AL-FARABI KAZAKH NATIONAL UNIVERSITY

MANUAL FOR LABORATORY CLASSES

IN BIOLOGICAL PHYSICS

ALMATY 2015

Foreword

Manual for laboratory work in biophysics is a set of teaching materials and guidelines for laboratory work on biophysics which prepared and held at the Al-Farabi Kazakh National University.

This manual has been tested for a number of years in a pedagogical setting in the Department of Biophysics and Biomedicine. They cover the main sections of the course in accordance with the types of programs in biophysics and are intended for university students.

The works may be included in the laboratory practical courses on "Biophysics", "Theoretical and Applied Biophysics", "Photobiology", "Biophysics Medical Biophysics with the basics," as well as during some sections Special practical.

The works presented here do not require complex and expensive equipment and can easily be reproduced in any university laboratory.

At the end of each Chapter are questions of self-control that will help more firmly and deeply understand the processes and phenomena observed in laboratory work.

SAFETY REQUIREMENTS
DURING BIOPHYSICS WORKSHOPS

Before carrying out any laboratory work, students are required to undergo safety training. The students will also have to sign the control sheets that indicate completion of the training.

During labs, instruments that meet standard requirements and have proper technical specifications and normative technical documentation could be used. The equipment is checked directly by the instructor before the laboratory.

To prevent electrical hazard in this workshop, follow these safety practices:

1. To prevent the hazard of electric shock, all devices must be grounded according to their documentation.
2. The wires used in the classroom must not be damaged.
3. The plug should not be pulled by holding the cord, the body of the plug should be held instead.
4. If there are any malfunctions in a device, the device has to be switched off and work has to be stopped immediately. Work can be resumed if and only if the device was fixed by a qualified personel.
5. Students have the right to start the work only after the instructor allows them to work independently, after the equipment and laboratory procedures are checked by the instructor.
6. Only class-1 lasers, which output radiation harmless to human eyes and skin, are going to be used for this workshop. To prevent retinal damage, the beam should not be observed without protective filters and the light should not be shined directly into the eye.
7. Upon completion, all of the devices must be switched off.

Chapter 1

THERMODYNAMICS

The temperature range on the Earth from - 80 C^o to +85 C^o significantly exceeds the borders within which the active life is possible. Temperature determines the activity level and distribution of animals. In an open ocean, the temperature of the surface water layers is -2 - +30 C^o . Vital processes are only possible at 0-40 C^o. In an inactive state, animals tolerate not only negative temperatures, but freezing. For example, small nematodes tissue cultures (epithelial, muscles, etc.), protozoa get frozen when placed in liquid air (-197 C^o) and comes to life if to warm them slowly. Some animals inhabit hot springs, like a few bacteria and algae that can reproduce at +70 C^o .

Temperature, as a measure of the velocity of molecular movement, determines the rate of chemical reactions, and is considered to be one of the factors limiting growth and metabolism. Animals that change their body temperature in accordance with the change in environmental temperature are called poikilothermic (changeable, labile). In this case, temperatures of bode and surroundings are not necessarily equal. Body temperature can be higher, especially, at the active state. Thus, fish of 40g have the muscle temperature higher by 0,44 C^o.

Animals able to regulate their temperature are homoeothermic (birds and mammals). This is due to the thermoinduction, protectional behavior, thermoisolation changes, circulation and other factors changing heat transfer. Periods of hibernation or lethargy, is accompanied by the decrease in body temperature. And the physiological thermostat switches to a lower temperature. Sensor mechanisms demonstrate the change in temperature, causing corresponding feedback reactions. Relatively few animals – heterothermic animals – can partially regulate body temperature that is limited by body regions or by environmental conditions. There are a lot of such animals among insects, for example, bees or ants, but most of arthropods are typical poykilothermic organisms. Temperature of any active cell must be higher than the temperature of the environment, as during oxidation processes and glycolysis heat is released. body temperature depends on several factors affecting the thermal balance in a contrary manner. The heat source can be metabotic thermogenesis (endothermy) or environment, mostly, solar energy (ectothermy). Heat transfer occurs by radiation, convection, heat conduction and water evaporation. Blood circulation from insite to outside of the body promotes heat losing, while thermo isolation obstacles it.

Thermal conductivity of water is 0,0014 kcal/(cm*c*degrees), it is lower than in metals but higher than in other liquids (for example, ethanol – 0,00042

kcal/(cm*c*degrees)). Specific heat capacity of water – 1 kcal/g*degrees, ethanol - 0,09 kcal/g*degrees, most of animal tissues – 0,07-0,09 kcal/g*degrees. The coefficient of temperature conductivity is equal to the coefficient of thermal conductivity divided by the product of specific weigh and specific heat capacity. Low temperature conductivity leads to slow cooling or heating of tissues and limitation in heat distribution within the organism. Fat is a good isolator for animals. Animals with a big tissue mass warm and cool themselves slowly, heat transfer is conducted by circulating liquids. Water evaporation cools any surface, the evaporation of 1 kg of water at 20C cost 585 kcal. Most of terrestrial animals use this to avoid body overheating.

Biokinetics studies the rate of biological processes and their dependence from concentrations of substances which participate in biochemical conversions, and also the dependence from external conditions, especially, from temperature. Such dependence is comprehensible if to take into account that any chemical conversion occurs if chaotically moving molecules collide. As temperature rises, the mean free path of the molecules increases, and thus, the likelihood of their collision also increases. So, the relative number of molecules able to participate in the reaction, or active molecules, increases with the rise of temperature, and the rate of the reaction also increases.

The parameter indicating how many times the number of active molecules and the rate increased at the temperature rise by 10C is called temperature coefficient Q10.

$$Q10 = V_{T}2 / V_{T}1 \quad (1.1)$$

Where $V_{T}1$ – reaction rate at the initial temperature,
$V_{T}2$ –the rate increased at the temperature rise by 10C

There is a ratio between t coefficient and excessive energy that molecules should possess so that their collision could cause a chemical reaction. (so-called Activation Energy)

$$E = 0{,}46\ T1 * T2 * \lg Q10 \quad (1.2)$$

Where E – activation energy, kcal/mol
T1 и T2 – temperatures with the difference of 10°C , i.e. $T_2 = T_1 + 10°$
$\lg Q_{10}$– decimal log of Q_{10}.

It is clear that with the rise of temperature by 10°C, the number of molecules with the energy exceeding the critical value will double, although the

increase in kinetic energy proportional the absolute temperature will be much lower. In biological range, the temperature of Q_{10} values for most metabolic reactions lays in the interval between 2 and 2,5. Some complex changes in rates of physiological processes for example, circadian rhythms, are relatively independent from temperature, and Q_{10} for oxygen consumption of some poykilothermic animals is between 1 and 2. If to compare oxygen consumption at rest and at the active state, we can determine the characteristic exchange levels at different temperature conditions.

In *Cardium* mollusks Q_{10} values for active state and rest are equal to 1,84 and 1,20 respectively. For most of invertebrates Q_{10} values are small. Temperature parameters of enzymatic reactions can lower with the decrease in substrate concentration to the limiting level, thus the measurements of temperatures coefficient have no sense. So, in the case of complex reactions with parallel and consecutive stages, with the contrary influence on Q_{10} it is impossible to perform an elementary analysis of temperatures coefficient. Even though, in biological research, studies of adaptation and acclimatization of animals to different environmental conditions the study and determination of Q_{10} coefficient are widely used.

Laboratory work № 1
Determination of temperature coefficient and calculation of the activation energy of the frog's heart

Objective: Determination of temperature coefficient and calculation of the activation energy of the frog's heart

Tasks:

1. Measure heartbeat at the room temperature;
2. Measure heartbeat at the increase in temperature by 10 C^o;
3. Measure heartbeat at the decrease in temperature by 10 C^o;
4. Calculate the temperature coefficient and activation energy using the formulas;
5. Make conclusions of the observed phenomena and prepare a report.

Equipment and Materials:

Thermometer (0 - 30^0C), vessel for the heart (50 mL), thermostat, ice, hot water, Ringer-Lock solution for the cold-blooded, stopwatch, dissecting tools (scissors, scalpel, dissecting needle, tweezers, gauze.

Procedure:

In this lab work students should register the heartbeat of frog's heart at 2 or 3 different temperatures, with differences between each other by 10^0C).

After the immobilization of the frog, the heart should be taken from the chest and placed it in a vessel full by 2/3 with Ringer-Lock solution for the cold-blooded.

1st measurement is after 5-7 min. Heartbeat during 1 min is measured 3-4 times and the average is calculated.

Measure room temperature. Place the vessel with the heart in the thermostat with the temperature 10^0C higher than room T. After 3-4 min perform the 2nd measurement with the calculation of the average.

Return the vessel to the room conditions and observe restoring of the initial heartbeat. After 3-5 min the heartbeat during 1 min is measured 3-4 times and the average is calculated.

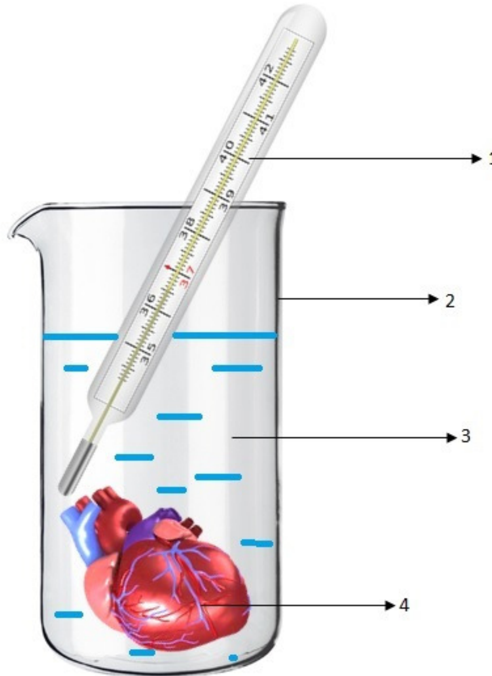
Put the heart into the camera being cooled by the mixture of ice and water. When the temperature would be 10^0C lower than room's temperature, heart rate should be measured again, the measurements should be done 3-4 times and calculated mean of heart rate.

Return the vessel to the room conditions and observe restoring of the initial heartbeat again. After 3-5 min the heartbeat during 1 min is measured 3-4 times and the average is calculated.

All data should be written in table 1.1.1

Table 1.1.1

Experiment's conditions, temperature, °C	Heart rate	Q10	Q10	Q10	E act
Room t =					
Low t =					
High t =					



Picture 1.1.1

1 - thermometer; 2 - vessel; 3 - Ringer-Lock solution; 4 - frog's heart.

