## Water

Life on earth originates in Ancient Ocean after water has reduced the temperature on earth to the permissive level to support vital biological activities. Most of the biological reactions operate in aqueous environment and use water as one of the reactant. It is making ~70% content of the organism and controls the enzymatic activity of major metabolic pathways. In higher mammals, it has been used as a medium for material transfer between different organs and a reactant to neutralize and facilitates excretion of toxic metabolites out of the body and temperature regulation. The enormous capacity of water to perform multitasking is the sole basis of life activities and lies in its unique structure and ability to interact with biological molecules.

## Structure of Water:

Water has a chemical formula of $H_2O$ with central oxygen covalently attached to two hydrogen atoms (Figure 2.1, A). It has two lobes of unpaired electron opposite to the hydrogen atom called as "lone pairs". The H-O-H bond angle is $104.50^\circ$ and over-all molecules adopt partial tetrahedral geometry. In the water molecule, oxygen is more electronegative than hydrogen; as a result, sharing electrons are placed towards oxygen and gives partial positive charge to hydrogen ($\delta^+$) and negative charge to oxygen ($2\delta^-$). Hence, water molecule develops a dipole and form hydrogen bonding involving electronegative oxygen of first molecule and positively change hydrogen of next molecule. In this manner, each water molecule can be in hydrogen bonding interactions to 4 neighboring molecules (Figure 2.1, B). As a result, water molecules exist in three different state of matter; liquid, solid (Ice) and gas (vapor). In liquid state, water molecules are under dynamic hydrogen bonding interaction to the neighboring molecules where as in solid state (Ice) molecules present in one plane are in hydrogen bonding interaction with molecules present in same plane and neighboring plane giving a quasi-crystal like packing (Figure 2.1, C).

## Water Ionization and pH:

Water weakly ionizes reversibly into hydrogen ion ($H^+$) and hydroxide ion ($OH^-$) as shown in Eq. 2.1. But $H^+$ thus formed gets hydrated to form hydronium ion ($H_3O^+$) due to hydrogen bonding between two water molecules which allows instantly hydration of ionized proton (Eq 2.2).

$H_2O \rightleftharpoons H^+ + OH^-$............................................(2.1)

$2H_2O \rightleftharpoons H_3O^+ + OH^-$......................................(2.2)
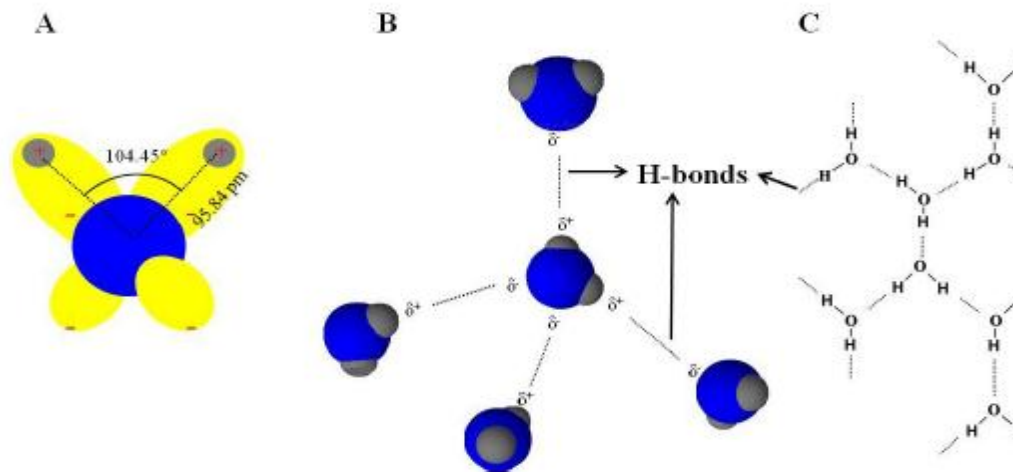


Figure 2.1: Structure of Water. (A) Chemical structure of water molecule. (B) Hydrogen Bonding between water molecules and (C) Hydrogen bonding pattern observed in Ice.

