MUNI
PHARM

Molecular Biophysics

Biophysics

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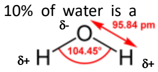
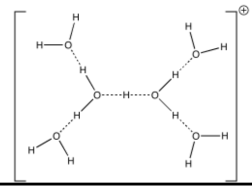
Water

- in the human body, the proportion of water is ca 60%
- water content in the blood is 90%, in the bodies of about 80% (depending on the fat content), 76% of muscles, skeleton 22%
- compound of fundamental importance: loss of 10% of water is a serious complication; the loss of 25% = death
- polar compound, bonding angle = 104,5°



- formation of hydrogen bonds (cluster structure of water): influence of physico chemical properties (e.g. boiling point, heat of vaporization, heat capacity)
- the hydrogen bond is the electrostatic attraction between polar molecules that occurs when a hydrogen atom bound to a highly electronegative atom: H_2O, HF, HCl



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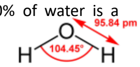

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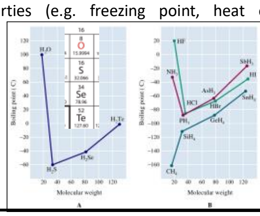

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Water and living organisms

1. Very good solvent (due to its polarity and H-bond formation)
2. Acid – base properties (dissociation, chem. reaction)
3. Mechanical function - fills cavities (joints) and body structures
4. Transport functions (transport of gases, nutrients, heat)
5. Thermoregulatory functions

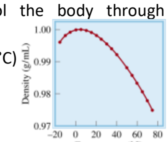
a) good thermal conductivity: heat exchange between the body and the surroundings (heat loss by conduction)

b) large heat capacity: heat storage - preventing climate change and reduces temperature fluctuation throughout the day

c) large latent heat of vaporization: cool the body through perspiration (evaporation of sweat)

6. Density anomalies of water (max. ρ at 3.98 °C)

– life under the ice



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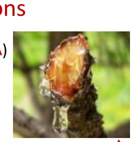
Colligative properties of solutions

- Lowering the vapor pressure (Raoult's law $p_A = x_A \cdot p^*_A$)
- Elevation of the boiling point

  the solution has higher T_B than the pure solvent



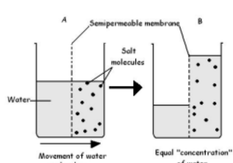
- **Depression of the freezing point**

  the solution has a lower freezing point than the pure solvent (coniferous trees - metabolism even at low temperatures, sap is saturated solution of biopolymers)

- **Osmotic pressure**

$$\pi = iRT \cdot c_{solute}$$

i - the correction factor on the number of dissociation particles



For intravenous administration must be used isotonic solutions:
e.g. 0.9% NaCl or 5% glucose

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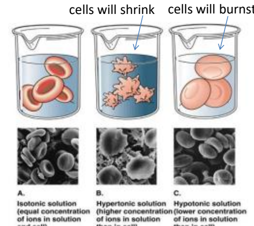
Colligative properties

Osmotic pressure

The osmotic pressure in the body **is controlled by the hypothalamus** (antidiuretic hormone, ADH). Increased resorption of water by the kidney is the result of ADH.

The basic osmotically active particles are plasma ions (mainly Na^+ and Cl^-).

cells will shrink   cells will burnst



Osmotic pressure: infusion preparations

The osmotic pressure of parenteral preparations directly affects the balance of body fluids in the extracellular and intracellular space.
Osmotic concentration (**osmolality**) is expressed in osmoles:

NaCl dissociation into 2 ions; 1 mol/kg NaCl = 2 osmol/kg

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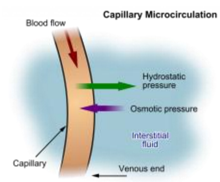
Colligative properties

Oncotic pressure

Osmotic pressure, which is caused by solutions containing particles with a high molecular weight (e.g. **proteins**).

- Oncotic pressure contributes to maintaining a sufficient circulating blood volume.
- Decreased protein content (hypoproteinemia) in the blood plasma cause edema.
- Endothelium is permeable to ions but poorly permeable to proteins => oncotic pressure is applied instead of osmotic pressure.



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Colligative properties

Osmotic pressure: The diagnostic significance

- Hyperosmolality of blood plasma

Cause: water loss, diabetic coma, burns, kidney failure, sepsis, drowning in salt water, acute intoxication by small molecules (e.g. ethylene glycol, methanol).

