V.N. Karazin Kharkiv National University
Medical Chemistry
Module 2. Lecture 6

The colloid stability.
Electrical properties of colloidal systems.
Surface phenomena. Adsorption.
Macromolecules

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Lecture topics

√ The colloid stability
√ Coagulation of lyophobic colloidal systems
√ Coagulation threshold
√ Schultze-Hardy rule
√ Kinetic and aggregate stabilities of colloidal systems
√ Protective action
√ Flocculation
√ Introduction to blood coagulation
√ Electrokinetic phenomena
√ Practical applicability of electrophoresis
√ The structure of the electric double layer
√ Electrokinetic potential or zeta potential
√ Adsorption
√ Surface tension
√ Gibbs's equation
√ The Langmuir adsorption isotherm
√ Adsorption of electrolytes
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√ Medical applications of activated carbon
√ Practical application of adsorption
√ Chromatography
√ Macromolecules in solution

Coagulation

Coagulation is the process in which colloidal particles come together to aggregate and form a **visible precipitate or coagulum**. **Coagulum** is an aggregate of colloidal particles having a relatively tight, dense structure formed as a result of the inability of the colloidal system to maintain its dispersed state. Such aggregates are normally formed irreversibly; that is, they cannot be returned to the colloidal state without significant input of work.

stable Fe(OH)$_3$ sol

Sol undergoes coagulation upon the addition of Al$_2$(SO$_4$)$_3$ solution

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Coagulation threshold

One of the most important ways of the coagulation of lyophobic sols is ***the addition of an electrolyte***.

A certain minimum value of electrolyte concentration in the 1 liter of sol at which coagulation begins is called ***coagulation threshold***, χ, or ***critical coagulation concentration (ccc)***: $\gamma = n/V$, mol/l

where n – electrolyte moles number where coagulation is observed; V – total volume of solution.

Investigations of the coagulation process of lyophobic sols by electrolytes have led to the formation of the so-called ***Schultze-Hardy rule*** (H. Schultze, 1882; W.B. Hardy, 1900):

Schultze-Hardy rule

(1) <u>Coagulation of the sol is caused</u> by the ions carrying ***the charge opposite*** to that of sol particles.

(2) <u>Coagulating power of ions</u> causing coagulation is ***directly proportional to the valence of these ions***.

For example, to coagulate **negative particles of sol of As$_2$S$_3$**, the coagulation power of different cations has been found to decrease in the order as:

Al^{3+} > Mg^{2+} > Na$^+$.

Similarly, to coagulate **positive particles of sol such as Fe(OH)$_3$**, the coagulating power of different anions has been found to decrease in the order:

[Fe(CN)$_6$]$^{4-}$ > PO$_4{}^{3-}$ > SO$_4{}^{2-}$ > Cl$^-$.

Schultze-Hardy rule

According to the theory of coagulation of hydrophobic sol by the electrolytes the coagulation threshold varies as the inverse **_sixth power_** of the valence of ions causing coagulation:

$$\gamma = \frac{\text{const}}{z^6}$$

For monovalent ions, the effectiveness for coagulating negatively charged colloids has the order:
$Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$,
while for divalent cations the order is:
$Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+}$.

At increasing of the concentration of added electrolyte to the sol _the diffuse layer of electrical double layer is compressed and zeta potential is decreased._ This effect leads to instability of colloidal system and to coagulation process.

Stability of colloidal systems

Colloidal systems differ widely _**with respect to stability**_. Some of them can be preserved unchanged for long periods of time; others are comparatively unstable being more sensitive to various influences.

**There are two kinds of processes which lead to the disintegration of colloidal systems** and which under certain conditions can take place spontaneously. These are _sedimentation processes_ and _coagulation processes_. _**Kinetic and aggregate stabilities**_ characterize colloidal systems stability with respect to sedimentation process and the changing in particle size (coagulation).
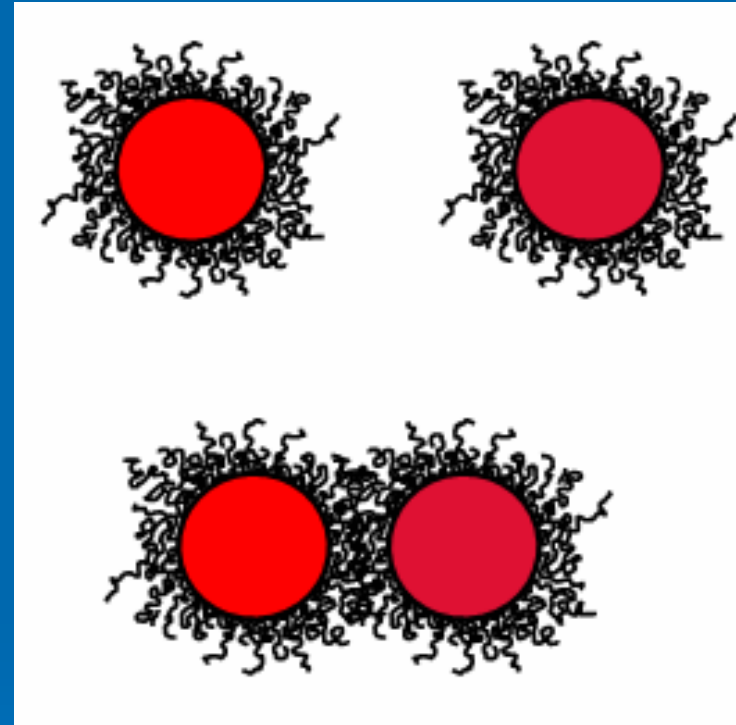
Stability of colloidal systems

Kinetic stability is determined by two conflicting processes: sedimentation of the particles and their thermal motion.

The *aggregate stability* is a measure of the ability of a colloidal system to preserve its degree of dispersion. It is due to the fact that the particles of the dispersed phase are electrically charged and are surrounded by a solvate (hydrate) shell.
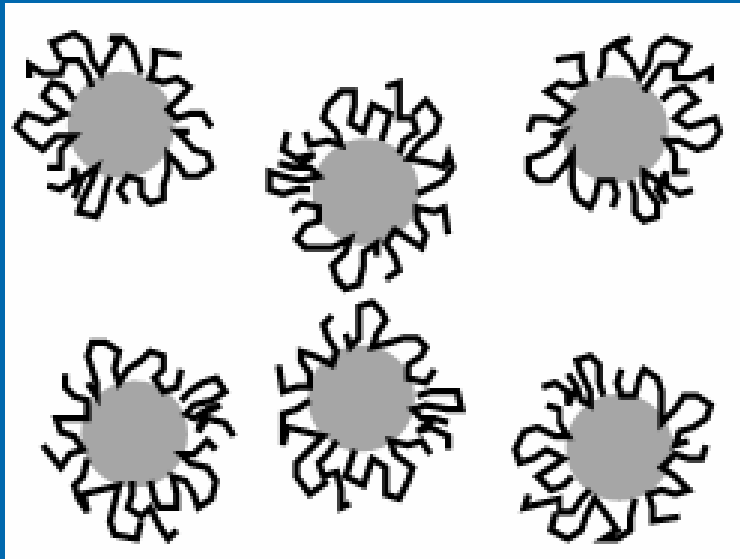
Protective action

The addition of <u>a lyophilic substance (protein, surfactant, starch, gelatin, etc.) to a lyophobic sol</u> frequently renders the latter less sensitive _**to the precipitating effect of electrolytes**_.
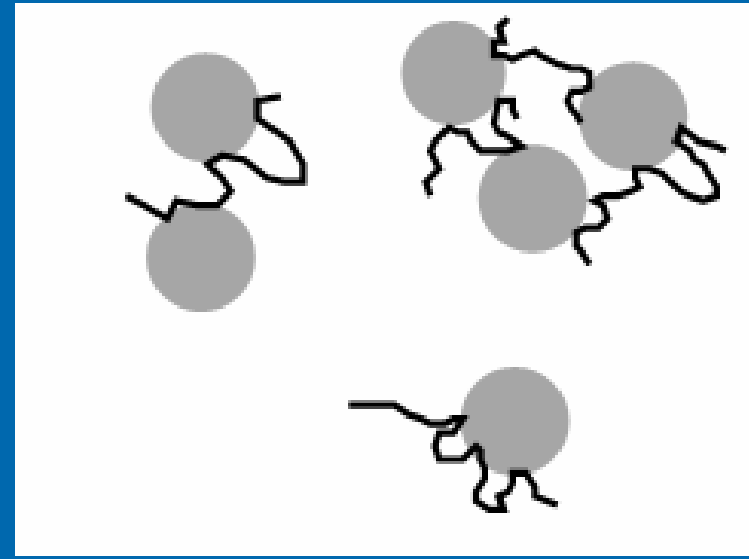
This is an illustration of the phenomenon of _**protective action**_: <u>macromolecules of lyophilic substances are adsorbed onto the colloidal particles of lyophobic sol</u> and provide _steric_ or _entropic_ _stabilization_.

Protective action

<div align="center">(a)   (b)</div>

Figure. Sterically stabilized systems: a given adsorbed macromolecule is associated with one particle – a protective action (**a**). Process of flocculation (**b**)

Flocculation

In colloidal systems containing ***a low concentration of lyophilic substances***, macromolecules can become adsorbed onto two or more particles leading to phenomenon, termed ***flocculation*** (see Figure above).

Flocculation is the process of *flocs* forming.

Flocs are an aggregate of individual colloidal particles associated by lyophilic substance to a coagulum but generally having a rather loose, open structure.

Flocs may sometimes be formed reversibly and returned to the dispersed state with minimal energy input.

Protective action

The relative protective effects of different substances can be expressed quantitatively in terms of what is known as the *__gold number__*. This is defined as the dry weight in milligrams of protective material which when added to 10 ml of a standard gold sol is just sufficient to prevent color change from red to blue on the addition of 1 ml of a 10 per cent solution of sodium chloride.

The color change referred to is due to coagulation of the particles, and hence the gold number is a measure of the **quantity of protective colloid which just fails to prevent precipitation by the electrolyte** (sodium chloride).

That *__the smaller the gold number the greater the protective action of the given substance.__* Gelatin has a very small gold number, and hence is a very good protective substance; egg albumin and gum arabic are less effective, while potato starch has relatively little protective action.

Introduction to blood coagulation

The ability of the body to control the flow of blood following vascular injury is paramount to continued survival. <u>The process of blood clotting and then the subsequent dissolution of the clot</u>, following repair of the injured tissue, is termed _hemostasis_. _Hemostasis, composed of four major events that occur in a set order following the loss of vascular integrity_:

1. The initial phase of the process is vascular constriction. This limits the flow of blood to the area of injury.

2. Next, platelets become activated by thrombin and aggregate at the site of injury, forming a temporary, loose platelet plug. The protein fibrinogen is primarily responsible for stimulating platelet clumping. Platelets clump by binding to collagen that becomes exposed following rupture of the endothelial lining of vessels. In addition to induced secretion, activated platelets change their shape to accommodate the formation of the plug.

Introduction to blood coagulation

3. To insure stability of the initially loose platelet plug, a fibrin mesh (also called the clot) forms and entraps the plug. If the plug contains only platelets it is termed a white thrombus; if red blood cells are present it is called a red thrombus.

4. Finally, the clot must be dissolved in order to normal blood flow to resume following tissue repair. The dissolution of the clot occurs through the action of plasmin.