The equilibrium constant, $K_{eq}$ for the reversible ionization of water is:

$K_{eq}$= [H$^+$] [OH$^-$]/ [H$_2$O] …………………………2.3

The concentration of pure water, [H$_2$O] = 55.5M while concentration of [H$^+$]and[OH$^-$] is very low=10$^{-7}$M at 25C$^o$

$K_{eq}$=10$^{-7}$Mx10$^{-7}$M / 55.5M

$K_{eq}$= 1.8x10$^{-16}$M

Rearrangement of

(55.5M) (K$_{eq}$) = [H$^+$] [OH$^-$]=K$_w$ ……………2.4

(55.5 M) (1.8x10$^{-16}$M) = [H$^+$] [OH$^-$]

99.9x10$^{-16}$M$^2$ = [H$^+$] [OH$^-$]

1.0x10$^{-14}$M$^2$ = [H$^+$] [OH$^-$]=K$_w$

Hence [H$^+$] [OH$^-$] in pure water is always equal to 1.0x10$^{-14}$M$^2$ (neutral pH=7)

[H$^+$] [OH$^-$] can be calculated from water ionization constant $K_w$= [H$^+$] [OH$^-$]=[H$^+$]$^2$

H$^+$=√Kw=√1.0x10$^{-14}$ or [H$^+$]=[OH$^-$]=10$^{-7}$M

Ion product is a constant and allows us to calculate [H$^+$], if [OH$^-$] is known or vice-versa. Hence, it is used to develop pH scale to define concentration of H$^+$ or OH$^-$ in any aqueous solution. pH is defined as "**negative logarithm of hydrogen ion concentration**" and it ranges from 1-14 (Figure 2.2, A). Solution with pH=7 is neutral whereas solution with pH lower than 7 is acidic and pH above 7 are considered as alkaline. Biological samples of different origin and products of daily use are examples of solution with variable pH (Figure 2.2, B).

pH= -log [H$^+$]……………………2.5

Hence strong acids (hydrochloric acid, sulfuric acid, etc.) and bases (sodium hydroxide, potassium hydroxide, etc.) are those that are completely ionized in dilute aqueous solutions. In biological systems one generally encounters only weak acids and bases. Weak acids and bases do not completely dissociate in solution. They exist instead as an equilibrium mixture of undissociated and dissociated species. For example, in aqueous solution, acetic acid is an equilibrium mixture of acetate ion, hydrogen ion, and undissociated acetic acid.

**A**                                                                                           **B**
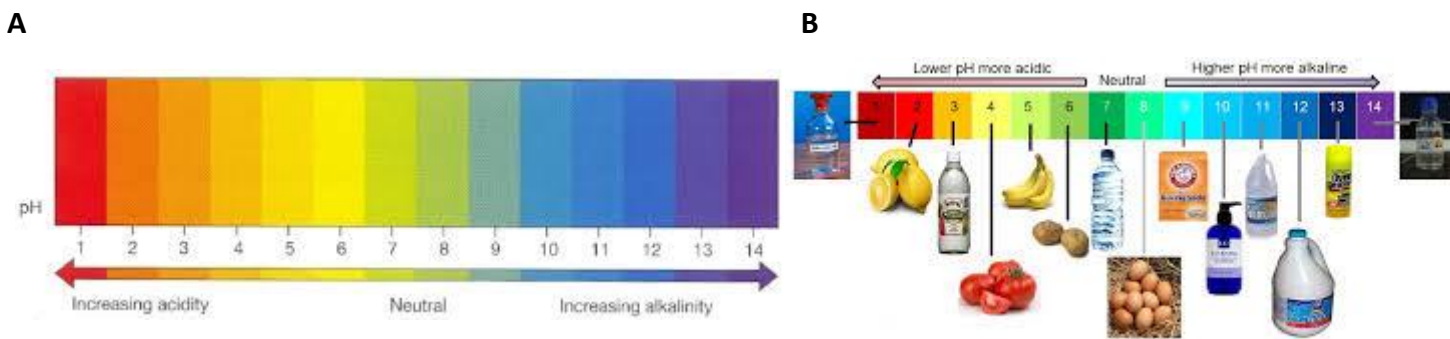


