Subject 13. Adsorption processes and ionic exchange in biosystems. Chromatography

1. Importance

Adsorption from solutions on solid surface is of great importance for the life of the organism. Many processes in the organism employ molecular adsorption from solutions. This is adsorption of substrates on the surface of enzymes, adsorption on cell membranes (15000 м^2 in the organism), adsorption of proteins on the surface of hydrophobic particles for their transport in blood and transport of oxygen by hemoglobin of erythrocytes.

Such solid adsorbents as activated carbon and ion-exchangers are used to remove foreign substances (xenobiotics, poisons, harmful metabolites, excessive medicines) from the organism. The surface area of the phase interface of a pill of activated carbon (0.25 g) is about 125 m^2. Enterosorbents are widely used in medical practice.

There are a variety of methods of adsorption therapy, that include hemosorption (removal of toxins from blood), plasmasorption, lymphosorption, liquorosorption (purification of the spine liquor), applicative sorption, enterosorption.

Adsorption on solid adsorbents is widely used to purify vitamins and antibiotics.

Immobilized preparations of enzymes, hormones, antibiotics on inorganic and organic polymers are used in medical practice.

Chromatography is a group of methods for separation and analysis of mixtures of gases, vapours, liquids or solutes with sorption processes. It is used for diagnostics, monitoring of treatment, control of detoxification of the organism at poisonings. It helps to make decisions about prophylaxis and treatment of diseases.

Chromatography is used in toxicological chemistry, forensic medicine, criminalistics and hygiene.

Competences

Be able to analyze information, make informed decisions, establish appropriate relationships to achieve objectives.

Be able to apply knowledge in practical situations.

Know methods of application of knowledge in solving practical problems.

Know standard methods of physical and chemical (laboratory) research, be able to analyze and evaluate obtained results.

Use English language for professional and business communications and preparation of documents.

2. Concrete aims

Draw conclusions about surface activity of substances based on their structure.

Analyze structural features of the surface layer of adsorbed molecules of surface-active compounds, explain principles of structure of biological membranes.

Analyze adsorption equations and their applications, distinguish between monomolecular and multimolecular adsorption.

Explain concepts of adsorption of substances from solutions on solid surfaces.

Explain physical and chemical principles of adsorption methods of therapy.

Distinguish between selective adsorption and ion exchange adsorption of electrolytes.

Explain methods of chromatographic analysis and their role in biomedical research.

Explain adsorption methods of therapy.

3. Basic knowledge, skills necessary for studying the subject (interdisciplinary integration)

Previous subjects	Obtained skills
1. Chemistry (school course)	Understand polarity of molecules.
2. Mathematics (school course)	Be able to draw plots.
3. Medical Biology	Have an understanding of cell membranes, their structure and function.
4. Medical and Biological Physics	Possess knowledge about structure and function of biological membranes, permeability of biological membranes. Have an understanding of surface phenomena.
5. Foreign language for professional purposes	Have fluent knowledge of English. Use English language for professional and business communication and preparation of documents.

4. Tasks for independent work during preparation for the class and in class.

4.1. The list of key terms, parameters, characteristics which the student is to learn while preparing for classes:

Term	Definition
1. Surface phenomena	**Surface phenomena** are processes that take place on the interface of heterogeneous systems.
2. Surface tension	**Surface tension** is the Gibbs energy per one unit of area.
3. Surface-active compounds (surfactants)	**Surface-active compounds** are substances that reduce surface tension of water.
4. Surface-inactive compounds	**Surface-inactive compounds** are substances that increase surface tension of water.
5. Duclos-Traube rule	**Duclos-Traube rule**: in a homologous series of carboxylic acids, alcohols, amines with increasing length of the hydrocarbon chain in one —CH_2— group surface activity of the substance increases 3 - 3.5 times.
6. Adsorption	**Adsorption** is a spontaneous process of concentrating a dissolved substance on the

