Chapter 1



Plant–Water Relations

1.1 Water – A Universal Solvent

Keywords

- Adhesion
- Aquaporins
- Chemical potential
- Cohesion
- Dialysis
- Electrochemical gradient
- Gated channel
- Hydration shell
- Hydraulic conductivity
- Hydrogen bond
- Ion channel
- Proton motive force
- Relative water content
- Tensile strength
- Transport proteins
- Water potential

Water is the most abundant resource for nearly all organisms. Covering over 70 per cent of the earth's surface and making up as much as 95 per cent of the matter of living organisms, it is virtually unique among all liquids. The water content of plants is in a continuous state of flux, based on the degree of metabolic activity, the water status of the surrounding air and soil, and a variety of other factors.

Water plays a crucial role in the physiological processes of plants; roles for which it is well-suited because of its special physical and chemical properties, some of which are:

- Water is an excellent solvent, making it a suitable medium for the absorption and translocation of mineral elements and other solutes necessary for normal plant growth and development.
- Most of the 'biochemical reactions', that are so characteristic of life, occur in water and water itself participates directly or indirectly in all the metabolic reactions.
- The 'thermal properties' of water, i.e., high specific heat, high latent heat of vaporization and high heat of fusion, ensure that water remains in the liquid form over the range of temperatures where most biological reactions take place.
- The combined properties of **'cohesion'** (capacity to bind strongly with itself), '**adhesion**' (capacity to bind strongly with other molecules containing oxygen such as glass and cell walls) and **'tensile strength'** (a measure of the maximum tension a material can

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withstand before breaking) are especially significant for maintaining the continuity of water columns in plants.

- The 'transparency' of water facilitates penetration of sunlight into the aqueous medium of cells thus enabling photosynthesis and other physiological processes to function in the cells.

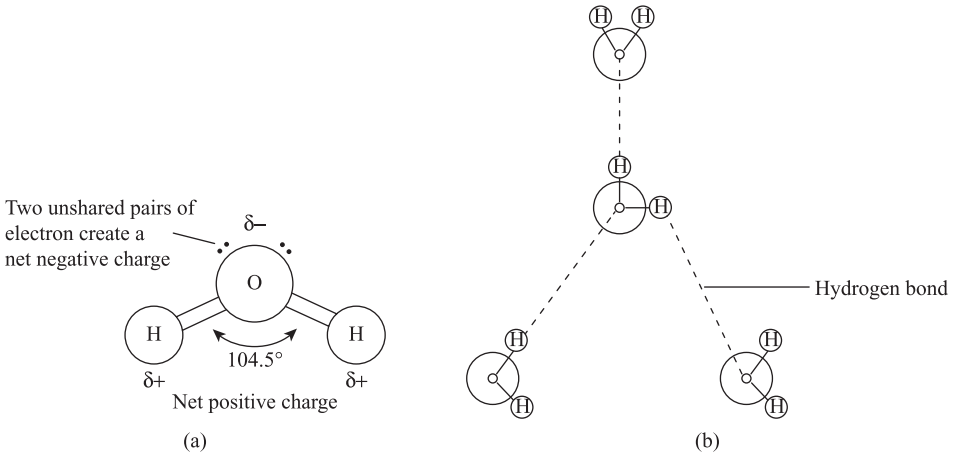


Fig. 1.1 (a) Structural configuration of a water molecule. Because of asymmetrical distribution of charge, the water molecule is polar. The bonding angle between the two hydrogen atoms is 104.5° rather than 180°. The oxygen atom shares one electron with each hydrogen atom. (b) Hydrogen bonds among the water molecules resulting from the electrostatic attraction between the partial positive charge on one molecule and the partial negative charge on the next.

These properties derive primarily from the '**polar**' structure of the water molecule (Figure 1.1a). The water (H_2O) molecule (molecular weight, 18) is composed of an oxygen atom covalently bonded to two hydrogen atoms that are attached asymmetrically to one side. The two O–H bonds form an angle of 104.5° rather than 180°. The oxygen atom being more electronegative than hydrogen, has a tendency to attract the electrons of the covalent bond thus resulting in a partial negative charge at the oxygen molecule and a partial positive charge at each hydrogen. Due to equal partial charges, the water molecule has no net charge.

The polarity and the tetrahedral shape of water molecules permit them to form **hydrogen bonds** that give water its unique physical properties (Figure 1.1b). Hydrogen bonding is a key feature that makes certain compounds soluble in water. Besides interactions among water molecules, hydrogen bonding is also accountable for the attraction between water and other molecules. However, when the groups responsible for hydrogen bonding are linked to a group other than water, they lose their solubility. This commonly occurs when hydrogen bonding exists within the same molecule, i.e., intra-molecular hydrogen bonding. For example: cellulose-a polymer of glucose found in plant cell walls. Hydrogen bonding is the basis for formation of **hydration shells** that develop around biological macromolecules such as proteins, lipids, nucleic acids and carbohydrates.

When water freezes below 0°C, it produces crystal 'lattice work', which we know as snow and ice. Ice is much less dense (a density of about 0.9179 g cm^{-3}) than liquid water (0.999 g cm^{-3}). This enables the ice to float on the lakes and ponds surfaces rather than

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sinking to the bottom where it might remain there throughout the year . This is extremely important for the survival of all types of aquatic life. If it were otherwise, it would have disastrous consequences, such as killing aquatic life. This unusual feature enables icebergs to float. The maximum density of water is 1.0 g cm^{-3} at 4 °C. This happens because molecules in the liquid state are tighter packed than in the crystalline state of ice.

The water expands when it freezes, resulting in the bursting of water pipes, and in plants, the inter- or intra-cellular risk of tissue damage increases manifold during extreme cold conditions.

The '**surface tension**' – the tendency of the surface to contract and resemble an elastic membrane–is high for water (Figure 1.2). And so, water droplets tend to form spheres as the water molecules at the surface are subjected to unequal forces and are pulled in towards the centre. Because of surface tension, the molecules tend to occupy the least possible space. In a deeper part of a mass of water, the molecules of water are attracted with equal force in different directions while at the air–water interface the water molecules, however, are attracted downward and sideways by other water molecules. Here, all of the hydrogen bonds in water face downward, causing the water molecules to cling together, resembling an elastic membrane. Some insects and spiders can glide on water surface due to the surface tension property of water, which is stronger than the force that one foot brings to bear.

The various physiological activities of the cell take place in an aqueous medium. They actually operate in dilute aqueous solutions, suspensions and colloidal phases. A study of their properties is, therefore, necessary for a better understanding of the different physiological processes.

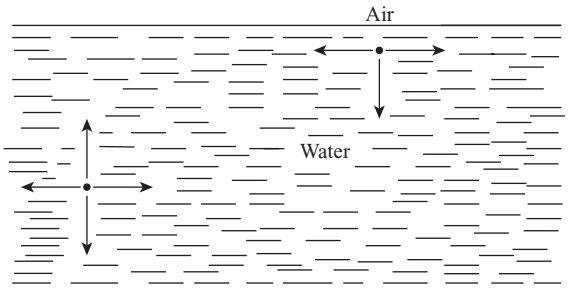


Fig. 1.2 Diagrammatic representation explaining surface tension and forces in a liquid. The particle is pulled downward because of unbalanced forces. A particle deeper in the liquid is attracted by forces from all directions.

1.1.1 Solution

A 'solution' is a homogenous mixture composed of two or more components. A component that is present in greater proportion is termed the 'solvent' while the other component that is present in smaller amounts is called the 'solute'.

A solution may exist in gaseous, liquid or solid state. It is convenient to distinguish solutions in terms of the physical states of the mixing components. The various types of solutions with examples are given in Table 1.1.

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Most significant types of solution are: (i) solids-in-liquids; (ii) liquids-in-liquids; and (iii) gas-in-liquids.

Table 1.1 Various types of solutions.

Solute	Solvent	Examples
Gas	Gas	Air
Gas	Liquid	CO_2 in aerated water
Gas	Solid	H_2 in platinum or palladium
Liquid	Gas	Moisture in air during mist
Liquid	Liquid	Alcohol in water
Liquid	Solid	Jellies, mercury in silver
Solid	Gas	Camphor in air
Solid	Liquid	Sugar in water
Solid	Solid	Solid solutions, alloys, etc.

Terminology connected with the concentration of solutions

The concentrations of solutions are expressed in different units.

Molarity (M): The 'molarity' of a solution gives the number of moles of the solute present per litre of the solution. Thus if one mole of a solute is present in one litre of solution, the concentration of the solution is said to be one molar. The term molar is generally used for salts, sugars and other compounds.

$$\text{Molarity} = \frac{\text{Weight of a solute in g/L of solution}}{\text{Molecular weight of a solute}}$$

Molality (m): The 'molality' of a solution gives the number of moles of the solute present in one kilogram (1000 g) of the solvent. Thus, if one mole of a solute is dissolved in 1000 grams of the solvent, the concentration of the solution is said to be one molal. Molality is used in situations, where it is desirable to express the ratio of solute to solvent molecules, for example, in discussions of osmotic pressure.

$$\text{Molality} = \frac{\text{Weight of a solute in g/kg of solvent}}{\text{Molecular weight of a solute}}$$

Normality (N): In volumetric work, we express the concentrations in terms of normality of the solutions.

The normality of a solution gives the number of gram equivalents of the solute per litre of the solution. Thus, if one gram equivalent of a solute is dissolved per litre of the solution, the concentration of the solution is said to be one normal. A normal solution is generally used for acids and bases.

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$$\text{Normality} = \frac{\text{Amount of a solute in g/L of solution}}{\text{Equivalent weight of solute}}$$

Per cent Solution (%): The concentration of substances that do not have a uniformly defined composition, such as proteins, nucleic acids, and such, is expressed in weight per unit volume rather than moles per unit volume. Thus, a 5 per cent solution would imply 5g of solute dissolved in solvent to give a final volume of 100ml of the solution. When either solute or solvent is taken by weight or volume, then per cent solution results in v/v (volume/volume), v/w (volume/weight), w/w (weight/weight) or w/v (weight/volume).

ppm Solution (mg/L or µg/ml solution): ppm solution needs to be prepared when a solute is present in a very small quantity. It represents one gram of a solute per million grams of the solution or one gram of a solute per million ml of the solution.

$$\text{ppm} = \frac{\text{mass of the component}}{\text{total mass of the solution}} \times 10^6$$

or

$$\text{ppm} = \frac{\text{g or ml of solute}}{\text{g or ml of solution}} \times 10^6$$

When 1 mg of a solute is dissolved in water and the volume is made up to 1000 ml, it results in 1 ppm (parts per million) solution.

In the true solution, the molecules of the solute do not settle down and cannot be visibly distinguished from the solvent even under the highest power of microscope. A true solution is transparent and has a higher boiling point, and lower freezing point as well as water potential, as compared to pure water. The number of solvent and solute molecules is constant in a sample of given solution. If to a given amount of solvent maintained at a constant temperature, a solute is added gradually in increasing amounts, a stage will be reached when some of the solute will remain undissolved, no matter how vigorously we stir. The solution is then said to be **saturated**. The excess solute is in dynamic equilibrium with the solute that has gone in solution.

1.1.2 Factors affecting solubility

1. ***Nature of Solute and Solvent*:** In general, if a solute and solvent have similar chemical characters, the solubility is very high and if they have entirely different chemical character, the solubility is extremely low. For example, sugars are largely soluble in water but not in benzene.
2. ***Temperature*:** The solubility of a solute in a given solvent varies greatly with temperature. It may increase or decrease with rise in temperature. Most of the salts (e.g., KNO_3, NH_4Br) show marked increase in solubility with rise in temperature, whereas others (such as, NaCl) show only a small increase. There are only a few substances (e.g., anhydrous sodium sulphate, Na_2SO_4, caesium sulphate) which show decrease in solubility with rise in temperature.

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'Standard solution' contains a known weight of the substance dissolved in a known volume of the solvent.

1.1.3 Suspension

A 'suspension' is a two-phase system where solute particles are not dispersed as molecules or ions. The larger solute particles (>0.1 μm in diameter) remain suspended throughout the liquid. The system is unstable and the particles settle down with the passage of time.

1.1.4 Emulsion

Unstable emulsions are prepared by vigorously shaking two immiscible liquids together. Small droplets (dispersed phase) of one of the liquids will be dispersed throughout the other (dispersion medium). An emulsion can be made stable by the addition of an emulsifying agent. These substances generally function either by decreasing the surface tension of the liquids or by forming a protective layer around the droplets, thus making it impossible for them to combine with each other. Milk is an example of emulsion containing butter fat dispersed in water, with casein acting as an emulsifying agent.

1.1.5 Acids, Bases and Salts

Acids are ionizable compounds that give up protons. Strong acids like HCl, H_2SO_4 and HNO_3 tend to ionize completely when in solution.

$$HCl \rightarrow H^+ + Cl^-$$

Weak acids are the organic acids and tend to ionize only partially when in solution. Thus, acetic acid ionizes to form acetate ions and protons, but some acetic acid still remains unionized.

$$\underset{\text{Acetic acid}}{CH_3COOH} \rightleftharpoons \underset{\text{Acetate ion}}{CH_3COO^-} + \underset{\text{Hydrogen ion}}{H^+}$$

Bases are ionizable compounds that tend to take up protons. Strong bases such as sodium hydroxide (NaOH) dissociate into its ions, when in solution.

$$NaOH \rightarrow Na^+ + OH^-$$

In contrast, ammonia is a relatively weak base. Occasionally, substances that yield OH^- ions are considered bases. For example: acetate (CH_3COO^-) is the conjugate base to acetic acid (CH_3COOH).

Salts are compounds formed from the conjugate base of an acid and the conjugate acid of a base, e.g. on mixing a solution of NaOH with acetic acid, the salt of sodium acetate is formed.

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1.1.6 Electrolytes and Non-electrolytes

Electrolytes are those substances that can conduct an electric current and undergo ionization when dissolved in water. Acids, bases and salts are electrolytes. Some common strong electrolytes with complete ionization in plant tissues are the inorganic ions K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Cl^-, SO_4^{2-} and PO_4^{3-}. Common weak electrolytes with incomplete ionization are the organic cations and anions, amino acids, organic acids and the complex charged polymers, such as proteins and nucleic acids. The behaviour of electrolytes was first discussed by Arrhenius in 1880.

Non-electrolytes are substances that do not undergo ionization on dissolving in water. Examples are: gases (oxygen and hydrogen) and most of the organic compounds such as sugars and alcohols and others. However, they do carry groups having positive or negative potential, such as, amino NH_2, imino NH, amide $CONH_2$, carbonyl CO, carboxyl COOH, hydroxyl OH and so on. These groups combine with water molecules which protect them from coming together by forming water shells.

1.1.7 Polar and Non-polar Compounds

Jacobs (1924) emphasised the importance of recognizing two classes of compounds, polar and non-polar, from the stand point of permeability. Polar compounds are compounds that have slight charges (+ve as well as –ve) within the compound. For example, water (H_2O), hydrogen fluoride (HF), hydrogen chloride (HCl), and ammonia (NH_3). Slight charges are due to electro-negativity differences between 0.3 and 1.4. In polar molecules, two atoms do not share electrons equally in a covalent bond. In addition to water, acids, bases and salts, there are many polar organic compounds such as sugars and alcohols.

Non-polar compounds do not have slightly negative and positive charges within the compound (in other words, the electrical charges are evenly distributed across the molecule), such as hydrocarbons, organic molecules: benzene, methane, ethylene, carbon tetrachloride, and the noble gases: helium, neon, argon, krypton, xenon and so on. Electronegativity differences of non-polar compounds lie between 0 and 0.2. In non-polar molecules, electrons are shared equally by the atoms of the molecules and there is no net electrical charge. Some molecules such as those of the fatty acids are mostly non-polar (the long hydrocarbon chain) but have polarity at one end (–COOH group).

