Ministry of Health of Ukraine
Ukrainian Medical Stomatological Academy

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Methodical instructions
for students' self-preparation work at preparation for a practical lesson
at home and at the classroom

Subject matter	**Medical and biological physics**
The unit	2. Bases of medical physics
Theme of lecture:	**Electrokinetic appearances. Electrophoresis.**
Year	1
Faculty	Medical
Speciality	Medicine

Poltava - 2020

The topic significance:

A great number of diagnostic and therapeutic methods used in modem medicine are based on effects developing in human body tissues under the influence of electric, magnetic, and electromagnetic fields, accompanied by electrical currents. These currents and fields action and accompanying phenomena depend upon current nature and electrical characteristics of biological tissues.

Specific targets:

To have general knowledge of the topic studied;
To understand, to remember and to use the knowledge received;
To form the professional experience by reviewing, training and authorizing it;
To be able to carry out laboratory and experimental work.

Basic knowledge, experience, skills necessary for studying the topic in connection with other subjects:

Disciplines	**Obtainable skills**
Previous (providing disciplines): physics, mathematics, chemistry, biology	To know concepts: electric field, potential, potential difference, gradient. Ohm's law, electrolytic dissociation, diffusion potential (electrochemical potential). To describe them. To describe electrokinetic appearances.
The subsequent disciplines: Normal physiology	To know role of electric processes in cell, tissues and whole organism functioning.

Materials for the before-class self-preparation work:

List of main term, parameters, characteristics, which student have to learn at preparation to class:

Term	Definition
Electrophoresis	Motion of charged particles under influence of electric field
Electroosmosis	It is locomotion of a dissolvent in an electrical field if molecules of a dissolvent are charged, or owing to the osmosis accompanying locomotion of dissolved charged particles.
Sedimentation potential	Electric field generated by sedimenting colloid particles
Sedimentattion	Movement of charged particles through a liquid by gravity
Streaming potential	Electric potential generated by fluid moving through porous body, or along a flat charged surface.
Direct electrokinetic phenomena	Motion of particles as result of influence of electric field
Reverse electrokinetic phenomena	Electric potential generated by moving particles as result of other causes (gravity, hydrostatic pressure, mechanical oscillations an others).

Theoretical questions to class:

1. What are electrokinetic appearances?
2. What is electrolytic dissociation?
3. What is ions mobility?
4. What is electrophoresys?
5. What is electroosmosis?
6. Using of electrophoresys in diagnostics, medical and biological research.
7. Using of electrophoresys in clinical purposes.
8. Explain ion mobility. What does it depend on? What role does different ion mobility play in tissues of human organism?
9. Role of electrophoretic processes in organism.
10. How to define the ions velocity and mobility by electrophoresis in practice?
11. Primary action of direct electrical current on human organism tissues.
12. Apparatus for galvanization and medicinal electrophoresis design and principle of operation.
13. Technique of providing of galvanization and medicinal electrophoresis.

Practice work executed at class.

Task. Determine hydroxyl ions [OH^- ions] movement speed dependence on the intensity of electrostatic field (v = *f(E))*:

- place a glass or plastic plate (bridge) on edges of partition of vessels with electrolyte (electrophoretic cell);
- moisten a strip of filter paper with Na_2SO_4 and phenolftalein solution and place it on a glass plate so that the ends were 1-2 cm submerged into the solution;
- measure distance ***X*** between the electrodes;
- switch the power supply of direct electric current and establish the voltage between electrodes *U*=60 V;
- put cotton thread moistened by *NaOH* solution approximately in the middle of the filter paper perpendicular to the line between electrodes;
- start a stopwatch and after the time *t*=10 min switch off the direct current power supply;
- remove the glass plate with filter paper and measure the way *S (m)* passed by OH^--ions (by colored zone);
- repeat measurements at voltage between electrodes *U* = 80 V and 100 V,
- calculate hydroxyl ions movement speed for each case
- by the formula: $V = S/t$;
- calculate the intensity of electric field *E* between electrodes by the formula: $E = U/X$;
- calculate hydroxyl ions mobility for each *E* value by formula: $v_0 = V/E$;
- put the data received to the table.