Example:

During the experiment we have been seeing that the frog's heart at 18°C (T_1 = 273 + 18 = 291) has HR (heart rate) = 31 beat/minute, and at 28°C (T_2 = 273 + 28 = 301) - 60 beat/minute. So, Q10=60/31=1,9.

In the table Bradis value of lgQ10 is 0,27875. By inserting obtained data into the formula (1.2) calculate the energy of activation:

$$E=0,46 * 291*301*0,27875= 11,239 \text{ kcal/mol.}$$

Report design. The results are inserted into the table and the calculations are made. Define measurement deviations and make conclusions.

Laboratory work № 2

Determination of the temperature coefficient and calculation of the action energy of respiration of elodea plant branch

Objective: to get sure with the experiment that physical chemical laws are maintained in the living systems.

Tasks:

1. Count the amount of gas bubbles at room temperature;
2. Count the amount of gas bubbles during the rise of the temperature by 10 C^o;
3. Count the amount of gas bubbles during the declination of the temperature by 10 C^o;
4. To calculate the temperature coefficient and the energy of activation using formulas.
5. To make a conclusion and process the results.

Equipment and materials: Elodea plant in the small vessel, two big vessels with water more and less than room temperature by 10 C^o; thermometers, timer, Bradice table.

Procedure:

To complete this work you need observe the gas bubbles emission by the elodea plant in the 3 different vessels with waters with temperatures which differ from each other by 10 ^0C.

Elodea is a typical alga of most of the aquariums, which with sufficient amount of light emits the oxygen bubbles from the tips of its leaves. You can count the quantity of emitted bubbles by watching the plant.

The first count of gas bubbles is done after 5-7 minutes after termostation of the vessel with plant. To make it, you should place the vessel with elodea into the bigger vessel with fixed temperature of the water. Then, you should count the emitted quantity of gas bubbles during 1 minute. You should repeat it for 3-4 times and the find an average quantity. Also you should define the temperature under the normal (room) conditions.

Then you should transport the vessel with plant into a thermostat with water hotter by 10 ^0C than in the first one. After 3-5 minutes count again the quantity of bubbles, repeat it 3-4 times and the find the average.

After that, you should transport the vessel with elodea into the first one with room temperature to retain the initial gas emission state. Then after 3-5 minutes repeat the counting like previous times.

Repeat all the same but with the vessel with colder water.

Fill the table 1.2.1.

EXAMPLE.

During the experiment following was obtained: at the temperature 18°C (T_1 =273 + 18=291) 31 gas bubble was emitted but at the 28°C (T_2 =273 + 28=301) 60 bubbles per minute. Thus, Q_{10} =60/31=1,9. Using the table Bradis find lgQ_{10}=0,27875. By inserting obtained data into the formula (1.2) calculate the energy of activation:

E=0,46 * 291*301*0,27875= 11,239 kkal/mol.

Table 1.2.1.

Conditions of the experiment, temperature	Number of bubbles					Q_{10} 1.room.-low.	Q_{10} 2. low -room.	Q_{10} 3.room.-hot.	Q_{10} 4.hot.- room	$E = 0,46\ T_1 * T_2 * lgQ_{10}$, Enerdy of activation
	1			4	V					
1.room.t=										
2. low.t=										
3.room.t=										
4. hot.t=										
5.room.t=										E average =

Report design: Fill the table the obtained results and calculate the energy of activation using formulas. Find the error of calculations and make conclusion.

Chapter 1 «THERMODYNAMICS» questions:

1. How the dependence, which is expressed via this formula, is called?
   $E = 0,46\ T_1 * T_2 * lgQ_{10}$
2. To which type of thermodynamic systems living organisms belong?
3. Why our planet is considered an open thermodynamic system?
4. Which animals regulate their body temperature by homeostatic regulation (change of the metabolic processes) and change in the behavior.
5. What is an energy of activation – E?
6. Give an examples of warm-blooded and cold-blooded animals.

CHAPTER 2.
WORKING PRINCIPLE OF PH-SENSOR

Chemically clear water partially dissociates on ions:

$$H_2O \leftrightarrow H^+ + OH^-$$

Thereby forming a H + ion rapidly hydrated (attaches to itself a water molecule) to form hydronium ion H_3O^+. However, for simplicity instead of hydronium talk about hydrogen ion. The law of mass action applied to the dissociation of water of any aqueous solution expressed in this equation:

$$K = \frac{[H^+]\cdot[OH^-]}{[H_2O]}, \qquad (2.1)$$

where, K - a constant of dissociation of water or its solution.
The constant of dissociation of water at 22°C is equal 1,8°C10-16, that is degree of dissociation of water is very small. Therefore concentration of not dissociated molecules [H2O] can be considered a constant. At its expression in moths on 1 l it turns out:

$$[H_2O] = \frac{1000}{18} = 55{,}56 \; mol \; in \; 1l.. \qquad (2.2)$$

From the formula (2.1):

$$[H^+]\cdot[OH^-] = [H_2O]\cdot K = 55{,}56\cdot K = K_{w.} \qquad (2.3)$$

Thus, water and any aqueous solutions at a constant temperature, product of the concentrations of hydrogen and hydroxyl ions is a constant value, called the ion product of water. If the equation (2.3) instead of K substitute its value equal $1{,}8\cdot10^{-16}$, the product ion at 22°Cwe have:

$$K_w = 55{,}56\cdot1{,}8\cdot10^{-16} = 10^{-14} \qquad (2.4)$$

At dissociation of water the identical quantity of ions of hydrogen and a hydroxyl is formed; therefore, concentration of both ions in the distilled water at 22°C will be:

$$[H^+] = [OH^+] = \sqrt{10^{-14}} = 10^{-7}\,(g\cdot ion/l) \qquad (2.5)$$

ie 0.1 microgram per 1 liter.

Since the concentration of hydrogen ion and hydroxyl ion-linked product, they are of the conjugate. Therefore, any increase in the concentration of H + is suitable entails a decrease in the concentration of OH and vice versa. The concentration of one of the ions can be determined by knowing the concentration of the other. From the formula (2.5)

$$[H^+] = \frac{10^{-14}}{[OH^-]};\; [OH^-] = \frac{10^{-14}}{[H^+]} \;. \qquad (2.6)$$

Consequently, acidity, alkalinity, and the degree of oxidation and the alkalinity of the solution depends on the presence and concentrations of H + ions. If concentration of H+ of ions is equal to concentration IT - ions, solution is called neutral. In case $[H^+]>[OH^-]$, solution is called sour, at $[H^+]< [OH^-]$- alkaline.

For the characteristic of acidity or alkalinity of solution it is enough to calculate concentration of one ion. Usually (as it is accepted the international convention) define concentration of H+ (g·equiv/l). As concentration of ions of hydrogen with what deal in practice, is small, for convenience at records and calculations reaction of solution express a symbol pH.

$$pH = -lg[H+] \quad (2.7)$$

ie pH value - is the negative logarithm of hydrogen ion concentration. The concept of pH scale was first introduced by Danish chemist Soren Peder Lauritz Sorensen at the Carlsberg Laboratory in the year 1909.The reaction among all the solutions from highly acidic to highly alkaline can be expressed in a single pH scale ranging from 0 to 14.

For example, pH = 0 means that [H +] = 100 = 1, i.e. in 1 liter of solution containing 1 g of hydrogen ions in 1L etc.

In determining the reaction of the environment should not be forgotten that at the pH of the solution significantly affected by temperature, which depends on the increase in the degree of dissociation of water with it an increase.

Each ion plays a role in the biological and chemical processes. The special position among other ions take up hydrogen ions. They are essential for enzymatic activity. Each enzyme has an optimum of the action at a certain pH when the enzymatic reaction rate is at a maximum. Sometimes, the optimum pH is expressed very strongly, and the action of the enzyme appears only in a narrow range of pH values. Enzymes are proteins have an electric charge, so their structure is dependent on the pH. The concentration of hydrogen ions has a huge impact for the vital activity of organisms, the functional activities of individual animals and tissues of higher animals and humans. Diphtheria microbe better developed at a pH in the range 7.3-7.6; microbe Escherichia coli - at pH 6-7; active nitrogen fixation by soil microbes observed at pH = 7.2. Death of warm-blooded animals there comes a shift of blood pH at 0.03-0.04 units (normal blood pH 7,35±0,02). Reaction urine (urine pH) normal diet mixed with acidic or neutral (pH range 5.0-7.0). The response should be determined in fresh urine. Predominance of the animal protein in the diet gives a shift toward acidic, and the prevalence of plant food - towards the alkaline reaction. By fevers, diabetes, starvation, kidney failure, etc. urine has very acidic reaction. Alkaline urine is observed in cystitis and pielitah, hematuria, after vomiting and diarrhea, with resorption of exudates, when taking soda, mineral water.

Under the influence of hydrogen ions may change the basic physical and chemical properties of substances and solutions: solubility, surface tension, viscosity, stability, osmotic pressure, swelling, and others. All this is the cause of frequent determination of the concentration of hydrogen ions in chemistry, biology, medicine, agriculture and technology.