1. Polar substances tend to be soluble in water and other polar solvents while non-polar substances are soluble in fats and organic solvents.
2. Polar compounds, with the exception of water, penetrate cells with difficulty, whereas non-polar compounds penetrate cells rapidly.
3. Polar compounds ionize readily and are highly reactive (except sugars and alcohols), whereas non-polar compounds do not ionize and are electrically neutral.

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1.1.8 pH and Buffers

pH

The term 'pH' has been derived from the French word 'Pouvoir Hydrogene' (power of hydrogen) and is used to quantitatively measure the acidity or alkalinity of aqueous or other liquid solutions. The concept of 'pH' was introduced by a Danish chemist, Søren Peder Lauritz Sørenson (1909), to express concentration of the hydrogen ion ($H^{+)}$ in an aqueous solution. The 'pH' can be defined as the 'negative logarithm' of the hydrogen ion concentration.

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

or

$$\text{mathematically speaking, } pH = \log \frac{1}{[H^+]}$$

Water dissociates into equal numbers of H^+ and OH^- ions and is therefore neither an acid nor a base. At ordinary temperature (22 °C), the actual concentration of H^+ (and of OH^-) ions in pure water is very small, i.e., 10^{-7} mol/L each of H^+(protons) and OH^- (hydroxyl) or [0.0000001 M]. The product of the two $(H^+) \times (OH^-)$ is 10^{-14} and this remains constant for all aqueous solutions. By knowing the concentration of H^+, the concentration of OH^- can be calculated or vice-versa.

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

$$[H^+][OH^-]$$

$$[10^{-7}][10^{-7}] = 10^{-14} \text{ (to multiply } 10^{-7} \text{ by } 10^{-7}\text{), exponents are added to give } 10^{-14}.$$

The pH of pure water, (with H^+ concentration of 1.0×10^{-7} M), will be 7 as calculated below:

$$pH = \log \frac{1}{[H^+]} = \log \frac{1}{1 \times 10^{-7}}$$

$$= \log [1 \times 10^7]$$

$$= \log 1.0 + \log 10^7$$

$$= 0 + 7$$

$$pH = 7$$

Hence, pure water with equal numbers of protons and hydroxyl ions will have a pH of 7. However, the pH of water is lowered down at higher temperature, e.g., at 50 °C, the pH of water is 6.55 ± 0.01.

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The pH scale covers a range of values from 0 to 14, in which the midpoint is 7.0 (Figure 1.3). Solutions having pH less than 7.0 are acidic (having more H^+ ions), while those having pH greater than 7.0 are basic or alkaline (having more OH^- ions than H^+ ions). Thus a solution of 6.0 pH will have 10 times more H+ ions than that of a 7.0 pH solution. Similarly a solution of 8.0 pH will have 10 times less H^+ than that of a 7.0 pH solution.

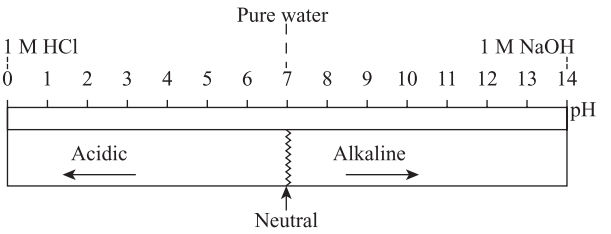


Fig. 1.3 The pH scale.

There is always an inverse relationship between the H^+ and OH^- ions concentration in a solution (Table 1.2). One needs to know only the value $[H^+]$ to determine $[OH^-]$ as well. Even a very strong acid of say pH 1 contains some OH^- ions (10^{-13}N) and a strong base of pH 14 has a few H^+ ions (10^{-14}N).

Table 1.2 Relationship between H^+ and OH^- ions concentration in a solution and the corresponding pH values.

H^+ mol/L	OH^- mol/L	Log $[H^+]$	$-$Log $[H^+]$ = pH
1×10^{0}	1×10^{-14}	0	0
1×10^{-1}	1×10^{-13}	−1	1
1×10^{-4}	1×10^{-10}	−4	4
1×10^{-7}	1×10^{-7}	−7	7
1×10^{-10}	1×10^{-4}	−10	10
1×10^{-13}	1×10^{-1}	−13	13
1×10^{-14}	1×10^{0}	−14	14

Thus, 1N HCl or any other strong acid that dissociates (100 per cent) shall have a pH of 0 because

$$\begin{aligned} pH &= -\log [H^+] \text{ or } [H] \text{ would be equal to } 1. \\ &= -\log [1] \\ &= 0 \end{aligned}$$

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Actually, the dissociation is not quite complete and 1.0 N HCl gives a pH of 0.10.
A 5N solution of hydrochloric acid, for example, would have a pH value of a little less than 0; correspondingly a 5N solution of sodium hydroxide would have a pH value a little greater than 14.

However, exceptionally acidic or basic solutions may have pH less than 0 or more than 14. For example, the acid mine 'run off' has a molar concentration of 3981 M, and the pH can be as low as –3.6.

While the pH of 1N NaOH would be 14 (since the log of $1 \times 10^{\circ}$ $[OH^-]$ would be –14).

The most important consequence of this system of measurement is that each unit decrease in pH indicates a 10-fold increase in H^+ ion content. Thus, pH 6 is ten times more acidic than pH 7, and pH 1 is 1,00,000 times more acidic than pH 6.

Because the amount of water ionized to form H^+ and OH^- is very small, there will be essentially no reduction in the total amount of $[H_2O]$ present.

A weak acid is one in which only a small fraction of the molecules are dissociated. An example is acetic acid, in which the 1N acid gives a pH of 2.37 because only 0.42 per cent of the molecules are dissociated.

$$[H^+] \text{ in 1N acetic acid} = \frac{0.42}{100} \times 1 = \ 0.0042 \text{ or } 10^{-2.37}$$

Thus pH = 2.37 because –2.37 is the log of 0.0042.

Many of the chemical and physical reactions occurring in plant cells are regulated by pH. Virtually all enzymatic reactions have rather narrow pH optima and many of the chemical reactions of cells either give up or take up protons. The pH drives many of the transport and energetic reactions in the cells. Thus, pH and its regulation in the cells are intimately tied to metabolism. The usual range of pH in plant fluids is from 5.2 to 6.2 (acidic). It is well known that the soluble pigments of the anthocyanin group change colour with pH. Thus, the floral colours of many plants are pH dependent.

Buffers

Buffers are the solutions that resist changes in pH by absorbing or reacting with either protons or hydroxyl ions. These are generally mixtures of either weak acids and their salts or weak bases and their salts. In biological systems, the vast array of amino acids and proteins operate as primary buffers.

A solution of acetic acid and sodium acetate will form an effective buffer. These compounds ionize in solution as follows:

$$CH_3COOH \rightleftharpoons CH_3COO^- + H^+$$
$$CH_3COONa \rightleftharpoons CH_3COO^- + Na^+$$

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On addition of a small amount of NaOH, the OH^- ions released in solution are neutralized by the free H^+ ions in the buffer solution. This causes more of the acetic acid to ionize and thus restores the original H^+ ion concentration. As more NaOH is added, more acetic acid ionizes until all of the acetic acid in solution is ionized. At this point, any further addition of NaOH will cause an abrupt rise in pH (Figure 1.4).

When a small amount of HCl is added to the acetic acid–sodium acetate buffer, the released H^+ ions rapidly unite with free acetate ions to form undissociated acetic acid.

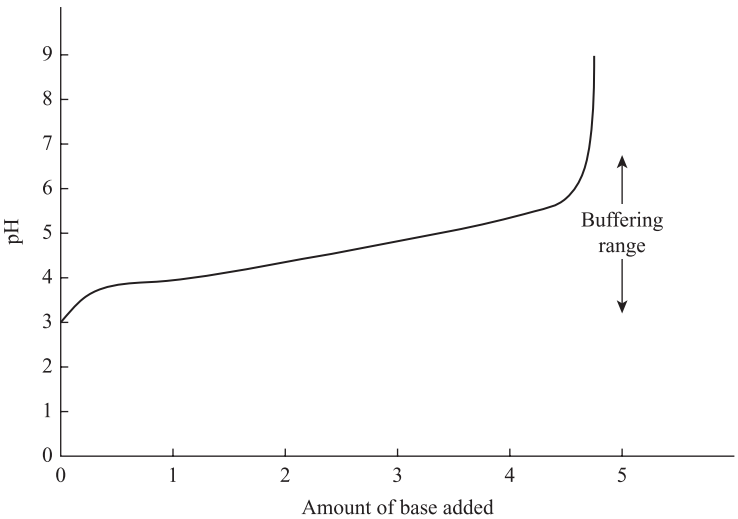


Fig. 1.4 Buffering range of a solution on addition of a base.

Therefore, there is no change in H^+ ion concentration. As more of HCl is added to the buffer, more H^+ ions will be available to combine with free acetate ions to form acetic acid, until all of the acetate ions have been converted. When this happens, any further addition of HCl will cause an abrupt drop in pH.

Buffer solutions are used to maintain specific pH of solutions in biochemical experiments. These are abundant in living plant cells and play vital roles in their existence. Enzymes generally function within a narrow pH range. A deviation of any magnitude impairs or completely inhibits their function. The living systems cannot tolerate any large increases or decreases in H^+ ion concentration. The biochemical reactions continuously produce acids and bases within cells. Moreover, we all consume foodstuff that are either acidic or basic; for example, a cola drink is a moderately strong acidic fluid with a pH of 3.0. In spite of such variations in the H^+ and OH^- ion concentrations, the pH of all living organisms is maintained relatively constant with the use of buffers solutions.

1.1.9 Colloids

If ordinary clay soil is agitated thoroughly with water and the resulting murky liquid uniformly brown in colour is allowed to stand, it clears rapidly. The larger particles settle out first followed by the smaller particles. Very minute soil particles still remain suspended in

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water and the stable, heterogeneous mixture that results is called a **colloid.** The suspended phase is called the **dispersed phase** (discontinuous phase) and the medium in which the dispersion takes place is called the **dispersion medium** (continuous phase). The dispersed particles range between 0.001 and 0.1 µm.

The term 'colloid', first suggested by T. Graham in 1861, derives its name from the Greek words 'Kolla' (= glue) and 'eidos' (= like). He used the term to describe glue-like preparations, such as solutions of certain proteins and liquid preparations of vegetable gums, such as gum arabic. But it is more than likely that there are multiple ions or molecules making up each colloidal particle, resulting in large surfaces. It is these surfaces of the colloidal particles that make them so interesting and reactive in the biological sense. The stability of the colloid results mainly because of the tendency of the particles to clump together and thereby reduce their surface area.

Colloids of solid particles in liquid are called **sols.** A solid colloidal system is called a **gel**. Reversible sol-gel transformations are common in biological systems: Colloids of solids or liquids in gas are termed '**aerosols**' whereas liquids in liquids are called **emulsions.**

Types of colloidal systems with examples:

1. Gas in liquid (foam)
2. Gas in solid (pumice stone)
3. Liquid in gas (fog)
4. Liquid in liquid (emulsion)
5. Liquid in solid (wet cell walls)
6. Solid in gas (smoke)
7. Solid in liquid (suspension)
8. Solid in solid (copper into zinc, if the bars of two metals are kept pressed with one another).

Depending on their properties, colloids can be lyophilic (show an affinity for solvent) or lyophobic (no affinity) or, if specifically aqueous, hydrophilic or hydrophobic. Hydrophilic colloids are those in which the particles have an affinity for water; hydrophobic colloids lack such affinity. It is believed that hydrophilic sols base their stability on water hydration of the particles and hydrophobic sols are stable because of water exclusion and repulsion of particles due to charged surfaces. Plant gums and mucilages, starch and proteins tend to form hydrophilic sols or colloids, whereas metallic sols are frequently hydrophobic.

Particles of colloidal dimension range in size from 1 to 200 nm. Colloidal particles are so tiny that they cannot be viewed even with the light microscope. The size of the colloidal particles is somewhere between the size of the particles that form true solutions and the size of particles that are found in unstable suspensions.

Properties

The most important properties of colloids are based on their large surface area and sol-gel transformations:

1. ***Surface area*:** Several biochemical reactions occur on the surface of colloids. Thus surface area is an important part in determining the physiological behaviour of protoplasm.

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2. ***Filterability*:** Colloids are considered to be filterable even though they will pass through the usual laboratory filters. Ultra-filters (composed of biologically inert cellulose esters) of the proper dimensions allow for the separation of colloidal particles from those in true solution. This property is used in separating a colloid from a true solution by the process of dialysis.
3. ***Tyndall effect*:** Colloids illuminated with a beam of light at right angles to the observer are seen to scatter or diffract light. The Tyndall effect (reported by M. Faraday in 1858), known to anyone who has observed dust in room air, is indicative of a colloid. The greater the index of diffraction, the greater will be the visual effect. Scattering of light entering a leaf is important for photochemical reactions in which light is absorbed by pigments.
4. ***Viscosity*:** Colloids decrease in viscosity with increase in temperature. Hydrophilic sols increase exponentially in viscosity when the colloidal particles are increased. For example plant gums, mucilages and protein sols become highly viscous when concentrated.
5. ***Electrical properties*:** The micelles of colloids are almost always charged because of the chemical groups associated with the particles. For example, the micelles of proteins have a net negative charge and therefore cations will always be attracted to the negative charge. The ionic double layer is one force giving stability to the colloid. Besides having an ionic double layer, hydrophilic colloids will also have a hydration shell associated with them resulting in even more stability.
6. ***Coagulation*:** Coagulation can be brought about by removing the water layer and neutralizing the charge. Proteins will tend to clump at their isoelectric points when their net charge is zero or upon treatment with solvents that remove their water shells. These properties are frequently manipulated in protein purification in which unlike proteins are separated.
7. ***Brownian movement*:** Colloidal particles show Brownian movements, first described by Robert Brown. The random motion of the particles is caused by their uneven bombardment by molecules of the dispersion medium. The smaller the suspended particles, the greater will be the amplitude of the Brownian movement. This is more active at higher temperatures because of greater kinetic activity and lower viscosity of the medium.

1.1.9.1 Protoplasm as a colloidal system

Physically, the protoplasm is a living, heterogenous polyphasic colloidal system, consisting of a thick or viscous (semi-fluid, jelly-like), translucent and odourless fluid matrix or ground substance surrounded by the cell wall. The protoplasm is a complex colloidal system of many phases where water is the dispersion medium (about 80 per cent) with dissolved inorganic ions and small molecules. The dispersed phase comprises mainly the large molecules of proteins (14 per cent), lipids (3 per cent), carbohydrates (1 per cent) and nitrogen-based derivatives of DNA and RNA and such, representing many phases. Much of the colloidal nature is due to the protein phase which forms the major part of the living protoplasm, about 79 per cent of the dry weight of protoplasm. Proteins are very large complex molecules which

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sometimes reach colloidal dimension. They are dispersed throughout the ground substance of the protoplasm where they are involved in such cell activities as respiration, digestion and secretion. Undoubtedly, the immense surface area provided by protein enzymes dispersed in the protoplasm is of extreme importance to the many enzyme–substrate reactions upon which all life depends.

