МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ’Я УКРАЇНИ
ХАРКІВСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ

SORPTION OF BIOLOGICALLY-ACTIVE SUBSTANCES AT A PHASE INTERFACE. ION EXCHANGE. CHROMATOGRAPHY

Methodical instructions for 1st year students’ self-work
in Medical Chemistry

СОРБЦІЯ БІОЛОГІЧНО-АКТИВНИХ РЕЧОВИН НА МЕЖІ РОЗПОДІЛУ ФАЗ. ІОННИЙ ОБМІН. ХРОМАТОГРАФІЯ

Методичні вказівки для самостійної роботи студентів 1-го курсу
з медичної хімії

Затверджено
Вченою радою ХНМУ.
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Sorption of biologically-active substances at a phase interface. Ion exchange. Chromatography: methodical instructions for 1st year students' self-work in Medical Chemistry / compiled by A.O. Syrovaya, S.V. Andreeva, O.S. Kalinenko, V.N. Petyunina, V.O. Makarov S.N. Kozub, L.V. Lukianova, T.S. Tishakova, O.L. Levashova, O.O. Zavada, E.V. Savelieva, N.N. Kopoteva, N.N. Chalenko, M.O. Vodolazhenko. – Kharkiv: KhNMU, 2017. – 16 p.

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Сорбція біологічно-активних речовин на межі розподілу фаз. Іонний обмін. Хроматографія: метод. вказ. для самостійної роботи студентів 1-го курсу з мед. хімії /уклад. Г.О. Сирова, С.В. Андрєєва, О.С. Каліненко, В.М. Петюніна, В.О. Макаров, Л.В. Лук’янова, С.М. Козуб, Т.С.Тішакова, О.Л. Левашова, О.О. Завада, О.В. Савельєва, Н.В. Копотєва, Н.М. Чаленко, М.О. Водолаженко. – Харків: ХНМУ, 2017. – 16 с.

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SUBJECT «SORPTION OF BIOLOGICALLY-ACTIVE SUBSTANCES AT A PHASE INTERFACE. ION EXCHANGE. CHROMATOGRAPHY»

1. **Hours** 4

2. **Material and methodological support.**

Multimedia support (presentations, scientific films)

Posters:

1. Adsorption from solutions
2. Chromatographic columns
3. Partition chromatography
4. Sorptographic analysis

Education literature

Education literature:

1. Medical chemistry: textbook / V.O. Kalibabchuk, V.I. Halynska, L.I. Hryshchenko et al.; – Kyiv : AUS Medicine Publishing, 2010. – 224 p.

2. Fundamentals of medical chemistry: manual for students’ self-work / A.O. Syrovaya, E.R. Grabovetskaya, L.G. Shapoval. – Kharkiv: KhNMU, 2015. – 196 p.

3. Medical chemistry. Adapted concise course: manual for students’ self-work manual for students’ self-work / A.O. Syrovaya, E.R. Grabovetskaya, L.G. Shapoval. - Kharkiv: KhNMU, 2013. – 160 p.

4. Medical chemistry: workbook for self-work of first-year students of medical and dentistry faculties / compiled by A. O. Syrovaya, V. N. Petunina, V. A. Makarov et al. – Kharkiv : KhNMU, 2017. – 72 p.

5. Sorption of biologically-active substances at a phase interface. Ion exchange. Chromatography. - Methodical instructions for 1st year students' self-work in Medical Chemistry / compiled by A. O. Syrovaya, S.V. Andreeva, O.S. Kalinenko et al. – Kharkiv : KhNMU, 2017. – 16 p.

6. Individual tasks for students’ self-control of knowledge in Medical Chemistry / A.O. Syrovaya, L.G. Shapoval, et al. – Kharkiv: KhNMU, 2014. –50 p.

7. Text of lecture.

3. Substantiation for the subject. Living systems are characterized by the presence of a developed surface, which separates one parts of the system from another. Therefore, the phenomena taking place on the interface play an important role in the processes of vital activity. The properties of many biologically active substances are determined by their behavior on the phase interphase. The location of phospholipid molecules in the cellular membranes, capability of salts of fatty acids to emulsify fats, these and other phenomena are explained by superficially active properties of the substances participating in them. The capability of some compounds to accumulate the substances from the environment on their surface is used for blood purification from toxic substances, elimination of poisons and toxins from the gastrointestinal tract, treatment of burns and other superficial pathologies.