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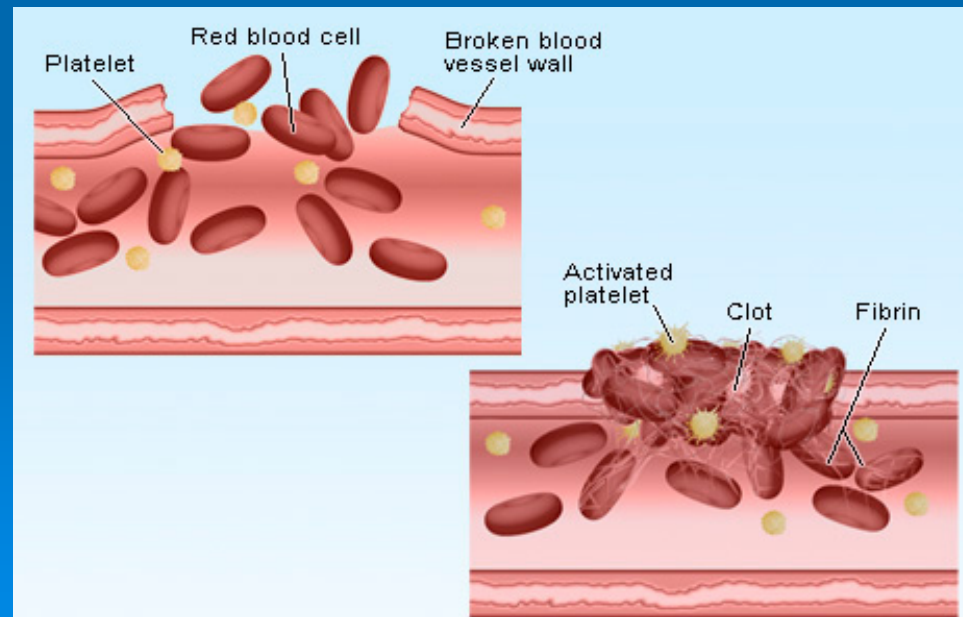
Electrokinetic phenomena

When *a hydrophobic sol* *is placed in an electric field* the **particles become moving definitely in one direction or another**.

This means that **colloidal particles are electrically charged with respect to the dispersion medium**.

The phenomenon of *the migration of colloidal particles under the influence of an electrical potential* **is called (1)** *electrophoresis*.

The movement of particles in an electric field can be easily observed in the apparatus constituting the U-tube with two electrodes. **The velocity of the particles** under a fall of potential of 1 volt per meter, i.e., **the electrophoretic mobility**, may be calculated. **The electrophoretic mobilities for aqueous sols** almost always lie within the range of 2 to 4×10^{-4} cm per sec.

The sign of the particles charge can be determined by observing the direction in which the boundary moves.

Electrokinetic phenomena

Other electrokinetic phenomena:

(2) electroosmosis (a liquid flows along a charged surface when an electric field is applied).

(3) sedimentation potential (Dorn effect) – an electrical potential created by the movement of charged particles through a liquid by gravity. *This effect may be regarded as the reverse of electrophoresis.*

(4) streaming potential (Quincke effect) is *the production of a potential difference when a liquid is forced through a porous membrane or capillary tube.* The streaming potential effect may thus be regarded as the reverse of electroosmosis.

Practical applicability of electrophoresis

Electrophoresis is a method used in clinical and research laboratories for separating molecules according to their size and electrical charge.

Electric current is passed through a medium that contains the mixture of molecules. Each kind of molecule travels through the medium at a different rate, depending on its electrical charge and molecular size. Separation of the molecules occurs based on these differences.
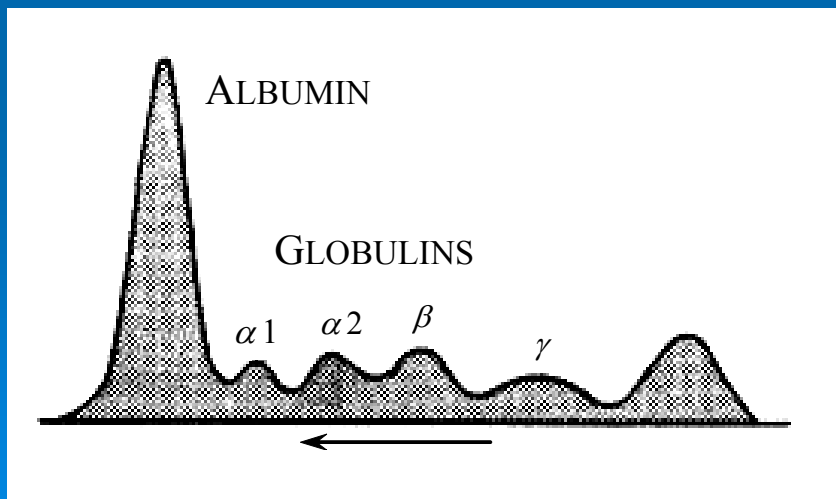
From the position of the peak (see Figure), which is determined by the electrophoretic mobility,
√ the nature of the protein can be identified;
√ the area under of the peak is a measure of the amount present in the sol.

Figure. Electrophoretic diagram (ascending) for human blood serum

# The structure of the electric double layer

Electrical double layer of positive and negative ions exist between two phases at the surface.

According to modern views, developed in works of Gouy, Chapman and Stern, *the electrical double layer at a solid-liquid interface is made up of two parts*:

the first part is formed by ions (either positive or negative) coincide with the solid surface (*potential determining ions*), **the second part** is layer of *counter-ions*.

The layer of counter-ions can be divided on two parts: 1) layer of ions in aqueous solution, which are firmly held to the solid, so-called *Stern layer*, and 2) more mobile *diffuse layer*, extending into the solution.

The structure of the electric double layer

Figure. Schematic representation of the structure of the electric double layer
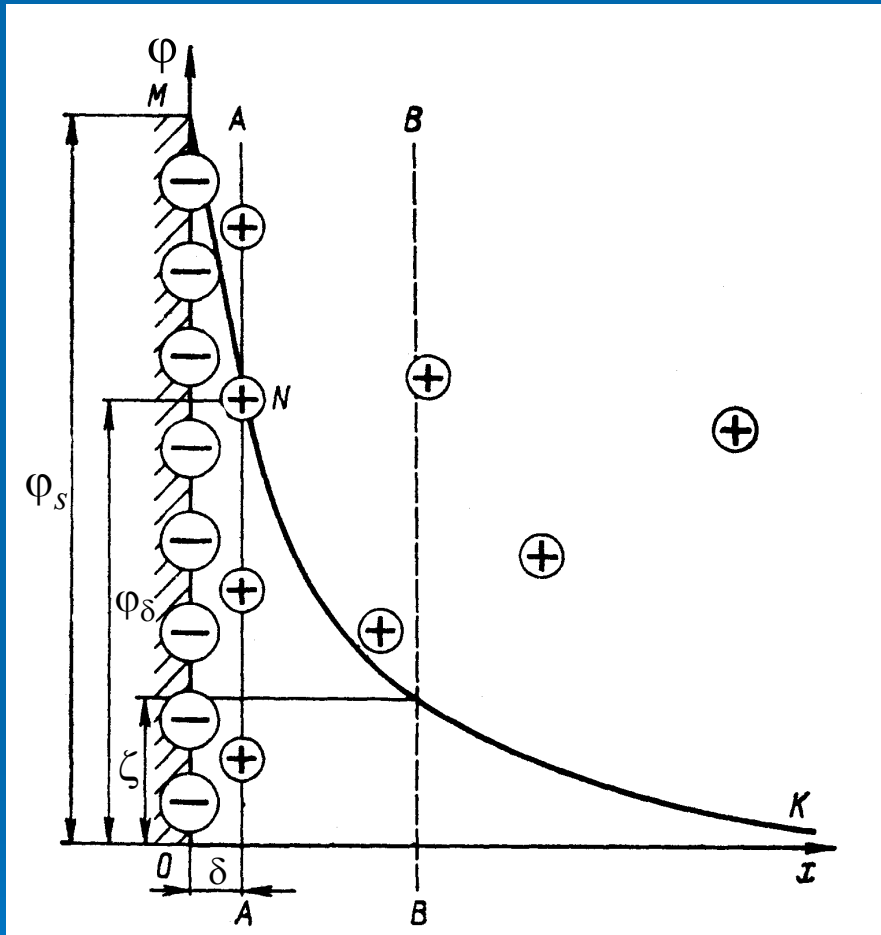
Because of existence of spatial separation of electrical charges, there is a difference of potential between solid and liquid phases. The electric potential (φ_s) in the Stern layer varies linearly (φ_δ, section *MN*) with the distance (δ); the potential in diffuse layer varies exponentially (section *NK*) (see Figure).

The structure of the electric double layer

When an electric field is applied to an electrical double layer there must be a displacement of the oppositely charged layers relative to one another; <u>the actual movement will presumably take place in the diffuse layer at the region indicated by the dotted line (*BB*)</u> in Figure (see above) , this line represents the slip boundary that denotes the separation between two moving parts.

In the case of a sol, the layer closely attached to the colloidal particle is free to move, together with the particle itself, in an applied field, thus producing the phenomenon of electrophoresis, described above. It is to be expected that there should be a connection between the velocity of electrophoresis and the potential acting at the slip surface of the moving colloidal particle.

Electrokinetic potential
or zeta potential

    This potential has been called the **_electrokinetic potential_ or _zeta potential_**, because it is represented by the Greek letter zeta – ζ (see Figure above):

$$velocity = \frac{E\varepsilon\varepsilon_o}{\eta}\zeta$$

    where E – electric field intensity; ε – dielectric permittivity of dispersed medium; ε_0 – dielectric permittivity of vacuum; η – viscosity of medium.

    The velocity of the particles under a fall of potential of 1 volt per meter, i.e., the electrophoretic mobility, may be calculated. The electrophoretic mobilities for aqueous sols almost always lie within the range of 2 to 4×10^{-4} cm per sec.

Adsorption

__Adsorption__ is a process that occurs when a gas or liquid solute accumulates <u>on the surface of a solid or a liquid</u>, forming **a molecular or atomic film**.

It also can be defined as *the preferential concentration* (i.e., location) *of one component of a system at an interface*, where the local (i.e., interfacial) <u>concentration of one or more components of one or both phases is different from those in the bulk phases</u>.

Adsorption should be clearly differentiated from '*__absorption__*', in which physical penetration of one phase into another is involved. The term *__sorption__* encompasses both processes.
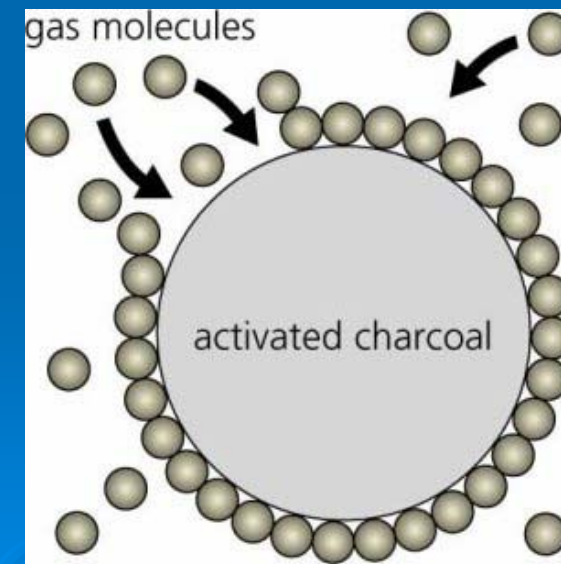
Adsorption on the surface of activated charcoal

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Adsorption

Where the <u>interfacial concentration</u> of the adsorbed species <u>is greater than that in the bulk phase</u>, one can refer to **'*positive*' *adsorption*.** In a situation of **'*negative*' *adsorption***, the concentration of a system component in the region of the interface <u>will be less than that in one or both bulk phases</u>.