Table

№	*U (V)*	X (m)	S (m)	t (s)	*V (m/s)*	v_0 *(m^2/ Vs)*
1	60					
2	80					
3	100					

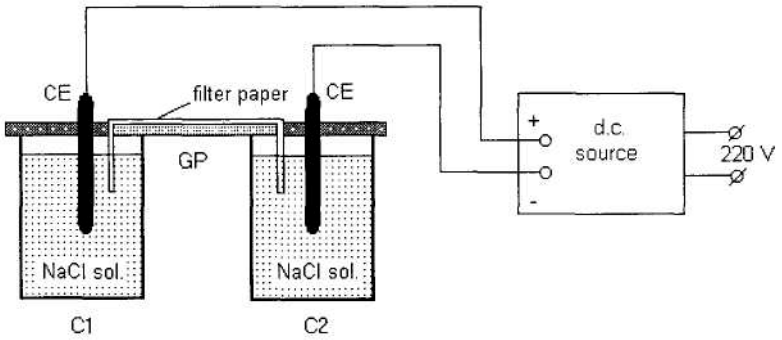

Fig. Scheme of arrangement. CE – electrode, GP – glass plate (plastic plate).

Contens of the topic.

Biological objects are complex heterogenous systems with a great number of boundaries: cells membranes, cell compartments mambranes, globular peptides, and other structures of tissues.

Electrokinetic phenomena is a family of several different effects that occur in heterogeneous fluids (heterogeneous mediums) or in porous bodies filled with fluid. Surface phenomenas show in objects with an advanced surface: the surface layers, threads, fibers, a film, capillars, fine particles. Disperse particles together with medium in which they distributed, makes a disperse system.

The family of electrokinetic phenomena includes:

direct electrokinetic phenomena –

- **electrophoresis**, as motion of particles under influence of electric field;
- **electro-osmosis**, as motion of liquid in porous body under influence of electric field;

reverse electrokinetic phenomena –

- **sedimentation potential**, as electric field generated by sedimenting colloid particles (sedimentattion – movement of charged particles through a liquid by gravity);
- **streaming potential**/current, as either electric potential or current generated by fluid moving through porous body, or along a flat charged surface;

and some others.

Electroosmosis is locomotion of a dissolvent in an electrical field if molecules of a dissolvent are charged, or owing to the osmosis accompanying locomotion of dissolved charged particles.

Ordered movement of charges is an electrical current. The electrical current in electrolytes is an unidirectional movement of ions. Ions are formed at dissolving of salts, acids or alkalis (bases). Two types of ions are forms at dissociation: positive cations and negative anions. If electric field will be applied to the electrolyte (for example, at immersion of electrodes, connected to direct voltage source), cations will move to the negative cathode, anions will move to the positive anode.

Ions movement is in full volume of solution between electrodes. Electric field strength (electromotive intensity) between electrodes is equal to $E = U / x$, where U – voltage, x – distance between electrodes. Friction force brakes ions. The movement of ions in electrolytes under the action of force of an electrical fieid F_E and friction force F_f will be uniform, when $F_E = F_f$.

Ions rate **v** in this case will be:

v = vE, where **v** is ionic mobility.

The unit of the ion mobility is $m^2s^{-1}V^{-1}$.

The ions mobility at a given temperature depends on their mass, charges and viscosity of electrolyte. In solutions with small concentrations mobility is specific for different ions. The ions of sodium Na^+, potassium K^+, calcium Ca^{++}, magnesium Mg^{++}, chlorine Cl^- and others have the different mobility.

In medicine the electrophoresis is applied to divide various fractions of albumens. This process of division is possible due to the fact that different fractions of albumens in an electric field will move with different velocities. It has the diagnostic value, as at some diseases the ratio between peptide fractions of human blood changes sharply.

