It is surprising to note the small difference between the number of hydrogen bonds in ice (85%) and liquid water (50%), comparing the fluidity of water and the rigidity of ice. The explanation lies in the rapidity with which the hydrogen molecules are formed and separated in liquid water, with the halve time of each bond being approximately 10^{-11} seconds.

There are numerous models outlining the structure of liquid water, but none of them was able to be totally verified through experiments. However, the simplest models suggest that liquid water consists of the same associated molecules as ice, in the labile equilibrium with the free molecules of water.

The presence of hydrogen bonds between water molecules explain why the heat of vaporization is so great. The liquid state of water made it possible for life to exist.

THE PHYSICAL PROPERTIES OF WATER:

Hydrogen bonding of water gives the liquid state a wide temperature range (0-100°C), while at the same time a unique role in conducting biochemical processes of live cells - specific heat, high melting and boiling points, heat of vaporization, increased thermal conductibility, superficial tension and very high dielectric constant.

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Specific heat

Specific heat of water is the necessary heat to vary the temperature unit of mass by one degree, which is greater than that of any other liquid. Due to this property, water avoids sudden changes in body temperatures.

Heat of vaporization

The amount of heat, in calories, necessary for vaporization of a unit of mass, is considerably higher for water than for any other ordinary liquids.

Thermal Conductibility

The thermal conductibility of water is very high, which contributes to the spread of heat lying deep inside the body outwards.

Dielectric Constant

Dielectric constant makes water an ideal and universal solvent. Water is an excellent solvent for both ionic compounds and those with low molecular weight - non-ionic polar compounds such as sugars, urea and alcohols. Ionic compounds are soluble, because water is capable of overcoming electrostatic attraction between ions through ion solvatation. The polar non-ionic compounds are soluble, because water molecules are capable of forming hydrogen bonds with polar groups thereof.

Osmotic Pressure

Osmotic pressure is a measure of the tendency of water molecules to migrate from a diluted solution into a concentrated solution through a semipermeable membrane. This migration of water molecules is called osmotic. A solution which contains one molecule of dissolved particles per one kilogram of water is a mono-osmotic solution. In blood plasma, normal total concentration of solutes is remarkably constant (275-295 osmoles). The osmolality changes in certain pathological conditions, such as dehydration, kidney failure, diabetes insipidus, hyponatremia and hypernatremia, hyperglycemia.

WATER IONIZATION. IONIC PRODUCT OF WATER. THE $_p$H NOTION:

The mass of the hydrogen atom is very small, its only atomic electron is attracted by the electronegative atom: oxygen. The formed proton can be separated from oxygen into a separate molecule and passed to the oxygen molecule of the neighbouring molecule. Said another way, water has the ability to ionize.

$$H_2O + H_2O \rightleftharpoons OH^- + H_3O^+$$
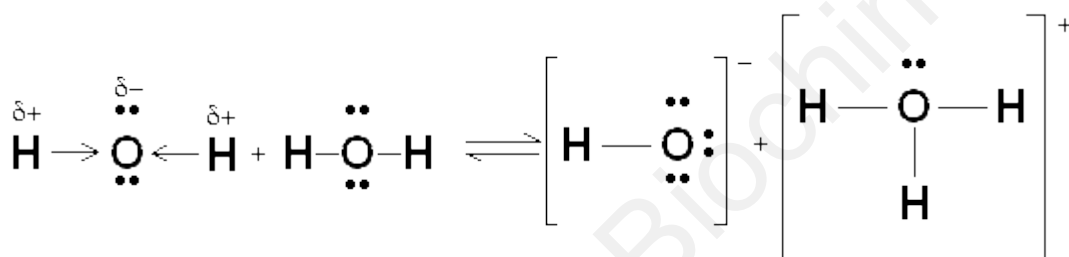
Hydronium ion, noted as H_3O^+, is often abbreviated as H^+. Both notations mean the same thing.

The above reaction is called auto-ionization of water. The fact that the reaction equilibrium is moving far to the left, is of big importance for living systems. It has been found that the rate of migration of hydronium ion H_3O^+ into an electric field is much higher than that of the monovalent cations - a quite curious fact given the large volume of H_3O^+ ions versus that of the cations. This anomaly occurs because a proton can quickly jump from a hydronium ion to the neighboring water molecule. In other words the translocation of protons occurs at a speed greater than the speed of migration of ion H_3O^+. The phenomenon explains why ice, in spite of its rigid structure, has the same electric conductibility as liquid water.