4. The purposes of the subject:

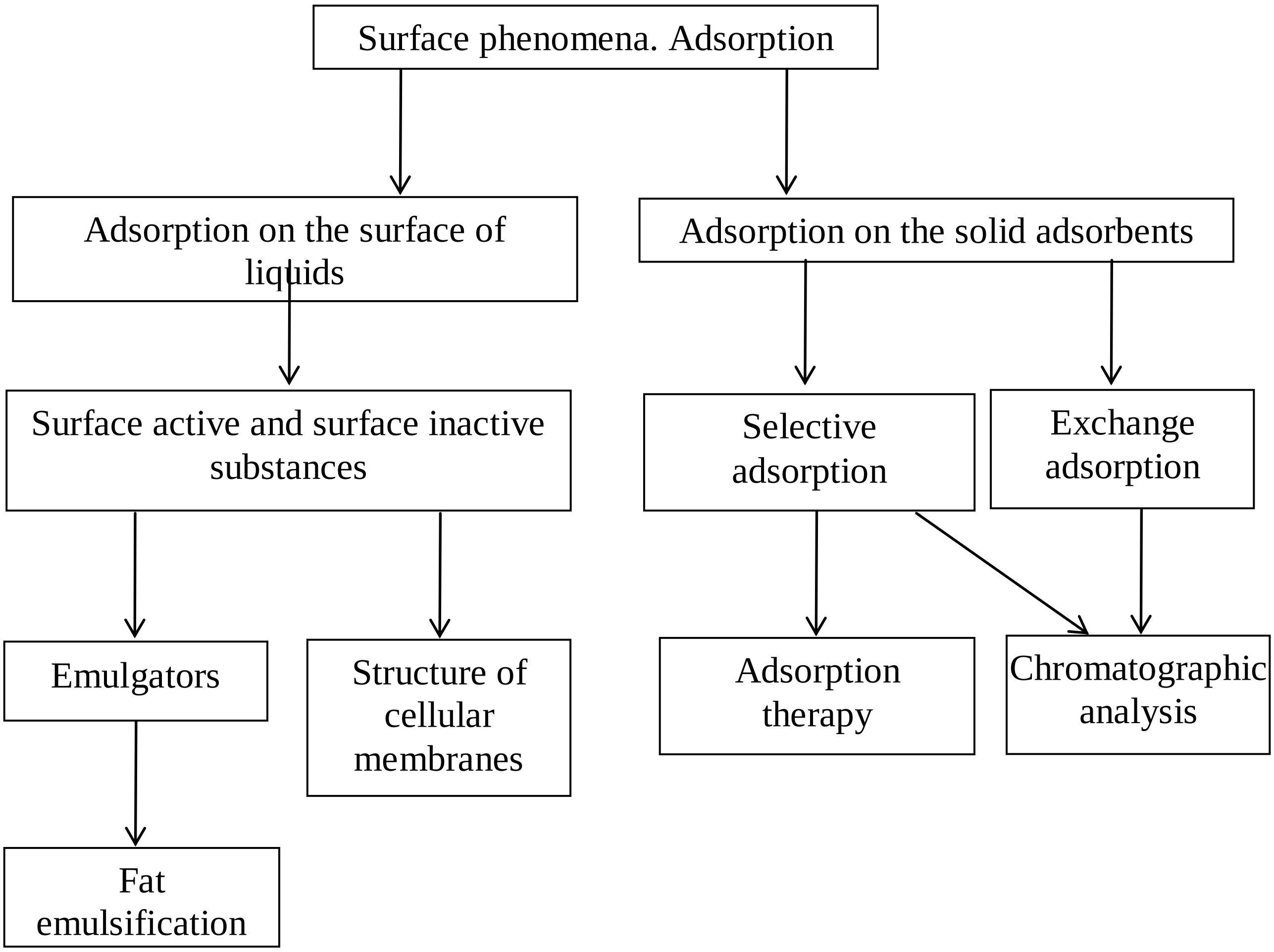
- general: to learn expound pysico-chemical phenomena of adsorption in relation to its biological function and to know characteristic properties of chromatography as the method used for medical-biological researchers.