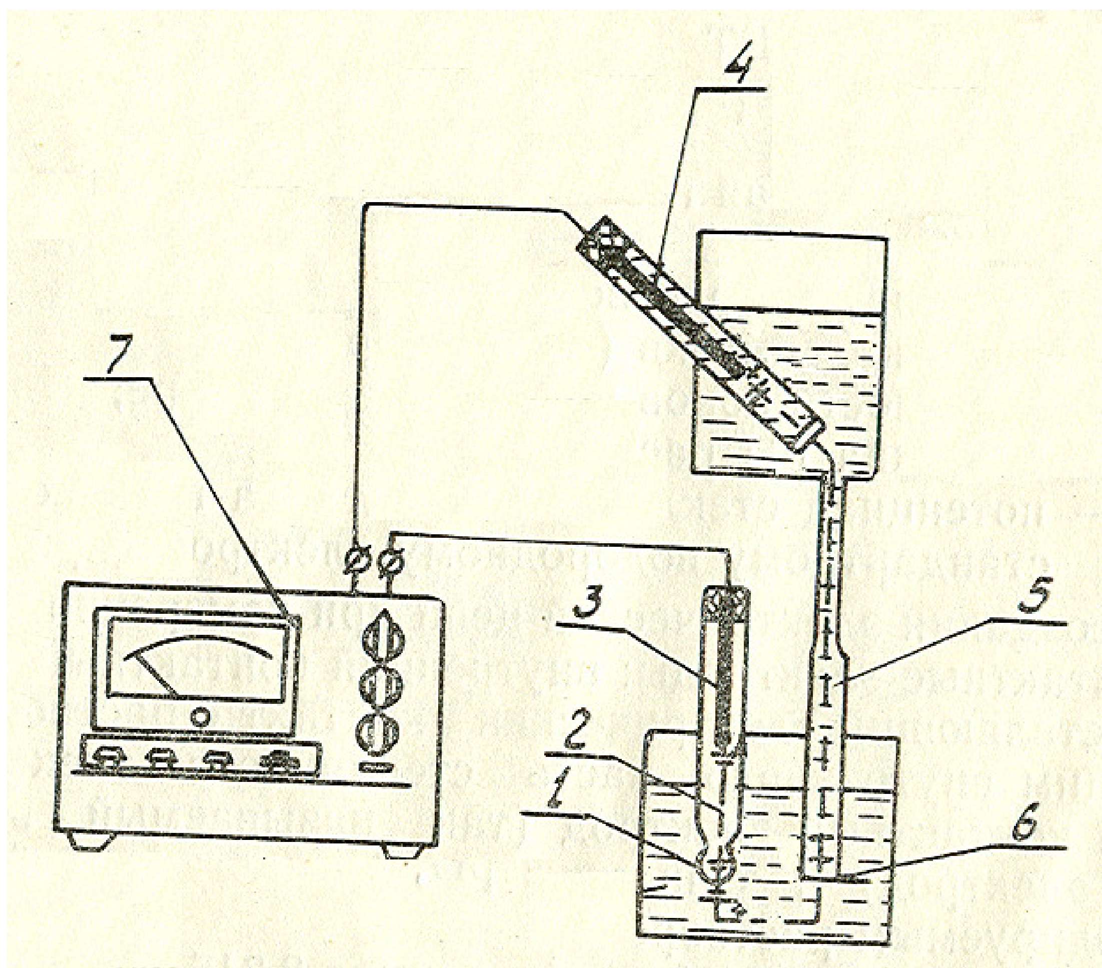


Figure 2.1 - Scheme of measuring the pH of the solution

1 - hollow ball of electronic glass; 2 - glass electrode;
3 - internal contact electrode; 4 - auxiliary electrode;
5 - Electrolytic key; 6 - a porous wall; 7 - pH meter

Under the influence of hydrogen ions the main physical and chemical properties of substances and solutions can change: solubility, superficial tension, viscosity, stability, osmotic pressure, swelling, etc. All told is also the reason of

frequent determination of concentration of hydrogen ions in chemistry, biology, medicine, agriculture and equipment.

Between a surface of glass and controlled solution there is a potential difference of E_x which size is defined by activity of hydrogen ions in solution and its temperature.

$$E_x = E_0 + \frac{RT}{F}\ln\alpha_h = E_0 - 2{,}3\frac{RT}{F}pH \qquad (2.8)$$

Where $\alpha_н$- the activity of hydrogen ions in solution; E_0 - glass electrode potential relative to a standard hydrogen electrode at $\alpha_н$=1.

Contact electrodes are applied to creation of an electric chain at measurement: the internal contact electrode 3 which is carrying out electric contact with the solution filling internal part of a glass electrode, and an external contact electrode (a so-called auxiliary electrode) 4, the carrying-out electric contact with controlled solution.

For protection against influence of high temperatures (at measurement pH solutions which temperature is higher than air temperature) the auxiliary electrode is placed out of controlled solution, and communication with it is carried out by means of an electrolytic key 5 - the tube which is coming to an end with a stopper with glass fiber 6.

Solution of chloride potassium continuously filters through glass fiber of a stopper, preventing penetration from controlled solution into system of an electrode of 4 foreign ions which could change electrode potential size.

The electromotive force of the electrode system is equal to the algebraic sum of the potentials of the contact electrodes E_c and E_{aux}, potential occurring on the inner surface of the glass electrode and a defined pH internal solution E_{int} and potential arising on the outer surface of the glass electrode E_c. The values E_c,E_{aux} and E_{int} does not depend on the composition of the control solution and only change when the temperature changes. The total electromotive force of the electrode depends linearly on the pH of the solution. By measuring the emf an electrode system with an electronic millivoltmeter 7, a scale which is graduated in units of pH, determine the pH-controlled solution.

Laboratory work № 3

pH of various solutions and biological liquids

Objective: Experimentally find pH values of various solutions and biological liquids.

Tasks:

1. Calibrate the device on standard solutions.
2 Measure pH of various solutions and biological liquids.
3. Draw graphs of pH values of various solutions, and biological and non-biological liquids.
4. Make conclusions about the observed phenomena and prepare a report.

Equipment and Materials: pH meter, nitric acid, alkali, solutions of proteins, gastric juice, urine, distilled water, vessels.

Procedure:

Rinse thoroughly with electrodes; the purity of the electrodes is judged by the pH of distilled water. Electrodes may be used for research in case, if pH value of distilled water close to 6,0-6,3.

Determine pH value of the following solutions: gastric juice diluted (5-fold) gastric juice protein solution, an acidic solution, an alkaline solution, tap water, distilled water. Measuring the pH of the sample solution should be performed at least in triplicate. Before replacing of the sample solution the electrodes should be thoroughly washed. Record these pH values in the table 2.3.1.

Table 2.3.1

Solution	Hydrogen ionization value			
	First measurement	Second measurement	Third measurement	Average measurement
The distilled water Tap water Alkaline solution Sour solution Protein solution Juice The diluted juice Urine				

Based on these data, you must specify what determines the specific pH of each solution.

Report design. The results bring to the table and to construct graphics (histograms). Define measurement error and to draw conclusions.

Laboratory work № 4
Determination of pH of fermented milk products using a pH meter

Objective: pH of various solutions and of biological fluids.

Tasks:

1. Perform calibration standard solutions.

2. To measure the pH of various solutions and biological fluids.

4. Build a graphic pH of various solutions, and biological and non-biological fluids.

5. Make conclusions about the observed phenomena and prepare a report.

Equipment and materials: pH meter burette 1 and 50 ml, 8-10 cups 50 ml of 0.01N. sulfuric acid, distilled. water, yogurt (or any other dairy products)

Task 1. Determination pH of the solution with sulfuric acid of various concentrations

Procedure: you need to determine the pH of 0.01 n. sulfuric acid solution. Then prepare a dilution at two, four and eight times, and carry out measurements. Plot the pH of the test solution concentration.

Task 2. Determination pH of fermented milk products with different concentrations

Procedure: Measure the pH of the original dairy products. Then prepare a dilution at two, four and eight times, ten times, and carry out measurements. Plot the pH of the test solution concentration.

To explain the observed differences in the results and graphs.

Table. pH values

	Initial value	Dilution 1/2	Dilution 1/4	Dilution 1/8	Dilution 1/10	Dilution 1/20
Sulfuric acid						
Dairy product						

Chapter 2 "Physical-chemical methods" questions

1. What is the pH of solutions and how to determine its value?
2. Why does the pH change?
3. What instruments allow us to estimate the pH
4. How big limits could change pH in living objects?
5. Explain, how ph-meter measure pH value.
6. What substances are buffering?
7. Describe the dissociation of the peptide.
8. Describe the dissociation of sulfuric acid, for some reason it does not have buffer properties?

9. Explain biological significance for constancy of pH within each cell, and in all body fluids.
10. Why diluted fermented milk product solution's pH does not change much, while a dilution of sulfuric acid (or salt) in 10 times pH changes a lot?

CHAPTER 3

BUFFER PROPERTIES AND BUFFER CAPACITY OF BIOLOGICAL LIQUIDS

The constancy of the hydrogen ion concentration of blood and other body fluids is provided by a number of mechanisms. Despite the many processes that result can change the internal environment of the reaction, the pH is constant. In warm-blooded animals, constancy of pH of the internal environment is maintained during the life of a very narrow range, the deviation does not exceed 0,03-0,04 units. (in human blood pH is 7,35±0,02).

The shift reaction medium leads to a change in many physical and chemical indicators of protein nuclein, and protein lipid complexes - the magnitude of the charges, the extent of hydration, stability of colloids, viscosity, electrical conductivity, etc. Changes in biochemical reactions lead to disruption of conjugation of many biochemical processes that may be the cause of death of the organism.

However, in a normally functioning blood in the living body continuously it receives the acid products of working organs and the gastrointestinal tract. Alkaline reaction products enter the body mainly in food. To maintain a constant internal environment include buffers which neutralize both the acid and alkali in the received blood products stably maintain the reaction medium (hydrogen ion concentration) at a predetermined level. Buffer systems are presented with proteins or systems consisting of a weak acid and a strong base salt of this acid (H_2CO_3 and $NaHCO_3$; H_3PO_4 and Na_2HPO_4; H_3PO_4 and Na_3PO_4 etc.).

The main role of proteins in the body member which are amphoteric compounds, and proteins from the blood hemoglobin plays a specific role, in which human blood contains up to 14% of its weight. In second place is the carbonate system in the third - a phosphate buffer system.

Of course, each buffer system may be kept constant pH within certain limits, which depend on its buffer capacity. Under the buffering capacity to understand the amount of 0.1N acid or alkali which is needed to shift the pH unit. An alkaline buffer is contained in the serum is about 5 times more than the acid, i.e. the acid can be neutralized to 5 times greater than the alkali. This means that the body is better protected from disturbances in blood entering the acidic products.

Laboratory work № 5

Definition of alkaline and acid buffers of serum blood

Objective: Buffer properties of serum

Tasks:

1. Carry out calibration of the device on standard solutions.
2. Measure pH of blood
4. Draw graphs of pH values
5. Make conclusions on the observed phenomena and do the report.

Equipment and Materials: pH-meter burette 1 and 50 ml, 50 ml cups, 0.1N solutions of hydrochloric acid and potassium hydroxide or sodium, 0.02% methyl orange solution, 0.1% ethyl alcohol solution phenolphthalein, serum.

Task 1. Acid buffer.

Procedure. Pour into a glass 5 ml of the distilled water and add 2-3 drops of methyl orange indicator, which has a transition zone pH = 3,1-4,4. Titrate drop by drop from a burette 0.1 N hydrochloric acid solution until a slight red coloration (1 drop of acid).