The substance that adsorbs is the **_adsorbate_** and the underlying material is the **_adsorbent_** or **_substrate_**. The reverse process of adsorption is **_desorption_**.

Types of adsorption: physical and chemical adsorptions.

The forces involved in adsorption processes are nonspecific Van der Waals forces, ionic or electrostatic forces, and specific forces involved in the formation of chemical bonds. Because the nonspecific interactions are orders of magnitude smaller than the chemical forces, **adsorption processes that involve only nonspecific Van der Waals** (weak intermolecular) **interactions** are generally referred to as **'*physical adsorption'*** while those in which **stronger chemical interactions** occur are termed **'*chemical adsorption or chemisorption'*.**

Adsorption

*The process of **physical adsorption** is reversible* and equilibrium is attained rapidly. Physical adsorption is generally a multilayer process (see Figure).

Chemisorption is limited to the formation of a monomolecular adsorbed layer (see Figure). Chemisorption processes may be much slower than physical adsorption and *are not readily reversible*.
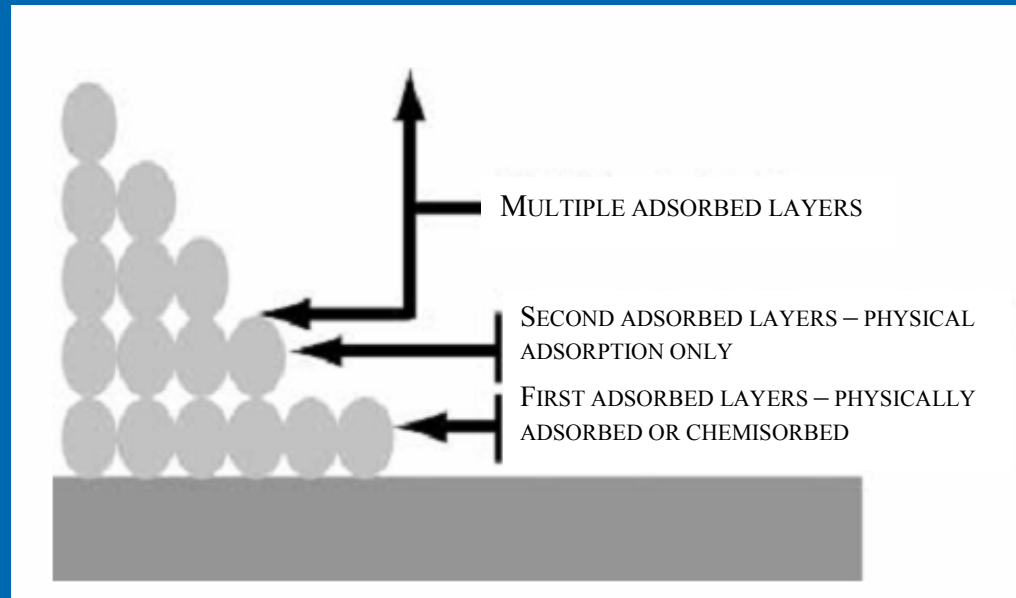

MULTIPLE ADSORBED LAYERS

SECOND ADSORBED LAYERS – PHYSICAL ADSORPTION ONLY

FIRST ADSORBED LAYERS – PHYSICALLY ADSORBED OR CHEMISORBED

Figure. Multilayer adsorption on a solid surface: the first adsorbed layer may be physically adsorbed or chemisorbed; subsequent layers will be physically adsorbed

Adsorption

Absolute adsorption, A, is quantitative characteristic of adsorption process. It is equal the amount of adsorbed substance (in moles or kg) per unit area of the surface layer or per unit mass of the adsorbent:

$$A = n / s \quad or \quad A = n / m$$

where n – the amount of adsorbed substance; s and m – the adsorbent area and its mass respectively.

Adsorption in the surface layer of a solution is described quantitatively by ***Gibbs's adsorption***:

$$\Gamma = n^s / s$$

where n^s – moles excess of adsorbate in the surface layer

with area as compared to the bulk of solution.

Adsorption

The *A* and Γ values are related by the following equation: $A = \Gamma + c\delta$,

where *c* – concentration of a component in the bulk phase; δ – a thickness of the surface layer.

A molecule *in the inner layers* of a substance experiences *averaged forces* of attraction in all directions by surrounding molecules, so that *the net force on it is zero*. *For a molecule in the surface layer*, however, *these forces are unbalanced* (see Figure).
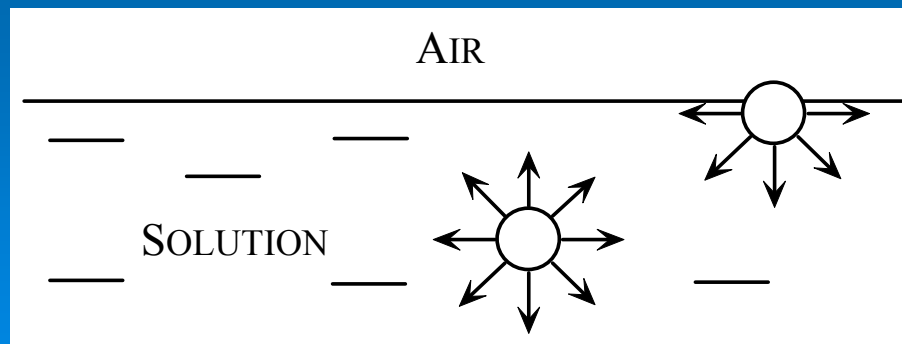

Figure. The surface layer of a liquid

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Surface tension

Owing to the unique environment of the molecules in the surface layer an increase in surface area requires the work. This work referred to 1 m^2 is called the **_surface tension_, σ**. Its unity is N/m or J/m^2 (numerically the values in both units are equal).

Hence **_the surface tension_** may be regarded as force per unit length ($\sigma = F / l$) or energy per unit area ($\sigma = A / s$).

Substances which greatly reduce the surface tension of a solvent are called **_surface active agents_** or **_surfactants_**. The quantity

$$g = -(d\sigma/dc)_{c \to 0}$$

is usually taken as **the _measure of surface activity_**.

Surface tension

With rise in temperature the thermal motion of the molecules becomes more energetic and, hence, *adsorption decreases*.

Surfactant molecules content usually hydrophobic hydrocarbon chains and hydrophilic groups: –COOH, –OH, –NH$_2$, –SH, –CN, –NO$_2$, –SCN, –CHO, –SO$_3$H, –SO$_3$Na, for instance sodium *n*-dodecylsulfate C$_{12}$H$_{25}$OSONa$^+$ or cetyltrimethylammonium bromide C$_{16}$H$_{33}$N(CH$_3$)Br$^-$.

The longer the hydrocarbon chain, *the greater is the tendency for surfactant molecules to adsorb at the air-water surface and, hence, lower the surface tension*. A rough generalisation, known as **Traube's rule**, is that for a particular homologous series of surfactants the concentration required for an equal lowering of surface tension in dilute solution decreases by a factor of about 3 for each additional −CH$_2$− group.

Gibbs's equation

Adsorption of a solute in the surface layer of a solution is described quantitatively by ***Gibbs's equation***:

$$\Gamma = -\frac{c}{RT} \cdot \frac{d\sigma}{dc}$$

where $d\sigma/dc$ is the change in the surface tension of the solution with concentration c; R – gas constant; T – absolute temperature.

This equation shows that when σ takes off with increasing concentration, i.e., $d\sigma/dc < 0$, then $\Gamma > 0$, and the concentration of solute is greater in the surface layer than in the bulk of solution (*positive adsorption*). It is observed for surfactant solutions. In the opposite case, i.e., when $d\sigma/dc > 0$, $\Gamma < 0$, and the concentration is lower in the surface layer than in the bulk of the solution (*negative adsorption*).

The Langmuir adsorption isotherm

Chemisorption does not extend beyond *a single layer of gas molecules on the surface of the solid*.

By supposing that *a unimolecular layer only of gas can be adsorbed on the surface of a solid*, in 1916 Langmuir was able to derive *an adsorption isotherm* relating the *pressure of the gas to the extent of adsorption, since it is applicable at constant temperature*.

The simplest physically plausible isotherm is based on four assumptions:

1. All sites are equivalent and the surface is uniform (that is, the surface is perfectly flat on a microscopic scale).

2. The ability of a molecule to adsorb at a given site is independent of the occupation of neighbouring sites (that is, there are no interactions between adsorbed molecules).

The Langmuir adsorption isotherm

3. Adsorption cannot proceed beyond monolayer coverage.

4. The free gas and the adsorbed gas are in dynamic equilibrium. The dynamic equilibrium is

$$G \text{ (gas)} + M \text{ (surface)} \leftrightarrow GM \text{ (surface)}$$

with rate constants k_a for adsorption and k_d for desorption. The constant of adsorption equilibrium is equal

$$K = \frac{k_a}{k_d}$$

The rate of change of surface coverage due to adsorption is proportional to the partial pressure p of gas and the number of vacant sites. The *fractional coverage*, θ = number of adsorption sites occupied / number of adsorption sites available of the surface depends on the pressure of the overlying gas.

The Langmuir adsorption isotherm

The variation of *θ* value with pressure at constant temperature is called the *Langmuir adsorption isotherm*:

$$A = A_\infty \frac{Kp}{1+Kp}$$

$$A = A_\infty \frac{Kc}{1+Kc}$$

where A_∞ – limiting monomolecular adsorption; *K* – constant of adsorption equilibrium; *p* – the partial pressure of gas; *c* – the substance concentration in the bulk phase; the *p* and *c* values are equilibrium ones.

The linear form of Langmuir adsorption isotherm is following:

$$\frac{1}{A} = \frac{1}{A_\infty} + \frac{1}{A_\infty K}\frac{1}{c}$$

The $1/A_\infty$ value is intercept on Y-axis (1/*A*); $tg\alpha = 1/(A_\infty K)$ – angular coefficient of the line to X-axis.