Lipids constitute only a small fraction of the protoplasm as compared to the proteins. They are important structural components of the cell. Being insoluble in water (a polar solvent), they occur in the protoplasm as minute globules (or droplets) or form an emulsion. However, they are soluble in the non-polar organic solvents, including chloroform, ether, acetone and benzene.

The colloidal nature of protoplasm was suggested by Wilson Fisher (1894) and William Bate Hardy (1899).

Although protoplasm exhibits the properties of a 'sol', it sometimes becomes gel-like rigid. The change from sol to gel consistency takes place during cell division in the formation of nuclear fibres and during the formation of plasma membrane. The plasma membrane and cell walls are gel-like and so are the chromosomes and centrosomes.

1.2 Permeability

Permeability is a fundamental phenomenon for the functioning of living cells and for maintaining satisfactory intracellular physiological activities. Every cell and organelle needs some sort of physical barrier to keep its contents in and external materials out, as well as some means of controlling exchange between its internal environment and the extracellular environment. Ideally, such a barrier should be impermeable to most of the molecules and ions found in cells and their surroundings. At the same time, it must be readily permeable to water because water is the basic solvent system of the cell and must be able to flow in and out of the cell as required. As expected, the membranes that surround the cells and organelles satisfy these criteria admirably.

The three types of membranes found in plants are **permeable, semipermeable** and **impermeable**. The cellulosic cell wall is a permeable membrane, which allows both water and dissolved substances to pass through. The cutinized and suberized cell walls are impermeable membranes which allow neither water nor solutes to pass through. The cell membrane and vacuolar membrane (tonoplast) behave as semipermeable membranes allowing water to pass through but impermeable to most of the solutes. Although the term 'semipermeable' is often used, it is not descriptive of biological membranes. Semipermeable implies that the membrane is permeable to many substances but without specificity and the term fails to describe the true dynamic nature of such membranes. Instead, the term 'differentially permeable' is widely used to describe a membrane that regulates the passage of diverse materials in and out of the cell, organelles and vacuoles at different rates, depending on the relative solubility of those materials for the lipid and protein constituents.

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1.2.1 Cell membrane as permeability barrier

Permeability may be passive if it obeys only physical laws, as in the case of diffusion. If a concentrated solution of soluble substance, e.g., sugar is placed in water, there will be a net movement of the solute along a concentration gradient. However, if the plasma membrane is interposed, the diffusion process is greatly modified and the membrane acts as a barrier to the movement of water-soluble molecules.

At the end of nineteenth century, Overton demonstrated that substances that are soluble in lipids pass more readily into the cell. Further Collander and Bärlund in their classic experiments with the cells of *Chara* demonstrated that the rate at which substances penetrate depends upon their solubility in lipids and the size or dimension of the molecule. The molecules that are more soluble in lipids pass across the lipid layer more quickly and further, with equal solubility in lipids, the smaller molecules traverse at a faster rate.

The permeability (P) of molecules across the membrane is expressed as follows:

$$P = \frac{KD}{t}$$

where K, is the partition coefficient; D, the diffusion coefficient (determined by the molecular weight); and t, is the thickness of the membrane.

Various theories or hypotheses have been proposed over a period of time to describe the permeability of the cell membrane: Retention pressure theory (Traube, 1867); Solution theory (Overton, 1895); Fluid-mosaic theory (Nathansohn, 1904); Sieve or ultrafiltration theory (Ruhland, 1912; Hoffman, 1925; Kuster, 1911, 1918); Colloidal theory (Sparth, 1916); Electro-capillary theory (Michaelis, Scarth and Lloyd).

The permeability attribute depends upon the chemical composition of the membrane and the molecular arrangement of its constituents. Our current understanding of membrane structure is the culmination of almost a century of studies, beginning with the recognition that lipids are an important membrane component and moving progressively from a lipid monolayer to a phospholipid bilayer (Figure 1.5). Once proteins were recognized as important components, Davson and Danielli (1935) proposed their 'Sandwich model', with the lipid bilayer surrounded on both sides by layers of proteins. Their model was acknowledged as the basis of the 'unit membrane' put forwarded by Robertson (1950) but was later discredited because it could not account for key features of membrane proteins. In its place, the **fluid-mosaic model** (S. J. Singer and G. L. Nicolson, 1972) has emerged as the generally accepted description of membrane structure (Figure 1.6). The membrane is envisioned as a mosaic of proteins discontinuously embedded in a bimolecular lipid layer. Two principal types of lipids – phospholipids[1], and sterols,[2] especially stigmasterol - exist in the plasma membrane of plant cells. The phospholipid bilayer structure gives the membrane three properties that are particularly significant. These are: being highly fluid, very stable, and impermeable to most molecules of biological interest.

[1] Phospholipids and transmembrane proteins with hydrophilic as well as hydrophobic groups are referred to as 'amphipathic molecules' (the Greek prefix *amphi* means 'of both kinds'). The term 'amphipathy' was coined by Hartley in 1936.

[2] Cholesterol is the major sterol in animal tissues.

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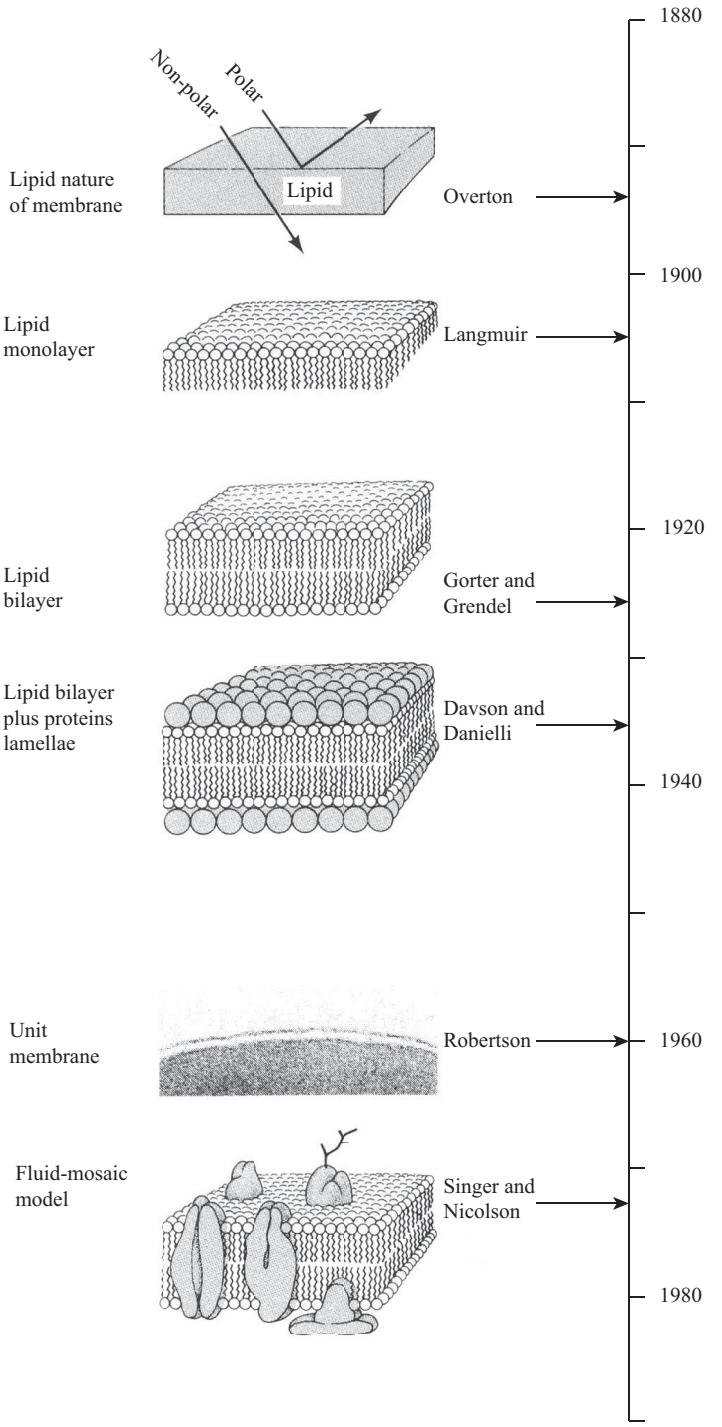


Fig. 1.5 Timeline for the development of our understanding of cell membrane structure.

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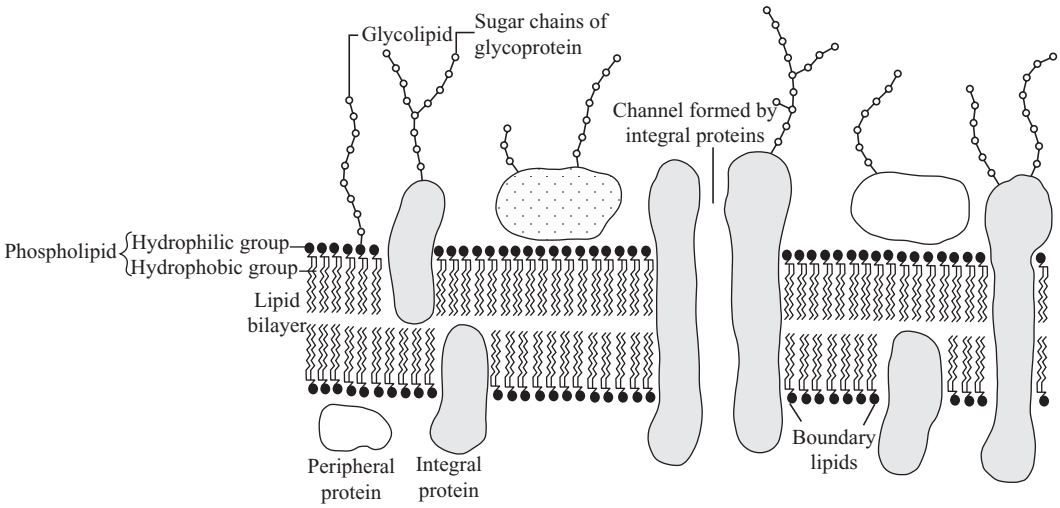


Fig. 1.6 The fluid-mosaic model of cell membranes.

This model recognizes two broad categories of membrane proteins that differ in their affinity or attraction for the inside of hydrophobic region of the membrane. **Integral membrane proteins** (intrinsic proteins) have one or more hydrophobic regions with an affinity for the hydrophobic region of the bimolecular lipid layer. These proteins are therefore intimately associated with the membrane and cannot be readily removed. In contrast, **peripheral membrane proteins** (extrinsic proteins) lack distinct hydrophobic segments and therefore do not enter into the lipid bilayer. Instead, they associate with the membrane surfaces through weak electrostatic forces, binding either to the polar heads of membrane lipids or to the hydrophilic portions of integral proteins that extend out of the membrane. Peripheral proteins are much more readily removed from the membrane than integral proteins and can usually be rendered soluble by aqueous salt solutions, without resorting to detergents. Proteins of the plasma membrane play a variety of roles in plant cells. They may serve as enzymes, or as anchors for structural elements of the cytoskeleton, or as, transport proteins for movement of specific ions and hydrophilic solutes, receptors for specific chemical signals that impinge on the cell from its environment and need to be transmitted inward to the cell.

The peripheral proteins and the parts of the integral proteins that occur on the outer membrane surface are attached to short-chain carbohydrates (oligosaccharides), thereby forming glycoproteins. Some of the phospholipids (specifically the hydrophilic region) at the outer membrane surface are linked to carbohydrates to form glycolipids.

1.2.2 Factors affecting permeability

Permeability of the membrane primarily depends upon the nature of the solute as well as of membrane. Three primary properties of the solutes must be considered: relative size, relative polarity and the ionic nature of solutes.

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***Relative size*:** Generally speaking, a membrane is more permeable to smaller molecules (mol. wt. below 100 D) regardless of whether they are polar (such as CO_2, ethanol and urea) or non-polar (such as O_2) as shown in Figure 1.7. Thus, both CO_2 and O_2 can move readily in and out of cells and organelles. Water is an important example of a very small molecule that, although polar, diffuses very rapidly across membranes through water-selective channels called **aquaporins.**

***Relative polarity*:** Because of its hydrophobic interior, a membrane is relatively permeable to non-polar molecules and is quite impermeable to most polar molecules.

***Ionic nature of solutes*:** Broadly, two types of substances can be recognized by their ability to cross the cell membrane:

- Ionic (electrolytes), e.g., mineral salts.
- Non-ionic (non-electrolytes), e.g., sucrose, alcohol, aldehydes.

As a general rule, a membrane is highly impervious to all ions. This is because a huge amount of energy (~40 kcal/mol) is needed to transport ions from an aqueous environment into a non-polar environment.

The impermeability of membranes to ions is very important for cell activity, because nerve cells, mitochondria and chloroplasts must maintain large ion gradients in order to function. Even the smallest ions (Na^+, K^+) are excluded from the hydrophobic interior of the membrane due to the charge on an ion and the sphere of hydration that surrounds the ion. Indeed, cells have ways for transferring ions such as Na^+ and K^+ as well as a wide variety of polar molecules across membranes that are otherwise not permeable to these substances. These membranes are equipped with **transport proteins** to serve this function. A transport protein is a specialized transmembrane protein that serves *either* as a hydrophilic **ion channel** (K^+, Ca^{2+} channels) through an otherwise hydrophobic membrane (Figure 1.7) *or* as a carrier that binds a specific solute molecule towards either side of the membrane accompanied by change in its conformation and thus transporting the solute across the membrane. Whether a channel or a carrier, each transport protein is specific for a particular molecule or ion. Diffusion through a channel depends on the size of the hydrated ion and the charge carried by it. Channels may exist in two different configurations – open and closed, i.e., **gated channels.** As a result, biological membranes can be best described as 'selectively permeable'.

1.2.3 Cell wall as permeability barrier

The presence of a wall around a plant cell poses a significant permeability barrier, though only for large molecules. In a typical cell wall, the diameter of the pores is about 5 nm, big enough to allow the passage of globular molecules with molecular weight up to 20,000 D. Not surprisingly, the hormones that serve as intercellular signals in plants are without exception small molecules, with molecular weight well below 1000 D.

Plasmodesmata are cytoplasmic channels (40–50 nm in diameter) through relatively large pores in the cell walls, connecting protoplasts of adjacent plant cells and allowing cytoplasmic exchange between the cells (Figure 1.8).

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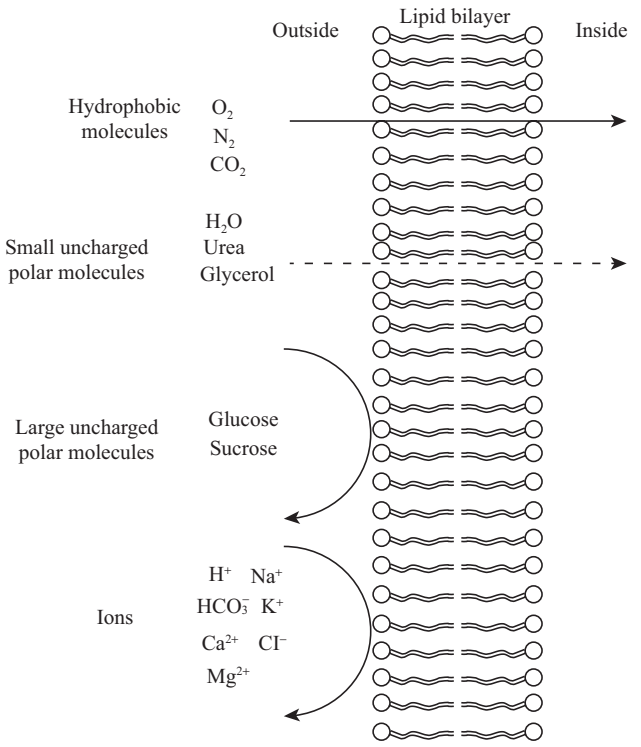


Fig. 1.7 The relative permeability of the lipid bilayer to different types of molecules (hydrophobic molecules, small and large uncharged polar molecules, various types of ions, and such).