Peptides have not color. After division they must be colored by special stain. Other method of determination is quantitative: peptides displayed by ultraviolet light. They absorb ultraviolet. After peptide electrophoresis on transparent gel plates, plates are photographed in ultraviolet light, and separated peptides are shown like dark spots. Intensity of color is proportional to a quantity of peptide. Photometry of plates gives exact values.

Medicinal electrophoresis is a method of treatment, based on administration of the of ions of medicinal substances into human organism through undamaged skin and mucous tunic under the action of electric field.

The advantages of medicinal electrophoresis are:

— joint action of direct electric current and medicinal substance;
— local influence at a superficial allocation of pathological focus;
— absence of unnecessary effects on other tissues;
— reduction of probability of side effects;
— small doze of medicinal substance;
— prolonged influence of medicinal substance due to its slow coming from skin depot into blood.

The primary action of direct electric current is connected with the movement of ions, their division and change of their concentration in different elements of biological tissues owing to different mobility.

The treatment by direct electric current refers to as *galvanization.* In galvanization direct electric current with the voltage of U= 60–80 V is used.

Galvanization and medicinal electrophoresis is made with the help of galvanization apparatus. Apparatus for galvanization consists of direct current rectifier with electric filter for smoothing pulsations output regulations potentiometer and milliammeter.

Galvanization is done from electroplating apparatus with the help of sheet electrodes with the help of pad (flannel, thick flannelette), moistened by warm water, which are imposed on the surface of a body.

The purpose of pads is to create a uniform density contact of the electrode with the patient's body, to lower down high ohmic resistance of dry skin, to remove harmful influence of electroplating products. There must be no burn on the skin under the anode from hydrochloric acid HC1, and under the cathode -from sodium hydroxide NaOH (alkali).

At medical electrophoresis ions of medicinal forms enter the organism with the help of linings, moistened with solutions of these forms and put under electrodes.

Thus, with the help of the apparatus for electroplating the following procedures are carried out:

1) Treatment by direct electric current (electroplating),
2) Increase of sells excitation under the cathode owing to accumulation of Na^+ and K^+ ions, that leads to skin hyperaemia under the cathode,
3) Decrease of sells excitation under the anode with the

purpose of reducing pains. It occurs owing to relative prevalence of Ca^+ and Mg^+ ions in this region, as they are less mobile than Na^+ and K^+ ions.

4) Medicinal electrophoresis.

Medical electrophoresis is performed similar to galvanization but one of hydrophrlic layers is wet not with water but with a certain medicine solution.

Introducing of medical subslances by means of electrophoresis is possible if a medicine dissolving in water forms ions. In this case anions are introduced to patient when a layer under cathode is wet with medicine solution, cations are introduced to patient when a layer under anode is wet with medicine solution.

Electrophoresis method of introducing medicines has a lot of advantages in comparison with other methods. Electrophoresis does not hurt skin and ensures local effect in a required place. The medicine is introduced in ion form, and il is ions that make therapeutic effect. The medicine introduced by means of electrophoresis accumulates in hypodermic (subcutaneous) cellular tissue and is washed out from it slowly that ensures prolonged uninterrupted medicine action on a pathological center.

Electrophoresis can be used for diagnostic purposes, for example, for plasma protein fraction separation and detection that is highly informative for diagnostic of a number of diseases.

Direct current can cause a phenomenon of *galvanism in a human mouth.* This phenomenon is possible if some stomatological structures in the mouth are made of different metals. Urtdenthe influence of electrolytes (saliva, components of food) the EMF induced between melal structures causes flowing of small galvanic currents. Those currents gradually cause metal corrosion that makes metal structures worse.

There is a common source of all these effects — the so-called interfacial 'double layer' of charges. Influence of an external force on the diffuse layer generates tangential motion of a fluid with respect to an adjacent charged surface. This force might be electric, pressure gradient, concentration gradient, gravity. In addition, the moving phase might be either continuous fluid or dispersed phase.

Double electrical layer

Double electrical layer (**DEL**) is a result of heterogeneous system tendency to diminution of a surface energy that produces an orientation of polar molecules and ions in the surface layers therefore adjoining phases get quantity equal charges with opposite signs.