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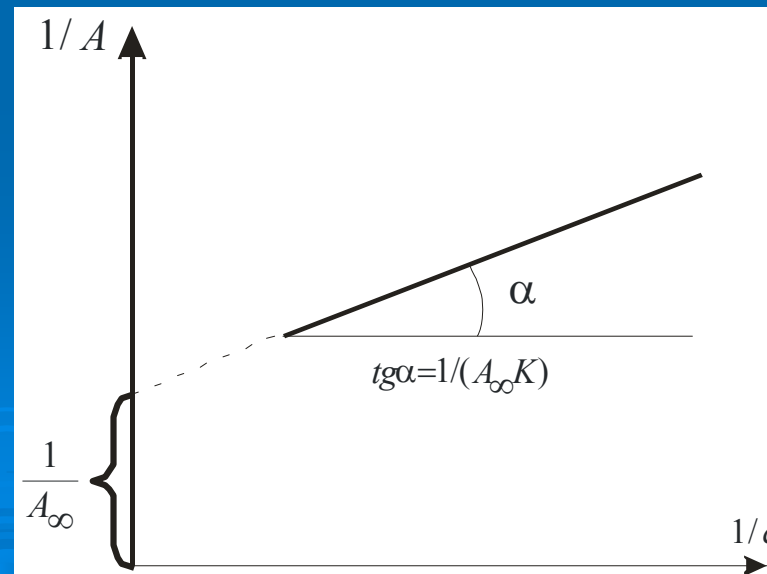
The Langmuir adsorption isotherm

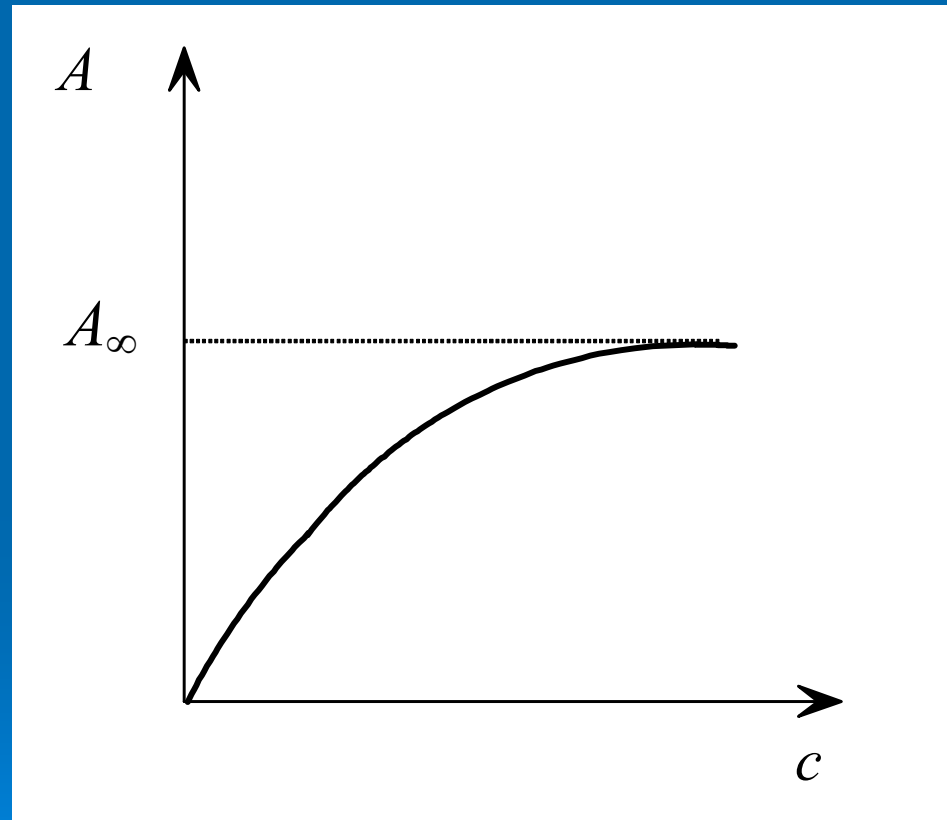*The plot of Langmuir adsorption isotherm* is presented in the Figure.

Figure. The Langmuir adsorption isotherm

Adsorption of electrolytes

Strong electrolytes in aqueous solutions exist in the form of ions, therefore adsorption from such solutions is called an *__ion adsorption__*. The ion adsorption proceeds due to a chemical interaction between ions of dissolved substance and solid surface of adsorbent.

Adsorption of ions may be classified by two types:

1) adsorption of ions on solid surfaces;

2) ion exchange adsorption.

Adsorption on solid surfaces is caused by the adsorption of cations or anions and is described by *__Paneth-Fajans-Hahn adsorption rule__* for predicting which of several ions in solution will be preferentially adsorbed as counterions.

Adsorption of electrolytes

First rule. Of two ions present at equal concentration, the ion of higher charge is preferentially adsorbed. Of two ions of equal charge, the ion present at higher concentration is preferentially adsorbed.

Second rule. Of two ions of equal charge at the same concentration, the ion most strongly attracted by the lattice ions is preferentially adsorbed. Stronger interionic attraction between adsorbed lattice ions and counterions is indicated by (a) lower solubility, (b) lesser degree of dissociation, (c) greater covalency, or (d) greater electrical polarizability of the anion and greater polarizing character of the cation.

Adsorption of electrolytes

Solid phase of adsorbent absorbs cations or anions from the solution and gives equivalent amount of ions of the same sign instead of them. If the process of equivalent ion exchange has place, the adsorbents are called *ionites*.

Ionites classified according to different criteria:

1) by origin: natural and synthetic;

2) by the composition: the inorganic and organic;

3) by the type of ionic groups: cationite (acid ionite), anionite (basic ionite) and ampholyte. The latter can exchange both cations and anions it is depend from the conditions.

4) by the degree of ionization of ionogenic groups: strong and weak acid cationites or basic anionites.

Ion exchange

Ion exchange involves an electric double layer situation in which two kinds of counter-ions are present, and can be represented by the equation:

$$RA + B = RB + A$$

where R is a charged porous solid. Counter-ions A and B compete for position in the electric double layer around R, and, in this respect, concentration and charge number are of primary importance. R may be a cation exchanger (fixed negatively charged groups, such as $-SO_3^-$ or $-COO^-$) or an anion exchanger (fixed positively charged groups, such as $-NH_3^+$). A range of highly porous synthetic cation and anion exchange resins are available commercially. The porosity of the resin facilitates fairly rapid ion exchange.

Ion exchange

The most important applications of ion exchange are the softening of water and the 'deionisation' of water.

In the first of these processes, hard water is passed through a column of a cation exchange resin usually saturated with sodium counter-ions. The doubly charged (and, therefore, more strongly adsorbed) calcium ions in the water exchange with the singly charged sodium ions in the resin, thus softening the water. Regeneration of the resin is effected by passing a strong solution of sodium chloride through the column.

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Ion exchange

The 'deionisation' of water involves both anion and cation exchange. A cation exchange resin saturated with hydrogen ions and an anion exchange resin saturated with hydroxyl ions are used, often in the form of a mixed ion exchange resin. These hydrogen and hydroxyl ions exchange with the cations and anions in the water sample and combine to form water.

Ion exchange has many preparative and analytical uses; for example, the separation of the rare earths is usually achieved by cation exchange followed by elution of their complexes with citric acid.

Medical applications of activated carbon

The first place among the adsorbents used in practice belongs to the different kinds of specially prepared carbons with exceptionally high porosity and, hence, a huge surface area. For instance, in 1 g of a highly adsorbing carbon (_activated carbon, also called activated charcoal or activated coal_) the inner surface of the pores has a surface area of 400-900 m^2. Besides the general degree of porosity, its character, i.e., the numbers of pores of different diameters, is of high significance in adsorption processes.

In addition to activated carbon, other substances are used as adsorbents for different purposes. Activated carbon is frequently used in everyday life, in: industry, food production, medicine, pharmacy, military, etc. These are mostly highly porous or finely divided materials, such as silica gel, alumina, kaolin, and certain alumosilicates.

Medical applications of activated carbon

Activated carbon is used to treat poisonings and overdoses following oral ingestion. It is thought to bind to poison and prevent its absorption by the gastrointestinal tract. In cases of suspected poisoning, medical personnel administer activated charcoal on the scene or at a hospital's emergency department. In rare situations activated charcoal is used in intensive care to filter out harmful drugs from the blood stream of poisoned patients. Activated charcoal has become the treatment of choice for many poisonings, and other decontamination methods.

While activated carbon is useful in acute poisoning, it has been shown to not be effective in long term accumulation of toxins, such as with the use of toxic herbicides.

Tablets or capsules of activated charcoal are used in many countries as an over-the-counter drug to treat diarrhea, indigestion, and flatulence. Activated charcoal is also used for bowel preparation by reducing intestinal gas content before abdominal radiography to visualize bile and pancreatic and renal stones.

Practical application of adsorption

Adsorption has a wide variety of applications. In heterogeneous catalysis, both in the gaseous phase and in solution, adsorption of the reacting substances by a solid catalyst is of decisive importance. Solid adsorbents are also used in various processes of purification of gases or liquids from undesirable admixtures or impurities. Other examples are the many processes of, purification and drying of gases in industrial conditions, and the discoloration of solutions in the manufacture of sugar, glucose, petroleum products, certain pharmaceutical products, etc.

Adsorption is sometimes utilized to separate some valuable product from a gas mixture or solution; for example, in the processes of recuperation of volatile solvents air containing the vapor of the solvent to be recuperated (benzene, acetone, etc.) is passed through a layer of activated carbon or silica gel which adsorbs the vapor. By subsequently heating the adsorbent or sending water vapor through it, one obtains the solvent in the pure form.

Practical application of adsorption

Adsorption phenomena play an important part in dyeing processes. For example, when wool is dyed, the dye-stuff is first adsorbed, after which a chemical reaction takes place in the adsorbed layer.

The properties of many powdered materials, in particular building materials, can be radically changed by the adsorption of various substances. This phenomenon, for instance, is at the basis of the hydrophobization of cement upon treatment with solutions of high-molecular organic acids, etc. Soils adsorb various dissolved substances from natural waters.

Chromatography

Chromatography is a separation technique, which is used for the separation of complex mixtures into their individual components and for determining quantitatively the amounts of those components. The most important chromatographic methods are *gas chromatography, high-performance liquid chromatography*, and *thin-layer chromatography*.

Fundamentals of chromatographic separations. Efficient separation of chemically similar compounds is only possible through repeated use of separation steps.

Multiplicative separations can be undertaken using chromatographic methods. It is estimated today that approximately 60% of all analyses world wide can be attributed to chromatography.

Chromatography

The Russian botanist M.S. Tswett introduced column chromatography in 1903. Using a packed column containing finely dispersed calcium carbonate. He was able separate extracts of leaf pigments, such as chlorophylls and the xanthophylls spirilloxanthin. When doing so, he observed colored zones on the column, which prompted him to create **the word chromatography (Greek *chroma* for 'color' and *graphein* for 'writing').**

The principle of chromatography is based on the passage of the constituents to be separated between two immiscible phases. For this, the sample is dissolved in the **mobile phase**, which can be a liquid, a gas, or a supercritical phase, and moved across a **stationary phase**, which can either be a solid or a liquid in a column or on a solid surface. Due to the interactions of the constituents with the stationary and mobile phases they separate after sufficient running time.

Chromatography

A basic chromatographic process may be described as follows (see Figure below):

1. A vertical hollow glass tube (the column) is filled with a suitable finely powdered solid, the stationary phase.

2. At the top of this column is placed a small volume of the sample mixture to be separated into individual components.

3. The sample is then taken up by continuous addition of the mobile phase, which goes through the column by gravity, carrying the various constituents of the mixture along with it. This process is called *elution*. If the components migrate at different velocities, they will become separated from each other and can be recovered, mixed with the mobile phase.