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Ionic Product of Water

Water is a weak electrolyte that dissociates into ions, albeit in a very limited extent, according to the reaction:

$$H_2O + H_2O \rightleftharpoons OH^- + H_3O^+$$

The proof behind this is lies in the fact that even very pure water, which does not contain a single dissolved electrolyte, manifests a measurable eletrical conductibility, although very low.

This conductibility allows the calculation of the degree of dissociation of water. This value is very low. Thus, at room temperature (22ºC), only 1/10.000.000 mol·g or 10^{-7} mol·g of H_2O dissociates per litre. Because each dissociated molecule of H_2O releases one H^+ion and one OH^- ion. The concentration of these ions in pure water as follows:

$$\left[H_3O^+\right] = \left[HO^-\right] = 10^{-7} mol/l$$

Like ions of other weak electrolytes, the process of dissociation of water molecules in ions can be characterized by the value of the constant of corresponding dissociation:

$$K_c = \frac{[HO^-] \cdot [H_3O^+]}{[H_2O]^2}$$

This equation can be further simplified:

$$K_c \cdot [H_2O]^2 = K_w = \left[HO^-\right] \cdot \left[H_3O^+\right]$$

Because water is found almost exclusively in nondissociated H_2O molecules, the variation in the degree of dissociation will have virtually no influence on the concentration. In the previous equation, we can consider the concentration (H_2O) virtually constant. The **Kc** Value is also not variable. As such, the whole left side of the obtained equation and thus the product **[H⁺]·[OH⁻]** is constant. This constant is called the ion product of water and is noted by Kw.

$$K_w = [H_3O+] \cdot [HO^-] = 10^{-14} \, MOL^2 / L^2$$

The notation of the relation **[H⁺]·[OH⁻] = K$_w$** is explained by: whatever the variation of the concentration of the ions **H+** or **OH**, their product in any aqueous solution remains a constant, equal to 10^{-14} at ambient temperature (22°C). In pure water:

$$\left[H_3O^+\right] = \left[HO^-\right] = 10^{-7} mol/l$$

In relation to the ionic product of water, there are two clarifications:

1. Ionic product of water depends only on the temperature. The **K$_w$** value is modified considerably in correlation with the increasing of temperature
2. Ionic product of water is the same (is not modified) in neutral solutions, whether acid or basic.

Depending on the concentration of hydronium ions in a solution, they are divided into:

- Neutral solutions, where:

$$\left[H_3O^+\right] = \left[HO^-\right] \Rightarrow \left[H_3O^+\right] = 10^{-7}$$

- Acidic solutions, where:

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$$\left[H_3O^+\right] > \left[HO^-\right] \Rightarrow \left[H_3O^+\right] > 10^{-7}$$

- Basic solutions, where:

$$\left[H_3O^+\right] < \left[HO^-\right] \Rightarrow \left[H_3O^+\right] < 10^{-7}$$

As such, any aqueous solution, regardless of its reaction, has to contain the same amount of **H⁺** ions as **OH⁻** ions. Because their concentrations are inversely proportionate as this equation shows:

$$[H_3O^+] \cdot [OH^-] = \textbf{\textit{K}}_w$$

The reaction of any solution can be quantitatively characterized by knowing its ion concentration *[H₃O⁺]*.

The pH Notion

Instead of ion concentration **H+** it's much more convenient to use the decimal logarithm with the changed sign called hydrogen symbol, or **pH**.

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

Knowing that the proton does not exist on its own, but rather in the form of hydronium *[H₃O⁺]*,

$$\textbf{\textit{pH= -log[H}}_3\textbf{\textit{O}}^+\textbf{\textit{]}}$$

Results, for example, in the following:

$$\textbf{\textit{[H}}^+\textbf{\textit{]}} = 10^{-4} \text{ ion-g/L}$$

$$\textbf{\textit{pH}} = -(-4) = 4$$

Also, if:

$$[H^+] = 5 \cdot 10^{-10} \text{ ion-g/L}$$

$$pH = -\log 5 \cdot 10^{-10} = 10 - \log 5 = 9{,}30$$

It is evident then that the first solution (**pH** = 4) is an acid reaction, because **[H⁺]** is higher than 10^{-7} ion-g/L. On the other hand, the reaction of the second solution (**pH** = 9.3), is alkaline, because **[H⁺]** is smaller than 10^{-7} ion-g/L. In acidic solutions **pH**< 7, in alkaline solutions **pH**> 7 and in neutral solutions **pH** = 7.
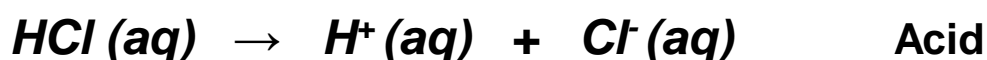
We can then easily deduce that acidity of a solution increases when the pH drops, and alkalinity increases as the pH increases.

For example, at pH of 2, the reaction of the solution is more acidic than at a pH level of 4, because in the first case the ion concentration **H⁺**(10^{-2} ion-g/L) is 100 higher than in the second case (10^{-4} ion-g/L). In acidic solutions the pH level is below 7, in alkaline solutions the pH level is above 7 and in neutral solutions the pH level is equal to 7.

The **pH** value has a significant importance for living systems, life being possible only in certain **pH** limits. The **pH** of blood for example has a value of 7.35-7.4. This is because enzymes operate within certain limits of **pH**. Maintaining a constant **pH** level is accomplished in several ways, in which an important role is played by buffer systems.

Acid-Base Balance

In order to understand the actions of the buffer systems we need notations of acid and base. The theory of Arrhenius states that acids are substances that produce **H⁺** ions as a result of dissociation, while bases are substances that produce **OH⁻** ions as a result of dissociation.

$$NaOH \ (aq) \ \rightarrow \ Na^+ \ (aq) \ + \ OH^- \ (aq) \quad \textbf{Base}$$

$$HCl \ (aq) \ \rightarrow \ H^+ \ (aq) \ + \ Cl^- \ (aq) \qquad \textbf{Acid}$$

The theory of electrolytic dissociation of Arrhenius defines:

Acids are substances that, in aqueous solutions release H^+ hydrogen ions. Bases are substances that, in aqueous solutions release HO^- hydroxide ions.

In a pure state (without water), acids and bases are not dissociated substances and not electrically conductive, or very weak conductors. In aqueous solutions however, are electrically conductive.

Johannes Brønsted and Thomas Lowry revised the acid-base theory of Arrhenius defining notions of acid and base as:

The acids are substances capable of yielding protons H^+. Bases are substances capable of accepting protons H^+.

These two reactions influence each other, the acid, yielding the proton, transforms in a conjugated base; base, accepting one proton from the acid, transforms in a conjugated acid:
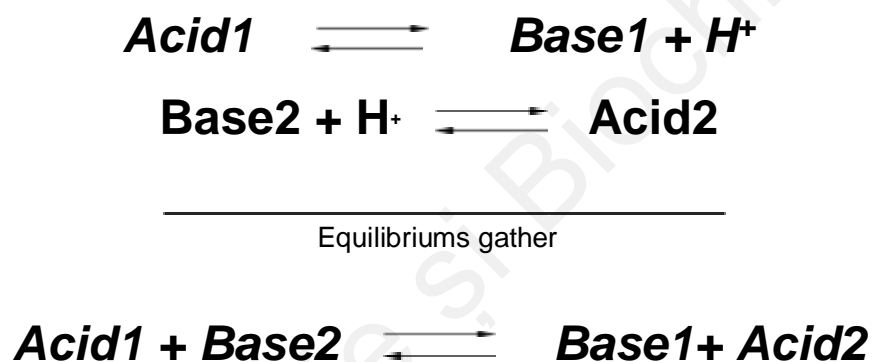
$$HA + H_2O \rightarrow A^- + H_3O^+$$
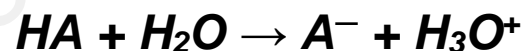
| ACID | WATER | BASE | CONJUGATED ION HYDRONIUM |

$$B + H_2O \rightarrow BH + HO^-$$
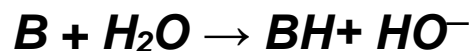
| BASE | WATER | BASE | CONJUGATED ION HYDROXYDE |