	phase interface.
7. Gibbs equation	**Gibbs equation**: $\Gamma = -\frac{C}{RT} \cdot \frac{\Delta\sigma}{\Delta C}$
8. Langmuir equation	**Langmuir equation**: $\Gamma = \Gamma_\infty \cdot \frac{C}{C+K}$
9. Freundlich equation	**Freundlich equation:** $\frac{x}{m} = k \cdot c^{\frac{1}{n}}$
10. Adsorption therapy a. hemosorption	**Hemosorption** is a direct method of removal toxins from blood by passing it through a column of an adsorbent.
b. plasmasorption	**Plasmasorption** is a process of passing blood plasma through a column of a sorbent; then the purified plasma is mixed with blood cells and returned to the bloodstream.
c. lymphosorption	**Lymphosorption** is a process of passing lymph through a column of a sorbent; then the lymph is returned to the vasculatory system of the patient.
d. enterosorption	**Enterosorption**: a sorbent enters the mouth, and adsorbs toxins and products of metabolism while passing through the digestive tract.
e. application therapy	**Application therapy** promotes healing of infected wounds and burns, restores integrity of the skin and mucous membranes by adsorption of toxins from the wound or burn area.
11. Paneth-Fajans rule	**Paneth-Fajans rule**: ions identical or isomorphic to those that build the lattice of the crystal adsorbent are preferentially adsorbed on the crystal surface from the solution.
12. Chromatography	**Chromatography** is a group of physical and chemical methods of analysis and separation of mixtures of substances based on their different distributions between two phases. Classification of methods of chromatography:
13. Classification of chromatography of analysis:	1) by aggregate state of phases: - gas chromatography - liquid chromatography 2) by mechanism of distribution: - affinity chromatography - partition chromatography - ion exchange chromatography - sedimentation chromatography - gel filtration

	3) by technique: - paper chromatography - thin layer chromatography - column chromatography - capillary chromatography

4.2. Theoretical questions to the lesson:

1. Surface phenomena and their importance in biology and medicine. Surface tension of liquids and solutions. Isotherm of surface tension. Surface-active and surface-inactive substances. Surface activity. Duclos-Traube rule.

2. Adsorption at the liquid-gas and liquid-liquid interface. Gibbs equation. Orientation of molecules of surface-active compounds in the surface layer. The concept of structure of biological membranes.

3. Adsorption at the solid-gas interface. Langmuir equation.

4. Adsorption from solutions on a solid surface. Physical and chemical adsorption. Freundlich equation.

5. Physico-chemical basis of adsorption therapy (hemosorption, plasmasorption, lymphosorption, enterosorption, application therapy). Immunosorbents.

6. Adsorption of electrolytes: specific (selective) and ion exchange. Paneth-Fajans rule.

7. Natural and synthetic ion exchangers. Role of adsorption and ion exchange processes in plants and animals.

8. Chromatography. Classification of chromatographic methods of analysis:

1) by aggregate state of phases;

2) by technique;

3) by distribution mechanism.

Application of chromatography in biology and medicine.

4.3. Practical work (task) done by students in class:

1. Measurement of static exchange capacity of sorbents (with an example of organic resins)

Place 1 ± 0.01 g of each of four samples for parallel measurements of previously prepared H-formula cation exchanger with a known humidity level in a 100 mL conical flask. Add 100 mL 0.05M solution of $CaCl_2$ to two of the flasks, and 100 mL 0.1 M solution of NaOH to the two other flasks, then close the flasks and allow to stand for at least 4 hours stirring periodically 2 times per hour. Take 25 mL of each solution with a pipette and titrate in the presence of methyl orange:

a) the solution containing calcium chloride with 0.1 M solution of NaOH;

b) the solution containing sodium hydroxide with 0.1 M solution of HCl.

The calculation is carried out with the formula:

$$CO\text{Є}_{NaOH} = \frac{100M_{NaOH} - 4\,V_{HCl}M_{HCl}}{m}$$

$$CO\text{Є}_{CaCl_2} = \frac{4M_{NaOH} - 4\,V_{NaOH}}{m}$$

where M_{HCl} is the molarity of HCl;

M_{NaOH} is the molarity of NaOH;
V_{HCl} is the volume of the solution of HCl that was used for the titration, mL;
V_{NaOH} is the volume of the solution of NaOH that was used for the titration, mL;
m is the mass of the dry cation exchanger, g.

To determine cation exchange capacity of weakly acidic cation exchangers in the H-form take a sample corresponding to about 0.1 g of a dry resin. Add 100 mL of 0.1 M CH_3COONa solution to the sample. After 24 hours of infusion with periodic stirring, titrate 25 mL of of the obtained solution with 0.1 M NaOH solution in the presence of phenolphthalein as an indicator.