Chromatography
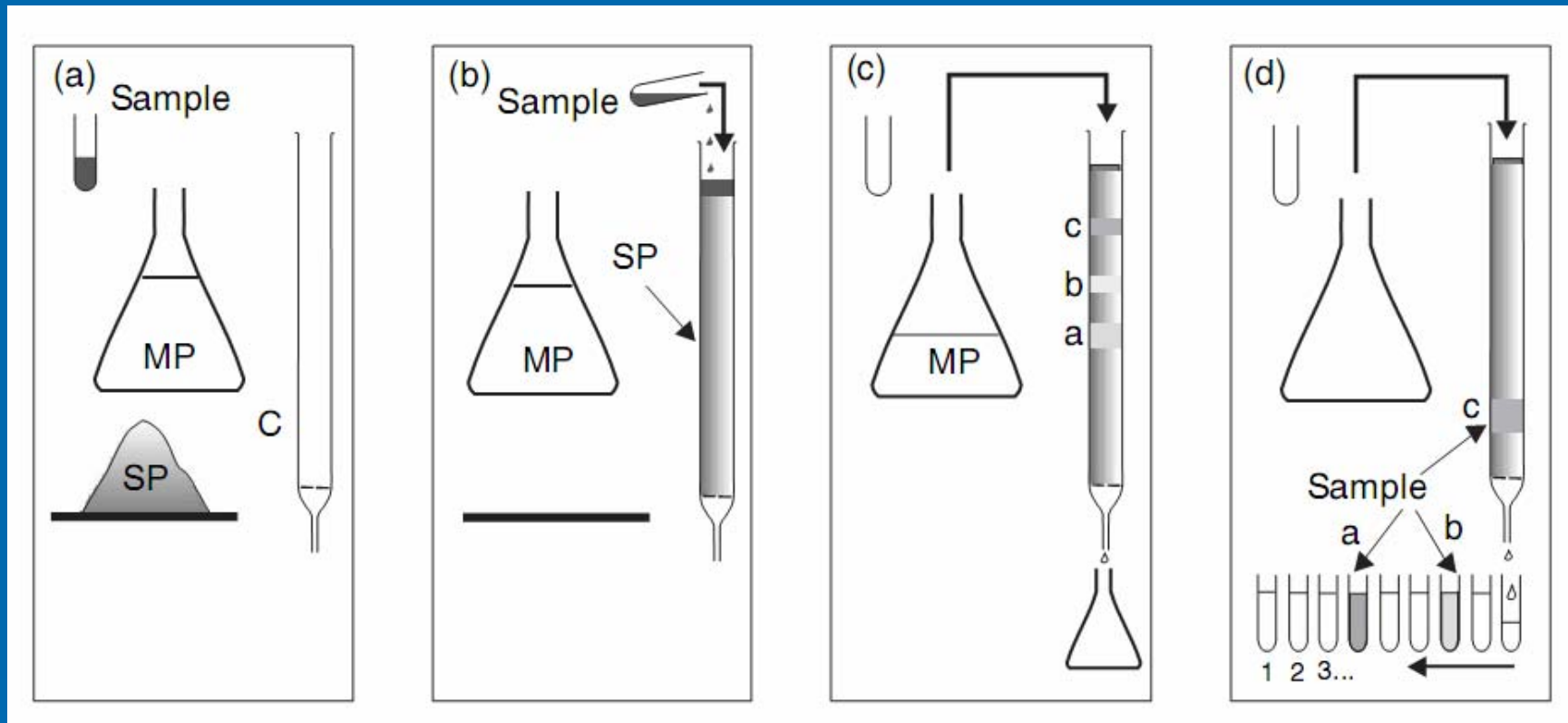
Figure. A basic experiment in chromatography. (a) The necessary ingredients (C, column; SP, stationary phase; MP, mobile phase; and S, sample); (b) introduction of the sample; (c) start of elution; (d) recovery of the products following separation.

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Chromatography

Classification of column chromatographic methods according to the mobile or stationary phase is set out in Table:

Stationary phase	Mobile phase		
	gaseous	fluid	liquid
Solid	GSC (gas-solid chromatography)	SFC (supercritical fluid chromatography)	LSC (liquid-solid chromatography)
Liquid	GLC (gas-liquid chromatography)		LLC (liquid-liquid chromatography)

Chromatography

The two most important separation principles in the passage between the mobile and the stationary phase are _**distribution** and **adsorption**_. Adsorption chromatography is based on the direct interaction of the analyte with the surface of the stationary back-up phase, as for instance in GSC or LSC. Distribution chromatography is linked to the presence of an immobilized liquid stationary phase (GLC, LLC).

Liquid chromatography can be undertaken on a column or on a plan. Thus, the basic physicochemical principles are also valid for methods of planar chromatography. Gas chromatographic methods are limited to column chromatography.

Chromatography

The identification of a compound by chromatography is achieved by comparison: to identify a compound which may be A or B, a solution of this unknown is run on a column.

Next, its retention time is compared with those for the two reference compounds A and B previously recorded using the same apparatus and the same experimental conditions.

The choice between A and B for the unknown is done by comparison of the retention times.

Chromatography

Nowadays chromatographic techniques are piloted by computer software, which operate highly efficient miniature columns able to separate nano-quantities of sample. These instruments comprise a complete range of accessories designed to assure reproducibility of successive experiments by the perfect control of the different parameters of separation.

Thus it is possible to obtain, during successive analyses of the same sample conducted within a few hours, recordings that are reproducible to within a second (see Figure below).

Chromatography
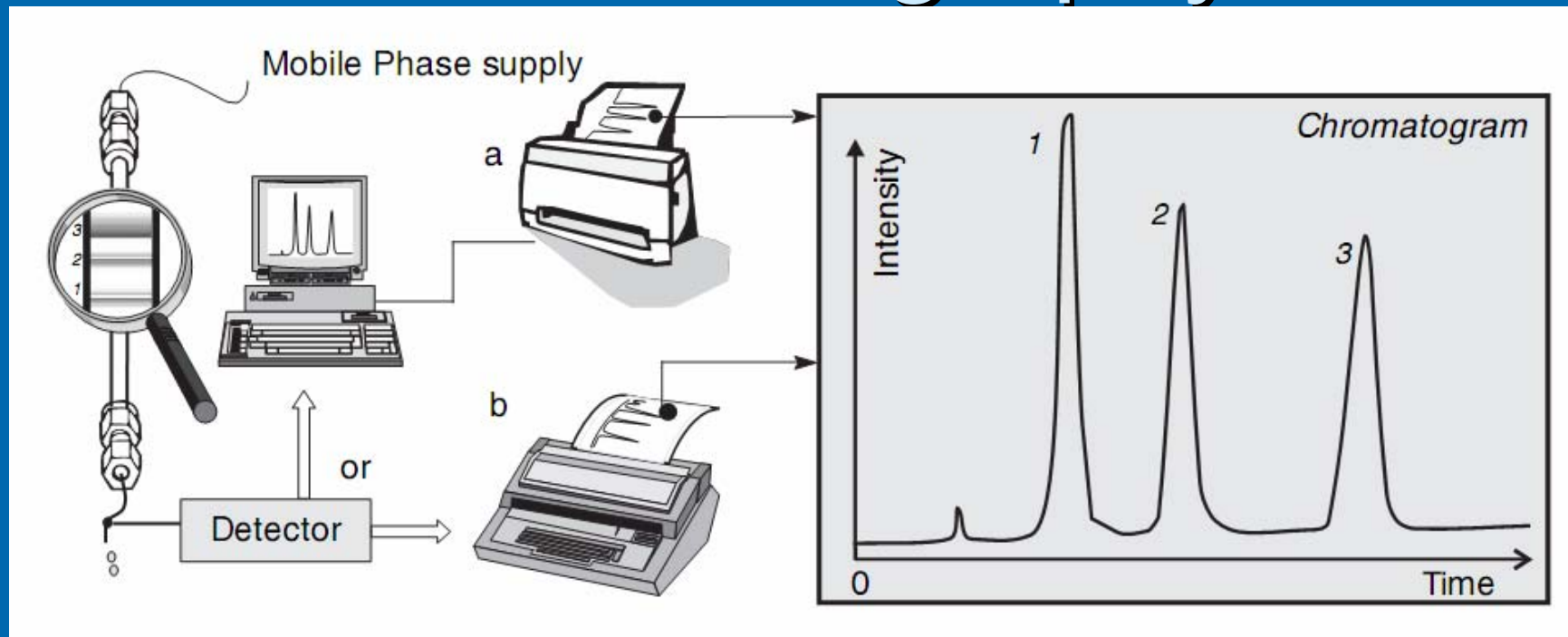
Figure. The principle of analysis by chromatography. The chromatogram, the essential graph of every chromatographic analysis, describes the passage of components. It is obtained from variations, as a function of time, of an electrical signal emitted by the detector. It is often reconstructed from values that are digitized and stored to a microcomputer for reproduction in a suitable format for the printer (a). For a long time the chromatogram was obtained by a simple chart recorder or an integrator (b). Right, a chromatogram illustrating the separation of a mixture of at least three principal components. Note that the order of appearance of the compounds corresponds to the relative position of each constituent on the column.

Chromatography

The essential recording that is obtained for each separation is called a ***chromatogram.*** It corresponds to a two-dimensional diagram traced on a chart paper or a screen that reveals the variations of composition of the eluting mobile phase as it exits the column.

To obtain this document, a sensor, of which there exists a great variety, needs to be placed at the outlet of the column. The detector signal appears as the ordinate of the chromatogram while time or alternatively elution volume appears on the abscissa.

Chromatography

Application of gas-liquid chromatography. It can be used to assess the purity of a substance, or for the preparative isolation of substances from a mixture. The analytical chemist is primarily interested in the application of gas chromatography for qualitative and quantitative analysis.

Size-exclusion chromatography (also known as gel chromatography, is a particular from of liquid chromatography: *it is based on the separation of molecules due to their size*) serves for the analysis of substances with relative molecular masses greater than 2000. Thus, proteins can be separated from amino acids and smaller peptides. Gel permeation serves for the separation of homologs and oligomers, for example fathy acids with relative molecular masses in the range between 100 and 350. For this, a polymer with an exclusion limit of mass 1000 is selected.

Chromatography

Supercritical fluid chromatography is used to analyze natural products, drugs, foodstuffs, pesticides, surfactants, polymers, additives, crude oil, and explosives.

Gas chromatography has been widely used in medicine for the determination of many drugs content, products of their metabolism, levels of fatty acids, cholesterol, steroids in the diseased organism, etc. These analyzes provide important information about the state of human health, disease, the effectiveness of the use of certain drugs.

As an example, in Figure below gas-liquid chromatogram of purulent lung secretions of the patient affected by anaerobic infection before and after treatment with cephalosporin antibiotic is shown.

Chromatography

a　　　　　　　　**b**
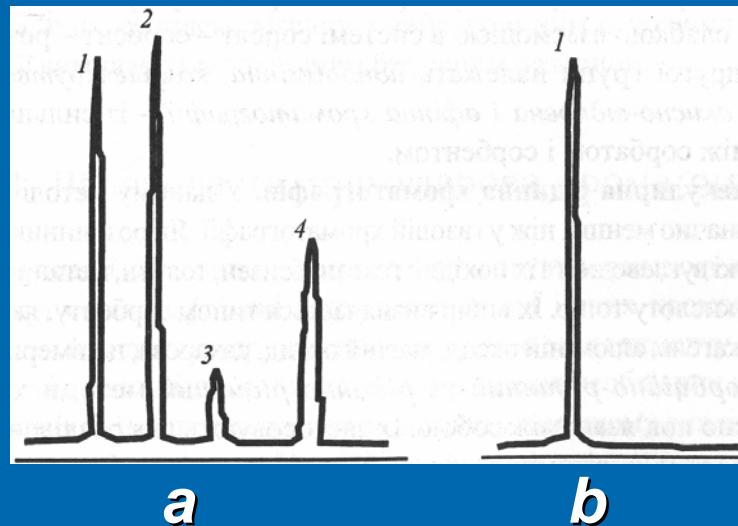
Figure. Chromatogram of pus from pleural cavity at anaerobic sepsis: *a* – before and *b* – after treatment; *1* – acetic acid; *2* – propionic acid; *3* – butyric acid; *4* – isovaleric acid.