Acid/base couples (or conjugated base / acid) are acid-base couples. The proton cannot exist in liberty in a solution because it is very small and extremely reactive. In all chemical reactions, the **H⁺** (having a basic property) proton is yielded from an acid / base couple (for example Acid1) and accepted by another chemical species, another conjugated acid / base couple (for example Base2):

$$Acid1 \rightleftharpoons Base1 + H^+$$

$$Base2 + H^+ \rightleftharpoons Acid2$$

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Equilibriums gather

$$Acid1 + Base2 \rightleftharpoons Base1 + Acid2$$

As such, equilibriums are formed with the exchange of protons, in which multiple protons can be transferred between the acid of one couple (Acid1 / Base1) and the base of another couple (Acid2 / Base2). Equilibriums with exchange of protons with the participation of acid from one couple and base of another couple is called acid-base equilibriums or acid-base reactions.

Buffer Systems

The solutions formed from

- a weak acid and its salt with a strong base, or
- a weak base and its salt with a strong acid

which have the tendency to resist high **pH** variations with the addition of acids or bases in limited quantities are called buffer systems

Buffer systems are characterized by two parameters:

1. **pH** level that a buffer system maintains
2. The buffering capacity of the buffer system.

pH& the Henderson-Hasselbalch Equation

In order to determine the optimal **pH** level which we maintain constant within a buffer system, we take a buffer system formed from a weak acid and its salt with a strong base. The weak acid is a little bit dissociated:

$$HA \leftrightarrow H^+ + A^-$$

The relative constant of acidity is:

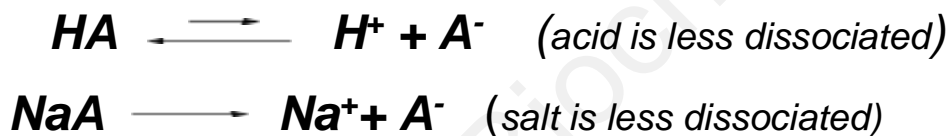$$K_a = \frac{[H^+][A^-]}{[HA]}$$

and by rearranging:

$$[H^+] = K_a \frac{[HA]}{[A^-]}$$

Convert the above equation into a logarithm and multiply by (-1):

The obtained equation is called the Henderson-Hasselbalch equation and represents the fundamental equation of buffer systems. In its most general form, it is written:

$$pH = pKa + \log_{10} \frac{[salt]}{[acid]}$$

This is possible because the acid is a little bit dissociated, while salt is fully dissociated.

$$HA \longrightarrow H^+ + A^- \quad (\textit{acid is less dissociated})$$
$$NaA \longrightarrow Na^+ + A^- \quad (\textit{salt is less dissociated})$$

It appears that **A⁻** comes mostly from salt, weak acid being very little dissociated. The total concentration of acid **HA** is equal with the concentration of the non dissolved acid for the same reason. This results in:

$$pH = pKa + \log_{10}1, \text{ or}$$
$$pH = pKa$$

because molecular concentrations are the same:

$$[salt] = [acid]$$

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As such, the optimal **pH** level maintained constant by a buffer system is equal with the evaporation of **pKa** of weak acid from the corresponding buffer system.

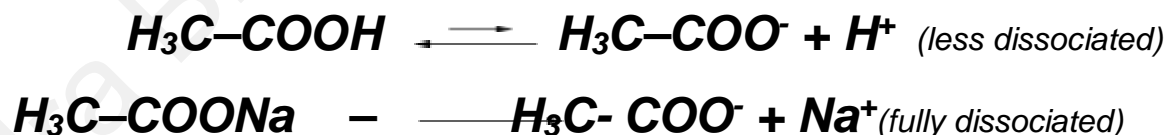In conclusion, a buffer system is more efficient at the following interval:

$$pH = pKa \pm 1$$

The Capacity of Buffers

The capacity of buffers in buffer systems depends on the effective molecular concentration of conjugated acid and base from the corresponding buffer system. As the concentration of components of the buffer system increases, so does the number of buffers capasity.