- specific: to come to conclusions about substances' surface activity on the basis of their structure, analyze adsorption equations and limits of their use, distinguish monomolecular and polymolecular adsorption, expound regularities of adsorption from the solutions on solid surface, explain physics and chemistry of the methods of application therapy, distinguish selective and ion-exchange adsorption of the electrolytes, expand the methods of chromatographic analysis and their role in medical-biological researchers.

a) **to know:** main concepts – adsorption, adsorption on the surface of liquid – gas and liquid – liquid, surface-active and surface-inactive substances, Gibbs equation, molecular orientation of SAS on the phase interphase, concepts of the cellular membranes' structure, emulsification of fats, adsorption on the surface of solids, Langmuir's theory, adsorption selectivity, adsorption therapy, exchange adsorption, ion-exchangers, main methods of methods of chromatographic analysis, chemical sorption;

b) **to be able:** estimate surface properties of the substances based on the structure of their molecules, explain surface active substances' behavior based on surface activity, expand the usage of adsorbents for analytical and medical purposes.

5. Graph structure of the subject.



6. Plan of students' work.

No	Stage	Time, min	Educational and visual aids	Location
1.	Motivational characteristics and plan of the subject. Answers to the students' questions	20	Text-book (work-book)	Class room
2.	Incoming control	20		
3.	Students' self-work with methodical literature, solution of learning tasks	50	Methodical instructions for students, lecture note, text book for students' self-work, reference data, posters	
4.	Laboratory work	45	Reagents, equipment	
3.	Final control	30		
4.	Analysis and conclusions	10		
5.	Home work	5		

7. Tasks for self-work:

- list of questions to be studied

1. Adsorption: concepts and definitions.
2. Adsorption on the surface liquid – gas and liquid – liquid. Surface–active and surface–inactive substances. Gibbs equations.
3. Orientation of the molecules on the phase interphase. Concept of the cellular membranes' structure. Fat emulsification.
4. Adsorption on the surface of solids. Langmuir's theory.
5. Adsorption selectivity. Adsorption therapy.
6. Exchange adsorption. Ion-exchanger.
7. Main methods of the chromatographic analysis.

1. Adsorption — concepts and definitions

Adsorption is an accumulation of one substance on the surface of the other. The substance on the surface of which adsorption takes place is called adsorbent, the accumulating substance is called adsorptive. Both molecules and ions can be adsorbed, thus molecular and ion adsorption are distinguished. Adsorption accompanied by chemical interaction of the adsorbent with adsorptive is called chemisorption.

Adsorption is a reversible process. Reverse adsorption is termed desorption.

Qualitatively adsorption is expressed as difference of mmol in the adsorptive in the volume of superficial layer with the area of 1 cm^2 and the number of its mol in the same volume inside the adsorbent (G = mmol/cm^2).

2. Adsorption on the surface of liquid — gas and liquid — liquid. Surfce active and surface inactive substances.

Gibbs equation

Adsorbents can be both liquid and solid. On dissolution one substances accumulate on the surface of the solvent, but the other tend to leave the surface and go inside the liquid. This is due to the different influence of the dissolved substances on the value of solvent surface tension.

From the course of physics we know that surface tension equals specific free energy of the surface. As, according to the second law of thermodynamics, free energy of any system tends to minimum, the substances on dissolving of which surface tension diminishes will accumulate in the surface layer, that is adsorbed positively (G>0). Such substances are called superfice active. The substances increasing the surface tension and tending to go inside the liquid are called surface inactive. Many organic substances are surface active with water (alcohols, carboxylic acids, and amines); inorganic electrolytes are surface inactive (acids, bases, salts).

Common for all SAS is their diphilic nature, that is presence of a polar group in the molecule (–OH, –COOH, –NH), etc.), hydrated on the dissolving in water and a hydrocarbon radical having no water shell. Diphilic molecules are marked by the symbol –O, where O corresponds to the polar group, a dash – hydrocarbon radical.

Depending on the sign of the charge of the particle possessing surface activity, anion, cation and nonionic SAS are distinguished. An example of anionic SAS is a soap, salt of higher fatty acids ($C_{17}H_{35}COONa \rightarrow C_{17}H_{35}COO^{-} + Na^{+}$), cationic SAS are amines and organic ammonium derivatives ($C_{12}H_{25}NH_2 + H_20 \rightarrow C_{12}H_{25}N^{+}H_3 + OH^{-}$), nonionic – alcohols, sucrose esters, oxyethylated compounds).