After two weeks of treatment all acids disappeared except acetate which is a natural metabolite. Thus, the gas-liquid chrochromatography becomes a valuable method for monitoring clinical course of the treatment process.

Macromolecules
Properties of Polymers

A polymer is a molecular compound distinguished by a high molar mass, ranging into thousands and millions of grams, and made up of many repeating units.

The physical properties of these so-called **_macromolecules_** differ greatly from ordinary molecules.

Naturally occurring polymers include proteins, nucleic acids, cellulose (polysaccharides), and rubber (polyisoprene).

Most synthetic polymers are organic compounds. Familiar examples are nylon, poly(hexamethylene adipamide); Dacron, poly(ethylene terephthalate); and Lucite or Plexiglas, poly(methyl methacrylate).

Synthetic Organic Polymers

Synthetic polymers are created by joining monomers together, one at a time, by means of (1) addition reactions and (2) condensation reactions.

Polyethylene, a very stable polymer is made *by joining ethylene monomers via an addition-reaction mechanism*. First an initiator molecule (R_2) is heated to produce two radicals:

$$R_2 \longrightarrow 2R \cdot$$
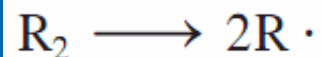
The reactive radical attacks an ethylene molecule to generate a new radical:

$$R \cdot + CH_2{=}CH_2 \longrightarrow R{-}CH_2{-}CH_2 \cdot$$

which further reacts with another ethylene molecule, and so on:

$$R{-}CH_2{-}CH_2 \cdot + CH_2{=}CH_2 \longrightarrow R{-}CH_2{-}CH_2{-}CH_2{-}CH_2 \cdot$$

Eventually, this process is terminated by the combination of two long-chain radicals to give the polymer called polyethylene:

$$R\text{---}(CH_2\text{---}CH_2)_n\text{---}CH_2CH_2\cdot \ + \ R\text{---}(CH_2\text{---}CH_2)_n\text{---}CH_2CH_2\cdot \ \longrightarrow$$
$$R\text{---}(CH_2\text{---}CH_2)_n\text{---}CH_2CH_2\text{---}CH_2CH_2\text{---}(CH_2\text{---}CH_2)_n\text{---}R$$
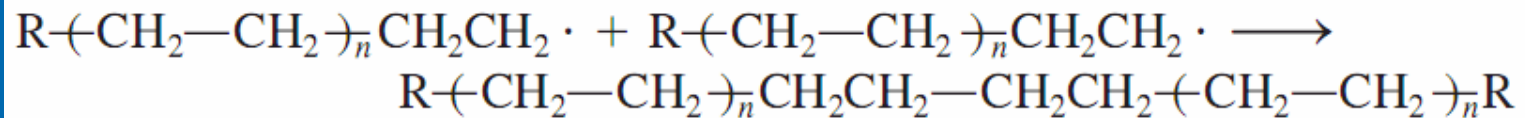
$(CH_2\text{---}CH_2)_n$ is a convenient shorthand convention for representing the repeating unit in the polymer. The value of n is understood to be very large, on the order of hundreds.

Polyethylene is an example of **_a homopolymer_**, which is a polymer made up of only one type of monomer. Other homopolymers that are synthesized by the radical mechanism are Telfon, polytetrafluoroethylene and poly(vinyl chloride) (PVC):

$$(CF_2\text{---}CF_2)_n \qquad\qquad (CH_2\text{---}\underset{\displaystyle Cl}{CH})_n$$

Teflon   PVC
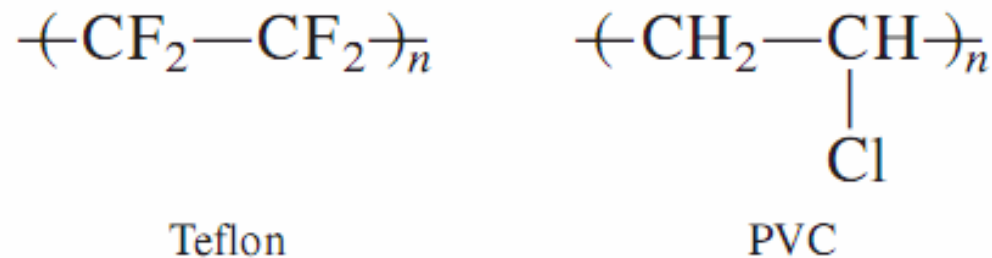
Natural rubber is poly-*cis*-isoprene, which is extracted from the tree Hevea brasiliensis (Figure).
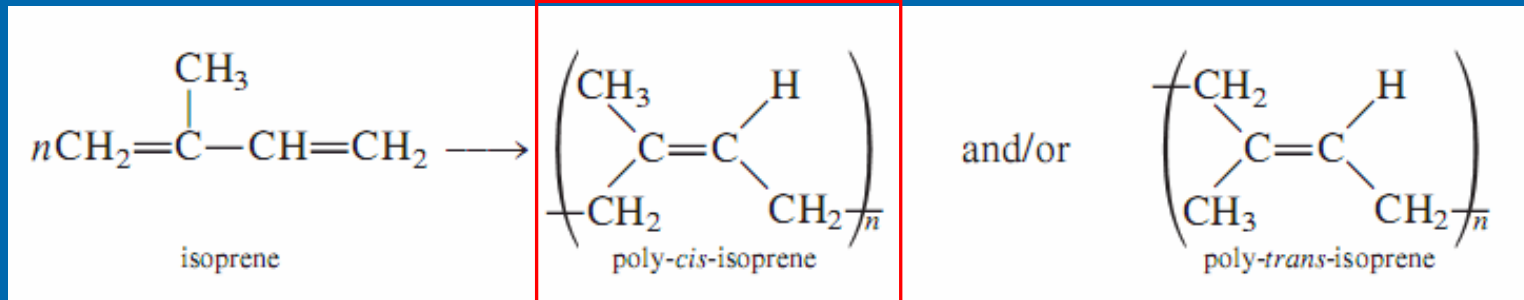
Figure. Latex (aqueous suspension of rubber particles) being collected from a rubber tree.

The elastic property of rubber is due to the flexibility of its long-chain molecules.

In the bulk state, however, *rubber is a tangle of polymeric chains*, and if the external force is strong enough, *individual chains slip past one another*, thereby causing *the rubber to lose most of its elasticity*.

In 1839, Charles Goodyear discovered that **natural rubber could be cross-linked with sulfur** (using zinc oxide as the catalyst) to prevent chain slippage (Figure). **Process, known as vulcanization**, paved the way for many practical and commercial uses of rubber, such as in automobile tires and dentures.

(a) (b) (c)
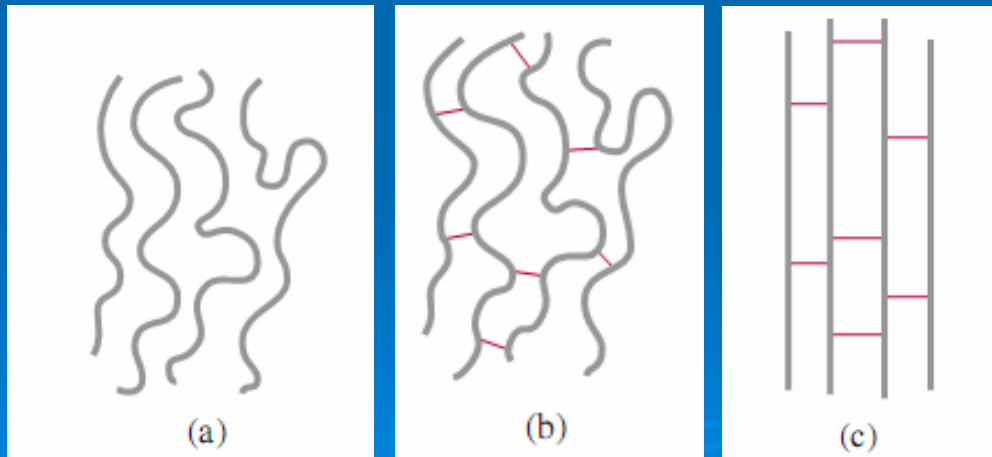
Figure. Rubber molecules ordinarily are bent and convoluted.

Parts (a) and (b) represent *the long chains before and after vulcanization*, respectively;

(c) shows the alignment of molecules when stretched.

Without vulcanization these molecules would slip past one another, and rubber's elastic properties would be gone.

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Proteins

Proteins are polymers of amino acids.

Enzymes, the catalysts of biochemical reactions, ***are mostly proteins***. Proteins also facilitate a wide range of other functions, such as transport and storage of vital substances, coordinated motion, mechanical support, and protection against diseases.

__The human body contains an estimated 100,000 different kinds of proteins__, each of which has a specific physiological function. The chemical composition and structure of these complex natural polymers are the basis of their specificity.

Proteins have high molar masses, ranging from about 5000 g to $1 \cdot 10^7$ g, and yet the percent composition by mass of the elements in proteins is remarkably constant: carbon, 50 - 55 %; hydrogen, 7 %; oxygen, 23 %; nitrogen, 16 %; and sulfur, 1 %.

Amino Acids

The basic structural units of proteins are amino acids.

An amino acid is a compound that contains at least one amino group $(-NH_2)$ and at least one carboxyl group $(-COOH)$.

amino group   carboxyl group
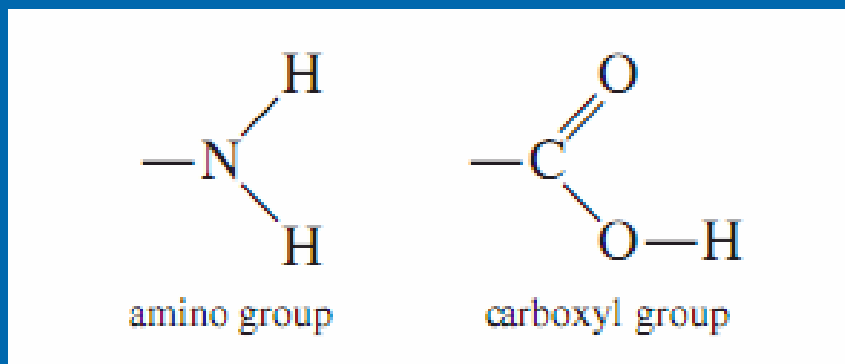
Twenty different amino acids are the building blocks of all the proteins in the human body.

Amino acids in solution at neutral pH exist as dipolar ions, meaning that the proton on the carboxyl group has migrated to the amino group.

Glycine, the simplest amino acid.

un-ionized form   dipolar ion

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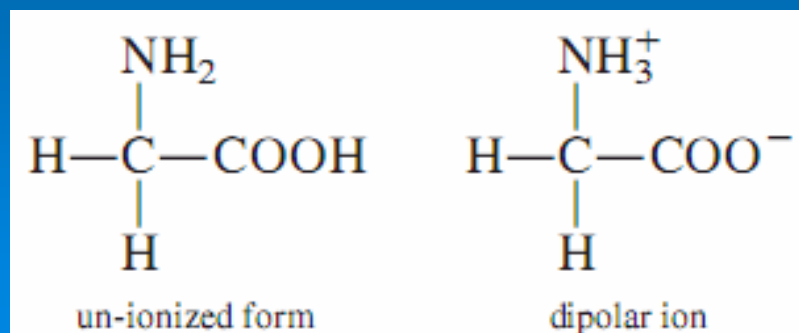
__The first step in the synthesis of a protein molecule is a condensation reaction__ between an amino group on one amino acid and a carboxyl group on another aminoacid.