Figure 2.2: pH Scale and pH of different fluids. (A) pH Stripe, (B) pH in different biological fluids and daily use items

**Handerson-Hasselbalch Equation-**

Titration is the method to determine the content of acid or base present in a solution. In a typical titration experiment, a fixed amount of acid is titrated with a solution of strong base, such as NaOH in the presence of pH indicator dye. The purpose of adding pH indicator dye is to monitor the change in pH during the course of titration. A typical plot of change in pH against the amount of NaOH added for acetic acid is given in Figure 2.3.

In the beginning, acetic acid is present majorly as $CH_3COOH$ but with the addition of NaOH, $OH^-$ will neutralize $H^+$ to form $H_2O$ and promotes further dissociation of acetic acid to give $H^+$ (Figure 2.3). At the midpoint of where 0.5 equivalent NaOH has been added, solution has an equal concentration of $CH_3COOH$ (proton donor) and $CH_3COO^-$ (proton acceptor). At the end of titration, all undissociated $CH_3COOH$ will be converted into the $CH_3COO^-$.
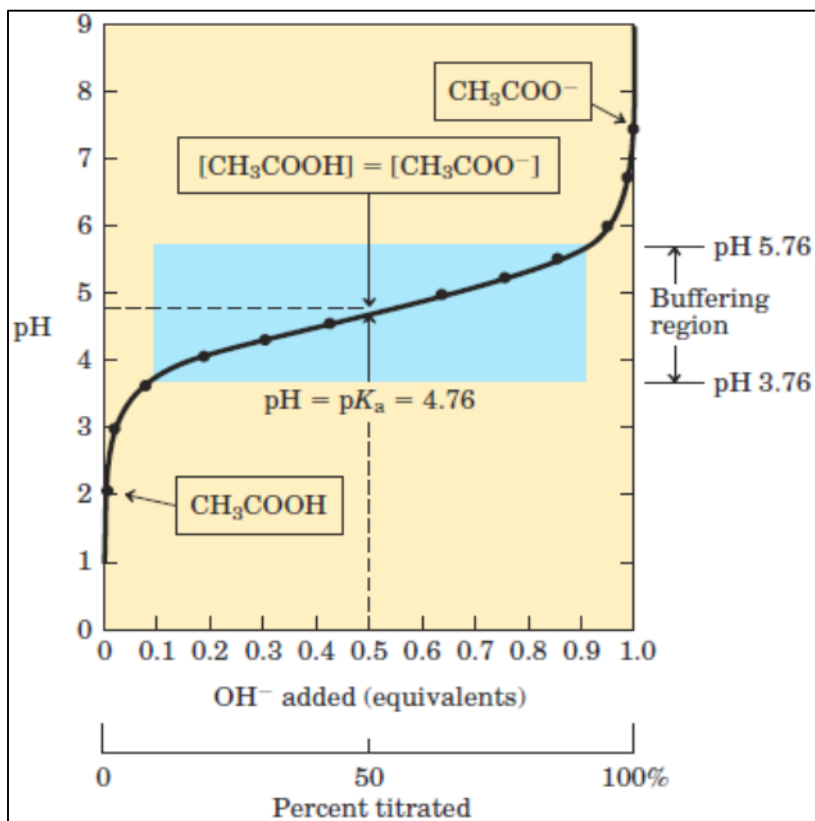

Figure 2.3 Titration curve of acetic acid

During a titration experiment, two reversible equilibrium exists.