Dependence between adsorption (G), SAS concentration in the solution and the change of surface tension is expressed using the Gibbs equation:

$$G = -(C / RT)(\Delta\delta / \Delta c)$$

$\Delta\delta/\Delta c$ – is the change of surface tension per a unit of concentration and is called surface activity. With the increasing of the length of hydrocarbon radical of the SAS by $-CH_2-$ group (homological difference) surface activity increases 2-3,5 times (Duclot-Traube rule).

3. Orientation of the molecules on the phase interphase. Concept of the cellular membranes' structure. Fat emulsification.

When the surface of the liquid is filled with SAS molecules they are located in such a way that polar groups of diphilic molecules because of hydrophility and ability to hydrate will be sunk into the water. Hydrocarbon radicals are located over the water forming a so-called monomolecular layer (Langmur picket-fence).

After filling up the surface layer (at the concentrations of $10^{-5} - 10^{-3}$ mol/l) surface active substances will accumulate in the inner layers of the solution forming aggregates (surfactant micellas), the inner portion of which consists of hydrophobic hydrocarbons chains, hydrophilic groups of the molecules are located on the external surface of the micella.

Phospholipid molecules in the cellular membrane have the same orientation. Hydrophilic groups of phospholipid molecules are phosphoric acid residues and amino alcohols, but hydrophobic are hydrocarbon radicals of fatty acids.

There are two layers of phospholipid molecules in the membrane. Their hydrophilic groups are oriented outside and hydrophobic inside the molecule. Proteins and polysaccharides are adsorbed on the polar groups of phospholipids. Besides, the proteins are built in the lipid base in such a way that their hydrophobic portions are in the inner surface of the membrane and polar groups of amino acids are on its surface.

If surface active substances are added to water – oil emulsion, their hydrophilic groups will be in the water and hydrophobic – in the oil phase.

As a result, joining the drops in the emulsion will be difficult, its stability will increase. In the process of digestion fat emulsion is formed in the intestine. It is stabilized by the salts of bile acids present in the bile.

Solutions of bile acid salts have a very low surface tension. Spontaneous fat crushing takes place without a prior mechanical breaking. This determines the importance of bile for digestion and absorption of fats in the gastrointestinal tract.

4. Adsorption on the surface of solids. Langmuir's theory.

Gases as well as molecules and ions of the dissolved substances may be absorbed on the surface of solids. Main statements of adsorption theory were formulated by an American scientist Langmuir in 1915. They are as follows:

1. Adsorption is caused by the forces of residual chemical valence. The particles (atoms, molecules, ions) of solid substances located on the surface combine with smaller number of the particles of this substance then the particles in the inner layers. Interaction with adsorptive molecule is possible due to the unrealized bonds.

2. Adsorption takes place in active centers which are always available on the adsorbent surface. These centers are bulges which are present on any (even very smooth) surface. Thus, a finely polished surface of a mirror has bulges up to $3 \cdot 10^{-7}$ cm.

3. Every active center can bind only one molecule of the adsorptive. That is why the theory was called theory of monomolecular adsorption.

4. The adsorbed molecules are kept by the active centers only for a particular period of time, after that they are desorbed and replaced by new particles. This results in dynamic equilibrium between free and adsorbed particles.

Adsorption value (G) depends on the chemical and physical nature of the adsorbent and adsorptive, temperature, concentration of the adsorbed substance.

As the surface of a solid adsorbent is unknown, G is expressed as the amount of adsorptive in mols per unit of mass of adsorbent.

Dependence of the amount of adsorbed substance on its concentration in the solution at a constant temperature is called adsorption isoterm. Based on the above ideas Langmuir deduced the equation of adsorption isotherm:

$$G = G_{max}C/(C+K)$$

G is a quantity of adsorbed substance (mol/g of adsorbent); G_{max} is a maximal adsorption, when all active centers of the adsorbent are taken by the adsorbed substance; C is adsorptive concentration after equilibrium has established; K is adsorption coefficient.

If $G = G_{max}/2$, then $C = K$, that is K equals the concentration at which adsorption equals a half of maximally possible. Adsorption coefficient depends on the nature of the adsorbent and adsorptive.

At low concentrations of adsorbed substances C can be neglected because C<<K. Then $G = G_{max}/2$, that is the amount of adsorbed substance is directly proportional its concentration in the solution. If the concentration of the adsorbed substance is higher, we can neglect K because C>>K, then $G=G_{max}$.