Pour into another glass 5 ml of the distilled water, add 1 ml of serum and 2-3 drops of methyl orange and titrated to the same stain; count the number of drops, or determine the amount of 0.1N hydrochloric acid solution that spent to the titration.

Calculation: for acidification 5 ml of water was spent **A** ml of 0.1 N hydrochloric acid (1 drop - 0.03 ml of 0.1N hydrochloric acid or alkali). By adding 1 ml of serum was spent **B** ml of hydrochloric acid. Thus, for 5 ml serum would be spent: (B-A × 5ml 0.1N Hydrochloric acid). In the calculations of the buffer capacity of the water can be ignored. Make a calculation for 100 ml of serum, which would be its buffer capacity. The results bring to the table 3.4.1.

Normally, for serum it is necessary to add 250-300 times more hydrochloric acid than for water.

Task 2. Alkaline buffer.

Procedure.

Pour into a glass 10 ml of the distilled water and add 2-3 drops of a phenolphthalein; transition zone pH =8,9-9,8. To titrate 0,1 N solution of caustic sodium before appearing weak-violet coloring (usually 1-2 drops).

In the second glass to pour 10 ml a dist. waters to add 1 ml of serum and 2-3 drops of a phenolphthalein. To titrate 0,1 N solution of caustic sodium before the same coloring, as in the first case, counting quantity of drops or measuring volume. Write the results in table 3.4.1.

Table 3.4.1 - Acidic and alkaline buffers serum

	5 ml distilled	5 ml of a distilled water + 1 ml of	For 5 ml of serum	The alkaline buffer

	waters	serum		
Number of 0,1 N of the HCl solution which went for titration				
	10 ml distilled waters	10 ml of a distilled water + 1 ml of serum	For10 ml of serum	The acid buffer
Number of 0,1 N of the KOH solution which went for titration				

Calculation. For change (shift) reaction of 10 ml distilled water is necessary D drops of alkali. At addition of 1 ml of serum for neutralization of the acid buffer is required E ml alkali, so for 10 ml of serum will be required 10×(E-0,03D) ml alkali. Usually to serum it is necessary to add at 50-60 times more alkalis to cause identical change of reaction.

Report design. Write results in the table, make calculations and draw figures (histogram). Calculate standard error of measurements and make conclusions.

Laboratory work №6

Buffer capacities of biological liquids

Objective: the buffer capacity of serum of blood or urine

Tasks:

1. To carry out calibration of the device on standard solutions.
2. To take pH measurement of blood.
3. Draw figures of pH values.
4. Make conclusions on the observed phenomena.
5. Make conclusions and write report.

Equipment and materials: pH-meter, burette 1 and 50 ml, 50 ml cups, 0.1N solutions of hydrochloric acid and potassium hydroxide or sodium, 0.02% methyl orange solution, 0.1% ethyl alcohol solution phenolphthalein, serum.

Task 1. Determination of buffer capacity of serum or urine.

Procedure: measure pH of biological liquid, then titrate the studied solution (volume of 100 ml) in the small portions of acid or alkali and write down that quantity of a reactant which is necessary for change pH on 0,1 units of a scale. Titrate until reaction will change on 2 units of pH (concentration of ions of hydrogen changed in relation to initial by 100 times). For calculations of own buffer capacity take that quantity of a reactant which is necessary for change pH on unit, i.e. concentration of ions of hydrogen has to change by 10 times.

Change pH to 7,2 and 7,55 is a sign of the most serious condition which is usually coming to death of an organism. It is necessary to know not only the size of buffer capacity at a concentration deviation by 10 times from normal value, but it is much more important to know that amount of acid and alkali which is necessary for a deviation pH to value 7,2 and 7,5. Besides, it is important to know the course of curve dependence pH from amount of titrable substance (acid and alkali). For this purpose it is necessary to remove dependence of change pH solutions (protein, serum of blood, milk, urine) from amount of the added decinormal acid or alkali. Titration should be carried out before achievement of pH values 10-11,0 in alkaline area and to 2,0-1,5 in the sour.

According to the obtained data to construct the schedule of dependence of size pH from quantity of a reactant (fig. 3.5.1)

Determination of buffer capacity of blood

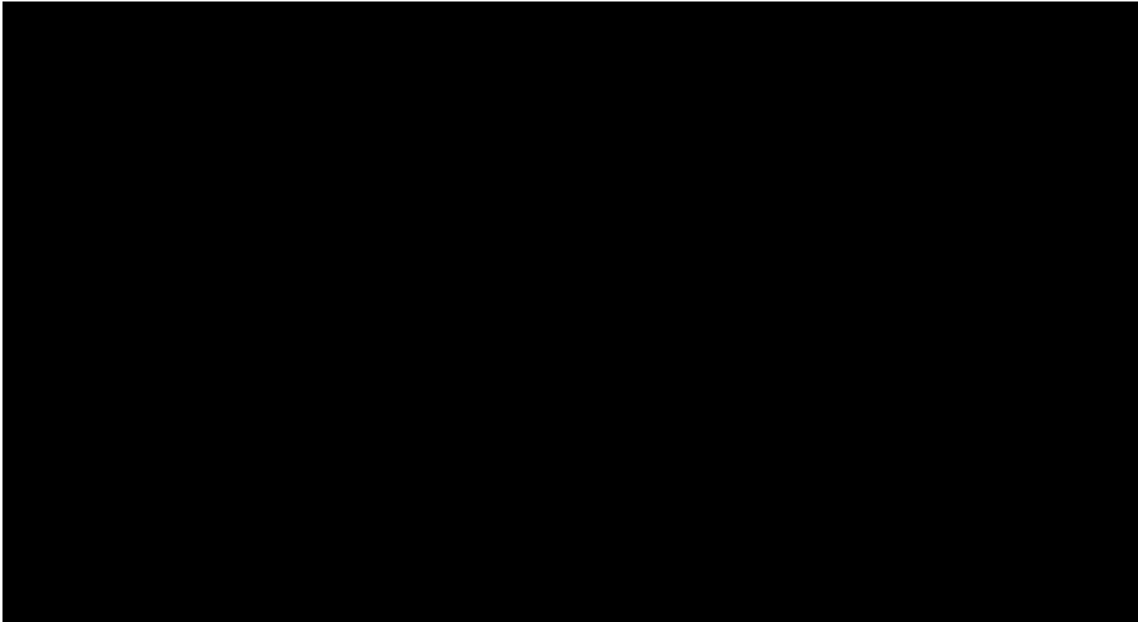


Figure 3.6.1.
Designations: on abscissa axis – amount of the NaOH or HCl solution, on ordinate axis – pH value

Task 2. Buffering capacity of hemoglobin.
Procedure: Take 1 ml of packed red blood cells (blood centrifuged 20 minutes at 5000 rpm), wash twice with saline or 5.4% glucose, and 10.3% aqueous solution of sucrose and add to 10 ml of distilled premeasured water with pH. Using a pH meter to measure the concentration of hydrogen ions after the full osmotic erythrocyte hemolysis (appears quite intense staining in the red, and the solution becomes completely transparent). If hemolysis occurs slowly, increase the temperature in the beaker to 40-50°C. Then produce titration with 0.1N. HCl solution. Take a new batch of packed red blood cells, hemolyze and titrate with 0.1 N NaOH solution to pH shift by 1. Perform per 100 ml of packed red blood cells. This will be the quantity of alkaline and acidic buffer capacity respectively.
Report design. Write these results in the table to make calculations and plot graphs (bar graphs). Define measurement error and draw conclusions.

Chapter 3 "Buffering properties and buffer capacity of biological fluids" questions

1. What is the pH of the solutions and how to determine its value?
2. Why does the pH change?
3. What instruments allow us to estimate the pH

4. How big limits of pH changes in living objects?
5. What substances are buffers?
6. Describe the dissociation of the peptide.
7. Describe the dissociation of sulfuric acid, for some reason it does not have buffer properties?
8. Explain biological significance for constancy of pH within each cell, and in all body fluids.

Chapter 4
THE OXYGEN CONTENT IN WATER

The concentration of oxygen determines the value of the redox potential and largely direction and rate of processes of chemical and biochemical oxidation of organic and inorganic compounds.

Oxygen regime has a deep impact on the life of the reservoir. The minimum content of dissolved oxygen that ensures the normal development of fishes is about 5 mg O_2/l. If this measure decreases until 2 mg/l, it will cause massive lethality of fishes. Supersaturation of the water with oxygen also adversely affects its condition. Threshold limited value of dissolved oxygen for the water objects of fishery purposes in the winter period is 4 mg/dm^3 , in the summer – 6 mg/dm^3.

Determination of oxygen in surface waters is included in the program of observations in order to assess the living conditions of aquatic organisms, including fish, indirect water quality characteristics, intensity of processes of producing and destruction of organic matter, self-purification of reservoirs, and so on.

Concentration of oxygen is expressed either in milligrams per liter or as a percentage of saturation, the calculation of oxygen is made according to the formula

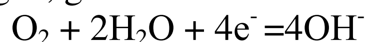
$$O_2\% = \frac{C_x 100 \cdot 760}{C_0 p}, \tag{4.1}$$

where, C_x - concentration of oxygen found experimentally, mg/l; C_o - normal concentration at a given temperature, normal and atmospheric pressure 760 mm (found from Table. 4.1); p - atmospheric pressure at the time of analysis.

The principle of measuring the concentration of oxygen.

Amperometric method of analysis forms the basis for measuring the concentration of dissolved oxygen. The oxygen concentration is determined by current intensity, flowing in the circuit of the electrode system of the sensor O_2.

Electrodes, cathode and anode, of O_2 sensor are located in the electrolyte solution and separated from the analyzed liquid by gas permeable membrane. The oxygen freely diffuses through the membrane and electrolyte to the electrodes, which are under the constant voltage coming from the source of polarizing voltage of the device. The current, which is caused by a reduction reaction of molecular oxygen, generates in the electrode circuit and flows by the following scheme:



$O_2 + 2H_2O + 4e^- = 4OH^-$

The current is converted into voltage, which is measured and, in turn, is converted into a value of concentration of oxygen.

Preparation and conducting of measurements.

Measurements of the concentration of dissolved oxygen are produced by special sensors. Sensors "generate" the current that is proportional to the oxygen concentration in the environment. The conversion coefficient depends on several factors and therefore requires periodic calibration of the sensor.

Calibration - put of parameters of the measuring channel sensor → converter, current-voltage of the device obtained in solutions with known concentrations of oxygen into the memory of the device.

Such relatively easy reproducible and available solutions are solutions with zero (0%) and one hundred percent (100%) of the dissolved oxygen concentration.