Plasmodesmata can be primary or secondary; depending upon whether they are created during cell division or after the cell division is completed. The endoplasmic reticulum network of adjacent cells is connected, forming the desmotubule that runs through the centre of the channel. Proteins assemble on the external surface of the desmotubule and the internal surface of the plasma membrane; both the surfaces are linked by spokes, the filamentous proteins, which divide the cytoplasmic sleeve into microchannels. Surrounding the necks of the channel at either end are valve-like wall collars, composed of the polysaccharide callose, which serves to restrict the size of the pore.

Calcium appears to regulate movement of molecules through plasmodesmata. Intercellular transport of water and solutes can occur through plasmodesmata, which provide high-resistance connections between almost all living cells in plants. Only exceptions are stomatal guard cells and embryonic cells which lack plasmodesmata. The frequency of plasmodesmata varies widely from about 0.1 to 10 per square micrometer. Cells that are interconnected by plasmodesmata make up the symplasm and all space outside the plasma membrane is called the 'apoplast'. Not only small molecules but also macromolecules including virus and endogenous mRNA and proteins have been reported to move from one cell to another within the symplasm.

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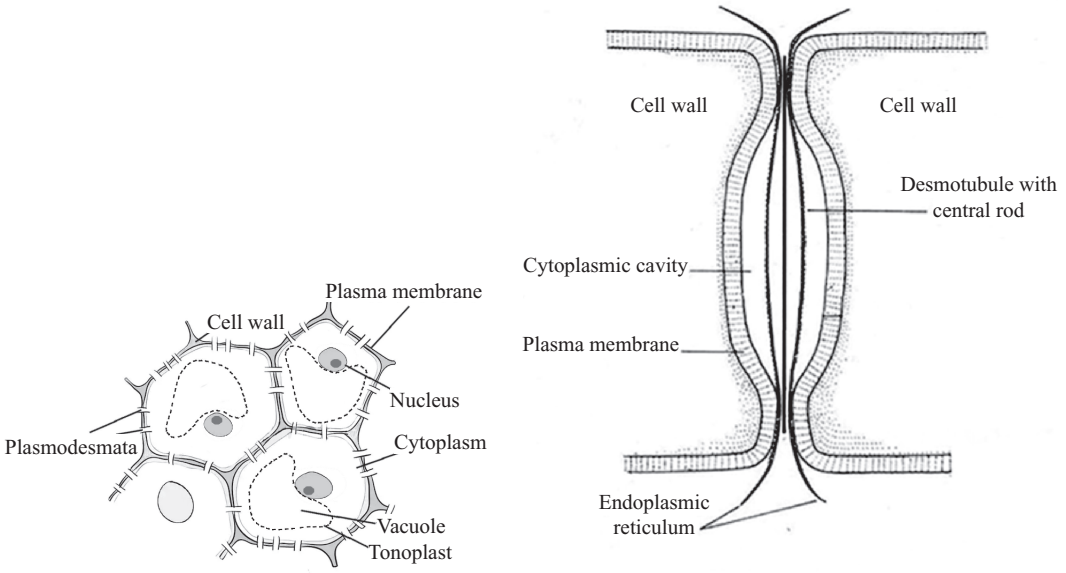


Fig. 1.8 (a) Plasmodesmata connect the cytoplasm of adjoining cells and facilitate communication between the cells. Symplastic movement of water and solutes occurs through the plasmodesmata. (b) Detailed structure of a plasmodesmata connecting two adjacent cells. The plasma membrane is continuous between the two cells and the endoplasmic reticulum goes through the centre of the plasmodesmata that forms the tubule.

The virus genome code for the '**movement proteins**' that facilitate movement of the virus particles by interacting with plasmodesmata, and this operates through one of the two mechanisms:

i. Viruses, such as cowpea mosaic virus and tomato spotted wilt virus, encode movement proteins that form a transport tubule within the plasmodesmatal channel that enhances the passage of mature virus particles through plasmodesmata.
ii. Movement proteins of tobacco mosaic can move between cells in leaves that are susceptible to the virus, where it recruits other proteins in the cell, thus increasing the size of the plasmodesmatal pore. So virus-sized particles can readily move through the plasmodesmata to a neighboring cell.

However, there is a restriction limit on the size of molecules that can be transported *via* the symplast (called **size exclusion limit**), which varies with cell type, environment, and developmental stage. The movement through plasmodesmata can be regulated or gated by altering the dimensions of wall collars, the cytoplasmic sleeve, and the lumen inside the desmotubule. In addition, adjacent plasmodesmata can form interconnections (branched plasmodesmata) that alter the size exclusion limit.

1.2.4 Plant vacuoles—multifunctional compartments

Vacuoles, the fluid-filled organelles or compartments surrounded by a single membrane, the **tonoplast,** occupy 30–80 per cent of the cell volume and enable plant cells to grow rapidly

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with the least amounts of energy and proteins. The number and size of the vacuoles in different plant cells vary greatly.

Plant vacuoles perform a multitude of functions such as:

i. **Plant cell expansion:** Cell expansion is controlled by the osmotic entry of water into the plant vacuoles followed by modified cell wall extensibility. Solutes (such as K^+, Na^+, Ca^{2+}, Cl^-, SO_4^{2-}, PO_4^{3-}, NO_3^-, amino acids, organic acids, and sugars) must be actively transported into the vacuole to maintain its osmotic concentration and also the turgor pressure of continuously growing cells. An electrochemical gradient across the vacuolar membrane, maintained by electrogenic H^+-ATPase pumps, provides the driving force for solute intake. Water movement across the tonoplast is mediated by tonoplast intrinsic proteins (TIPs)

ii. **Storage**: Plants store large amounts of proteins in their vacuoles, especially in seeds, which can be used in various metabolic pathways whenever required. Storage compounds in the vacuoles are also responsible for flavours of vegetables and fruits.

iii. **pH and ionic homeostasis**: Large vacuoles serve as reservoirs of protons and metabolically important ions, such as Ca^{2+}. Typically, the pH of plant vacuoles lies between 5.0 and 5.5 but vacuoles of lemon fruit and unactivated protein storage cells have pH of 2.5 and 7.0 respectively. Therefore, cells can regulate cytosolic pH, the enzyme activity, events such as assembly of cytoskeleton components and membrane fusion by regulating the efflux of protons and other ions into the cytosol.

iv. **Pigmentation**: Vacuoles of plant cells especially fruits and flower petals contain anthocyanin pigments which are used to attract pollinators and seed dispersers. In leaves, these pigments help in preventing photo-oxidative damage caused to the photosynthetic apparatus, by filtering out UV and visible light.

v. **Sequestration of toxic compounds**: Plants sequester heavy metals and metabolites like oxalate in vacuoles. Foreign compounds **(xenobiotics)** are transported from cytoplasm into vacuoles by an **ABC family of transporters** or specific cells develop vacuoles containing an organic matrix within which oxalate reacts with Ca^{2+} to form calcium oxalate crystals. Leaves are shed regularly because of accumulation of toxic compounds in leaf vacuoles.

vi. **Defence against microbial pathogens and herbivores**: Plant cells accumulate several toxic compounds in their vacuoles, which reduce feeding by herbivores and also destroy microbial pathogens. These compounds include:

- Phenolic compounds, alkaloids, cyanogenic glycosides, protease inhibitors
- Cell wall degrading enzymes, i.e., **chitinase and glucanase**, and defensive molecules such as **saponins** to destroy pathogenic fungi and bacteria
- Latexes and wound-clogging emulsions of hydrophobic polymers possessing insecticidal and fungicidal properties to serve as anti-herbivory agents.

vii. **Digestion**: Vacuoles have been shown to contain acid **hydrolases** such as proteases, nucleases, glycosidases and lipases, which degrade and recycle the cellular components. Such recycling is required for the retrieval of valuable nutrients during programmed cell death associated with development and senescence as well as for the normal turnover of cellular structures.

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1.2.5 Transport channels

Transport channels in plants can be classified into two categories**: Ion channels and Aquaporins**

Ion channels

An ion channel forms a selective, water-filled pore in the membrane which permits the transport of a large number of ions very rapidly because of little interaction between the ion and the channel. Ion channels are ubiquitously distributed in the membranes of plant cells (Figure 1.9). Movement through ion channels is passive and is dictated by the electrochemical potential for the particular ion. The membrane potential has a strong influence on the diffusion of ions across the channels. Because the membrane potential of the plasma membrane is usually negative, cations tend to diffuse into the cytosol and anions tend to diffuse out. Ion channels show strong selectivity for either cations or anions based on size exclusion. Most plasma membrane anion channels allow the passage of anions such as chloride, nitrate and organic acids whereas vacuolar anion channels are specific for malate. Similarly cation channels are specific for divalent and monovalent cations. Ion channels play important regulatory roles e.g. they regulate osmotic concentration by allowing the flux of K^+ into and out of cells, and set the concentration of ion important in cellular signalling, i.e., cytosolic Ca^{2+}.

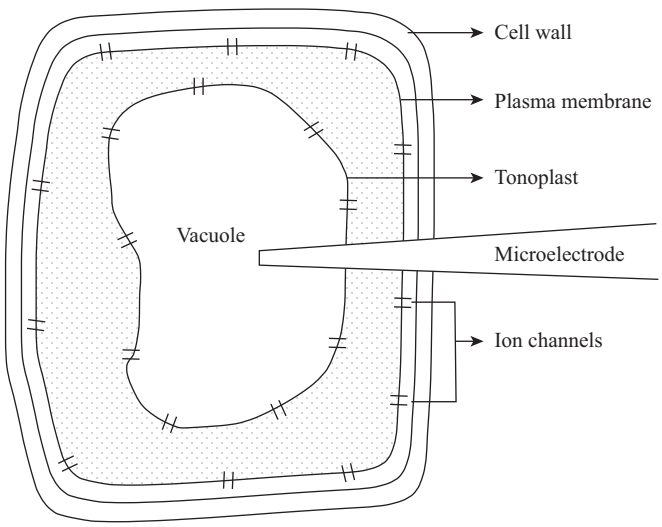


Fig. 1.9 Ion channels present in the plasma membrane and use of microelectrode to measure electrical properties of the cell.

The movement of ions through channels results in the flow of current, which can be used as a measure of the activity of a channel. Equation for Ohm's law can be used to model the movement of ions across the membrane.

I = V/R, where I = current, V = membrane potential, R = resistance of the membrane

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The resistance of a membrane to a particular ion depends on its selectivity and the number of channels. No current will pass through an impermeable membrane, whereas membranes that are freely permeable will behave as a resistance-free circuit.

Channel opening and closing, termed as **gating**, is regulated by membrane potential. Voltage-gated K^+ channels are important in maintaining the cell's membrane potential. The plasma membrane has channels that allow either inward or outward movement of K^+, referred to as 'inwardly rectifying channels' or 'outwardly rectifying channels'. These channels operate like valves and are said to rectify. By opening and closing in response to voltage, these channels can keep membrane potential constant. Some ion channels can also be gated by ligands such as hormones, Ca^{2+}, G-proteins and pH and others by changes in cell's turgor pressure. Complex regulatory circuits can be built up by the interaction of these various regulators of ion channel activity; for example, the closing of stomatal guard cells in the presence of abscisic acid (ABA). The tonoplast has at least four voltage-dependent Ca^{2+} ion channels, and these may be regulated by binding of ligands or covalent modification. The presence of multiple Ca^{2+} channels gated by different signals allows for dynamic changes in cytosolic Ca^{2+} in response to a variety of stimuli.

Patch-clamp technique allows the study of single ion channels in selected cellular membranes (Figure 1.10). The microelectrode tip (1 µm diameter) is held in contact with the membrane outside an isolated protoplast and with little suction, a tight seal between the electrode and the membrane is formed. The small section of membrane in contact with the electrode is known as the '**patch**'. Measurements can be done either with the whole cell attached or the electrode can be pulled away from the cell and then the patch sticking to the electrode tip can be bathed in solutions of known composition. The external surface of the membrane is in contact with the microelectrode solution-appropriately termed as 'inside-out' configuration. With a suitable electrical circuit, the potential difference can be held across the patch at some predetermined value. Simultaneously, the circuit will monitor any current flowing through the membrane patch.

The patch-clamp technique exemplifies the major advancements made in the field of electrophysiology and provides insights into transport mechanisms across cellular membranes in plants.

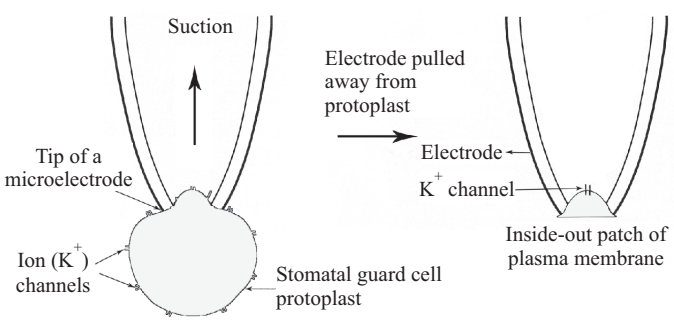


Fig. 1.10 Patch-clamp technique that is used to measure the flow of current through a single ion channel. A small section of membrane containing a individual ion channel can be isolated at the tip of the microelectrode. Sensitive amplifiers are used to measure the current carried by ions flowing through the channel.

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Aquaporins

Aquaporins are specialized membrane proteins controlling the selective movement of water. Plant aquaporins were first isolated from tonoplast but they are also localized to the plasma membrane as well as to some other endomembranes, excluding the chloroplast and mitochondria (Figure 1.11). An aquaporin consists of four subunits (tetramers), each with a mass of ~30 kD. Each of the four subunits has six transmembrane domains. The interior and the exterior of each aquaporin channel consists of hydrophilic and hydrophobic amino acids that interact with water molecules and hydrophobic fatty acids of the lipid bilayer respectively (Figure 1.12).

Aquaporins are divided into four subgroups:

1. Tonoplast intrinsic proteins(TIPs)
2. Plasma membrane intrinsic proteins(PIPs)
3. Nodulin intrinsic proteins(NIPs)
4. Small basic intrinsic proteins(SIPs)

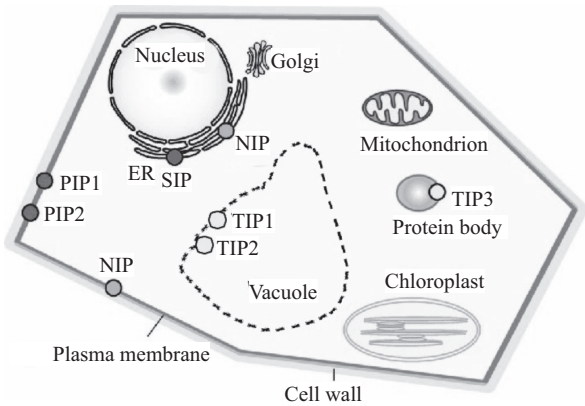


Fig. 1.11 Various types of aquaporins in a plant cell. Plasma membrane intrinsic proteins (PIPs), Tonoplast intrinsic proteins (TIPs), Nodulin intrinsic proteins (NIPs), and Small basic intrinsic proteins(SIPs).