The calculation is carried out with the formula:

$$\text{CO€} = \frac{8V_{\text{NaOH}}\text{K} \cdot M \cdot 100}{m(100 - \text{W})}$$

where V_{NaOH} is the volume of 0.1 M NaOH solution for titration in parallel samples, mL;
m is the mass of the sample of the air-dried resin, g;
M is the theoretical molarity of the NaOH solution;
W is the humidity of the resin, %;
K is the correction factor for 0.1 M NaOH solution.

2. Separation of mixtures with chromatography methods.

A. Adsorption chromatography of cations on aluminium oxide

Separation of cations $\mathbf{Fe^{3+}}$ and $\mathbf{Cu^{2+}}$ is done with adsorption column chromatography. Prepare the chromatography column as following: take a dry glass pipe (length - 12-15 cm, diameter - 1 cm) with a narrow end. Place some cotton wool into the end. Fill the pipe with 4-5 cm of aluminium oxide powder, tapping the pipe gently to avoid gaps. Fix the prepared column in a stand. Take 3 mL ferric chloride solution and 3 mL cupric sulfate solution of the same concentration; mix them in a test tube. Pour carefully the obtained mixture into the column. Place an empty flask under the column.

After some time the upper layer of the adsorbent becomes yellow (Fe^{3+} cations). A blue layer is formed under it (Cu^{2+}cations).

After all the solution has gone through the column, wash the adsorbent with a small amount of water and then pour a developer into the column for better spectacularity. The developer is a diluted solution of potassium ferrocyanide $\mathbf{K_4[Fe(CN)_6]}$. The top layer becomes dark blue, the bottom one becomes brown.

Make a conclusion about dependence of adsorbtion of cations on aluminium oxide on the charge of the cation. Draw the column with the coloured layers of the cations in the notebook.

B. Distributive chromatography of amino acids on paper.

Take a paper filter (diameter 12 cm), draw four sectors on it with a pencil. Mark the start points in three sectors at the distance 0.5 cm from the centre with a pencil . Cut out a narrow strip reaching almost to the centre in the fourth sector (see Picture 1). Mark the sectors on the edge: “Gly” – glycine, “Leu” – leucine, “Mix” – mixture.

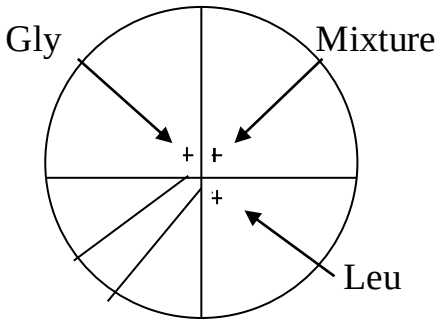


Figure 1.

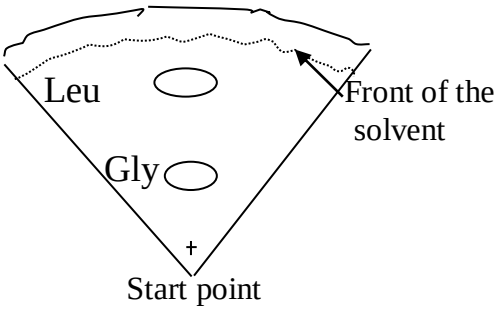


Figure 2. A chromatogram of mixture of amino acids

Place small drops (not bigger than 3-4 mm in diameter) of the corresponding solutions (glycine, leucine and their mixture) on the start points.
Pour the solvent in a Petry dish, place the filter so, that the strip were immersed in the solvent. Cover the cup with another Petry dish and place in the thermostate at temperature 45-50^{0}C. When the solvent almost reaches the edge of the paper, take the paper out, dry it up and develop it with ninhydrin solution. Dry it up again until violet stains appear.
With the chromatogram calculate the retention factors of leucine and glycine. For this measure the distance from the start point to the middle of the stain of each of the amino acids and from the start point to the front of the solvent (Picture 2).
Calculate the retention factors (R_f) of the amino acids with the formulas:

$$R_{f(glycine)} = r_1/r_s \qquad R_{f(leucine)} = r_2/r_s,$$

where r_1 is the distance from the start point to the middle of the glycine stain, cm;
r_2 is the distance from the start point to the middle of the leucine stain, cm;
r_s is the distance from the start point to the front of the solvent, cm.