Electrical charges on the colloid particles surface or on a surface of cells and cellular organoids can result from two processes: a dissociation of ionogenic groups and an adsorption of ions of dispersion medium on a disperse phase surface, which itself is not capable to form ions.

Originating of the surface charge due to ionization can to occur, for example, in proteins and other organic electrolytes containing carboxylic, amine and others polar dissociating groups.

In peptide molecules originating of a charge depends on presence of acid and alkaline groupings.

Due to presence of lead-acid and alkaline groups proteins represent bipolar ions.

In acidic solutions protein plays a role of a cation.

ELECTROKINETIC APPEARANCES USING

Electrophoresis on the carrier

It is possible to select and explore, for example, separate protein fractions of bloody plasma. t is necessary to effect qualitative and quantitative analysis of proteins fractions of bloody plasma for diagnostics of many diseases. In this connection methods of electrophoresis are widely applied in clinic-laboratory practice.

Now usually carry out gel-electrophoresis (which is enough precise and simple) on polyacrylamide or agarose gel plates. It is similar to simple, but not so precise method of paper electrophoresis.

Capillary electrophoresis (CE)

Capillary electrophoresis is formed in fused SiO_2 capillary tube long ~ 0.5 m, inner diameter: 25-75 mm.

The method of ***capillary electrophoresis*** is based on separation of builders of a complex mixture in the quartz capillary under activity of an electric field. Microvolume of explored solution is introduced into the capillary, the filled electrolyte.

Under activity of high tension (up to 30 kV), mixture components start to move on the capillary with the different rate dependent on a sign and quantity of a charge and quantity of ionic radius, and they reach detection zone in different time.

Rate of a concrete component is determined by a direction of electroosmotic stream (total fluid flow) and an electrophoretic stream dependent on charge sign of the conforming component

Capillary Electrochromatography (CEC)

At power-up (switching on) of current there is an electrophoretic movement of explored admixture components. Electrophoretic motility according to the equation (1) is proportional to quantity of ζ-potential of separate components. After experience terminal explored substances settle down on various distance from an application line (start lines) in dependence on electrophoretic motility value and quantity of interaction with a paper. A paper strip dry up and imbue by dye (stain) showing explored substances. Further separate components subject to the quantitative definition by photometred. Macromethods of electrophoresis apply in basic to separation and examination of electrochemical properties of colloidal solutions.

Electroosmotic flow has a flat profile while hydrodynamic flow has a parabolic profile. As a result, CE results in minimal band broadening.

Proteins and other charged macromolecules can be parted methods of electrophoresis. About 100 various proteins contains in human blood plasma. On motility at electrophoresis of them it is possible to separate them into five fractions: albumin, α1-, α2-, β- and γ-globulins. Among various electrophoretic methods the most prime is the electrophoresis on the carrier, is especial on acetyl cellulose film. Thus proteins which because of presence of exuberant negative charge move to anode are parted on five above mentioned fractions. After separation proteins can be dyed with the help of dyestuffs and to estimate quantities of proteins in obtained dyed strips. Change of concentration of separate proteins typically for some diseases (so-called disproteinemias).

After completion of the run, add a DNA staining material and visualize the DNA under UV light.

Micromethods of electrophoresis are applicable for studying electrochemical properties of suspensions of various cells (erythrocytes, leucocytes, bacteria, sex cells and so forth, and also cellular organoids).

Rate of cells movement under electric field activity is determined with the help of the microscope supplied with a scale micrometer.

The alive protoplasmatic surface is always charged negatively, i.e. all biological surfaces have negative electrokinetic potential. It is not known any example of clearly expressed positive potential of a surface of alive object.

Quantity of ζ-potential can have various values for different cells.

Quantity of erythrocytes ζ-potential at various mammalian changes from 7 up to 22 mV at pH 7,4.

At the human it is 16,3 mV in the given conditions. Erythrocytes ζ-potential is very stable quantity.