The four subgroups of aquaporins are known to be present in all land plants. The **tonoplast intrinsic proteins** (TIPs**)** and **plasma membrane intrinsic proteins** (PIPs) are localized to the vacuolar and plasma membranes respectively. **Nodulin intrinsic proteins** (NIPs) are confined to the peribacteroid membranes of symbiotic N_2-fixing nodules and are present in non-leguminous plants as well. The fourth class of aquaporins, the **small basic intrinsic proteins** (SIPs) are found in the endoplasmic reticulum and are encoded by a small family of two or three genes.

Aquaporin activity may be regulated by several factors, such as cytosolic pH, Ca^{2+} concentration, nutrient and water stress, hormones, regulatory molecules, and so on. Thus, they can have a profound impact on the hydraulic conductivity of a membrane. Phosphorylation of aquaporins has also been shown to regulate their opening. Ca^{2+}-

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dependent protein kinase may catalyze this phosphorylation, thereby suggesting a relation between Ca^{2+} signalling and the regulation of water flow between cells. They may also facilitate the movement of CO_2, NH_3, H_2O_2, boron and silicon.

Aquaporins are essential for osmoregulation in plant cells (intra- as well as inter-cellularly) and provide a least-resistance pathway for the movement of water thus enhancing its flow across the biological membranes.

Aquaporins are part of relatively large gene families in plants; for example, *Arabidopsis,* corn and rice have 35, 36, and 33 aquaporin genes respectively. There is high sequence homology (>97%) among members of each subgroup. The C- as well as N- terminal sequences of all aquaporins localize to the cytosol as do connecting loops II and IV. The I, III and V connecting loops of PIPs face the apoplast, whereas those of TIPs and SIPs face the lumen of the vacuole and endoplasmic reticulum, respectively. Four subunits (tetramer) make a functional aquaporin complex and an individual subunit of the tetramer forms a water channel (Figure 1.12). Post-translational modification of aquaporins occurs by phosphorylation and methylation of amino acids at the N- as well as C- terminals. Phosphorylation at multiple amino acids at the C terminus, and methylation of amino acids at the N terminus has been shown in PIP-type aquaporins.

1.2.5.1 Transport of molecules and ions across membranes

Three categories of transport of small organic molecules and inorganic ions have been identified:

Cellular transport involves the exchange of substances between the cell and its immediate environment. For example, the uptake of nutrients and other raw materials across the cell membrane and the removal of waste and secretory products.

Intracellular transport involves the exchange of substances through membranes of organelles within the cell. For example, traffic of molecules and ions in and out of organelles such as the nucleus, mitochondria, chloroplast, lysosome, peroxisome, Golgi complex and endoplasmic reticulum.

Transcellular transport involves the exchange of substances across the cell membrane, thereby accomplishing net transport across the cell, e.g., the cells of a plant root that are responsible for the absorption of water and mineral salts.

Transport of solutes across a membrane can be either passive or active, depending on the energy requirement of the process. Active transport is catalyzed by specialized membrane proteins, often referred to as '**pumps**'. Due to the energy input, active transport results in the generation of a 'concentration gradient' across the membrane. Active transport of H^+, K^+, Na^+ and Ca^{2+} across the membranes of the cells is quite important. Since ions have an electrical charge, the transport process and resulting concentration gradient can generate membrane potential across the membrane. The combined concentration gradient as well

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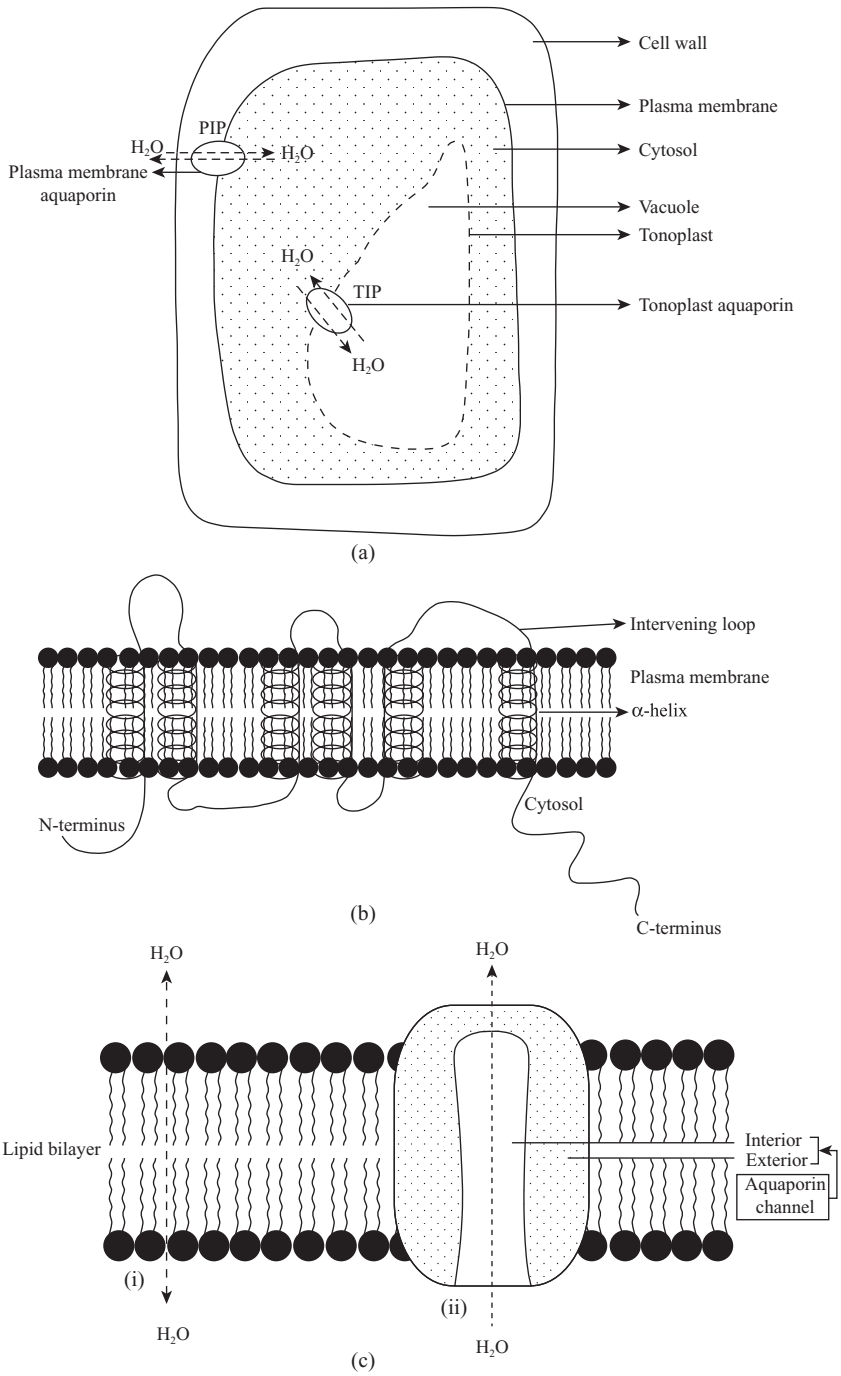


Fig. 1.12 (a) Aquaporins in the plasma membrane and vacuolar membrane in a plant cell. (b) Structure of a single subunit of a plant aquaporin. (c) Movement of water across the plasma membrane through (i) the lipid bilayer, and (ii) the aquaporin channel.

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as the membrane potential, are termed as an 'electrochemical gradient'. The stored energy of such gradients is used to drive a variety of processes in the cells. In mitochondria and chloroplasts, for example, the stored energy of an electrochemical gradient of protons is used to drive the synthesis of ATP by their F-type ATPases during cellular respiration and photosynthesis, respectively. H^+ -ATPase complexes (ATPase-proton pumps), localized to the plasma membrane, vacuolar membrane, and other organelle membranes, behave as a proton-translocating carrier protein. The free energy of ATP hydrolysis is utilized to establish a proton gradient across the membrane. The proton gradient (ΔpH), along with transmembrane electrical potential (ΔE) contributes to a **proton motive force** (pmf), which is the primary source of energy for several plant activities such as active transport of solutes, regulation of cytosolic pH, stomatal opening and closing, sucrose transport during phloem loading and hormone-regulated cell elongation.

$$\text{pmf} = \Delta E - 59\ \Delta pH$$

1.3 Diffusion, Imbibition, Osmosis and the Water Potential Concept

Plant physiology, as we know, deals with different life processes operating within the cell, and between the cell and its environment. The former includes processes like photosynthesis, respiration, fat metabolism, nitrogen metabolism, and such, whereas the latter includes diffusion of gases, absorption and translocation of water and minerals, transpiration, and so on. The mineral elements necessary for plant growth and development, the compounds essential for energy transfer and storage, and the components of structural compounds all require water as a translocation and reaction medium. These materials are dissolved in water, and in this form, distributed throughout the plant. The processes of **diffusion, osmosis** and **imbibition** are intimately associated with the essential function of the translocation of water and solutes from site of origin to site of activity.

1.3.1 Imbibition

Imbibition (derived from the Latin word imbibere, 'to drink in') is a first step towards seed germination and water uptake by the root hairs. The process of adsorption of water by hydrophilic colloids of dry seeds (or non-living wood) is called 'Imbibition'. It is a purely physical phenomenon requiring no expenditure of metabolic energy. It can be best demonstrated by placing dry gram seeds in water. A noticeable swelling takes place after some time and is accompanied by a considerable increase in volume. Dry seeds are a rich reservoir of storage food such as starch and proteins, as well as cell wall materials in the form of cellulose and hemicellulose which being colloidal in nature imbibe a large quantity of water. Similarly, gum tragacanth, agar–agar and raisins when put in water, swell up considerably. Swelling of wooden doors and windows following torrential rainfall is also a common example of imbibition.

Imbibition does not require a selectively permeable membrane and involves the chemical and electrostatic attraction of water to cell walls, proteins, and other hydrophilic cellular

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materials. An **imbibition pressure**[3] of great magnitude is generated when an adsorbent or 'imbibant' (rich in hydrophilic colloids), in an enclosed space, is placed in a liquid to be imbibed. In cocklebur (*Xanthium strumarium*), it may go up to 1000 atmospheres. Dry wooden stakes, driven into a small crack in a rock and then soaked with water can develop enough pressure to split the rock. This process has been used in the past by Egyptians to build pyramids. The ancient witch-doctors used to perform crude surgical operation by splitting open the sutures of the bones of a skull by putting soaked seeds on the head and wrapping all over. Another example of imbibition pressure can be demonstrated in an inverted plaster of paris cone with sandwiched dry seeds. When kept in water the cone will break after some time due to imbibition by the seeds and their subsequent swelling (Figure 1.13).

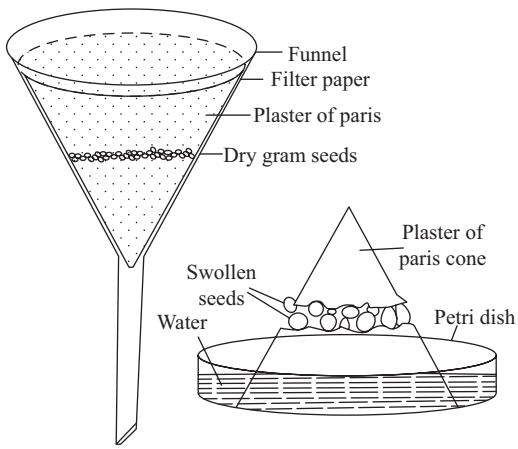


Fig. 1.13 Demonstration of imbibition pressure.

Conditions necessary for imbibition

1. A water potential gradient must be present between the surface of the imbibant and the liquid to be imbibed (water potential in dry seeds or wood is practically zero).
2. A certain amount of affinity or attraction must be present between the components of the imbibant and the liquid to be imbibed (e.g., dry seeds swell appreciably in water but not in ether).

Factors affecting imbibition

1. ***Temperature*:** The rate and extent of imbibition increases with an increase in temperature because of increase in kinetic activity of the imbibing molecules.
2. ***Osmotic potential of the substance to be imbibed*:** The rate of imbibition decreases with increasing solutes concentration in the medium as the water potential gradient becomes less steep.

[3] The old term 'imbibition pressure' has now been replaced by 'matric potential'.

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Consequences of imbibition

1. Although the volume of an imbibant always increases but the final volume of the system as a whole decreases. Water molecules in free-state occupy more space; while in a bound form they undergo reorientation or realignment on to the surface of colloidal materials, and thus are packed tightly and occupy less volume.
2. There is always an increase in temperature following imbibition. Some of the kinetic energy possessed by these molecules gets lost because of tight adsorption of water molecules and is liberated in the form of heat.

Importance of imbibition

1. It was used for the widening of shoes in earlier days.
2. It was used for breaking open sutures of bones for performing crude operations as done earlier by 'witch-doctors'.
3. Dry wooden stakes, driven into small crack in rocks, when soaked in water can develop enough pressure to split open the rock. This method of quarrying was practiced by the ancient Egyptians who built pyramids from rock structures split open by wetting dry wood wedges.
4. It is the initial step toward germination of seeds and absorption of water by the root hairs.
5. It causes swelling of seeds and results in cracking of seed coat, followed by growth and development, leading to flower and fruit formation.
6. It is an innovative biotechnological approach requiring no sophisticated expensive laboratory facility to deliver foreign DNA molecules, using pollen tubes as conduit. Alien genes of DNA can be absorbed by root hairs where they might integrate with the host genome.
7. Seed imbibition allows uptake of plasmid DNA by embryos.

1.3.2 Diffusion

Cellular processes depend on the transport of molecules both to the cell and away from it. Diffusion is the natural tendency of systems to proceed towards increasing entropy, or disorder and the lowest possible state of energy. The driving force for the movement of a molecule either in solution or in the gas phase, is a gradient in its potential energy. Diffusion is most effective way for molecules and ions to move short cellular distances, but it is ineffective for long-distance movement.

Diffusion can be defined as the net movement of molecules from a region of (their) higher concentration into another region of lower concentration, i.e., down the concentration gradient, because of the random, translational kinetic motion of molecules (or ions/atoms).

All components of matter are in a state of motion, at temperatures above absolute zero (0 °K or –273.18 °C), i.e., they possess a certain amount of translational kinetic energy and will distribute themselves uniformly in the space that they occupy. The molecules move randomly in all directions and collide with one another. Taking the example of the air we breathe: it is primarily a mixture of N_2, O_2 and CO_2 molecules. These gas molecules are mixed uniformly in the atmosphere by constant movement in a random manner. Similarly, if a

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bottle containing ammonia (or any scent) is opened in one corner of the room, its odour can be detected at another corner as time passes. This is because the gas molecules of ammonia (or scent) move constantly and diffuse into the air molecules. Among the three states of matter, the gases offer the least resistance to diffusing molecules. At normal pressure and temperature, gas molecules are widely separated. That is why diffusion through gases or air is most rapid and while it is the slowest through solids.

Since diffusion depends on molecular movement, it must be quantitatively related to two components:

(i) Thermal agitation (the rate of motion of an average molecule)
(ii) Number of molecules/unit volume.

The free energy of a substance is dependent upon these two components and is termed as the '**chemical potential**' of the substance.

Diffusion $\propto \mu$, (μ; chemical potential of the diffusing substance).

At constant temperature, chemical potential is directly proportional to the concentration of the diffusing substance at the two ends. The greater the difference, the more rapid is the diffusion. However, the rate of diffusion must also depend on spatial factors. The greater the area across which the substance is diffusing, the greater is the number of molecules diffusing per unit time.