Clinical manifestations: from mild neuropsychiatric disorders to coma

- Hypoosmolality of blood plasma

Cause: excess of water, the metabolic response to trauma, drowning in fresh water, inappropriate ADH secretion.

Clinical manifestations: weakness, nausea, lethargy, headache, brain edema.

- Urine osmolality

The diagnostic significance of renal disease (impaired ability to concentrate urine).

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Dissociation: Influence of pH on passage through biological membranes

- Many drugs are weak acids or weak bases
- These compounds are more water soluble in their ionized form, and vice versa more liposoluble in their non-ionized form
- pH in the GIT determines the degree of ionization of weak acids and bases, and thus determines their solubility and absorption of drug into the body

$HA \leftrightarrow A^- + H^+$ $\quad K_A = [A^-]\,[H^+] / [HA]$

$[H^+] = K_A\,[HA] / [A^-]$ $\quad -\log [H^+] = -\log K_A - \log [HA] / [A^-]$

$pH = pK_A - \log [HA] / [A^-]$ **Henderson-Hasselbach equation**

$pK_A - pH = \log$ [nonionized form] / [ionized form] **weak acid**

$pH - pK_A = \log$ [nonionized form] / [ionized form] **weak base**

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Dissociation: Influence of pH on passage through biological membranes

- **Stomach (pH = 1-3)**

Facilitate absorption of weak acids (nondisociated); bases are not absorbed.

Sample problem (pH = 3 or 1; pKa = 3):

$3 - 3 = \log [HA] / [A^-]$ => $\log [HA] / [A^-] = 0$ => $[HA] / [A^-] = 1$ — **50% of the drug is nonionised**

$3 - 1 = \log [HA] / [A^-]$ => $\log [HA] / [A^-] = 2$ => $[HA] / [A^-] = 100$ — **99% of the drug is nonionised**

- **Small intestine (pH = 5-7.5)**

A weak acids and bases are good absorbed; a strong acid and a strong base are absorbed less.

Sample problem (pH = 6; pKa = 4 or 5):

$4 - 6 = \log [HA] / [A^-]$ => $\log [HA] / [A^-] = -2$ => $[HA] / [A^-] = 0.01$ — **1% of the drug is nonionised**

$5 - 6 = \log [HA] / [A^-]$ => $\log [HA] / [A^-] = -1$ => $[HA] / [A^-] = 0.1$ — **10 % of the drug is nonionised**

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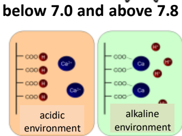
Dissociation: Acid-base ekvilibrium (ABR)

- dynamic balance between acidic and alkaline substances inside the body

- Due to metabolism pH is changed (CO_2 production) => regulation of ABR by buffers (substances capable of releasing or binding H^+).
  $H_2PO_4^- \leftrightarrow HPO_4^{2-} + H^+$ equilibrium => partial dissociation
- Precise adjustment of pH is important since pH changes influence the properties of proteins (including the activity enzymes), transport mechanisms, properties of the membrane channels etc.

- Blood plasma pH = 7.4 (7.35-7.45); **pH values below 7.0 and above 7.8** are incompatible with life.
- Blood plasma proteins act as buffers, mainly due to carboxyl groups and amino groups.



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Dissociation: Acid-base equilibrium

Buffers of boold plasma

- Phosphates
- Plasma proteins

} immediate effect but limited buffer capacity

Bicarbonate buffer

The most important system for controlling the acid-base equilibrium, because the body is able to actively change the concentration of $[HCO_3^-]$ (by metabolic process) and the concentration of $[CO_2]$ (by respiration) = the delayed but long-term effect, due to connection with metabolism

- **Bicarbonate** $H_2O + CO_2 \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-$

$pH = pK_A - \log [CO_2] / [HCO_3^-]$ **affected by metabolism**

- **Hemoglobin** (Hb released O_2 and bound H^+; Hb bound O_2 in the lungs and released H^+)

$HbH \rightleftarrows Hb^- + H^+$ $\qquad$ oxy-HbH $\rightleftarrows$ oxy-$Hb^- + H^+$

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Acid–base imbalance

Acidosis: decrease of blood pH below the physiological value
Alkalosis: increase of blood pH above the physiological value

- Acid-base imbalance caused by inappropriate regulation of respiration
  Acidosis (↑ pCO_2): hypoventilation (e.g. neuropathy), obstruction in the airway (inflammation, tumor), drugs (sedatives, hypnotics)
  Alkalosis (↓ pCO_2): hyperventilation (reflexively occurring in lung diseases), irritation of the respiratory center (e.g. stress, infection)
- Acid-base imbalance caused by inappropriate metabolic regulation
  Acidosis (↓ $[HCO_3^-]$): kidney damage, diabetic ketoacidosis, diarrhea
  Alkalosis (↑$[HCO_3^-]$): high intake of alkaline substances, loss acids (e.g. vomiting)

The state of bicarbonate buffer (determination of pH, $[HCO_3^-]$ and pCO_2) can be used to clinically assess the state of the patient ABR.