It is evident that absorbent binding of the adsorbed substance is not over after monomolecular layer formation. It goes on, that is polymolecular adsorption takes place. Adsorption forces act at comparatively long distances, we can suggest existence of adsorption volume formed by the molecules of adsorptive on the surface of the adsorbent. The action of adsorption forces decreases with the distance from the surface and becomes zero at a definite distance.

5. Adsorption selectivity. Adsorption therapy.

Sometimes adsorption of a definite substance or a group of substances by the adsorbent exceeds adsorption of the others, this is so-called selective adsorption. This phenomenon is of great practical significance as choosing needed adsorbents it is possible to extract definite substances from the complex mixtures.

In particular, ion adsorption is selective when adsorbents have ionic lattice. As it was established by Panet and Fayans the crystals are completed only by the ions which are present in the composition. E.g., AgI crystals in KI solution adsorb iodine ions. If AgI crystals are put to $AgNO_3$ solution, they adsorb silver ions. In these cases adsorption is caused by the forces of electrostatic attraction and the ions which complete the structure of crystal.

Selective adsorption is frequently observed in the organism. E.g., toxins are adsorbed selectively by the different tissues and cells. Tetanus toxins and botulism affect primarily the cells of the nervous system, dysentery - vegetative nervous system. Even small amounts of poisons with high adsorbtivity on the active centers of some enzymes and biologically active substances may block them. Thus, cyanides block iron-containing respiratory enzymes.

In some cases adsorption therapy is used in the process of treatment. Its main methods are hemosorption, enterosorption, and application therapy. Hemosorption is used for blood purification from toxic substances at poisoning, lever and kidney failure. The adsorbent is activated coal. Enterosorption is administration of granules of activated coal to remove the toxins from the gastrointestinal tract.

Application therapy is used to treat burns and other surface pathologies.

6. Exchange adsorption. Ion-exchangers.

Exchange adsorption is of great practical importance, at which exchange of one adsorbed substance with the other from the environment. At exchange adsorption the adsorbent adsorbing a definite amount of ions from the solution simultaneously displaces the same amount of other ions with the same charge. Weaker adsorbent is displaced by a stronger one, as a result both particles are accumulated on the adsorbent in the amounts proportional to their adsorption ability.

For example, if adsorption of one ion is 10 times higher then another, its amount in the adsorbent will be 10 times higher.

Not only earlier adsorbed ions but also the ions from the adsorbent itself formed in the result of molecule dissociation are capable of exchange.

If the exchange ions are H^+ or OH^-, the environment pH will change.

If the adsorbent absorbs a cation from the solution releasing H^+, pH will decrease:

$$\text{adsorbent}^-H^+ + Na^+ + Cl^- \rightarrow \text{adsorbent}^-Na^+ + H^+ + Cl^-$$

If the adsorbent changes any anion hydroxyl ion, pH will increase

$$\text{adsorbent}^{+}\text{OH}^{-} + \text{Na}^{+} + \text{Cl}^{-} \rightarrow \text{adsorbent}^{+}\text{Cl}^{-} + \text{Na}^{+} + \text{OH}^{-}.$$

Ion exchange resins are widely used in industry and in experiments. They are high molecular compounds capable of exchange sorption. They are divided into cation-exchangers and anion-exchangers depending on the charge of the exchanged ions. The functional groups of cation-exchangers are carboxyl, hydroxyl and sulfur groups ($-SO_3H$), exchanging a cation. The functional groups of anion-exchangers are amino groups.

Ion exchange resins are used to soften water and to extract metals from the complex mixtures etc. They can be used to bind poisons in the gastrointestinal tract.

7. Main methods of the chromatographic analysis.

Adsorption processes are widely used in separation of different substances. They are the basis of chromatography (M.S. Tsvet, 1903).

Chromatography is a physico-chemical method of mixture separation based on the different distribution of the components between two phases, one of which is stationary, the other is mobile filtered through the stationary phase.

Adsorbents in adsorption columns or placed by way of fine layer on glass plates as well as a special chromatography paper are used as the stationary phase. Column, thin-layer and paper chromatography are distinguished respectively.

Separation of the components may occur according to the different features: capability to be adsorbed on the adsorbent, capability to participate in ion exchange, difference in distribution between two immiscible liquids. These varieties of chromatography are called adsorption, ion-exchange, partition.