4. Writing a report of the laboratory work:

Draw the chromatogram, write the results of calculations. Make a conclusion about distribution of amino acids depending on the polarity of their molecules.

Contents of the subject (abstract):

1. Surface phenomena and their importance in biology and medicine. Surface tension of liquids and solutions. Isotherm of surface tension. Surface-active and surface-inactive substances. Surface activity. Duclos-Traube rule.

Surface phenomena are processes that take place on the interface of heterogeneous systems.

Surface tension is the Gibbs energy per one unit of area.

$$\sigma = \frac{G_s}{S}$$

Isotherm of surface tension is a graph of surface tension to concentration of solute at a constant temperature.

Surface-active compounds are substances that reduce surface tension of water**.** These include carboxylic acids, alcohols, amines.

Surface-inactive compounds are substances that increase surface tension of water. These include strong electrolytes (inorganic acids, alkalis, salts).

Duclos-Traube rule: in a homologous series of carboxylic acids, alcohols, amines with increasing length of the hydrocarbon chain in one —CH_2— group surface activity of the substance increases 3 - 3.5 times.

2. Adsorption at the liquid-gas and liquid-liquid interface. Gibbs equation. Orientation of molecules of surface-active compounds in the surface layer. The concept of structure of biological membranes.

Adsorption is a spontaneous process of concentration of a dissolved substance on the phase interface.

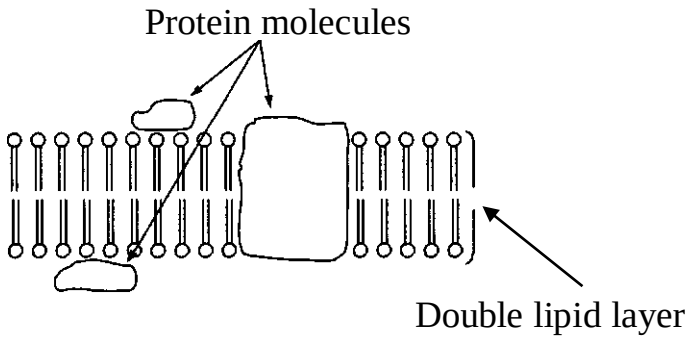
Adsorbent is a substance on which surface adsorption takes place.

Adsorbate is a substance that is adsorbed on the surface of the adsorbent.

Gibbs equation: $\Gamma = -\frac{C}{RT} \cdot \frac{\Delta\sigma}{\Delta C}$

Orientation of molecules of surface-active compounds in the surface layer: at low concentrations of surface-active compounds polar groups are immersed in a polar liquid while hydrocarbon chains almost lie on the surface; with increasing concentration of surface-active compounds the hydrocarbon chains rise and as a result, at a certain concentration form a monolayer on the surface ("Langmuir fence").

The cell membrane is a double layer of lipids (glycolipids, cholesterol and phospholipids). The double lipid layer contains protein molecules.



3. Adsorption at the solid-gas interface. Langmuir equation.

Langmuir suggested that:

- adsorption occurs only in certain sites on the surface;
- each site can adsorb only one molecule of the substance;
- energy of bonds formed between the surface and the molecules of the substance is the same for all sites.

Langmuir equation:

$$\Gamma = \Gamma_\infty \cdot \frac{C}{C+K}$$

4. Adsorption from solution on a solid surface. Physical and chemical adsorption. Freundlich equation.

Physical adsorption proceeds due to Van der Waals forces of intermolecular interaction.

Chemical adsorption (chemisorption) is caused by a chemical reaction between adsorbent and adsorbate that takes place at the phase interface.

Freundlich equation:

$$\frac{x}{m} = k \cdot c^{\frac{1}{n}}$$

5. Physico-chemical basis of adsorption therapy (hemosorption, plasmasorption, lymphosorption, enterosorption, application therapy). Immunosorbents.

Hemosorption is a method of direct removal of toxins from blood by passing it through a column of an adsorbent connected to the blood circulation system.