Calibration of electrochemical analyzers of oxygen.

Zero point calibration.

"Zero-solution" can be used as a standard sample with a zero content of oxygen. Beforehand, a solution of sodium sulfide (Na_2S) with temperature 20±0,5 °C and concentration of 80 g/l is prepared. The solution must stand after cooking for at least 8 hours. In another option for making "zero-solution 10±0,5 g of sodium sulfite (Na_2SO_3) is dissolved in 200 ml of tap water and add 20-50 mg of soluble salts of silver or cobalt (e.g. cobalt chloride - CoCl2). Addition of salts of heavy metals is used as the catalyst for the oxidation of sodium sulfite. After 10 minutes, "Zero-solution" may be used for calibration.

Place the sensor in an environment with zero oxygen content, and to calibrate the zero point of the device.

Calibrating by the air.

At this calibration atmospheric air, which is saturated with water vapor, can be used as a standard sample with known oxygen content.

Place the sensor into a bottle with a small amount of water so that the sensor does not touch the water. Calibrate the top point of the device.

The quality of the calibration and accuracy of the results of subsequent measurements is significantly affected by the temperature measurement accuracy. During measurement should not forget about another important factor - the pressure. Atmospheric pressure has a directly proportional effect on the solubility of oxygen in liquids, freely contacting with atmospheric air, so it must take into account the values during the calibration and measurement.

When working with the amperometric sensor it should be cautious, protecting the glass sleeve from impacts. It is not allowed to apply mechanical stress to the cable. For prolonged storage, when amperometric sensor is off (for more than 6 months) it is necessary to drain the electrolyte solution, wash the corps of the sensor with distilled water and put it on the amperometric sensor.

Table 4.1 - The solubility of oxygen in distilled water, saturated with air at a pressure of 101.3 kPa (760 mm Hg).

°C	mg/l	°C	mg/l	°C	mg/l	°C	mg/l	°C	mg/l	°C	mg/l
0	14,62	8,5	11,73	17,0	9,74	25,5	8,30	34,0	7,20	42,5	6,35
0,5	14,43	9,0	11,59	17,5	9,64	26,0	8,22	34,5	7,15	43,0	6,30
1,0	14,23	9,5	11,46	18,0	9,54	26,5	8,15	35,0	7,10	43,5	6,25
1,5	14,03	10,0	11,33	18,5	9,44	27,0	8,07	35,5	7,05	44,0	6,20
2,0	13,84	10,5	11,21	19,0	9,35	27,5	8,00	36,0	7,00	44,5	6,15
2,5	13,66	11,0	11,08	19,5	9,26	28,0	7,92	36,5	6,95	45,0	6,10
3,0	13,48	11,5	10,96	20,0	9,17	28,5	7,85	37,0	6,90	45,5	6,05
3,5	13,31	12,0	10,83	20,5	9,08	29,0	7,77	37,5	6,85	46,0	6,00
4,0	13,13	12,5	10,72	21,0	8,99	29,5	7,70	38,0	6,80	46,5	5,95
4,5	12,97	13,0	10,60	21,5	8,91	30,0	7,63	38,5	6,75	47,0	5,90
5,0	12,80	13,5	10,49	22,0	8,83	30,5	7,57	39,0	6,70	47,5	5,85
5,5	12,64	14,0	10,37	22,5	8,76	31,0	7,50	39,5	6,65	48,0	5,80
6,0	12,48	14,5	10,26	23,0	8,68	31,5	7,45	40,0	6,60	48,5	5,75
6,5	12,33	15,0	10,15	23,5	8,61	32,0	7,40	40,5	6,55	49,0	5,70
7,0	12,17	15,5	10,05	24,0	8,53	32,5	7,35	41,0	6,50	49,5	5,65
8,0	11,87	16,5	9,84	25,0	8,38	33,5	7,25	42,0	6,40		

When there are shorter work breaks, and between the series of analyzes, amperometric sensor is preferably stored in water vapor. In order to do this, select one flask, pour 10-15 ml of distilled water and set amperometric sensor without touching the sensitive part of the sensor to the surface of the flask. Storing the sensor in this position (in water vapor) between measurements and long periods of time can significantly increase the time of routine service of sensor.

Laboratory work №7

Determination of the oxygen content dissolved in water

Objective: Measurement of the oxygen content in the water.

Tasks:

1. To perform calibration of device on standard solutions.
2. To measure the amount of oxygen in the water
4. To build a graphic of values
5. To make conclusions about the observed phenomena and to prepare a report.

Equipment and materials: Laboratory amperometric analyzer, bottles of 300 ml, microcompressor, barometer, thermometer, 8% solution of sodium sulfide, salt $CoCl_2$, electro-mechanical mixing device.

Procedure:

1. To perform the necessary training and the setting of the device (see. Chapter 4);
2. To prepare the workplace, distilled and tap water. To rinse the sensor and detector with distilled water;
3. To lower the detector and the sensor into the solution, stir it to accelerate the achievement of temperature regime. Turn on the device;
4. To turn on the electromechanical mixing device and after 5-7 minutes remove the instrument readings;
5. To measure the temperature of the test sample;
6. To measure the atmospheric pressure;
7. To turn off the electromechanical mixing device.

Task:

1. To measure the oxygen content in distilled water;
2. To measure oxygen content in the distilled water saturated with oxygen in the air for 30 minutes – barbotage;
3. To measure the oxygen content in tap water.

Report design. To make all calculations by all measurements of the oxygen content of the samples taking into the factor of atmospheric pressure, using the formula 4.1 and Table. 4.1. Compare measurement results and make conclusions.

Laboratory work №8

Amperometric method of determining the rate of respiration of aquatic plants and yeast cells

Objective: To determine the intensity of respiration of aquatic plants, yeast suspension in normal conditions and in the action of various factors.

Tasks:

1. To carry out calibration of device.
2. To measure the amount of oxygen in water and the suspension of yeast cells.
3. To measure amount of oxygen in Chlorella.

4. To build a graphic of values

5. To make conclusions about the observed phenomena and to write a report.

Equipment and accessories: Device for determining the oxygen content in water samples, 300 ml bottles, the AEN-microcompressor type of 4-3 illuminator OH-32; 8% Na_2S solution, distilled water, a pipette for 1, 10 ml, 40% formalin solution, elodea, 5% suspension of yeast cells, chlorella, magnetic stirrer.

Respiration – an obligatory condition of life. It ensures metabolism and energy underlying the life of any organism. This is a process of oxidation of biosynthesis products and photosynthesis (chlorophyll in plants), which consumes oxygen and produces carbon dioxide.

For normal growth of yeast cells in aqueous medium requires the dissolved oxygen. Since yeasts are a group of facultative anaerobes, the process of respiration in these biological objects can be switched on fermentation depending on the conditions (absence of oxygen, the activity of reproduction). Intensity of respiration is expedient to estimate on oxygen consumption. In the chlorophyll-containing tissues, the measurement should be carried out in the dark, as in photosynthesis the gas exchange is in the opposite direction (CO_2 absorption, O_2 isolation). Since the processes of gas exchange involves molecular oxygen and water plants and yeast cells use exactly molecular oxygen, dissolved in water, the method of amperometric determination of oxygen is suitable.

Amperometric method for determining the concentration of substances is a special case of voltammetry at all. This method of determination is based on measuring the stationary current limit (depolarization) of oxygen diffusion. This method is applicable if the concentration of the test substance is not too small, and if there is no other substances in solution, which can react in the working range of potentials. For the analysis, the detector is used to determine the concentration of dissolved oxygen in aqueous solutions. The oxygen in the sample solution diffuses through the membrane, which, in turn, depends upon Fick's law the concentration of dissolved oxygen in the sample solution.

Procedure:

1. To expose the "0" of the device for 8% solution of Na_2S (see. Chapter 4). Configure the device.
2. To measure oxygen content in the water saturated with oxygen of the air during 30 minutes.
3. To measure oxygen content in the distilled water.
4. To add 2 ml to the water under study 5% suspension of yeast cells, the oxygen content is recorded every 30 seconds for 3 minutes. Create a table.
5. To increase the concentration of yeast cells (3 ml + 5% suspension) in the sample liquid, the oxygen content recorded every 30 seconds for 3 minutes.
6. To record the change in intensity of respiration of yeast cells by the action of

the inhibitor (40% formalin solution) for 3-5 minutes.
7. To record the oxygen content in water wherein water plants were kept in the dark (at least 1 day) and light. Carry an extra light. To build a graph of the intensity of the release of oxygen for 10-20 minutes.
8. Record the oxygen content in the Chlorella suspension, kept in the dark (not less than one day) in the light with additional illumination.

To calculate the oxygen content based on factors as atm. pressure and temperature (see. Chapter 4, the formula 4.1, Table 4.1).

Report design. Calculate the rate of absorption (release) of oxygen (respiration rate), to analyze the data. These results add to the table to make calculations and construct graphs (bar graphs). Define measurement error and to draw conclusions.

Laboratory work №9
Study of electrical parameters of the solutions and wash water of seeds

Objective: to measure electrical parameters of the solutions and washing water of seeds. To identify the correlation between the viability and electrical parameters of washing water of seeds.

Tasks:

1. To study the electrical conductivity of solutions depending on temperature (to measure the electrical conductivity of water at different temperatures (3-5°C, 18-20°C, 40°C);
2. To study the conductivity of solutions depending on the concentration of the KCl solutions (1 n and 0.1 n, and 0.01 n).
3. Record the results of measurements in the table.

Equipment and materials: conductivity meter type 5721, 1 N KCl solution, 0.1 N. KCl solution, 0.01 N. a solution of KCl, wheat seeds and soybean, laser HeNe, pharmacy scales, glass cups.

The study of electrical properties of seed is associated with a number of difficulties, chief of which is the high resistance of seeds, which can vary widely (from ten kOhm to mOhm), respectively, the resulting potential is characterized not only by small quantity but also large scatter. One way is to study the electrical parameters of the wash water of the seed, by which one can assess the degree of viability of the seeds. The vitality of seeds is understood as the content of viable seeds in seed material (expressed in percentages), able to germinate under favorable conditions. It is established that the loss of viability determined by the

ability of seeds to emit those or other substances when soaking. Thus, after conditioning of seeds of legumes for 6 h in distilled water, it was found to contain amino acids, sugars, organic acids, mineral substances.