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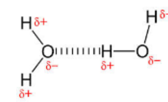
Hydrogen bond

- **Hydrogen bonds play an important role in physiological systems.**
- H-bonds have a much shorter lifetime than the covalent bonds (which are stable under physiological conditions); the lifetime of H-bonds between water molecules is about 10^{-11} s.
- H-bonds are about 10 times weaker than a covalent bonds.
- **Some structures, such as DNA, have many hydrogen bonds, which are mutually supportive => long-term stability of the whole structure.**
- Force acting between the two charges (Q_1, Q_2) at a distance r gives Coulomb's law:

$$F = (Q_1 * Q_2) / (4\pi * \varepsilon_0 * \varepsilon_{H2O} * r^2)$$

ε_0 permittivity of vacuum
ε_{H2O} permittivity of water



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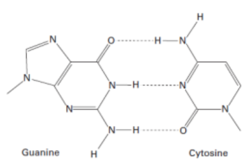
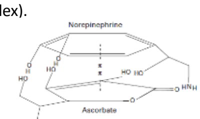
Hydrogen bond

- Hydrogen bonds influence the structure of biopolymers.
- H-bonds may be part of the internal structure of the molecule, or contribute to intermolecular binding.



- The intermolecular H-bonds also contribute to the formation and stability of the molecular complexes (e.g. vitamin C and noradrenaline form a bimolecular complex).



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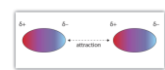
Non-bonded interactions

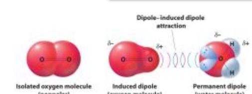
The motions of the electron around any atom produce instantaneous dipoles that in turn induce the formation of other attraction dipoles in neighboring atoms.

Dipole-dipole interaction: formed by the interaction of polar molecules



Van der Waals forces

Dipole-induced dipole interaction: formed by the interaction of polar and nonpolar molecules



London dispersion force: formed by the interaction of nonpolar molecules. London interactions are caused by random fluctuations in electron density in an electron cloud, which induces dipoles in other neighboring molecules.

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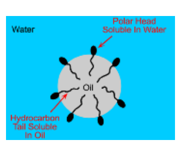
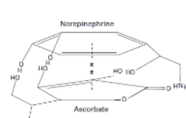
Non-bonded interactions

Hydrophobic interaction: formed by the interaction between the nonpolar parts of macromolecules in aqueous environments. The importance for the stability of the conformation of biopolymers - e.g. biological membrane, stabilizing pairs of aromatic amino acids.



π-π interaction: the ring structures have delocalized π-electrons produced by conjugated bond systems in the rings. When the rings are parallel to one another, π–π interactions occur between the p-orbitals. These interactions occur in supramolecular structures, like DNA (they provide substantial structural stability).



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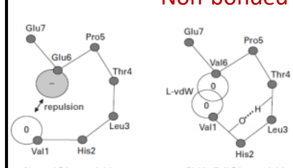
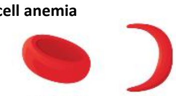
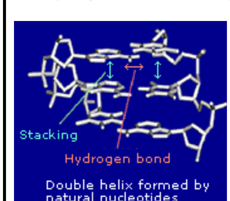
Non-bonded interactions



Sickle cell anemia



Models of the first seven amino acids in normal and sickle cell mutated β chain of hemoglobin.



DNA

π–π interactions (also called stacking forces) and H-bonds are important in nucleobase stacking within DNA molecules.

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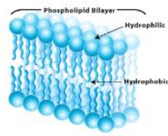
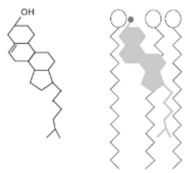
Non-bonded interactions: Cell Membrane

Non-bonded interactions between phospholipids **in cell membrane** (hydrophobic interaction; partially Van der Waals forces)



Cholesterol (stability of membranes)



The hydroxyl group will associate with the phosphate head groups of phospholipids. The steroid rings and hydrocarbon chain will associate with the fatty acid tails of phospholipids with the same London attraction forces that hold the fatty acids together. The presence of cholesterol in our membranes = protection of membranes from rupture.