$H_2O \leftrightharpoons H^+ + OH^-$ ............................................(2.1)

$CH_3COOH \leftrightharpoons H^+ + CH3COO-$ ........................ (2.6)

$K_a = [H^+][ CH_3COO^-]/[ CH_3COOH]$ ........................(2.7)

$pK_a$ = -log $K_a$, Strong acid will have lower $pK_a$. it is calculated by titration experiment and pH at the mid-point of the titration curve for acid or base (Figure 2.3).

After rearranging, $[H^+] = K_a [ CH_3COOH]/[ CH_3COO^-]$ ..........................(2.8)

Taking negative log on both side, -log $[H^+]$ = -log $K_a$-log $[CH_3COOH]/[ CH_3COO^-]$

∵ -log $[H^+]$=pH and −log $K_a$=$pK_a$

∴ pH=$pK_a$-log $[CH_3COOH]/[ CH_3COO^-]$ or

pH= $pK_a$+log $[CH_3COO^-]/[ CH_3COOH]$ or in general for any acid

pH= $pK_a$+log [proton acceptor]/[proton donor] ...............................(2.9)

The equation 2.9 is known as **Henderson-Hasselbalch equation** and it relates molar ratio of proton donor/acceptor with $pK_a$ at a given pH. It is also used to calculate the amount of proton donor, acceptor needed to prepare the solution of given pH, if $pK_a$ is given

**Buffers, Buffer Capacity and Range**

Buffers are aqueous systems that resist changes in pH when small amounts of acid or base are added. Buffer solutions are composed of a weak acid (the proton donor) and its conjugate base (the proton acceptor). Buffering results from two reversible reaction equilibria in a solution wherein the concentration of proton donor and its conjugate proton acceptor are equal. For example, in a buffer system when the concentrations of acetic acid and acetate ions are equal, additions of small amounts of acid or base do not have any detectable influence on the pH. This point is commonly known as the **isoelectric point**. At this point there is no net charge and pH at this point is equal to $pK_a$.

pH= $pK_a$+log $[CH_3COO^-]/[CH_3COOH]$

At isoelectric point $[CH_3COO^-] = [CH_3COOH]$ hence, pH = $pK_a$

Buffer capacity is a term used to describe the ability of a given buffer to resist changes in pH on addition of acid or base. A buffer capacity of 1 is when 1 mol of acid or alkali is added to 1 liter of buffer and pH changes by 1 unit. The buffer capacity of a mixed weak acid-base buffer is much greater when the individual $pK_a$ values are in close proximity with each other. It is important to note that the buffer capacity of a mixture of buffers is additive.

Buffers have both intensive and extensive properties. The intensive property is a function of the $pK_a$ value of the buffer acid or base. Most simple buffers work effectively in the pH scale of $pK_a \pm 1.0$. The extensive property of the buffers is also known as the buffer capacity. It is a measure of the protection a buffer offers against changes in pH. Buffer capacity generally depends on the concentration of buffer solution. Buffers with higher concentrations offer higher buffering capacity. On the other hand, pH is dependent not on the absolute concentrations of buffer components but on their ratio.

**Buffering in Cells and Tissues**

A brief discussion of hydrogen ion regulation in biological systems highlights the importance of buffering systems. Amino acids present in proteins in cells and tissues contain functional groups that act as weak acid and bases. Nucleotides and several other low molecular weight metabolites that undergo ionization also contribute effectively to buffering in the cell. However, phosphate and bicarbonate buffer systems are most predominant in biological systems. The phosphate buffer system has a pKa of 6.86. Hence, it provides effective buffering in the pH range of 6.4 to 7.4. The bicarbonate buffer system plays an important role in buffering the blood system where in carbonic acid acts as a weak acid (proton donor) and bicarbonate acts as the conjugate base (proton acceptor). Their relationship can be expressed as follows:

$K_1= [H^+][HCO_3^-]/[H_2CO_3]$

In this system carbonic acid ($H_2CO_3$) is formed from dissolved carbon dioxide and water in a reversible manner. The pH of the bicarbonate system is dependent on the concentration of carbonic acid and bicarbonate ion. Since carbonic acid concentration is dependent upon the amount of dissolved carbon dioxide the ultimate buffering capacity is dependent upon the amount of bicarbonate and the partial pressure of carbon dioxide. In air breathing human, the bicarbonate buffer system maintains pH near 7.4. This is possible due to the fact that carbonic acid in the blood is in equilibrium with the carbon dioxide present in the air. Figure 2.4 highlights the mechanism involved in blood pH regulation by the bicarbonate buffer system. Any increase in partial pressure of carbon dioxide (as in case of impaired ventilation) lowers the ratio of bicarbonate to $pCO_2$ resulting in a

decrease in pH (acidosis). The acidosis is reversed gradually when kidneys increase the absorption of bicarbonate at the expense of chloride. Metabolic acidosis resulting from the loss of bicarbonate ions (such as in severe diarrhea or due to increased keto acid formation) leads to severe metabolic complications warranting intravenous bicarbonate therapy. During hyperventilation, when excessive amounts of carbon dioxide are eliminated from the system (thereby lowering the $pCO_2$), pH of the blood increases resulting in alkalosis. This is commonly seen in conditions such as pulmonary embolism and hepatic failure. Metabolic alkalosis generally results when bicarbonate levels are higher in the blood. This is commonly observed after vomiting of acidic gastric secretions. Kidneys compensate for alkalosis by increasing the excretion of bicarbonate ions. However, an obligatory loss of sodium occurs under these circumstances. In case of severe alkalosis, the body is depleted of water, $H^+$, $Cl^-$ and to some extent $Na^+$.
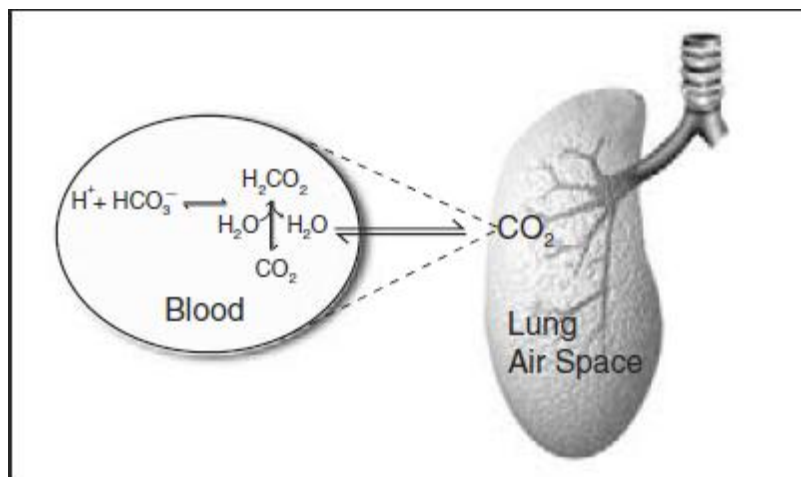