Plasmasorption is a process of passing blood plasma through a column of a sorbent; then the purified plasma is mixed with blood cells and returned to the bloodstream.

Lymphosorption is a process of passing lymph through a column of a sorbent; then the lymph is returned to the vasculatory system of the patient.

Enterosorption: a sorbent enters the mouth, and adsorbs toxins and products of metabolism while passing through the digestive tract.

Application therapy promotes healing of infected wounds and burns, restores integrity of the skin and mucous membranes by adsorption of toxins from the wound or burn area.

6. Adsorption of electrolytes: specific (selective) and ion exchange. Paneth-Fajans rule.

Paneth-Fajans rule: ions identical or isomorphic to those that build the lattice of the crystal adsorbent are preferentially adsorbed on the crystal surface from the solution.

Ion-exchange adsorption is a process in which the adsorbent and the solution exchange equivalent amounts ofcharged ions of the same name.

7. Natural and synthetic ion exchangers. Role of adsorption and ion exchange processes in plants and animals.

Ion exchangers are materials capable of exchanging ions with a solution.

Natural ion exchangers are soil, clay and various minerals.

Synthetic are ion exchange resins.

Various tissues of plant and animal organisms have cation exchange properties in the physiological pH range.

Ion exchange properties are characteristic for structural elements of cells, such as nuclei, mitochondria, membranes, microsomes, sarcolemma. Ion exchange is a part of functioning of enzymes and biological membranes.

8. Chromatography. Classification of chromatographic methods of analysis. Application of chromatography in biology and medicine.

Chromatography is a group of physical and chemical methods of analysis and separation of mixtures of substances based on their different distributions between two phases. One of them is stationary (solid or liquid), and another - mobile (gas or liquid).

Chromatographic methods of analysis are classified as follows:

1) by the aggregate state of phases: gas and liquid chromatography.

2) by distribution mechanism: adsorption, distribution, ion-exchange, sedimentation chromatography and gel filtration

3) by technique: paper, thin layer, column chromatography and capillary chromatography.

Adsorption chromatography separates mixtures of substances based on their varying ability to adsorb on a particular adsorbent (stationary phase).

Ion exchange chromatography is based on different abilities of ions in an analyzed mixture to exchange for ions in the ion exchanger (stationary phase).

Distribution chromatography is based on different distribution of substances between stationary phase (liquid) and mobile (gas or liquid) according to their distribution coefficients.

Chromatography is used for diagnosis, clinical monitoring of the course of treatment, control of detoxification process at poisoning. Chromatography is used in toxicological chemistry, forensic medicine, forensic science and sanitary applications.

Materials for self control:

A. Tasks for self control:

1. Which factors effect adsorption of an ion on a solid adsorbent from a water solution?

1 – ion charge;
2 – hydration degree of the ion;
3 – identity of the ion;
4 – nature of the adsorbent;
5 – mass of the adsorbent;
6 – total pressure.

2. Choose the processes on which chromatography methods are based:

1 – ion exchange;
2 – dissolving in water;
3 – adsorption;
4 – evaporation;
5 – crystallization;
6 – distribution between two phases.

3. Which of the methods of chromatography are based on distribution of components of a mixture between two liquid phases:

1 – gas adsorption chromatography; 4 – adsorption column chromatography;
2 – paper chromatography; 5 – thin layer chromatography;
3 – gel chromatography; 6 – ion exchange chromatography.

B. Practical tasks for self control:

1. From which solvent does activated carbon (non-polar adsorbent) adsorb a surface active compound better? The dielectric permeability of the solvents is given in brackets.

a) water (80);
b) ethanol (25.2);
c) acetone (20.7);
d) hexane (1.9).

2. Which ions are able to adsorb on the surface of crystalline silver iodide according to the Paneth-Fajans rule?

a) Ag^+; b) I^-; c) NO_3^-; d) Na^+.

Literature

Main:

1. Medical Chemistry: textbook / V.O. Kalibabchuk, V.I. Halynska, V.I. Hryshchenko et al.; edited by Prof. V.O. Kalibabchuk – Kyiv: “Medicine”, 2010 – 224 p. (P. 108 – 134).

Informational resources:

2. www. pdmu, edu.ua
https://med-chemistry.pdmu.edu.ua/

(Web page of Poltava State Medical University).