__The molecule formed from the two amino acids is called a dipeptide,__ and *__the bond joining them together is a peptide bond__*:

$$^+H_3N-\underset{\underset{R_1}{|}}{\overset{\overset{H}{|}}{C}}-\underset{}{\overset{\overset{O}{\|}}{C}}-O^- \; + \; ^+H_3N-\underset{\underset{R_2}{|}}{\overset{\overset{H}{|}}{C}}-\underset{}{\overset{\overset{O}{\|}}{C}}-O^- \; \rightleftharpoons \; ^+H_3N-\underset{\underset{R_1}{|}}{\overset{\overset{H}{|}}{C}}-\underset{}{\overset{\overset{O}{\|}}{C}}-\underset{\underset{H}{|}}{N}-\underset{\underset{R_2}{|}}{\overset{\overset{H}{|}}{C}}-\underset{}{\overset{\overset{O}{\|}}{C}}-O^- \; + \; H_2O$$

peptide bond
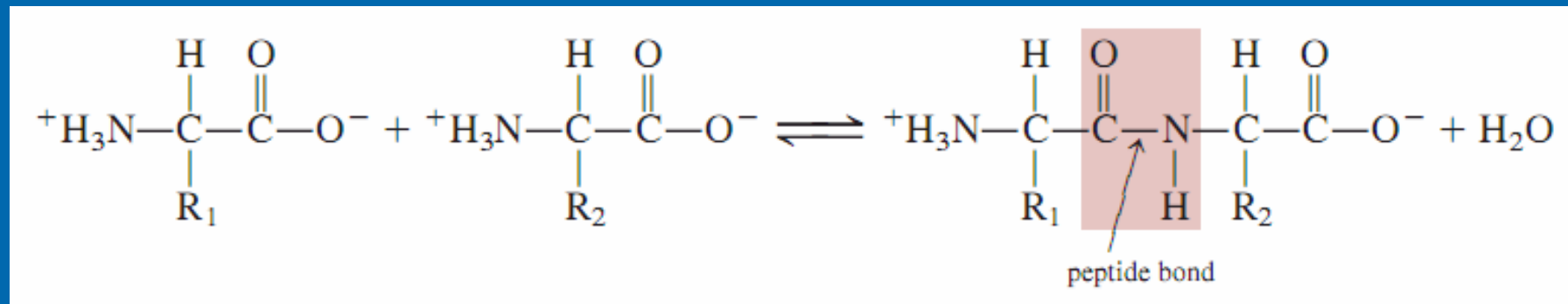
Protein Structure

The type and number of amino acids in a given protein along with the sequence or order in which these amino acids are joined together determine *__the protein's structure__*.

There are two common structures for protein molecules, called the α-helix and the β-pleated sheet. The α-helical structure of a polypeptide chain is shown in Figure.
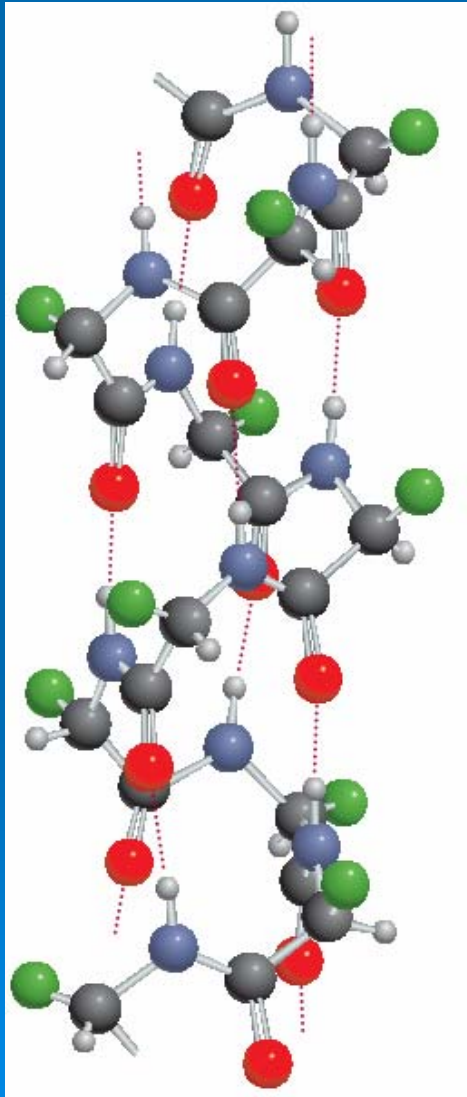
Figure. The α-helical structure of a polypeptide chain.

The gray spheres are hydrogen atoms.

The structure is held in position by intramolecular hydrogen bonds, shown as dotted lines.

The β-pleated structure is markedly different from the α-helix in that it is like a sheet rather than a rod.

The polypeptide chain is almost fully extended, and each chain forms many intermolecular hydrogen bonds with adjacent chains. Figure shows ***the two different types of β-pleated structures***, called ***parallel and antiparallel.***
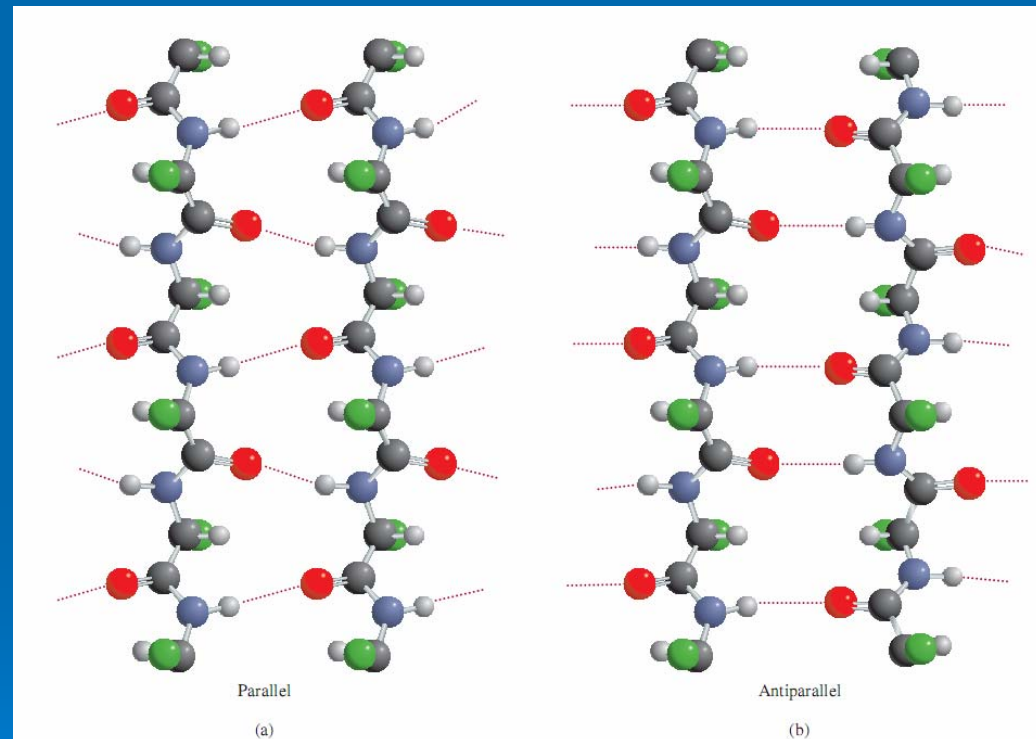
Figure. Hydrogen bonds (a) in a parallel β-pleated sheet structure, in which all the polypeptide chains are oriented in the same direction, and (b) in an antiparallel β-pleated sheet, in which adjacent polypeptide chains run in opposite directions.

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Protein structure is divided into four levels of organization.

The primary structure refers to the unique amino acid sequence of the polypeptide chain.

The secondary structure includes those parts of the polypeptide chain that are stabilized by a regular pattern of hydrogen bonds between the CO and NH groups of the backbone, for example, the α-helix.

The term tertiary structure applies to the three-dimensional structure stabilized by dispersion forces, hydrogen bonding, and other intermolecular forces. It differs from secondary structure in that the amino acids taking part in these interactions may be far apart in the polypeptide chain. A protein molecule may be made up of more than one polypeptide chain. Thus, in addition to the various interactions within a chain that give rise to the secondary and tertiary structures, we must also consider the interaction between chains.

The overall arrangement of the polypeptide chains is called *the quaternary structure*. For example, the hemoglobin molecule consists of four separate polypeptide chains, or subunits. These subunits are held together by van der Waals forces and ionic forces (Figure).
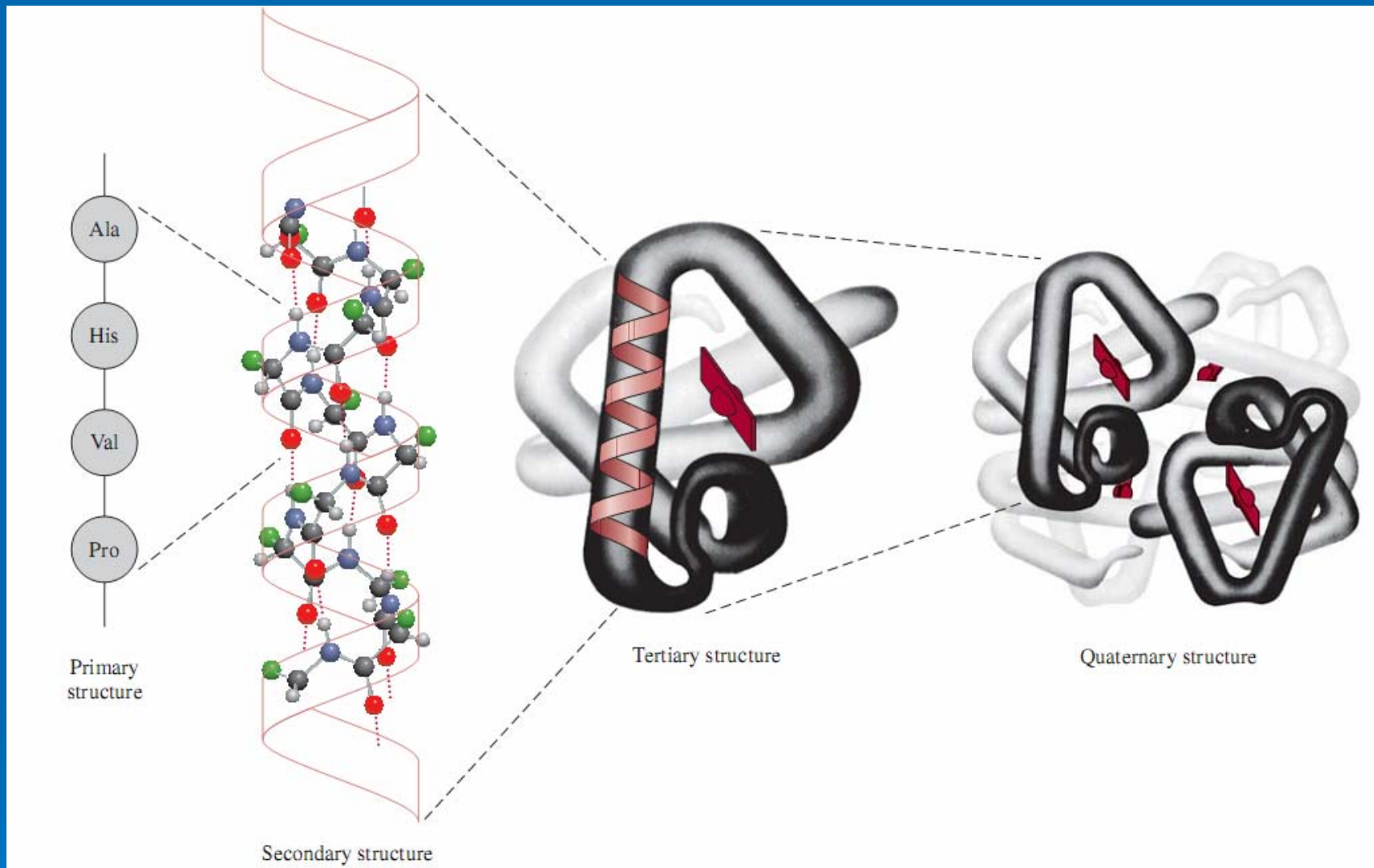
Figure. The primary, secondary, tertiary, and quaternary structure of the hemoglobin molecule.