Corn seeds, germinating in water for 4-36 h emit glucose, fructose, cystine, aspaRAGine, etc. into the environment. And the nature and intensity of substances emitted from the seeds depend on their viability and the availability of oxygen. Immature seeds and normal seeds under anaerobic conditions were characterized by the maximum number of emitted substances in the period between 24 and 48 h and 72-84 h. Obviously, the emition of significant amounts of free amino acids, organic acids and sugars from the seeds during their swelling is due to changes in the permeability of membranes and cell wall in the case of non-viability of seeds and metabolic disorder caused by prolonged anaerobiosis. However, the release of substances up to a certain limit is a physiological process regulating seed germination, stimulating or inhibiting them through the allocation of biologically active substances (phytohormones, inhibitors, etc.). The ability of seeds to selectively emit substances in the water depending on the degree of viability, chemical composition, structure and other properties allows its use as a diagnostic test. As a result of active transport of substances against electrochemical gradients the induction of the electric current occurs, the value of which can be measured.

Procedures:

Battery and network meter No. 5721 together with promote conductometric sensor No. 5981 designed for quick and easy changes of conductivity (electrolytic conductivity) aqueous solutions and their temperature.

1.To include the unit in and press the WL. The arrow should be on the right side of the measuring field. If the arrow is on the left, it indicates the insufficient battery level and the need to replace (1.5 V).

2.Press the button BAT. When you click the arrow of the meter must be marked BATERIA.

3.Press the keys T and K (the direction denoted KAL) and after stabilization of meter readings regulate the potentiometer through the hole on the right side of the side of the case so that the arrow will set at 100 divisions (upper scale of the meter).

4.Press keys T and K.

5.To the nest, marked WE, connect the sensor No. 5981.

6.Press the button K and by handle REG K set on the top (red) scale the value of the constant K=1.0 cm-1.

7.Overcome the button K.

8.Connect the TP Pt 100 temperature compensator , to lower it into the solution, press the button marked °C and make a counting on the 0-100 scale divisions of measured temperature.
9.Overcome the button °C.
10.Press the button T and set the measured temperature by handle REG T (scale 0-100 div.).
11.Start the measurement of inductance.
a) to place the sensor in the test solution so that the distance of the electrode from the bottom of the vessel will be more than 2 cm.
b) press the button S/m and to select the desired measurement range by a rotary switch.

For measurements in the range 0.3-30 M S/m (left side of the switch) the button indicated must be pressed, and for the measurement of 0.1-10/m – key must be overcame. The upper scale of the meter is (0-100 div) in the range of measurements 0,1; 1; 10; 100. The limits 0,3; 3; 30 are the lowest scale 0-30 divisions.

In case of failure of operation of the compensator measurements can be done without temperature compensation. To do this press T and by handle, marked T REG set the arrow of the meter on 25° (a scale of 0-100 div.).

To measure conductivity according to item 11b. The counted value corresponds to conductivity of measured solution at 25°C. After each measurement the sensor should be carefully rinsed with distilled water.

Report design: Enter the results into the table, make calculations and construct graphs (bar graphs). Determine measurement uncertainty and make conclusions.

Chapter 4 “The oxygen content in water” questions

1. What is the basis of amperometric method for determining the oxygen content?
2. In what kind of conditions the intensity of respiration of plants should be measured?
3. How does formaldehyde affect the kinetics of yeast cell respiration?
4. What types of indicators depends on the solubility of oxygen in the water?
5. What are the two basic methods to determine the concentration of oxygen?
6. What is the concentration of oxygen observed in natural bodies of water?
7. What effect does the change in the oxygen content on the aquatic organisms?
8. What is the concentration of oxygen which causes the death of aquatic organisms?

Chapter 5.
PHOTOMETRIC METHODS OF BIOLOGICAL SYSTEM'S RESERCH

When light passes through a layer of colored substance, part of it is reflected, absorbed, and part of the light passes through substance layer. The intensity of the incident light flux, passing through the absorbing solution, is decomposed into components:

$$I_0 = I_s + I_f + I_t + I_r$$

where, I_s - the scattered light (in the case of the dispersed system)
I_f - luminous flux absorbed by a colored substance;
I_t - the luminous flux passing through the layer of material;
I_r - reflected light.

During comparative measurements of light absorption by various solutions the same cuvette is used, for which the intensity of the reflected light flux is constant and small; the loss of light due to scattering in dealing with true solutions also becomes negligible. Light attenuation is mainly due to the absorption of light energy Ip by colored solution.

The absorption of light is manifested in the weakening of the light flux after passing through the test object, and it is greater due to higher concentration of the substance (s, moll-1), the thickness of the solution (l, cm), ability of substances to absorb. For monochromatic light these patterns are expressed by the law of Bouguer-Lambert:

$$D = \lg\frac{I_0}{I_t} = \varepsilon \cdot c \cdot l$$

Where, D- so called optical density of sample, I_0 and I- intensity of falling and transmittingof light. Value ε - ($l*mol^{-1}*cm^{-1}$) is called the *molar coefficient of absorption*. If we replace decimal logarithm to natural logarithm and express the concentration in number of molecules per 1 cm3, so instead of *εcross Chapter absorptions* is used (cm2), associated with ε relation: s=3.8*10-21ε. Physical sense of S— effective cross-Chapter of molecule during the investment of which, the absorption of photon at certain wave length occurs.

The dependence of optical density D of an object, such as a solution of the wavelength of light λis measuring the absorption spectrum of the object. The spectrum of the solution of the individual connections is made to normalize the concentration and the length of the cuvette, i.e. the absorption spectrum of the individual substance is called the dependence on the wavelength of the measuring molar light absorption coefficient ε.

The spectrum of the solution of the individual compounds made to normalize the concentration unit and the length of cuvette; ie, the absorption spectrum of the

individual substance called dependence on the wavelength of the measuring molar light absorption coefficient ε.

The absorption spectra of the amino acids are located in the ultraviolet region; in the visible region the amino acid residues do not absorb. In the near-UV range spectrum only some of the amino acids have absorption.

Among them - aromatic: tryptophan, tyrosine, phenylalanine, besides having a main maximum absorption in the far UV range of the spectrum (210-220 nm), the second - specific to each of the amino acids: the maximumabsorption is in the region of 260-280 nm.

In the near-UV range also characteristic absorption bands of sulfur-containing amino acids cysteine and cystine are located.Despite the strong overlap of the absorption bands of individual amino acids in the near UV range, they differ markedly from one another in the position of the maxima, band-width, extinction coefficient.

Amino acids in the free state contains free carboxyl groups and amino groups, and therefore their absorption spectra may vary with changes in pH.

So, in tyrosine due to pH change occurs the ionization of the phenolic group, which resulted in a maximum shift of the absorption spectrum and changes the molar extinction coefficient: in 0.1n HCl spectrum has a maximum value at 275 nm and E = 1300, then in 0.1 NaOH a maximum value is at 293 nm, and E = 2400.

Qualitative spectrophotometric analysis is based on the fact that each compound has a characteristic to it’s absorption spectrum. The following parameters are most important to identify the substance:

1) the number of maximums in the absorption spectrum; 2) position (wavelength) of each maximum; 3) absorption coefficients in each of the maximums (in units of s or ε); 4) the amplitudes ratio of maximums (ie, the ratio of the absorption coefficients at the maxima), if they are several (i.e.more than one)

Laboratory work№ 10
Working principle of photoelectrocolorimeter.

Objective: To study the work of the photoelectrocolorimeter.

Tasks:

1. To familiarize with the methodology of work with the photoelectric colorimeter CPC-2MP.
2. To tabulate the results.
3. To construct a calibration curve for an analyzing substances.

Equipment and materials: photoelectric colorimeter CPC-2MP, pipette for 5 and 1 ml, solution of methylene blue with following concentrations: 0.0005%, 0.0006%, 0.00075%, 0.001%, 0.00125%, 0.0015 %, 0.0025%, 0.005%.

Photoelectric colorimeter CPC-2MP is designed to measure individual Chapters of the wavelength range 315-980 nm, allocated by light filters, the transmission coefficients and optical density of liquid solutions and transparent solids, as well as the measurement of the substances concentration in solution after the preliminary determination of calibration characteristics by the user.

The principle of operation of colorimeter is based on alternately measuring the light flux transmitted through the solvent or control solution, in relation to which the measurement is made, and in relation to the flux passing through the analyzing medium.

Luminous flux photodetectors are converted into electrical signals which are processed by microcomputer colorimeter and reported on a digital display as transmittance, optical density, concentration, activity.

Description of the installation. Colorimeter consists of a colorimetric (1), computing (2) and power supply units (Fig.5.10.1).

The colorimetric unit includes: illuminator, the optical node, optical filters, compartment cuvettes, Cuvette Holder, the photometric device with direct current amplifier and elements of regulation.

And optical filters are entered into the light beam by handle 3.

Cuvette is settled in cuvette holder with the analyzing solution and control solution. Commissioning of one or another cuvette into light beam is made by turning the knob 4 completely to the left or right (to position 1 or 2).

In position "1" the cuvette with the solvent is entered into the light beam, in position "2" - with the analyzing solution. Sample compartment is closed by lid 4.

The photometric device includes a photoelements, photodiode, photosensitive plate amplifier. Switching of photodetector is carried by the handle 5.

The computer unit 2 includes a microprocessor "CMC Electronics 81201". The front panel is a keyboard, digital display and two signal LEDs. The keyboard has 24 keys.

Key START is used to start the microprocessor. Keys "Ь " and "C" –to call memory values on the digital display in corresponding to the coefficients to control or enter new values. СБР is key to erase the value caused by the coefficient. Keys "0", "1-9", "-", "" - set for the digital display of the new values of "Ь " or "C".

Key UTB (УТВ) is designed for recording in the memory of the new values dialed on the digital display. Keys "K (1)", "τ (2)", "D (5)" - for the measurement

of the transmittance, absorbance of the test substance, the concentration of the substance in solution, as well as the calibration of device.

Key "A (3)" is intended to measure the activity. Key "C / F" is designed to transfer one of the two modes of measurement: a mode of single measurements, or cyclic mode of measurements (with a period of 5 seconds). Key "W (0)" is designed to verify the measurement of "zero point".