In limens of one biological species, for example, the human, does not have any differences in quantity of ζ-potential of erythrocytes at representatives of various race and a sex. They are not observed also between representatives of different blood groups.

Electrophoretic motility of erythrocytes does not variate at series of blood diseases, including at many forms of anemias.

Erythrocytes ζ-potential preserves its quantity even after their complete haemolysis. It is possible to note, that electrochemical properties of a erythrocytes surface are distinguished major stability and a constancy. ζ-potential of erythrocytes depends from pH.

The isoelectric point of erythrocytes corresponds to pH 1,7, that does not coincide with an isoelectric point of a haemoglobin (pH 6,8), with an isoelectric point of plasma proteins (pH 4,7).

The electrokinetic potential of erythrocytes is caused by a dissociation of acid groups of phospholipids molecules on a erythrocytes surface and not connected to processes of adsorption of proteins and ions.

Quantity of electrokinetic potential of erythrocytes varies in the event that there is a change of the physicochemical composition of the cell surface. It is observed at some diseases, for example hemoblastoses, a lymphosarcoma.

Identification and analysis of cell populations and (micro)biological particles are essential in many practical applications ranging from cancer research to chemical analysis of environmental pollutants.

New methods (past decades): alternating current electrokinetic phenomena (electrorotation (ER), dielectrophoresis (DEP).

Both dielectrophoresis and electrorotation are based on dielectric properties of particles.

These properties depend on the nature of the surface, e.g., size, shape, and charge density. For example, since the composition and shape of cancer cells differ from those of healthy cells, these difference are reflected in their characteristic dielectric properties which can be exploited in identifying them. From a practical point of view, AC electrokinetic methods have the advantages of short detection times and high sensitivity.

Automated procedure for DNA sequencing

The computer scans vertically through each lane of the gel file, and converts the pattern of bands to an individual chromatogram with a series of "peaks" corresponding to the **DNA** sequence: **A C G** & **T**. **DNA** migration slows over the course of the electrophoresis, and multiple bases towards the end may appear as a single broad band instead of discrete "peaks".

Electroosmotic Flow (EOF)

A high potential (15 – 25 kV) is applied across a capillary column. At pH > 3, the inner capillary wall is negatively charged due to ionization of SiOH.

Cations in the buffer adsorb on the walls leading to an electrical double layer. The cations are attracted towards the cathode (negative electrode). Since they are solvated they drag the solvent molecules with them resulting in electroosmotic flow of the mobilephase.

Importance of Electrokinetic Phenomena in Porous Media

- Streaming potential used to map subsurface flow variations – faults, oil reservoirs;
- Monitoring and prediction of earth quakes;
- Water leakage from reservoirs;
- Monitoring of volcanoes and earthquakes.

Electrophoresis as way of regenerative processes

The electrokinetic phenomena play a determining role at curing damages of intrinsic surfaces of vessels - an endothelium.

Thrombocytes, as well as an endothelium in norm, have negative electrokinetic potential. However damage of an endothelium results in inverse of its potential on positive. It invokes a directional stream of thrombocytes to a place of damage and eliminates it.

Agglutination

Agglutination (agglutination, clumping) – process of a coalescing of such microparticles, as erythrocytes or suspended bacteria under activity of the serum antibodies termed agglutinins, with formation of visual accumulations. Any substance inducing formation of the agglutinin, is termed as an agglutinogen. Agglutination represents specific response.

Agglutinins (immune substances) are made in process of blood immunity formed; they capable to be adsorbed by certain bacteria. As a result value of bacteria cell ζ-potential appears less critical, as results in agglutination of bacteria and their prompt sedimentation. Agglutinating bacteria are not capable to show pathogenic activity.

Agglutination is a bonding and sediment of bacteria, erythrocytes and other cells carrier antigens from homogeneous suspension, under activity of specific substances (agglutinins); agglutinins can be antibodies, lectins, etc. The agglutination test apply to definition of blood groups, for revealing antigens and antibodies (identification of originators of contagions).