Action Mechanisms of Buffer Systems

Buffer systems have the role of resists big **pH** variations with the addition of acids or bases in limited quantities. In order to explain the action mechanism of buffer systems, we will examine the buffer system formed from a weak acid and its salt with a strong base **H₃C–COOH/H₃C–COONa**, which will form the following ions in a solution:

$$H_3C\text{–}COOH \rightleftharpoons H_3C\text{–}COO^- + H^+ \text{ (less dissociated)}$$

$$H_3C\text{–}COONa \longrightarrow H_3C\text{-}COO^- + Na^+ \text{(fully dissociated)}$$

We add acids, so **H⁺**. As a result, the process of consumption of **H⁺** protons takes place, which will be bonded with **CH₃ – COO⁻**, forming a weak acid little dissociated **CH₃ - COOH**, and at the same time the added protons will further mitigate the dissociation of acid by moving the equilibrium of the first reaction to the left.

We add bases, so **HO⁻**. As a result, the process of consumption of **HO⁻** takes place; these ions will be bonded with the protons (**H⁺**) from the first reaction, forming water. By consuming **H⁺**, the equilibrium of the first reaction is moved towards the right and therefore the **H⁺** result will be the measure of the neutralization of **HO⁻** ions.

Biological Buffer Systems

Blood pH is maintained between 7.36 and 7.44 with an intracellular **pH** of about 7.1 (between 6.9 and 7.4). By the excretion of the acid produced in the form of CO_2 in the breath or urine ion, the neutralization is needed in the main buffer systems of the body.

Functional buffer systems in the human body are:

- **$H_2CO_3/NaHCO_3$** - intercellular, plasma

- **Protein ofNA** - intercellular, plasma

- **NaH_2PO_4/Na_2HPO_4** - intercellular, plasma

- **Deoxi-Hb/Oxi-Hb** - intercellular, erythrocytes

- **KH_2PO_4/K_2HPO_4** - intercellular

The Bicarbonate Buffer System - The Most Important

The principal source of metabolic acids of the organism is CO_2, a principal product in the oxidation acetilCoA to the level of the Krebs cycle. In metabolic conditions, 13 moles of CO_2 are generated per day (approximately 0.5-1kg). Dissolved in water, it will react with acid thereby producing carbon dioxide, a carbonic anhydrase accelerated reaction. The

obtained carbonic acid is a weak acid which is partially dissolved in H^+ and HCO_3^-.

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

pKa of carbonic acid is 3.8. At the blood **pH** level of 7.4, the acid is almost completely dissociated and is theoretically incapable of neutralization and generation of bicarbonate, in other words to maintain a constant **pH** level. The reason for this is presented below.

Carbonic acid can be reformed from CO_2 because the concentration of dissolved gas in blood is 500 times greater than that of carbonic acid.

pKa of the buffer system of the bicarbonate combines **Kh** (constant of hydration or solubility of CO_2 in the water to form H_2CO_3) with the obtained chemical **pKa** value of 6.1.

$$pH = pK_{a\ H_2CO_3} + \log\left(\frac{[HCO_3^-]}{[H_2CO_3]}\right)$$

[HCO₃⁻] – Bicarbonate concentration in blood

[H₂CO₃] – Carbonic acid concentration in blood, which is calculated from the following relation

$$[H_2CO_3] = k_{H\ CO_2} \times pCO_2$$

Kh is approximately 0.03 (mmol/L) / mmHg, and **pCO₂** is the partial pressure of gas in arterial blood.

These two relations combine into one single one:

$$pH = 6.1 + \log\left(\frac{[HCO_3^-]}{0.03 \times pCO_2}\right)$$

in which **pH** is defined by the blood acidity.

From the last formula we can infer that the buffering power of the blood depends only on the concentration of dissolved bicarbonate in the blood and the carbon dioxide partial pressure in pulmonary alviole.

If the blood **pH** level falls, the concentration of **H⁺** grows, and so will the concentration of **H₂CO₃** for a short period of time; but it will revert back to the normal level by its decomposition and the generation of water and **CO₂**. Thus the increase of the concentration of **H⁺** is finally converted in the growth of **pCO₂**.

If on the contrary the blood **pH** level increases, the concentration of bicarbonate ion (**HCO₃⁻**) will also grow but temporarily, due to the dissociation of **H₂CO₃.** However, it will be restored quickly when atmospheric **CO₂** dissolves and hydrates the **H₂CO₃.**

As such, any modification to the **pH** level of blood at the pulmonary level will be quickly stabilize with the reserves of **CO₂** gas through modification of the pulmonary ventilation.