Further, small molecules are found to diffuse more rapidly than large molecules.

$$\text{Coefficient of diffusion, } D \propto \frac{1}{\text{molecular size}}$$

or,

$$\text{For small molecules, } D \propto \frac{1}{\text{mass}}$$

$$\text{For large molecules and colloidal particles, } D \propto \frac{1}{\text{radius}}$$

It is now obvious why the larger colloidal particles diffuse so much more slowly than the smaller molecules.

Diffusing particles also exert pressure, known as **diffusion pressure**. Actually it is a hypothetical term, which describes the potential ability of a gas, liquid or solid to diffuse from a region of its higher concentration to a region of its lesser concentration in a confined space. For example, in an inflated balloon, as the air gets concentrated, more molecules will be hitting the surface of balloon, resulting in an increased diffusion pressure. The walls of the balloon will stretch in order to compensate for the increase in pressure, giving visual evidence of the ability of a gas to exert pressure. If it is done excessively, the balloon bursts producing a bang because of the collision of gas molecules.

Law of Independent Diffusion

The direction of diffusion of a substance is determined entirely by the difference in diffusion pressure of that substance at the two ends and is completely independent of the diffusion

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pressure of the surrounding substances. For example, (i) in a balloon filled with nitrogen gas suspended in air, carbon dioxide from the air moves inside the balloon irrespective of high concentration of nitrogen gas; (ii) it is not desirable to sleep underneath a tree during night as more CO_2 diffuses out from the intercellular spaces of the leaf into the surrounding air, irrespective of oxygen concentration.

Factors affecting diffusion

1. ***Temperature*:** A slight increase in temperature results in an increased rate of diffusion due to increasing kinetic energy and thus the velocity of diffusing particles.
2. ***Density of the diffusing substance*:** According to **Graham's law of diffusion**, 'the rates of diffusion of gases are inversely proportional to the square roots of their densities'.

$$\frac{r_1}{r_2} = \frac{\sqrt{d_2}}{\sqrt{d_1}}$$

$$\text{For example, } \frac{r_H}{r_O} = \frac{\sqrt{16}}{\sqrt{1}} = 4$$

where r_1 and r_2 = diffusion rates of gases

d_1 and d_2 = densities of gases

   This shows that the rate of diffusion of hydrogen is four times more than that of oxygen.
3. ***Medium of diffusing substance*:** The more concentrated a medium is, the more slowly molecules will diffuse through it. For example, a gas diffuses more rapidly through a vacuum than through air. Similarly, a bromine gas vial when broken in a jar containing air or in an evacuated jar, bromine gas would fill the evacuated jar instantaneously.
4. ***Diffusion pressure gradient*:** In general, the steeper the diffusion pressure gradient, the faster is the diffusion rate.

Fick's law

The process of diffusion occurs spontaneously and no additional energy needs to be provided. It occurs in all biological systems—within cells or across the cellular membranes. The rate at which a molecule diffuses is dependent on four factors: size of the molecule, magnitude of its concentration gradient, viscosity of the medium, and temperature. The relationship between these factors is represented by **Fick's law**.

The German scientist Adolf Fick (1850s) noticed that the rate of diffusing molecule is directly proportional to its concentration gradient ($\Delta Cs/\Delta x$).

According to Fick's first law:

$$J_s = -D_s \frac{\Delta C_s}{\Delta x}$$

Js; amount of substance 's' crossing a unit cross-sectional area per unit time - mol m^{-2} s^{-1}
Ds; the diffusion constant

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$\Delta Cs/\Delta x$; the difference in the concentration of a substances between two ends separated by a distance x.

The negative sign in the above equation indicates that a substance moves down the concentration gradient in diffusion.

So a substance will diffuse rapidly when the concentration gradient (ΔCs)becomes more steep or the diffusion coefficient (Ds) increases. The importance of Fick's law is that one can calculate the time utilized by a molecule to diffuse a given distance. Small molecules in the gas phase diffuse rapidly, whereas large molecules, such as proteins and polysaccharides in solution diffuse slowly. The chemical potential and Fick's law describes the flux of an uncharged solute; which is governed only by its concentration.

Importance of diffusion in plants

Diffusion, mainly driven by concentration gradient, is a major factor governing the absorption of water as well as minerals through roots and their distribution throughout the plant. It is an important factor regulating the supply of CO_2 and O_2 during photosynthesis and respiration, respectively. It regulates loss of water vapour from the leaf intercellular spaces to the surrounding atmosphere during stomatal transpiration.

1.3.3 Osmosis

Plant and animal membranes are constructed in such a way that they are extremely permeable to water and are much less permeable to dissolved solutes. This is important because when a membrane separates two solutions having different solute concentration, there is an intrinsic tendency for water to move across the membrane towards the solution containing the greater solute concentration. In other words, there is a tendency for water to flow in a direction so as to equalize the solute concentration on each side of the membrane. The term for water movement in this manner is called 'osmosis', which may be thought of as a special type of diffusion. **Osmosis** is defined as the movement of water across a selectively permeable membrane from an area where its concentration is higher to an area where its concentration is lower.

The phenomenon of osmosis in non-living systems can be demonstrated by a U-tube glass apparatus having pure water and 5 per cent glucose solution separated by semi-permeable membrane. The water molecules will move from pure water to the 5 per cent glucose solution, till the concentration of water becomes equal on both the sides (Figure 1.14).

In such a physical system, there is only one force involved, i.e., difference between concentrations at two ends.

When an actively metabolizing plant cell is immersed in pure water or hypotonic solution,[4] water molecules tend to enter into the cell sap as a result of **endosmosis**. If the direction of movement is reverse, i.e., from cell sap to external medium as in hypertonic solutions, it is

[4] External solution is said to be hypotonic, hypertonic or isotonic with reference to cell sap if its osmotic pressure is less than, higher than or equal to osmotic pressure of the cell sap, respectively.

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termed **exosmosis**. In an isotonic solution, the cell will not lose or absorb any water, thus maintaining the net volume of water constant.

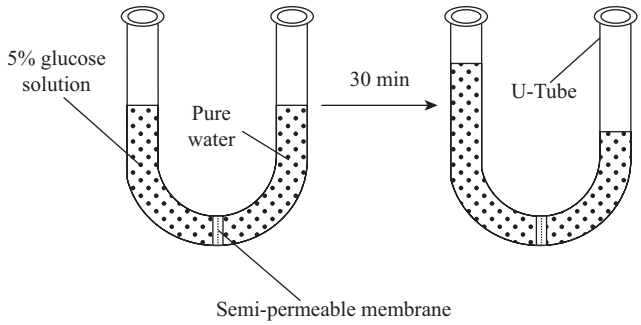


Fig. 1.14 Demonstration of osmosis.

Osmotic Pressure (OP): It is the maximum amount of pressure that can be developed in a solution separated from pure water by a semipermeable membrane.

$$OP = CRT$$

C = concentration of the solute
R = gas constant (0.082 L atm $K^{-1}mol^{-1}$)
T = absolute temperature (273.18 +°C).

This equation is applicable only in the case of non-electrolytes such as sucrose. In the case of electrolytes, such as NaCl, OP obtained is multiplied by its degree of dissociation.

Osmotic pressure (OP) of a solution is directly dependent upon the solute concentration. Therefore, it is a **colligative property**.[5]

Importance of osmosis in plants

1. The water uptake from the soil by the root hairs and its movement from one cell to another within the plant is partly controlled *via* osmosis.
2. This phenomenon is also involved in the movement of water from the non-living xylem elements into living cells.
3. The rigidity of plant organs is maintained through osmosis which helps in keeping the young stems erect and leaves expanded.
4. Growing points of root remain fully turgid due to osmosis and are, thus, able to move through the soil particles.
5. The osmotic uptake of water by the cells of the root and shoot generates the hydraulic pressure required for growth in these organs.
6. Many of the growth and turgor movements (opening as well as closing of stomata and flowers, nyctinasty of leaves, etc.) depend on this phenomenon.

[5] The other three colligative properties of solutions are freezing point depression, boiling point elevation and lowering of vapour pressure of the solvent.

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Diffusion Pressure Deficit (DPD): The term 'diffusion pressure deficit' was put forth by Meyer (1938). The amount by which the diffusion pressure of a solution is lower than that of its pure solvent, is termed as DPD. It denotes the net pressure that causes water to enter the cell. Osmosis takes place when the value of DPD is more than zero. Osmotic pressure of an 'unconfined solution' in a beaker is equal to DPD, i.e.,

$$OP = DPD$$

On the other hand, in a 'confined system' (living cell), the cell sap exerts a high osmotic pressure. Therefore, another force, 'Turgor Pressure' (TP) comes into the picture and the relationship, OP = DPD, does not hold good.

Turgor Pressure (TP): Turgor pressure is important in maintaining an erect habit of non-woody or herbaceous plants, that is, crispiness or rigidity of most soft tissues of plants such as leaves and young stems. However, when turgor pressure is lost, they become limp or wilted. It is a unique property of a plant cell that is surrounded by a rigid cell wall. If a living plant cell is immersed in water, it swells due to absorption of water. As a result, pressure is developed in the vacuole that presses the protoplasm against the cell wall, and this is called 'turgor pressure'. A cell experiencing turgor pressure is said to be **turgid**, whereas a cell that experiences water loss to the point where turgor pressure is reduced to zero is said to be flaccid. Indeed, one of the first outward signs of water deficit in plants is the wilting of leaves due to loss of turgor in the leaf cells.

The rigid and elastic cell wall exerts an equal and opposite pressure against the expanding protoplasm, termed as 'wall pressure' (WP).

$$\text{At a given time, } TP = WP$$

TP is exerted by the cell vacuole to the protoplasm against the cell wall, while WP is exerted by the cell wall to the protoplasm against the vacuole.

The net force with which the water is drawn into the plant cell depends upon the difference between two entities, osmotic pressure and turgor pressure.

$$DPD = OP - TP\ (WP)$$

Thus, in a living system or in other actively metabolizing cells, there is an interplay of only two forces: OP and TP.

1.3.4 Plasmolysis

In a turgid plant cell, the cell sap presses the protoplasm against the cell wall. When an actively metabolizing plant cell is kept in a hypertonic solution, water moves out of the cell due to exosmosis with a consequent shrinkage in the volume of the vacuole. With the result, the protoplasm is no longer pressed against the cell wall and begins to retract and ultimately separates away from the cell wall, assuming a more or less spherical shape in the centre of the cell. This phenomenon is called **plasmolysis** (Figure 1.15). In a plasmolysed cell, the space between the cell wall and protoplasm is occupied by the external solution. The immediate effect of plasmolysis is the retardation of metabolic activities in cells, leading to permanent wilting of tissues and eventually death of the plant if it continues for a longer period. That is why irrigation of the soil with a concentrated solution of salt or any other solute or chemical fertilizers causes burning of the plants.

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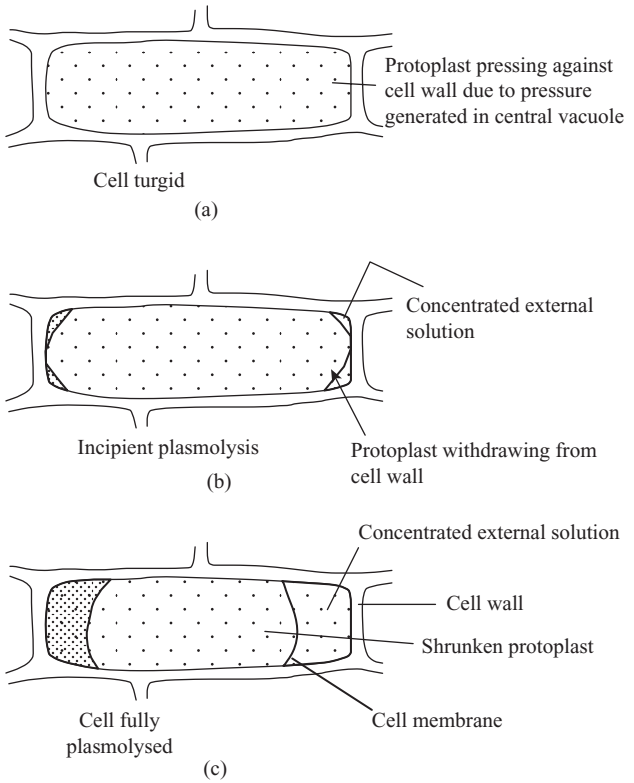


Fig. 1.15 The figures (a, b, c) show various stages of plasmolysis. In the fully plasmolysed state, the protoplast is rounded up in the centre of the cell. The space between the shrunken protoplast and the cell wall is occupied by the external hypertonic solution.

Importance of plasmolysis

1. The phenomenon of plasmolysis, first used by De Vries, is helpful in determining the approximate osmotic pressure of the plant cell.
2. It may be used to determine whether a cell is living or non-living. Plasmolysis never occurs in non-living cells such as cork or xylary elements.
3. It also proves that cytoplasm acts as a semipermeable membrane. If it were not so, the external bathing medium would just enter the vacuole, bringing about equilibrium between the vacuolar sap and the external solution. There will be no plasmolysis then.

So the phenomenon of plasmolysis provides important clues to the plant physiologists.

Applications of plasmolysis

Plasmolysis has some useful applications in our everyday life:

1. The salting of fresh meat and fish as in preserved or canned foods, and the addition of sugar to jams and fruit jellies – this is done to plasmolyse microbial spores of bacteria

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and fungi which could cause decay and spoilage of food through the secretion of extracellular enzymes.

2. Unwanted weeds and other obnoxious plants on clay courts are destroyed by sprinkling salt on the soil.
3. The growth of grass on clay tennis courts and in the cracks of brick walls is similarly checked.

Plasmolysis	Wilting
1. The shrinkage of cytoplasm from the cell wall under the influence of hypertonic solution.	The loss of turgor in plant tissues under water deficit conditions*. The cells become limp and its leaves and stem wilt.
2. It remains essentially a laboratory phenomenon, seldom occurring in nature (with the possible exception of saline environment).	This response is due to water-stressed environment.
3. The protoplast volume of the cell progressively decreases as plasmolysis progresses. Does not normally give rise to a significant negative pressure.	Never ever the collapsing protoplast separates from the cell wall but significant negative pressure (tension) may develop.
4. The space or void between the cell wall and the protoplast is filled-up by the external solution.	No space or void ever develops between the cell wall and the protoplast.
5. Plasmodesmata are broken as the protoplast pulls away from the cell wall.	Plasmodesmata are never broken between the adjacent cells.

* Resulting from excessive loss of water than water uptake or actual deficiency of water in the soil.

1.3.5 Water potential

The best way to express spontaneous movement of water from one region to another is in terms of the difference in free energy of the water between two regions.

Free energy is the energy of the system available for doing some work at constant temperature. Free energy per mole of any substance in a chemical system is known as **chemical potential** (μ). As the chemical potential of a substance increases, its tendency to undergo chemical reactions and other processes (such as diffusion and osmosis) also shows an increasing trend. Chemical potential of a substance, at constant temperature and pressure, depends on the number of moles of substance present, its electrical charge and hydrostatic pressure.

In plant–water relations, the chemical potential of water is known as **water potential** (ψ_w). The term 'water potential' was introduced by Australian scientists, Slatyer and Taylor in 1960. Water potential is defined as the chemical potential of water divided by the partial molal volume of water. Thus it is a measure of the free energy of water per unit volume ($J\ m^{-3}$; equivalent to pressure units such as pascal, which is the common measurement unit for water potential).

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$$\psi_w = \mu_w - \mu_w^\circ = RT \ln e/e^\circ$$

μ_w is the chemical potential of water at any point in a system; μ_w° is the chemical potential of pure water under standard conditions; *R*, is the gas constant (erg/mole/degree); *T*, the absolute temperature (°K); *e*, is the vapour pressure of the solution in a system at temperature, *T*; *e*°, the vapour pressure of pure water at the same temperature.