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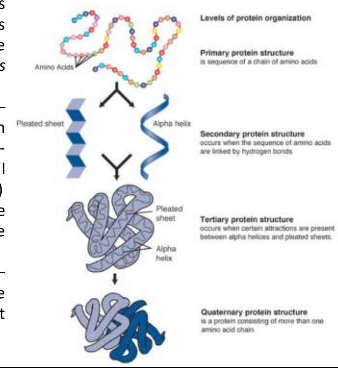
Non-bonded interactions: Protein structure

- **The primary structure** – is the exact specification of its atomic composition and the *chemical (covalent) bonds* connecting those atoms.
- **The secondary structure** – the pattern of hydrogen bonds in a biopolymer (three-dimensional form of local segments of the biopolymers)
- **The tertiary structure** – the three-dimensional structure of the macromelucule.
- **The quaternary structure** – the arrangement of multiple molecules in a multi-subunit complex (e.g. proteins, DNA).



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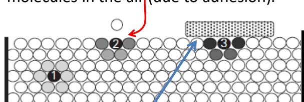
Biophysical interfaces

In physiological systems, interfaces occur in two areas, at **the air–tissue surface in the lungs**, and at **cell membranes**.

Surface tension

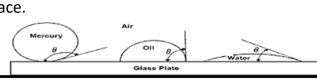
At liquid-air interfaces, surface tension results from the greater attraction of liquid molecules to each other (due to cohesion) than to the molecules in the air (due to adhesion).

$\sigma = F / \ell$ [N.m^{-1}]



σ tends to reduce the surface of phase (droplet formation, surface wetting).

The interfacial tension of the solid-liquid interface: affects the wettability of the solid surface.



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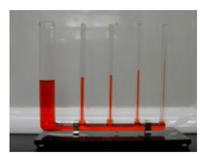
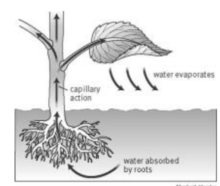
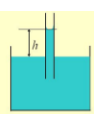
Biophysical interfaces: The capilary action

The capillary elevation (good wettability of the solid surface) allows the water to rise into the upper parts of plants.





$$h = \frac{2\sigma}{r\rho g}$$



$$r = 0,5\text{mm} \Rightarrow h = \frac{2 \cdot 76 \cdot 10^{-3}}{0,5 \cdot 10^{-3} \cdot 1000 \cdot 10} = 3\text{cm}$$

$$r = 0,5\mu m \Rightarrow h = \frac{2 \cdot 76 \cdot 10^{-3}}{0,5 \cdot 10^{-6} \cdot 1000 \cdot 10} = 30\text{m}$$

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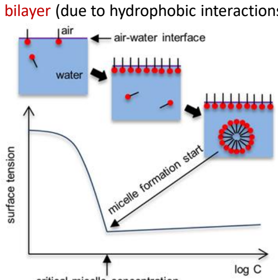
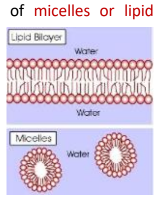
Biophysical interfaces: The micelles and lipid bilayer formation

The critical micellar concentration: Exceeding the the critical concentration of surfactant leads to formation of micelles or lipid bilayer (due to hydrophobic interactions).





The phospholipid bilayer form the building blocks of cell membranes

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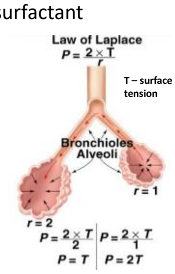
Biophysical interfaces: The pulmonary surfactant

- Pressure in alveoli is directly proportional to surface tension; and inversely proportional to radius of alveoli (law of Laplace).
- Pressure in smaller alveolus would be greater than in larger alveolus, if surface tension were the same in both => **Small alveoli are unstable**.
- How can they be stabilized?
  The pulmonary surfactants stabilize the alveoli.
- Premature babies not yet created a surfactant in the lungs - they need help to inflate the lungs (respiratory equipment).