Figure 2. 4: Relationship between bicarbonate buffer system and carbon dioxide

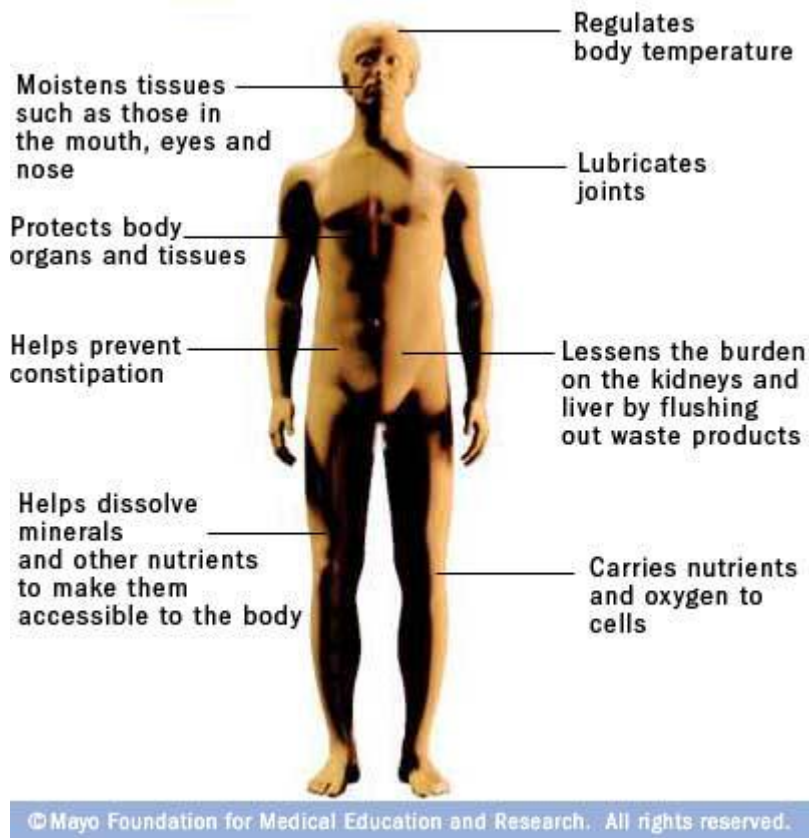Biological buffers should meet the following general criteria:

• Their $pK_a$ should reside between 6.0 to 8.0.

• They should exhibit high water solubility and minimal solubility in organic solvents.

• They should not permeate cell membranes.

• They should not exhibit any toxicity towards cells.

• The salt effect should be minimum; however, salts can be added as required.

• Ionic composition of the medium and temperature should have minimal effect of buffering capacity.

• Buffers should be stable and resistant to enzymatic degradation.

• Buffer should not absorb either in the visible or in the UV region.

*References:*

*NPTEL – Biotechnology – Fundamentals of Biotechnology by Vishal Trivedi. Joint initiative of IITs and IISc – Funded by MHRD.*

*Buffers (2003): A guide for the preparation and use of buffers in biological systems* **by Chandra Mohan.**

Regulates body temperature

Moistens tissues such as those in the mouth, eyes and nose

Lubricates joints

Protects body organs and tissues

Helps prevent constipation

Lessens the burden on the kidneys and liver by flushing out waste products

Helps dissolve minerals and other nutrients to make them accessible to the body

Carries nutrients and oxygen to cells

In chemistry, a lone pair refers to a pair of valence electrons that are not shared with another atom[1] and is sometimes called a non-bonding pair. Lone pairs are found in the outermost electron shell of atoms. They can be identified by using a Lewis structure. Electron pairs are therefore considered lone pairs if two electrons are paired but are not used in chemical bonding. Thus, the number of lone pair electrons plus the number of bonding electrons equals the total number of valence electrons around an atom.
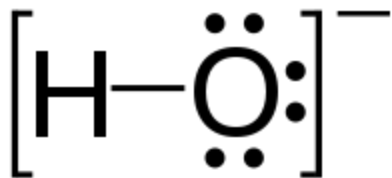


Lone pairs (shown as dots) in the Lewis structure of hydroxide



A          B          C
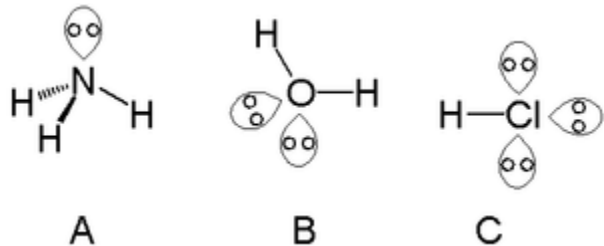
Lone pairs in ammonia (A), water (B) and hydrogen chloride (C)

Water has a simple molecular structure. It is composed of one oxygen atom and two hydrogen atoms. Each hydrogen atom is covalently bonded to the oxygen via a shared pair of electrons.