Water potential in a system is measured in bars [1 bar = 0.987 atm = 10^6 dynes/sq cm].

Pure water, at atmospheric pressure, has zero potential. Water potential of a solution or in a living cell is always less than zero because the addition of solutes to water increases the disorder or entropy of the system and thereby reduces the free energy of water, thus decreasing the water potential. The direction of water flow will be 'energetically downhill', i.e., from region of greater water potential (pure water) to the region of lesser water potential (solution). The water flow continues until the water potential of the two regions become equal.

In terms of the free energy concept, there is an interplay of three major forces –the osmotic potential (ψ_π), the pressure potential (ψ_p), and the matric potential(ψ_m).

$\therefore$ Water potential (ψ_w) is the algebraic sum of potentials of these components :

$$\begin{array}{cccccc} \psi_w & = & \psi_\pi & + & \psi_p & + & \psi_m \\ & & (-ve) & & (+ve) & & (-ve) \quad \text{(Values)} \end{array}$$

Osmotic potential[6] (ψ_π) is defined as the amount by which the water potential is reduced as a result of the presence of solutes. The osmotic potential of a solution is directly proportional to the concentration of dissolved solute but completely independent of the specific nature of the solute. Osmotic potential is equal to osmotic pressure but carries a negative sign. It is expressed in bars.

Pressure potential (ψ_p) refers to turgor pressure exerted by the vacuole. It is usually positive and tends to maintain turgidity of cells. In young unvacuolated cells and slightly plasmolysed cells, ψ_p is zero. ψ_p becomes negative in the xylem.

Matric potential (ψ_m) refers to decrease in water potential as a result of adsorption of water to colloidal substances, i.e., soil, protoplasm, cell wall, etc. It is especially important in the initial stages of imbibition, soil–water relations, as well as succulents with high mucilage content. ψ_m is always negative and becomes negligible (<–0.1 bar) in vacuolated plant cells.

$$\therefore \quad \psi_{soil} = \psi_\pi + \psi_m$$

Besides these three forces, two other forces namely, gravitational potential and electrical potential, also contribute to water potential.

[6] The term 'solute potential (ψ_s)' was used earlier in place of osmotic potential.

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Gravitational potential (ψ_g) may become a significant factor in tall trees. As the water column increases in height, the water potential increases correspondingly. Gravitational force causes water to flow in the downward direction, unless it is opposed by an equal and opposite force.

Therefore, ψ_g depends on the height (h) of the water column above the reference-state water, the density of water (ρ_w) and the acceleration due to gravity (g), as shown below:

$$\psi_g = \rho_w g h$$

($\rho_w g$ has a value of 0.01 MPa atm^{-1})

Gravitational forces are generally ignored or cancel out in plant–water relations, especially when considering adjacent cells at the equal level.

The water potential upstream is greater than the water potential downstream by virtue of the position in a gravitational field, one of the factors affecting water potential. We know there is substantial free energy present because the flow of water can be used to turn a paddle wheel, which in turn can be used to drive a turbine for the production of hydroelectric power.

Electrical potential (ψ_e): Electro-osmosis is a process by which water moves along electrical gradients, and could be of some significance since water is a dipole and can readily move in an electric field. Potentials across living membranes, however, are only of the order of 100 mV. This voltage is probably insignificant as a force for water transport in plants and as a consequence, ψ_e is usually not considered.

The relationship of volume, water potential, osmotic potential, and pressure potential of a living cell with elastic cell wall

If a flaccid cell with ψ_p zero (the point 1.0 on the abscissa) is kept in pure water, water starts moving into the cell, thereby increasing its volume. Hence, the osmotic potential (ψ_π) becomes less negative but there is an increase in ψ_p (Figure 1.16).

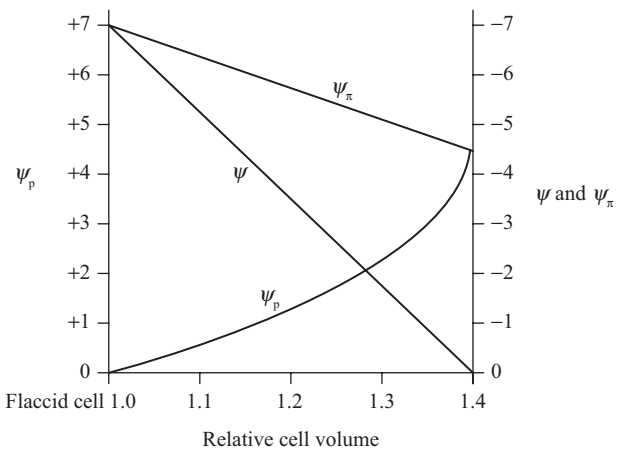
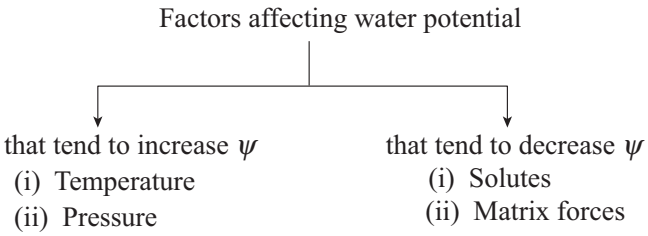


Fig. 1.16 Diagrammatic representation of relationship between volume changes and variations in ψ, ψ_p, ψ_π.

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At the point where the osmotic potential equals the ψp (but opposite in sign), the ψ reaches zero and the cell is said to be fully turgid and there is no further increase in the volume of cell. The system would be in equilibrium with water outside and hence no more water will enter (i.e., no net water flow).



Significance of water potential in plants

1. Water potential is the quantity that regulates the direction of movement of water across cell membranes by osmosis.
2. Water potential is a measure of the water status of a plant. Water deficits lead to inhibition of various activities in plants such as cell division, wall and protein synthesis, solute accumulation, stomatal opening, photosynthesis, etc.
3. Water potential also indicates how 'wet' or 'dry' a plant is and thus provides a relative index of the water stress the plant is going through.

Overall, water potential is a good indicator of plant health and has a strong influence on crop yield. Water potentials can be measured either by psychrometer or pressure chamber.

Water potential is not the only parameter that determines how rapidly water moves into the cells, but the **hydraulic conductivity** (Lp) of the plasma membrane also influences how quickly cells equilibrate with their local environment. It is a measure of the ease with which water crosses the membrane and is expressed in units of volume of water per unit area/per unit time/per unit driving force ($m^3\ m^{-2}\ s^{-1}\ MPa^{-1}$).

The rate of water flow across a membrane (Jv) is given by:

$$Jv = Lp \cdot \Delta\psi_w$$

where, Lp is the hydraulic conductivity and $\Delta\psi_w$, the driving force.

Diffusion pressure deficit (DPD)	Water potential (ψ)
1. The term 'DPD' was coined by Meyer in (1938) but was described originally under the name 'Suction Force' (Saugkraft) or 'Suction Pressure' (SP) by Renner (1915).	The term 'water potential (ψ)' was given by Slatyer and Taylor (1960).

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Diffusion pressure deficit (DPD)	Water potential (ψ)
2. The diffusion pressure deficit is abbreviated as DPD.	Water potential is symbolized by the Greek letter or uppercase psi, ψ (pronounced 'sigh').
3. It represents the difference in diffusion pressure between a solution and pure water (solvent).	It represents the difference in free energy between water molecules in pure water and in a solution.
4. The magnitude of DPD is usually expressed in atmospheres (with a positive sign).	The ψ is expressed in bar units (with a negative sign).
5. The direction of water movement within cells is from low DPD to high DPD.	The movement of water between cells is from high ψ to low ψ, i.e., in an energetically downhill direction or from less negative ψ to more negative ψ.
6. The DPD of a cell is equivalent to: DPD = OP – TP	The water potential (ψ) is equal to the algebraic sum of three forces:
	$\psi = \psi_\pi + \psi_p + \psi_m$
	(–ve) (+ve) (–ve)— (Values)

Water potential is expressed in terms of pressure units, most commonly mega-Pascals (MPa), although some other units of pressure are also used.

1 mega-Pascal (MPa) = 10 bars = 9.87 atm

1 bar = 0.987 atm

Review Questions

RQ 1.1. Prepare 1 molar solution of sucrose.

Ans. Dissolve 342.2 g (mol. wt.) of sucrose in about 500 ml of water and make the final volume to 1 L (1000 ml).

RQ 1.2. Prepare 1 molal solution of NaCl.

Ans. Dissolve 58.35 g (mol. wt.) of NaCl in 1000 ml of water.

RQ 1.3. Prepare 1N hydrochloric acid (HCl).

Ans. 36.5 g of HCl/L makes 1N solution. In terms of volume, $V = M/D = 36.5/1.16 = 31.4$ ml. [specific gravity of HCl is 1.16]. The purity is only 28 per cent; hence, $31.4 \times 100/28 = 112.1$ ml (approximately 115 ml will give strength a little more than 1N).

RQ 1.4. Prepare 1N H_2SO_4 and dilute it to 0.1N H_2SO_4.

Ans. 49 g of H_2SO_4 in 1 L of water = 1N H_2SO_4. Mol. wt. of H_2SO_4 is 98 and it has two replaceable hydrogen atoms; therefore, a normal solution of H_2SO_4 contains 49 g (98/2) of H_2SO_4 per litre.

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In terms of volume, $V = M/D = 49/1.84 = 26$ ml [1.84 being the specific gravity of H_2SO_4]. As the purity of H_2SO_4 is about 8 per cent, about 28 ml of conc. H_2SO_4 dissolved in 1000 ml gives a solution of a little more than 1N H_2SO_4. 0.1N $H_2SO_4 = 26/10 = 2.6$ ml of concentrated H_2SO_4 added to a litre of distilled water will yield a solution of about 0.1 N.

RQ 1.5. Prepare (i) 10 per cent solution of NaCl (weight/volume) (ii) 5 per cent solution of glycerine (volume/volume).

Ans. (i) 10 g of NaCl with water to make the final volume 100 ml → 10 per cent solution of NaCl.
(ii) 5 ml of glycerine with 95 ml of water→5 per cent solution of glycerine.

RQ 1.6. Prepare (i) 100 ppm and (ii) 10 ppm solution of auxin (IAA).

Ans. For making 100 ppm sol: (i) 100 mg of IAA added to distilled water to make the final volume to 1 L (1000 ml).
100 mg IAA/1000 ml of solution.

For 10 ppm sol: (ii) Stock solution (S) = 100 ppm,
concentration of the required solution (R) = 10 ppm
Total volume required = 10 ml.

Amount of stock solution required

$$= \frac{\text{Concentration of required solution (R)}}{\text{Concentration of stock solution (S)}} \times \text{Total volume required}$$

$$\text{Amount of stock solution} = \frac{10}{100} \times 10 = 1 \text{ ml}$$

So 1ml stock solution is added to 9 ml distilled water to make 10 ml of 10 ppm solution

(i) Distilled water should be used for the preparation of various solutions.
(ii) Acids/alkalies should be added to water but not the *vice versa.*

RQ 1.7. Why is the pH value only in between 0 and 14?

Ans.

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

$$K = \frac{[H^+][OH^-]}{H_2O}$$

$$K\,[H_2O] = [H^+] \times [OH^-]$$

$$= [H^{-7}] \times [OH^{-7}] = 10^{-14}$$

Hence, the pH values will range only in between 0 and 14.

RQ 1.8. Why in a given volume of pure water, are the number of H^+ ions exactly equal to the number of OH^- ions?

Ans. Pure water molecules have a 'slight' tendency to ionize, separating into H^+ and OH^- ions. The proportion of H^+ and OH^- ions is constant since water tends to dissociate into ions and this is exactly balanced by the tendency of the ions to combine again. Thus, even as some water molecules are ionizing, an equal number of others are forming covalent bonds.

RQ 1.9. What effect do you expect on pH when carbon dioxide gas or limestone is dissolved in distilled water?

Ans. The presence of dissolved CO_2 in distilled water may raise the hydrogen ion concentration so that it may reach 10^{-4} M (mol/L), i.e., pH 4. The hydroxyl ion concentration is then 10^{-10} M.

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$$CO_2 + H_2O \rightarrow \underset{\text{Carbonic acid}}{H_2CO_3}$$

$$H_2CO_3 \rightarrow H^+ + HCO_3^-$$

If tap water contains much dissolved limestone ($CaCO_3$), the hydrogen ion concentration can reach close to neutral or even slightly basic (pH 7 to 8).

However, the pure water when exposed to the atmosphere will take up CO_2, some of which reacts with water to form carbonic acid, thereby reducing or lowering pH to about 5.7.

RQ 1.10. How is the pH of cytoplasm kept near 7?

Ans. The pH of cytoplasm is kept near 7 by buffers, and by pumps operating in the plasma membrane and vacuolar membrane (tonoplast) that transport H^+ into the vacuole and out of the cytoplasm into extracellular spaces (apoplast) (refer to RQ 4.46). The vacuole of a plant cell is a reservoir of organic acids, so that its pH is usually in between or in the region of 3.5–5.5, although in certain fruits like lemon it may reach as low as 1.0.

RQ 1.11. How many H^+ and OH^- do you expect in a solution of pH 3? Compare it with respective solutions having pHs of 1 and 2.

Ans. A solution having a pH of 3 will have 10^{-3} or 0.001 H^+ ions. It will also have 10^{-11} OH^- (0.00000000001). On the other hand, a solution of pH 1 and pH 2 will have 10^{-1} or 0.1 and 10^{-2} or 0.01 hydrogen ions respectively, while their OH^- concentration will have a corresponding value of figures 10^{-13} and 10^{-12}.

The negative logarithm of a number is the logarithm of its reciprocal. Therefore, the pH corresponding to a hydrogen ion concentration of 10^{-3} is 3.

$$pH = \log \frac{1}{10^{-3}} = \log 10^{-3} = 3.00$$

RQ 1.12. List the different methods that are used for estimating the pH of an aqueous solution.

Ans. The methods used for measuring pH of a solution are as follows:

- By using various indicator dyes, such as phenolphthalein and phenol red. The dyes ionize at a specific pH, producing coloured ions.
- By using litmus paper (that changes colour with change in pH) which gives approximate idea of pH.
- pH can be measured by an instrument called pH meter. In most pH meters, there is an ion selective electrode that is sensitive only to H^+ ions .The signal from this electrode is amplified and then compared to the signal originating from another electrode dipped in a solution of known pH strength.

One molar solution of HCl or other acid, producing 1.0 M H^+ has a pH of 0 (as log 10 of 1 is 0). On the contrary, 1.0 M NaOH or other alkali will produce 1.0 M OH^- and 1 $\times 10^{-14}$ M H^+ and the pH will be 14. It is noteworthy to mention that the pH scale is logarithmic, and not arithmetical. Thus, a solution of 6.0 pH will be 'ten times' more acidic than a solution having pH value 7.

RQ 1.13. Milk curdles heavily when lemon juice is poured into boiling milk. Comment.

Ans. Milk is a common colloid (to be more exact an emulsion), composed of butter and fat dispersed in water, with casein as an emulsifying agent. All the particles in a colloidal system bear similar charges and thus, repel each other. This keeps the particles dispersed.