Nucleic Acids

Nucleic acids are high molar mass polymers that play an essential role in protein synthesis.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are the two types of nucleic acid.

DNA molecules are among the largest molecules known; they have molar masses of up to tens of billions of grams. On the other hand, RNA molecules vary greatly in size, some having a molar mass of about 25,000 g.

Compared with proteins, which are made of up to 20 different amino acids, nucleic acids are fairly simple in composition. ***A DNA or RNA molecule contains only four types of building blocks***: purines, pyrimidines, furanose sugars, and phosphate groups (Figure). Each purine or pyrimidine is called a base.
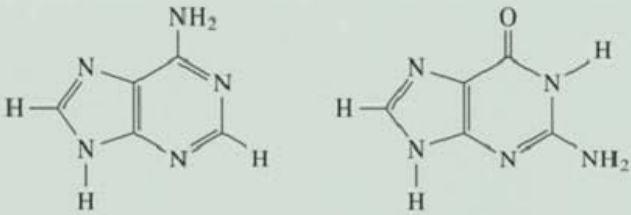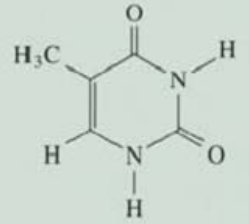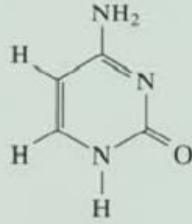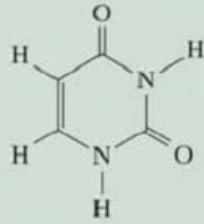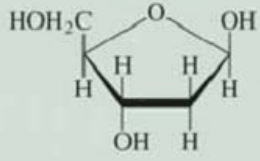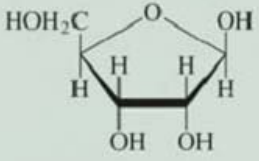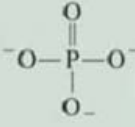
Figure. The components of the nucleic acids DNA and RNA.

The DNA molecule has two helical strands. *Each strand is made up of nucleotides, which consist of a base, a deoxyribose, and a phosphate group linked together* (Figure).
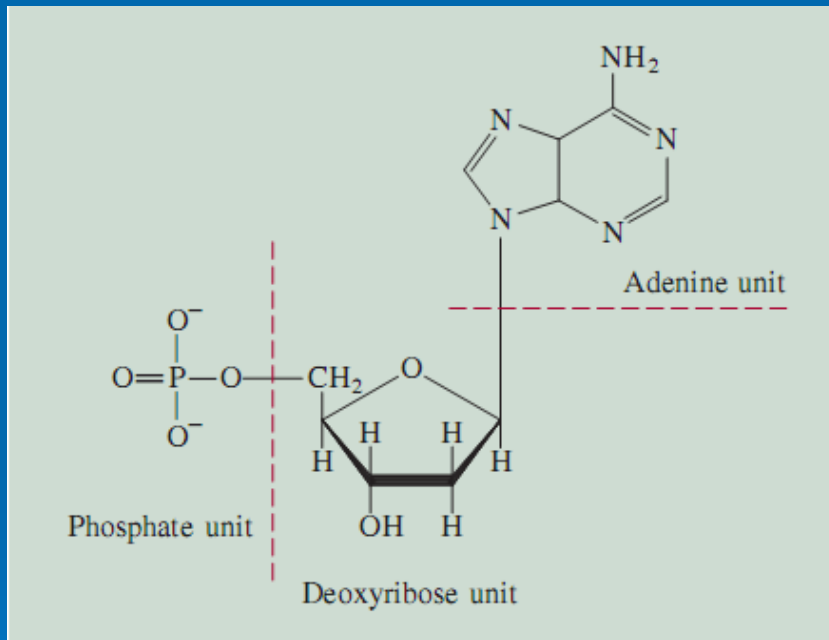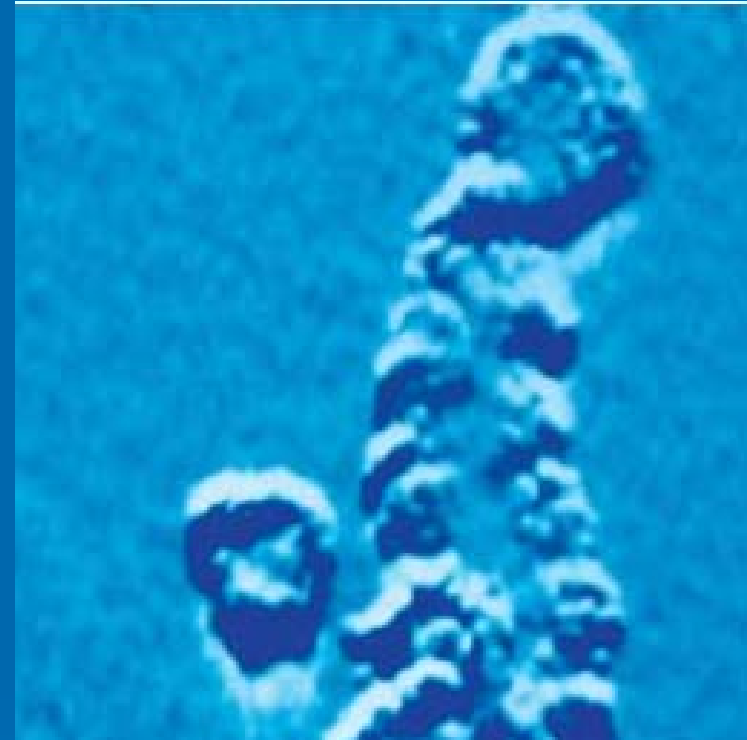

Figure. Structure of a nucleotide, one of the repeating units in DNA.

An electron micrograph of a DNA molecule. The double-helical structure is evident. If the DNA molecules from all the cells in a human were stretched and joined end to end, the length would be about 100 times the distance from Earth to the sun!

The key to the double-helical structure of DNA is ***the formation of hydrogen bonds between bases in the two strands of a molecule.***

Although hydrogen bonds can form between any two bases, called base pairs, ***the most favorable couplings are between adenine and thymine and between cytosine and guanine*** (Figure).

Note that this scheme is consistent with Chargaff's rules, because every purine base is hydrogen-bonded to a pyrimidine base, and vice versa (A+G = C+T). Other attractive forces such as dipole-dipole interactions and van der Waals forces between the base pairs also help to stabilize the double helix.
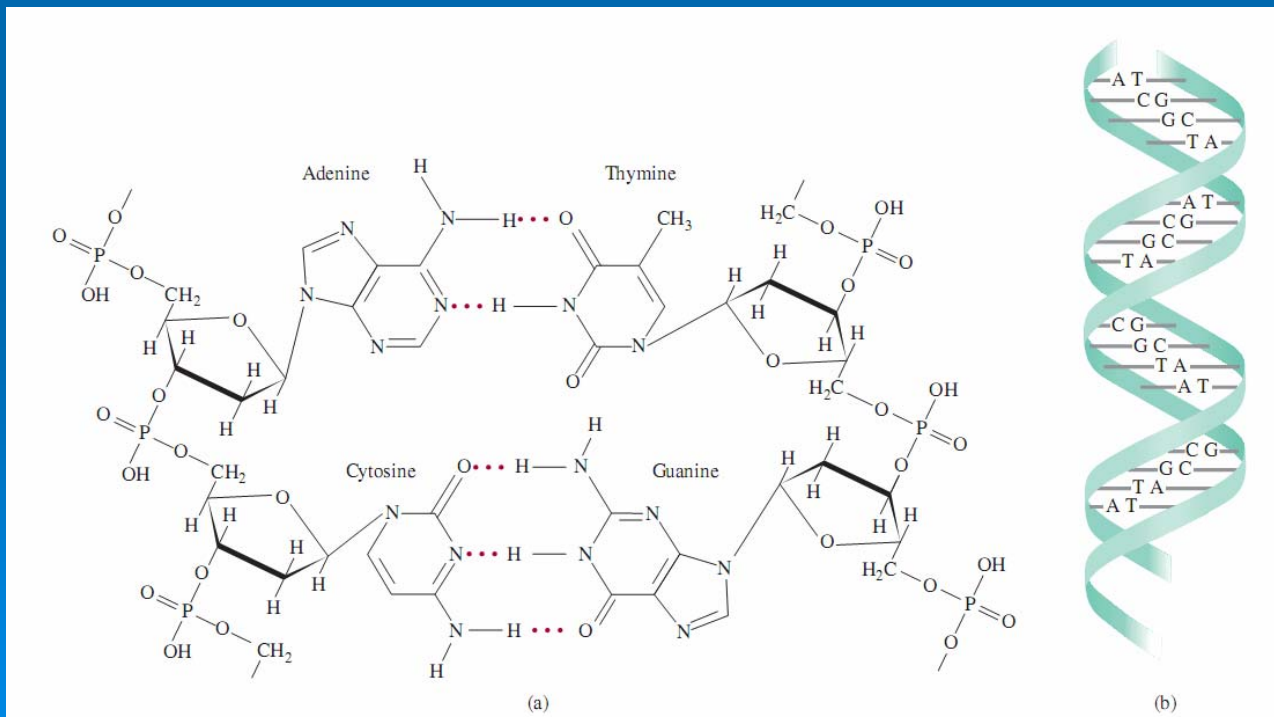

Figure. (a) Base-pair formation by adenine and thymine and by cytosine and guanine. (b) The double-helical strand of a DNA molecule held together by hydrogen bonds (and other intermolecular forces) between base pairs A-T and C-G.

***The structure of RNA differs from that of DNA in several respects.
First,*** as shown in Figure above (Figure. The components of the nucleic acids DNA and RNA), the four bases found in RNA molecules are adenine, cytosine, guanine, and uracil.

Second, RNA contains the sugar ribose rather than the 2-deoxyribose of DNA.

Third, chemical analysis shows that the composition of RNA does not obey Chargaff's rules. In other words, the purine-to-pyrimidine ratio is not equal to 1 as in the case of DNA. This and other evidence rule out a double-helical structure.

In fact, the RNA molecule exists as a single-strand polynucleotide. There are actually three types of RNA molecules- messenger RNA (*m*RNA), ribosomal RNA (*r*RNA), and transfer RNA (*t*RNA). ***These RNAs have similar nucleotides but differ from one another in molar mass, overall structure, and biological functions.***

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