At adsorption chromatography during passing of the mixture through the adsorbent the substances are placed consequently in accordance with their ability to absorption. More easily adsorbed substances are located in the upper layer, and so on with the decreased in the ability to absorption. When a solvent is passed through the adsorbent you can wash out the components of the mixture consequently.

At ion-exchange chromatography adsorbents are ion exchange resins. The most adsorbable ions exchange in the upper portion, then less absorbable and so on. As a result the ions are released in turns.

Partition chromatography uses differences in substance distribution between immiscible liquids. For example, if a mixture of substances with different solubility in water and benzene is consequently treated by the mixture of these two solvents, you can completely separate the substances according to their solubility.

Gas-liquid chromatography is a variant of partition chromatography. In this case a solid adsorbent (charcoal, celite) is saturated with nonvolatile liquid (glycerin, polyethylene glycol, lanolin) to the degree that it remains dry on touch and easily blown throw by gas. The adsorbent is placed to the column and a gas (nitrogen, argon), to which the separated mixture in the form of gas or vapor has been added, is blown through it. Substance separation takes place due to different adsorption capability of the components.

To perform chromatographic analysis chromatographs, specially designed equipment, are used. They allow automatizing the process and promoting quick separation of the components.

Chromatographic analysis in combination with other physical and chemical methods is widely used now in biology and medicine. It allows to separate mixtures of amino acids, nucleic bases, carbohydrates. Chromatographic analysis is used to determine the primary structure of proteins, nucleic acids, polysaccharides, quantitative analysis of vitamins, hormones and other biologically active substances.

LABORATORY WORK

Adsorption of acetic acid on charcoal

The purpose of the work: to study dependence of acetic acid adsorption from solution on concentration.

Experimental procedure:

Place 0.5 g of charcoal in each flask and add 25 ml of acetic acid solutions of the different molar concentrations using the burette. Adsorption takes 30 min. shake up the content of flasks periodically. In 30 min filter solutions through filter paper. pour away first 2-3 ml of filtrate. Determine the concentration of acetic acid after adsorption by titration against NaOH (c_{NaOH} = 0.1 mol/l). Titrate each solution 3 times in order to obtain the average value. Note down the data to the table:

№	c (CH_3COOH)	V (CH_3COOH)	c (NaOH)	V (NaOH)
1.	0.2	5 ml	0.1	
2.	0.1	5 ml	0.1	
3.	0.05	5 ml	0.1	
4.	0.025	5 ml	0.1	

Calculate equilibrium concentrations of acetic acid according to the equation:

$$\mathrm{C_p(CH_3COOH)} = \frac{C(NaOH)\cdot V_{cep}(NaOH)}{V(CH_3COOH)}$$

Calculate the value of absorption according to the equation:

$$\mathrm{G} = \frac{[C_0(CH_3COOH) - C_{eq}(CH_3COOH)] \cdot V(CH_3COOH)}{m(carbon)}$$

Plot the graph of adsorption isotherm

To make conclusions about the dependence of acetic acid adsorption from solution on concentration.

8. Tasks for knowledge control.

1. The functions of some biologically active substances (phospholipids, bile acids) are due to their superficial activity. The following can be superficially active substances:

A. Slightly soluble substances
B. Substances with a developed surface
C. Substances molecules of which contain hydrophilic and hydrophobic groups
D. Ions of dissolved inorganic electrolytes

2. Which of the given ions will be selectively adsorbed on the particles of barium sulphate?

A. SO_4^{2-} B. Ca^{2+} C. PO_4^{3-} D. Na^+

3. Which phenomenon is responsible for fat emulation by bile salts?

A. Chemical interaction between emulgator and fat

B. Considerable diminution of surface tension

C. Mechanical fat grinding

D. Formation of aggregates from emulgator molecules

Answers: 1 - C; 2 - A; 3 - B.

9. Recommendations for the work results design

Algorithms for solving educational problems of class work and self-work, and conclusions of laboratory work should be recorded in the workbook and come to conclusion in relation to physico-chemical phenomena of adsorption and about characteristics of chromatographic methods of analysis in medical practice.

10. Suggested readings

V. Kalibabchuk, V. Halinska, L. Hryschenko, S. Hodzynski, T. Ovsyanikova, V. Samarski. Medical chemistry. –Kyiv AUS Medicine Publishing, 2010. − 224p.

Навчальне видання

Сорбція біологічно-активних речовин на межі розподілу фаз. Іонний обмін. Хроматографія

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