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Addition of lemon juice (or for that matter any acid) into boiling milk would increase the hydrogen (H^+) ion concentration. The +vely charged ions in the lemon juice cause the -vely charged milk particles to lose their charge. As a result, the dispersed particles in the milk would collide, aggregate, and finally settle out in the form of precipitates. That is why milk curdles heavily on addition of lemon juice that contains citric acid. The pH at which the positive charge exactly neutralizes the negative charge (no net charge) is termed as the '**isoelectric point**', or **pK**. At the pK, proteins do not have the protection from agglomeration provided by the presence of an electrical charge. The proteins will thus tend to clump together when the 'net charge' is **zero**.

RQ 1.14. Why are deltas often found at the mouth of a river? Explain.

Ans. Weathering of parent rock yields sand, silt, clay and soluble mineral nutrient ions. Colloidal clay consists of much finer particles (0.001–0.1 μ) than may be seen by the naked eye. However, they remain in a suspended form in water and will not settle out. Clay particles consist primarily of aluminium silicates, predominantly 'negatively charged' because of 'exposed carboxyl and hydroxyl groups'. Positively charged cations are preferably adsorbed on to clay particles.

The clay particles are carried downhill in a suspended form by the river water and as it meets the ocean or sea water, the 'charged cations' present in the saline water (predominantly NaCl, $NaNO_3$) cause the negatively charged clay micelle of river water to lose their charge and settle out. Destruction of the electric charge will cause the dispersed particles of a colloidal suspension to collide, aggregate, and finally settle down in the form of precipitate.

This phenomenon leads eventually to the formation of a delta – a fertile stretch of land which is often produced at the mouth of a river.

RQ 1.15. What is common and what is different between clay and humus? Why are they so important for plants?

Ans. Both clay and humus are hydrophilic colloids. Clay is inorganic material consisting of aluminium and silicon oxides while humus is organic material, derived from the dead and decomposed remains of plants and animals. The surface of the colloidal particles is negatively charged. The negative charge arises from exposed carboxyl and hydroxyl groups. As a result, cations like calcium, potassium, magnesium and others are attracted or preferentially absorbed. The roots secrete H^+, which due to great affinity for the negatively charged colloidal surface, tend to displace cations into the soil solutions.

So this colloidal fraction of clay and humus, owing to their large surface area per unit mass, becomes a principal nutrient reservoir for release into the soil solution.

RQ 1.16. Beetroot loses its red colour when boiled in water but does not do so in benzene. Why?

Ans. The anthocyanin or betacyanin pigments in beetroot are located in the vacuolar sap which is surrounded by tonoplast. However, the pigments are lost when beetroot is boiled in water as the membrane structure is completely lost because of denaturation of membrane proteins and it becomes permeable, allowing the pigments to diffuse. In benzene also, the permeability is lost but the solution remains colourless as the pigments are insoluble in benzene. However, the colour will appear if water is added to this benzene solution as the pigments are water soluble.

RQ 1.17. Although beetroot grows underground, it does not lose its red colour in the soil solution. Explain.

Ans. Beetroot does not lose its red colour in the soil solution as the plasma membrane and the vacuolar membrane or tonoplast (enclosing the anthocyanin) behave like differentially or selectively permeable membrane, preventing the diffusion of pigments in the soil solution.

RQ 1.18. Animal cells do not possess a cell wall, how then these cells are able to maintain strength and rigidity of tissues and organelles?

Ans. True. The strength and rigidity of animal tissues and organs is provided by the extracellular matrix consisting of collagen, glycoproteins and other compounds.

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RQ 1.19. List the cell organelles that are bounded externally by (1) a single membrane and (2) a double or paired membrane.

Ans. Organelles with a single membrane: Microbodies such as Glyoxysomes and Peroxisomes. Organelles with a double membrane: Chloroplast, Mitochondria, Endoplasmic Reticulum and Nucleus with numerous pores.

RQ 1.20. Where do you find some unusual structural membranous features: (1) three-layered plasma membrane and (2) single lipid bilayer.

Ans.
1. Three layered (trilaminar) structure of plasma membrane: Erythrocytes
2. Single lipid layer: Liposomes (are being tested as vehicles to deliver drugs or DNA molecules within bodies).

RQ 1.21. What are amphipathic molecules?

Ans. The amphipathic molecules such as phospholipids have a hydrophilic (water-soluble) polar head and two (lipid-soluble) hydrophobic tails.

RQ 1.22. List the various functions attributed to proteins embedded in the membrane.

Ans.
1. Channel Proteins: form small opening for molecules to diffuse through.
2. Carrier Proteins (also called Transport Proteins): binding sites on the protein surface 'grab' certain molecules and pull them into the cells (Gated Channels),
3. Receptors Proteins: for attachment to suitable raw materials required for various functions in the cells. Acting as molecular triggers that set off cell responses (such as release of homones or opening of channel proteins).
4. Enzymatic proteins: carry out metabolic reactions.

RQ 1.23. Although the amount of CO_2 in the air is very low yet it enters the leaf tissues in spite of very high concentration of O_2 inside. Explain how?

Ans. The CO_2 level in the atmosphere is just only 0.03 per cent by volume yet it enters the leaf tissue because its molecular movement is controlled by its 'own concentration gradient' irrespective of concentration of other gases inside. After entering the leaf through stomata, it (CO_2) is immediately consumed during photosynthesis during the day and the CO_2 level remains low as compared to the air. Thus, the CO_2 gas will continue to enter as long as there is a positive gradient and it will not be affected by the oxygen concentration brought about by photosynthetic activity. This is in accordance with the 'principle of independent diffusion of gases'.

At night time there is build-up of CO_2 because of respiration and secondly because of lack of photosynthesis. CO_2 readily 'passes out' of the leaf tissue as its concentration outside is much less. That is why it is injurious to sleep under a tree at night. However, during daytime, O_2 level inside is much higher than outside, it will tend to diffuse outward from the leaf and helps to purify air. The molecular movement of oxygen is controlled by its own gradient, irrespective of very high concentrations of N_2 gas (which is most abundant, constituting 78 per cent of air).

RQ 1.24. What is plasmolysis? How does it differ from wilting?

Ans. Plasmolysis is the shrinkage of cytoplasm from the cell wall under the influence of 'hypertonic concentration' outside in the bathing medium. The protoplasm begins to retract from the cell wall and ultimately separates out, forming a more or less spherical mass. If it continues for a longer time, it will eventually lead to death of the cell. Plasmolysis *per se* does not occur in plant cells in the atmosphere.

Wilting is in response to loss of turgor pressure inside the leaf tissue, occurring during water stress conditions. There are three types of wilting: incipient, temporary and permanent. Here the cytoplasm never ever separates from the cell wall. A single severe wilting often results in 50 per cent or more reduction of yield.

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When the plants begin to wilt there is sudden increase in the level of ABA in leaf cells, i.e., from about 20 μg/kg fresh weight to 500 μg/kg and the stomata close down.

RQ 1.25. Excessive use of chemical fertilizers is dangerous. Comment.

Ans. Chemical fertilizers, such as nitrogenous, phosphatic and potash (NPK) are used to increase productivity in our crops. However, if they are used in excessive amounts and indiscriminately, these have deleterious effects because they lead to plasmolysis of roots (especially the root hairs) and these will no longer be functional in absorbing water and minerals. Plants will soon wilt. Even the burning of plants (often turning brown) will be caused by residues of fertilizers. That is why these fertilizers are applied to the soil quite judiciously in appropriate quantities.

The cells of the plants especially roots would be plasmolysed and the plants would undergo wilting and finally death unless the excess salts are washed away by rain or irrigation.

RQ 1.26. What occupies the space between the cell wall and cytoplasm in a plasmolysed cell? Explain.

Ans. In a plasmolyzed cell, the space between the cell wall and the protoplasm is occupied by the 'bathing medium' or the 'external hypertonic solution' in which tissues were immersed. The stage at which initial pulling away of the plasma membrane from the cell wall begins is called **incipient plasmolysis** (the magnitude of turgor pressure here is zero). Here the cell wall is 'just left behind alone', and being permeable it will allow the external solution to move freely towards inside as there is no barrier to the solute movement, thus filling the space as the cytoplasm shrinks.

As a result of outward diffusion of water (exosmosis), the vacuole shrinks and the protoplasm and plasma membrane are pulled away from cell wall.

RQ 1.27. Explain the terms diffusion, chemical potential and water potential.

Ans. Diffusion is the unequal movement of the molecules (be it a gas, liquid or solute) from an area of higher concentration or high diffusion pressure to an area of low concentration or low diffusion pressure and is controlled by their kinetic activity. The molecules are constantly moving at random and occasionally colliding with one another. The rate of diffusion depends upon concentration gradient under conditions of constant pressure and temperature: (i) concentration difference at the two regions and (ii) the distance through which the diffusing molecules have to move. The smaller the distance between the two concentrations, the more the number of molecules that would cross over per unit time.

Quantitatively speaking, the process of diffusion has two aspects – the rate of motion of average molecules (thermal agitation) and the number of molecules per unit volume, both parameters controlling the free energy. This free energy of any substance in a chemical system is called 'chemical potential'.

Hence,

Diffusion $\alpha\ \mu$

(μ = chemical potential of the diffusing substance).

$\mu = RT$ ln a Joules/g

$R = 0.082$ (gas constant)

T = temperature (degrees absolute)

a = 'effective' concentration or activity of the substance

ln = natural logarithm

At standard or constant temperature or pressure, the molecules diffuse from a region of high concentration or chemical potential to regions with lower chemical potential.

Thus, the water potential (ψ; pronounced psi) is the chemical potential of water and is equivalent to the algebraic sum of three forces operating or interplaying at the same time.

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Water potential = osmotic or solute potential + pressure potential + matric potential

$$\psi = \psi_\pi + \psi_p + \psi_m$$
$$(-ve) \quad (+ve) \quad (-ve) \qquad \text{values}$$

Other forces like gravitational, electrical, etc., play a negligible role and are often overlooked.

Water will flow from a region of high ψ to low ψ, i.e., in an energetically downhill direction or from a region of less negative ψ to more negative ψ.

RQ 1.28. Tabulate *only* the water potential (ψ) values in different plant systems.

Ans. The range of different types of 'potentials' found in plant systems is as under:

ψ_w = water potential
zero for pure water
zero (at field capacity) or negative for cells

ψ_π = osmotic potential
always negative – becoming more or more negative with increasing concentrations or with more and more addition of solutes.

ψ_p = pressure potential
usually positive in living cells,
but negative in xylem cells which are under tension.
zero at incipient[7] plasmolysis
negative (in plasmolysed cells).

For details of ψ in soil, root hairs, stem, leaf and the atmosphere (see RQ 3.6, page 97)

RQ 1.29. The osmotic relations of a cell depend upon the state of activity or physical state of the cell. Explain by assuming the magnitude of OP and TP, according to the 'old concept' of DPD.

Ans. Assuming cells with OP = 10 units – 'kept in water', and at 'incipient stage of plasmolysis' or 'plasmolysed conditions'

1. In the case of fully turgid cell

OP = 10 and TP = 10
DPD = OP –TP
= 10 –10
= 0 (no net movement of water)

Animal cells often burst if placed in pure water, a process called 'lysis', but plant cells do not or seldom burst, bursting occurring only under heavy downpour or torrential rains.

2. In the case of flaccid cell (showing slight or incipient plasmolysis)

OP = 10 and TP = 0
DPD = 10 – 0
= 10

Water will enter into the cell (endosmosis).

3. In the case of plasmolysed cell

OP = 10 and TP = –2 (negative turgor pressure)
DPD = 10 – (–2)
= 10 + 2
= 12 (endosmosis will occur)

[7] Incipient plasmolysis is defined as the point at which 50 per cent of the cells are plasmolysed.

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RQ 1.30. The osmotic relation values in plant cells depend upon the physical state. Discuss the earlier example in terms of the 'new concept' of water potential (ψ).

Ans. **1.** In the case of fully turgid cell:

assuming osmotic potential value = 10

Water potential = osmotic potential + pressure potential

$$\psi_w = \psi_\pi + \psi_p$$
$$\psi = -10 + 10 \text{ bars}$$
$$= 0 \text{ bar (cell in a state of dynamic equilibrium)}$$

2. In the case of a flaccid cell:

$$\psi_\pi = -10 \text{ bars and } \psi_p = 0 \text{ bars}$$
$$\psi = -10 + 0$$
$$= -10 \text{ bars}$$

ψ negative as compared to water

3. In the case of a plasmolysed cell (in a state of tension):

$$\psi_\pi = -10 \text{ bars and } \psi_p = -2 \text{ bars}$$
$$\psi = \psi_\pi + \psi_p$$
$$= -10 + (-2)$$
$$= -10 - 2 = -12 \text{ bars (more negative unlike above cited two examples)}$$

RQ 1.31. The adjacent cells A and B have osmotic pressure 12 and 10 atmospheres, respectively. Their turgor pressures are 9 and 4, respectively. In which way will the water flow and why?

Ans.

A	B
OP = 12 TP = 9	OP = 10 TP = 4
DPD = OP −TP = 12 −9 = 3	DPD = OP −TP = 10 −4 = 6

Water will flow from cell A to B, i.e., from low DPD to high DPD.

RQ 1.32. Two adjacent cells A and B have osmotic potential 12 and 10 bars. Their respective pressure potentials are 9 and 4 bars. In which way will the water flow and how? Explain.

Ans.

A	B
$\psi_\pi = -12$ $\psi_p = +9$	$\psi_\pi = -10$ $\psi_p = +4$
$\psi = \psi_\pi + \psi_p$ $= -12 + 9$ $= -3$ less negative ψ	$\psi = \psi_\pi + \psi_p$ $= -10 + 4$ $= -6$ more negative ψ

Water will move from cell A to B, i.e., from less negative ψ (less concentrated) to more negative ψ (more concentrated).

RQ 1.33. Explain how the ψ gradient concept is useful in hydroelectric power generation?

Ans. Let us visualize a river flowing downstream. The water potential (ψ) upstream is more than the ψ of the water downstream on account of its position in a gravitational field, one of the factors

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controlling ψ (gravitational potential). Water will flow downhill and this can be used to turn a paddle wheel, which in turn can be used to drive a turbine for hydroelectric power generation.

However, this flow of water downhill can be prevented by constructing a barrier (or a dam). From there water could be pumped upward against the potential gradient but this would need energy. The water so diverted can be used for irrigation or for power generation elsewhere.

RQ 1.34. Where do you experience negative pressure potential in plant tissues?

Ans. Pressure potential is usually positive but tends to become negative in its magnitude under following situations:

- Xylem elements of the stem during ascent of sap (under tension)
- Substantial negative pressure potential may develop in wilted leaves or fully plasmolyzed tissues

Hence, the water potential of wilted or fully plasmolysed tissues becomes increasingly negative as it is the total sum of the negative osmotic potential (ψ_π) plus the negative pressure potential (ψ_p).

RQ 1.35. Where do you find the value of water potential (ψ) equivalent to zero.

Ans. Water potential has a zero value as in:

(i) Pure water

(ii) Fully turgid cells where ψ_π (osmotic potential) is equal to ψ_p (pressure potential) as given here:

$$\begin{aligned}\psi &= \psi_\pi + \psi_p + \psi_m \\ &= -10 + 10 \\ &= 0\end{aligned}$$

(the value of ψ_m is negligible)

When turgor pressure of the cell is reduced to zero it is said to be flaccid as it happens at incipient plasmolysis – the protoplast exerts no pressure against the cell wall but neither is it withdrawn from the walls.

RQ 1.36. Under which situations do you expect the magnitude of the pressure potential to be zero?

Ans. The magnitude of pressure potential is zero in:

(i) Unvacuolated cells as occurring in meristematic regions

(ii) Cells at incipient plasmolytic stage

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