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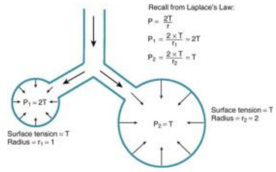
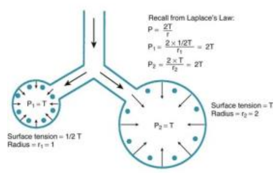
Biophysical interfaces: The pulmonary surfactant



Alveoli without pulmonary surfactant:
$P_1 > P_2$ => Small alveoli are unstable.



Alveoli with pulmonary surfactant:
$P_1 = P_2$ => **Alveoli are stable.**

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Dispersion

- a system in which particles (solid, liquid or gas) are dispersed in a continuous phase.
- any aqueous environment in the human body (e.g. blood) are dispersions (the extracellular and intracellular environment)

Classification of disperse systems according to the particle size:
- coarse dispersion (particle size 1 μm to 1 mm)
- colloid dispersion (particle size 1 μm to 1 nm)

Continuous medium	Dispersed phase	Coarse dispersion	Colloid dispersion
gas	gas	-	-
	liquid	Fog	aerosol
	solid	dust	aerosol
liquid	gas	foam	foam
	liquid	**emulsion**	**sol**
	solid	**suspension**	**sol**
solid	gas	solid foam	solid foam (aerogel)
	liquid	wet sponge	gel
	solid	solid mixture	solid sol

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Dispersion

Dispersed phase / Continuous medium	Coarse dispersion (1 μm to 1 mm)	Colloid dispersion (1 nm to 1 μm)	Analytical dispersion (less than 1 nm)
solid / liquid	blood (blood cells)	blood plasma	true solutions (electrolytes, non-electrolytes)
liquid/ liquid	milk (fat globules in solution of proteins)	solutions of macromolecules	mixtures of miscible liquids
The properties			
observability	light microscope	electron microscope	you can not observe individual particles
osmotic effect	no	small	big
diffusion	no	slow	fast
sedimentation	in the gravitational field	ultracentrifuge	no
optical properties	opaque	opalescence - due to the diffraction of light	clear

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Dispersion

Dispersed phase / Continuous medium	Coarse dispersion (1 μm to 1 mm)	Colloid dispersion (1 nm to 1 μm)	Analytical dispersion (less than 1 nm)
solid / liquid			non-
liquid/ liquid			scible
The properties			
observability			serve icles
osmotic effect			
diffusion			
sedimentation	field		
optical properties	opaque	opalescence - due to the diffraction of light	clear

Oncotic pressure is a form of osmotic pressure exerted by proteins (albumin, globulins, fibrinogen) – is relatively small but **significant** (basic physiological parameter of blood).

The diffusion of small molecules and ions from the colloidal solution through a **dialysis membrane** into pure solvent. **Hemodialysis** – replacement of kidney function.

Sedimentation rate of blood (erythrocytes) is basic laboratory tests - the first warning signal indicating some disease (blood sedimentation is accelerated especially in inflammations or infectious diseases).

Determination of **particle size** (laser diffraction) and **particle concentration** (turbidimetry, nefelometry).

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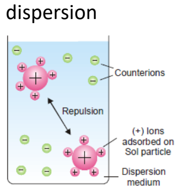
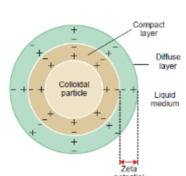
Electrokinetic properties of colloid dispersion

The electrical double layer
The colloids particles carry on their surface **electric charge**, which affect the **stability** of the sol. The charge is formed e.g. by **dissociation** of groups on the particle surface.



Zeta potential: around the charged colloidal particles are grouped ions of opposite sign - charge neutralization. Part of the ion atmosphere is fixed to the particle (compact layer), part continuously passes into solution (diffusion part). There is a potential difference (zeta potential) the compact part and the diffuse part. Zeta potential affects the stability of colloidal solutions.



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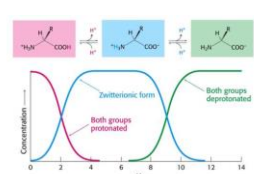
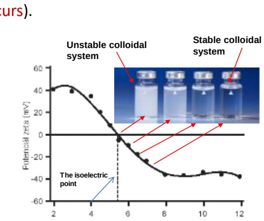
Electrokinetic properties of colloid dispersion

The isoelectric point
The pH of the solution, in which the same number of basic and acidic groups is dissociated (positive and negative charges are equalized; the electrical double layer is canceled; zeta potential = 0; the colloidal system is not stable - coagulation occurs).





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