Oxygen also has two unshared pairs of electrons. Thus there are 4 pairs of electrons surrounding the oxygen atom, two pairs involved in covalent bonds with hydrogen, and two unshared pairs on the opposite side of the oxygen atom. Oxygen is an "electronegative" or electron "loving" atom compared with hydrogen.

Water is a "polar" molecule, meaning that there is an uneven distribution of electron density. Water has a partial negative charge ($\delta^-$) near the oxygen atom due the unshared pairs of electrons, and partial positive charges ($\delta^+$) near the hydrogen atoms.

An electrostatic attraction between the partial positive charge near the hydrogen atoms and the partial negative charge near the oxygen results in the formation of a hydrogen bond as shown in the illustration.

The ability of ions and other molecules to dissolve in water is due to polarity. For example, in the illustration below sodium chloride is shown in its crystalline form and dissolved in water



Hydrogen bonding between water molecules
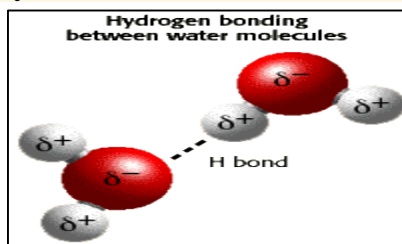
electronegativity, symbol $\chi$, is a chemical property that describes the tendency of an atom or a functional group to attract electrons (or electron density) towards itself.[1] An atom's electronegativity is affected by both its atomic number and the distance at which its valence electrons reside from the charged nucleus. The higher the associated electronegativity number, the more an element or compound attracts electrons towards it.
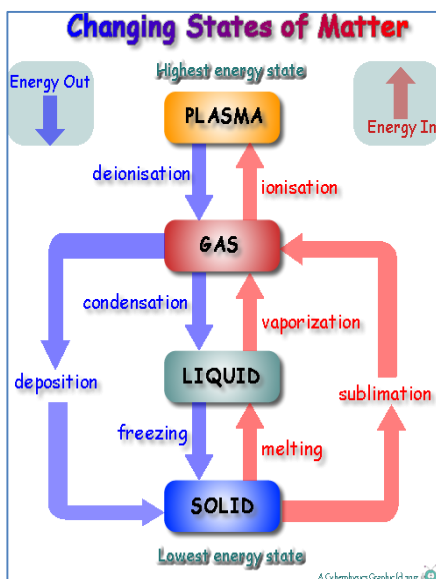


Deposition is a thermodynamic process, a phase transition in which gas transforms into solid. The reverse of deposition is sublimation. One example of deposition is the process by which, in sub-freezing air, water vapor changes directly to ice without first becoming a liquid. This is how snow forms in clouds, as well as how frost and hoar frost form on the ground or other surfaces. Another example is when frost forms on a leaf.

For deposition to occur, thermal energy must be removed from a gas. When the leaf becomes cold enough, water vapor in the air surrounding the leaf loses enough thermal energy to change into a solid. Even though the air temperature may be below the dew point, the water vapor may not be able to condense spontaneously if there is no way to remove the latent heat. When the leaf is introduced, the super cooled water vapor immediately begins to condense, but by this point is already past the freezing point. This causes the water vapor to change directly into a solid. Another example is the soot that is deposited on the walls of chimneys. Soot molecules rise from the fire in a hot and gaseous state. When they come into contact with the walls they cool, and change to the solid state, without formation of the liquid state. The process is made use of industrially in combustion chemical vapor deposition. Another example of physical deposition is the artificial process of physical vapor deposition, used to deposit thin films of various materials onto various surfaces. Deposition releases energy and is an exothermic phase change.

Quasicrystal:  a form of solid matter whose atoms are arranged like those of a crystal but assume patterns that do not exactly repeat themselves.