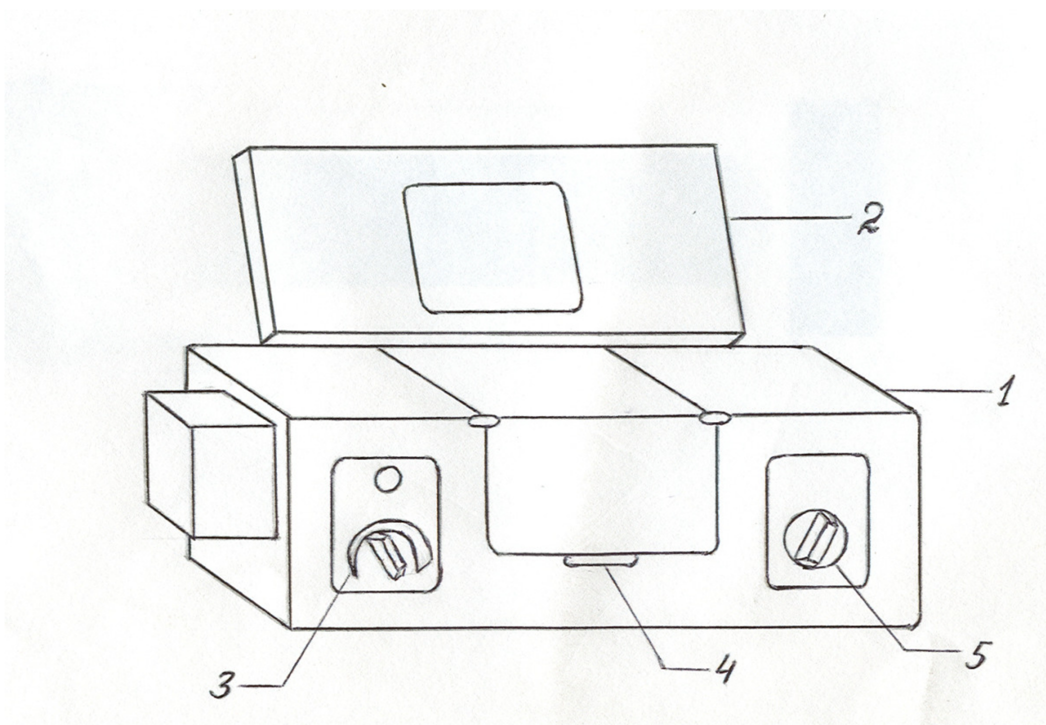


Figure 5.10.1.

Procedure:

Put the colorimeter to the current 220V, and turn on the button POWER, an indicating lamp must be lightened up. Push the key START (PUSK)- on the digital display twinkling comma will appear and “P” indicator will lighten up**.** Keep the colorimeter turned on 15 minutes with open lid of cuvette compartment. Then it is necessary to make measurement and accounting for the "zero point", for this: the sample compartment lid should be closed and opened, after 5 seconds, press the “Ш (0)." The digital display will show the symbol n0 on the right and on the left site "0". Value - n0, and on the left of "0". n0 value should be not less than 0.001 and not more than 1,000.

Selection of a light filter:

Pour the solution into a cuvette and determine the optical density for all of the 11 filters. The data obtained construct curve where the horizontal axis is the wavelength and the vertical - the respective values of optical density. Filter is

chosen by the principle that the wavelength, corresponding to the maximum transmittance of filter, is accounted for roughly parallel to the horizontal portion of the spectral curve. This work is done with a light filter with λ= 670 nm.

Selection of the cuvette.

Produced visually, respectively on the color intensity of the solution. If the solution is colored intensively (dark), you should use a cuvette with a small working length (1-3 mm), in the case of a weakly colored solutions – cuvette with a greater working length (30-100 mm).

Measuring of the transmittance:

- In the cuvette compartment set the cuvette with a solvent (nearest nest) and control solution (farthest nest). Knob 4 (fig.5.10.1) set to position "1", close the lid, press the key "τ2". On the left side of digital display appears symbol "2", indicating that the measurement of transmittance provided, and on the right side the numerical value will be shown. This operation performs 3-5 times, and transmittance coefficient is determined as the arithmetic mean of obtained values.

Measurement of optical density:

- Perform the same operation (at position 1, the handle 4), as for measuring the transmittance. Then, turn the knob 4 to the position "2", press the key "D (5)" on the left side of the digital display appears symbol "5", which indicates that an optical density is measured, and on the right site of the display its numerical value will be shown. This operation performs 3-5 times, and optical density is determined as the arithmetic mean of obtained values.

Construction of the calibration curve for the substance and the determination of the coefficients C and B:

- Prepare a series of solutions of the substance with known concentrations, encompassing the range of possible measurements of the substance concentration in analyzed solution.

To construct the calibration curve it is necessary to measure the absorbance of methylene blue with following concentrations:

0,0005%, 0,0006%, 0,00075%, 0,001%, 0,00125%, 0,0015%, 0,0025%, 0,005%.

Preparation of solutions of methylene blue:

Initial concentration - 0.005%. To prepare 50 ml of 0.0005% methylene blue it is necessary to take 5 ml of 0.005% and bring it up to 50 ml with distilled water. To 0.0006% - 6 ml, 0.00075% - 7.5 ml 0.001% - 10 ml, 0.00125% - 12.5 mL,

0.0015% - 15 ml, 0.0025% - 25 ml ,0.005% - 50 mg of methylene blue stock solution is taken and dissolved in 1 liter of distilled water.

Measure the transmittance and optical density of the solutions, store the data in Table 5.10.1 and construct the calibration curve, the horizontal axis -known concentration, and the vertical - the corresponding values of optical density.

Table 5.10.1.

Concentration, %	Coefficient of transmittance, %	Optical density

According the calibration curve determine the coefficients C and Ь. C - the value of optical density at C = 0 (i.e., at the interChapter of the calibration curve with the axis of optical density D).

$Ь = tg\alpha = \frac{D_i - C}{C_i}$, where α- angle between the calibration line and the axis of the concentration C; (Ci), (Di) - the current points of the schedule.

Enter into memory of computing block the coefficients of C and Ь, on the digital display to the right of the flashing point appears typed value of coefficient. Then press UTV (УТВ) - information on the digital display will disappear.

Report design: Obtained results put into the table, make calculations and draw a curve. Define the relative error of measurement and make conclusions.

Laboratory work №11
Measurement of optical density of different solutions

Objective: To investigate the change in optical density depending on the changes in the concentration of various solutions

Tasks: 1. Determine the optical density of the stock solutions and prepared dilutions at different wavelengths

2. Construct the graphs for the analyzed substances.

Equipment and materials: photoelectrocolorimeter, set of cuvettes of different thicknesses (working length), beakers, 1 ml micropipette and 5 ml of 0.02% solution of methyl orange, neutral red.

Procedure:

1. Prepare the dilutions

а) neutral red 1/4, 1/7, 1/14, 1/20.

б) methyl orange 1/4, 1/7, 1/14, 1/20.

2. measure the optical density of initial (stock) solutions and prepared dilutions at different wavelengths. (cuvette-5ml). Put obtained results into the table 5.11.1.

Table 5.11.1.

Dilutions	Wavelength, λ, nm							
1. Initial (stock) solution	400	440	490	540	590	670	750	870
2.								
3.								
4.								
5.								

Report design:

Measure the optical density of solutions in cuvette with volume-5ml. Put the results into the table. According to the obtained results make a graph. Determine the light filter type and size of the cuvette appropriate for each of the dyes. Make conclusions.

Laboratory work №12
The method of vital staining of tissues

Objective: determine the amount of alcohol soluble fraction of the dye in the flakes of onions due to heat treatment.

Tasks:

1. Learn to work with analytical balance and weigh the samples;
2. Learn to stain biological objects;
3. Learn to work with CPC-2 (КФК-2) and determine optical density;
4. Learn to mathematical analysis and relevance determination of mean differences between control and experimental sample.

Equipment and materials: analytical scales, photoelectric colorimeter CPC-2MP, tweezers, test tubes, methylene blue (concentration of 360 mg per liter), 70% solution of alcohol, onion.

The method of intravital (vital) dyeing, widely used in histological and serological studies, were analyzed and theoretically described by denaturation theory of damage and excitation by D.N.Nasonov with employees in 1934. The essence of this theory is as follows.

Under the action of in almost any environmental factor a certain degree of intensity to the cell, non-specific set of changes is observed in the cytoplasm of the same type, which are based on changes in cellular protein denaturation. Outwardly, this nonspecific response of the cells is shown next to the same type of symptoms that do not depend on the nature of altering agent, namely: an increase in dispersion of protoplasm colloids, increasing its viscosity, changes in the sorption properties of the cytoplasm in all cells of the same type as a set of changes under the influence of a variety of factors. These changes are caused by the irreversible denaturation of protoplasm proteins. Denudation reaction, according D.N.Nasonov, is primary occurs in the process of interaction between irritants and the living system, and it is one of the oldest and most common attributes of living matter. Such changes in the environment, which are enhanced by a living system, Nasonov called *irritant*, and the ability of living substrate to respond to the action of the irritant by certain complex changes is *irritability*. Signs of excitation of various cells associated with a pair of necrotic changes of protoplasm, which, in turn, serve as the trigger for a chain of complex biochemical reactions.

V.Y.Aleksandrov 1974 concludes that the exchange rate of the cells is associated with processes that use reversible denaturation. With increasing intensity of the denaturation agent changes may become irreversible and result cell death. This paranecrotic change reversibility allows to use paranecrosis as a criterion for the functional state of the cells, disruption of normal function, but is not an indicator of cell death.

The method of vital staining has for decades been successfully used to identify non-specific change of the protoplasm of animal and plant tissues under the influence of agents and diversified chemical and physical nature. Generally sorption capacity of the tissues can be an important feature of the functional state of the object.

Typical intravital dyes - are organic aromatic compounds having a relatively low toxicity to living cells. Chemical properties differ in basic and acidic dyes. Inside the cell, they are connected chiefly with cytoplasmic proteins and due to this first all cytoplasm acquires diffuse color. Some of them may interact with other proteins in the composition or organelles' membranes, therefore with time series of dye deposited in the cytoplasm in the form of granules. Staining of living cells makes it possible to detect changes in cells and tissues under different external influences. In the latter case, it is extremely important that the amount of dye absorbed by intact or damaged cells by any impact can be accurately identified and quantified. The difference in the amount of dye absorbed by intact and

damaged cells indicates the nature and extent of changes that occur due to various external influences.

Procedure:

1. Mastering the techniques staining. Object-unpainted part of onions. Dye-methylene blue, eosin. Extracted substance - aqueous solution of 70% industrial alcohol.

2. Sequence of stages:

- Prepare onion sample for the experiment. Total 10 weighed portions of slightly more than 1 gram.

5 Control - native onion, i.e. not scalded, is filled with distilled water at room temperature and leave for 5-10 minutes.