Electrokinetic's phenomena as way of cell nutrition

An ionophoresis (ions electrophoresis) play the important role in entering nutrients to osteal cells - to osteocytes. Osteal cells are posed rather far from blood vessels on which nutrients for their building are delivered. Besides that proper canals in a bone make only 3% of cross-sectional area that also impedes a diffusion of nutrients.

The essential help in delivery of nutrients is rendered with the electrokinetic phenomena caused by piezoelectric properties of a bone. These properties consist that at strain of a bone in compression section there is a negative potential, and in distension region - positive. Positively ionized atoms and plus charged molecules of nutrients are drawn to negative the charged region, providing its with necessary structural substances.

Constantly incipient physiological strains of an skeleton serve thus as the original pump for delivery of necessary substances. The specified phenomenon can be used and for stimulation of formation of an osteal callositas in a fracture place, by synthetic building the conforming potential.

Therapeutic electrophoresis

Therapeutic use of electrophoresis is very important in treatment and rehabilitation. Electrophonophoresis of medicines [officinal electrophonophoresis] is method of local administration of the medicine, which dissociates forming ions. Electrodes-plates are applied on treated areas of skin or mucous. One with pad wetted by solution of medicine must have charge identical of charge of medicine ion.

Self-control material:

B. Test tasks

1. Indicate what systems are colloid systems?

A) Sol
B) Suspensions
C) Gel
D) Solution
E) Emulsions

2. Indicate reverse electrokinetic phenomena

A) Potential of a sedimentation
B) Double electrical layer
C) Electrophoresis
D) Potential of fluxion
E) Electroosmosis

3. What is potential of sedimentation?
A) Electrical potential difference produced on a boundary between dispersion medium and disperse phase in time of disperse phase movement.
B) Electrical potential difference produced as result of the mechanical motion of dispersion medium in relation to disperse phase
C) Electrical potential difference produced as result of the mechanical motion of disperse phase in relation to dispersion medium
D) The mechanical motion of dispersion medium in relation to disperse phase as result of an outer electrical potential difference
E) The mechanical motion of a disperse phase in relation to dispersion medium as result of an outer electrical potential difference
4. What is double electrical layer?
A) Electrical potential difference produced on a boundary between dispersion medium and disperse phase as result of disperse phase movement.
B) Electrical potential difference produced on a boundary between dispersion medium and disperse phase as result of orientation of polar molecules and ions in the surface layers.
C) Electrical potential difference produced on a boundary between dispersion medium and disperse phase as result of adsorption of ions of dispersion medium on a disperse phase surface.
D) Electrical potential difference produced on a boundary between dispersion medium and disperse phase as result of dissociation of ionogenic groups of disperse phase.
E) Neutral as a whole system of ions produced on a boundary between dispersion medium and disperse phase as result of charged particles reallocation.
5. Rate of cells movement in time of electrophoresis can be found from Helmgolz-Smoluhovsky equation:

A) $\boldsymbol{v} = \frac{4\varepsilon \boldsymbol{E}\zeta}{\pi\eta}$; B) $\boldsymbol{v} = \frac{4\pi\eta}{\varepsilon \boldsymbol{E}\zeta}$; C) $\boldsymbol{v} = \frac{\boldsymbol{\varepsilon E \zeta}}{\boldsymbol{4\pi\eta}}$; D) $\boldsymbol{v} = \frac{\varepsilon \boldsymbol{E}}{4\pi\eta\zeta}$; E) $\boldsymbol{v} = \frac{\varepsilon \boldsymbol{E}\,\eta\zeta}{4\pi}$

6**.** What metabolic processes provided with electrophoretic appearances?

A) vision in dark;	B) vascular wall damages reparation;
C) osteal reparation;	D) locomotion of leucocytes to inflammation zone;
E) locomotion of erythrocytes along vessels.	

7. What are causes of erythrocytes sedimentation rate changing?
A) presence of proteins of acute phase;
B) changing of concentration of albumines;
C) changing of composition of erythrocytes membranous phospholipide;
D) changing of degree of a hemolysis;
E) some diseases.

Literature recommended

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