5 Experiment samples - onion is treated with boiling water for 5 minutes. The prepared onion flakes are dried using filters and weighed, making portions - 1 g each. Each sample is placed in a separate dry tube.

-10 ml of the dye (concentration of 360 mg per liter) are poured into each tube and left for 20-30 minutes.

-Then the dye is drained, washed 3 times with distilled water, then poured 10 ml of alcohol and left again for 30 minutes. The eluate (alcoholic extract dye from onion cells) is filtered and removed all pieces of onion, and then optical density determination is implemented.

-Measurement of the optical density of the eluate is carried out on the upper scale by fluorimeter CPC-2 (КФК-2) (percentage, the density of distilled water taken as 0, and is used as a control). Filter at 490 and 540 nm.

-Eluate from each tube is measured at least 2-3 times. The results are tabulated (Table 5.12.1.-5.12.2).

-Calculate the mean and error of the mean for the control and experiment; determine the accuracy of mean differences.

Table 5.12.1 -The optical density of the eluate for control

Tube	1			2			3			4			5			Average mean
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
490 nm																
540 nm																

Table 5.12.2 - The optical density of the eluate for experiment

Tube	1	2	3	4	5	Average

															mean
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
490 nm																
540 nm																

Report design: The measurement results should be entered in the table, also carried out the mathematical analysis, construction of histogram and making conclusions.

Laboratory work №13
Studying of sorption of dyes by bio objects depending on the action of various physical factors

Objective: To investigate the change of sorption activity of fabrics depending on the action of different physical factors.

Tasks:

1. To paint over the live and damaged fabrics organic dyes.
1. To prepare the dye and to take measurement of optical density
2. To bring data in the table and to construct schedules of the absorbed substances.

Equipment and materials: analytical scales, the colorimeter photo-electric concentration CPC-2MP, tweezers, penicillinic bottles, filter paper, pipettes on 5 and 10 ml, the laser helium - neon, the infrasound generator, Chizhevsky's chandelier, the thermostat, the rangette, hair or wool, a hair-dye, 70% alcohol solution.

Procedure:

1.1. For experience to prepare the following options:

• Control (without processing)

• Heat treatment (at a temperature of 105 □C in a drying cabinet within 50 minutes);

• Processing helium - the neon laser for 1 min. 5 times with a dark interval of 1 min;

• Infrasonic processing of hair of 25 minutes.

1.2. To prepare hinge plates of hair: on analytical scales to weigh with an accuracy of 0,001 g hair (wool) on 500 mg.

1.3. To place the prepared hinge plates of hair in 5-fold frequency in pure penicillinic bottles, to fill in 4 ml of the paint prepared according to the instruction, to bring to 10 ml the distilled water. To mark bottles. Time of a coloring of 30 minutes, in the thermostat at a temperature of 24 ^{0}C, previously having closed a stopper.

2. To define the light filter for the used dye (see a lab. work 9 - studying of operation of the photoelectrocolorimeter). To construct a calibration curve, to define coefficients C and B.
3. To measure the optical density of dye from bottles in which hair were painted and to write down results in table 5.13.1).
4. To measure concentration of solutions of dye by means of CPC of coefficients entered into memory C and B. For this purpose:
in cuvette site establish ditches with the control and studied solution;
- (see fig. 5.10.1) install the handle 4 in situation 1;
- close a cover of cuvette office, press the “K (1)” key, on a digital board to the left of the blinking comma the symbol 1 lights up;
- then install the handle 4 in situation 2;
- press the “C (4)” key, on a board there is a 4 symbol meaning that there was a measurement of concentration of the studied solution at the left. Counting on a digital board to the right of the blinking comma corresponds to value of concentration of the studied solution;
- take measurements 3-5 times and define an arithmetic average as final value of the measured size.
Enter results of measurements in table 5.13.1.
5. To wash out the painted-over hair in the small portions of the distilled water (5-multiply) to a total disappearance of coloring of washing waters. Place the hair to pure marked bottles and to fill in 5 ml of solution of 70% of the acidified alcohol. Eluating time 1 hour in the thermostat at a T temperature = 24°C.

Table 5.13.1 - Optical density and concentration of dye

Hair and wool	variance	Experiment variants							
		Control		Laserprocessing		Heattreatment		Electro - processing	
		D	C	D	C	D	C	D	C

6. To measure the optical density and concentration of spirit extracts. C and B to make determination of coefficients for spirit extracts according to the calibration schedule.
Enter results of measurements in table 5.13.2.

Table 5.13.2 - Optical density and concentration of spirit extracts

Hair and wool	variance	Experiment variants							
		Control		Laserprocessing		Heattreatment		Electro - processing	
		D	C	D	C	D	C	D	C

7. Calculation of the general sorption ability:

a) to calculate amount of dye in solution of initial concentration in the studied volume on a formula: $WC_1 = \frac{C_{исх} \cdot V_1}{100}$; where $C_{исх}$ – concentration of the dye used at a coloring (0,0025%); V1 – dye volume.

b) to determine the concentration of solutions which remained after a coloring of hair (C2) by a calibration curve.

c) To calculate amount of the dye which remained after a coloring in the studied volume on a formula:

$WC_2 = \frac{C_2 \cdot V}{100}$; where C_2 – concentration of dye, determined by a calibration curve; V – dye volume.

d) to calculate amount of the dye connected by hair:

$WC_3 = WC_1 - WC_2$

e) determination of the general sorption ability $A_{общ}$

$A_{общ} = \frac{WC_3}{P}$; where P - a hinge plate of hair

e) definition of alcohol-soluble fractions of dye on a calibration curve of C_x

g) To calculate WC_x dye weight

$WC_x = \frac{C_x \cdot V}{100}$; where V – the studied volume

h) to calculate amount of chemically connected dye on 1 g of hair

$A_X = \frac{WC_3}{P}$;where P - a hinge plate of hair

Enter results of measurements and calculations in table 5.13.3.

Table 5.13.3.

Variant	WC_1(г)	C_2 %	WC_2(г)	WC_3(г)	P(г)	$A_{общ}$	C_x	WC_x(г)	P(г)	A_x

Report design. To determine value of concentration of the studied solutions by the graduated schedules; To compare both ways of determination of concentration; To analyze data and to draw conclusions;

Laboratory work №14
Water sorption studying by fabrics depending on action of various physical factors

One of criteria of activity level of live systems is their ability to absorb water. Especially plants, whose vitality completely depends on the sorption abilities of the water necessary for development and existence of plants.

For swelling seeds at the time of their germination an important stage is water sorption. Any action of various physical factors changes sorption activity of seeds that connect first of all with conformational changes of proteins that are shown in deployment or compression protein globules, leading to change of number of the active centers lying on a surface of molecules and their ability to connect water molecules. Proteins in cells are in the hydrated state and it is many times more than connected water in cells, than free.

Research consists in definition of an amount of water, the absorbed seeds which various physical factors influenced. As factors influence by lasers – helium - neon and infrared (semiconductor), temperature (cooling and heating) and control is taken at the room temperature.

Objective: To investigate change of sorption ability of seeds depending on action of various physical factors.

Tasks:

1. To learn to work with analytical scales and to weigh bio objects.
2. To investigate sorption of water at the room temperature at the raw (native) seeds (control).
3. To investigate sorption of water at seeds after heat treatment at action by high or low temperatures.
4. To investigate sorption of water at seeds after radiation electro- magnetic waves in the infrared and red visible range.
5. To analyze the obtained data, to carry out mathematical processing and to construct histograms. To define a factor with the maximum influence on sorption activity of seeds.

Equipment and materials: scales pharmaceutical, tweezers, penicillin bottles, filter paper, pipettes on 5 and 10 ml, the laser helium - the neon, infrared (semiconductor) laser, the thermostat, the rangette, seeds, the distilled water.

Procedure:

1. For experience to prepare the following options:
• Control (without processing)
• Heat treatment (when heating on the rangette within 5 minutes);
• Heat treatment (cooling in the thermostat within 50 minutes);
• Processing helium - the neon laser for 1 min. 5 times with a dark interval of 1 min.;
• Processing by the infrared laser for 1 min. 5 times with a dark interval of 1 min.;
• Each option is prepared in 10 frequency.
a. To prepare hinge plates of seeds on pharmaceutical scales with an accuracy of 0,01 g on 2 g.
b. To place the prepared hinge plates of seeds in 10-fold frequency in pure penicillin bottles, to fill in with the 5th distilled water. To mark bottles.
c. To leave seeds for water sorption.
2. To define changes of weight of seeds after water sorption for what each hinge plate is dried separately on filter paper, and then weigh. Enter results of measurements in table 5.14.1.

Table 5.14.1 – Change of weight of seeds after sorption

control D	C	Experiment variants							
		Control		Laser		Heating		Cooling	
		D	C	D	C	D	C	D	C

Report design: Results of measurements enter in tables, carry out mathematical data processing, build the histogram and write down conclusions in a notebook.

Laboratory work № 15
Spectrophotometer

Objective: To investigate a range of absorption of amino acids and protein. To measure a range of absorption of amino acids.

Tasks:
1. To define a range of absorption of amino acids.
2. To define a range of absorption of protein.
3. To fill in the table.

4. To construct schedules of the studied substances.

Equipment and materials: the SF-26 spectrophotometer, pipettes on 1 ml, 5 ml, 10ml, measured test tubes on 5 ml, the distilled water, filters, solutions of a tyrosine, phenylalanine, egg white, a water bath.

Procedure: The SF-26 spectrophotometer is intended for measurement of coefficient of a transmission of liquid and strong substances in the field of a range from 186 to 110 nanometers. The coefficient of a transmission of the studied sample of T is equal to the relation of intensity of a stream of the radiation of I which passed through the measured sample to intensity of the stream of radiation of I_0 which is falling on the measured sample or passed through a control sample which coefficient of a transmission is accepted to unit and is expressed by a formula

$$T = \frac{I}{I_0}$$

Measurement is performed on a method of electric auto compensation. Are serially entered into a monochromatic stream of radiation the control and measured sample. At introduction of a control sample of the shooter of the measuring device is established on division by "100" adjustment of width of a crack, and value of the light stream which was established thus is taken for 100% of a transmission. At introduction to a stream of radiation of the measured sample of the shooter of the measuring device deviates in proportion to change of a stream, the size of coefficient of a transmission is counted on a scale as a percentage of a transmission.

For ensuring operation of the spectrophotometer in the wide range of a range two photo cells and two sources of radiation of a continuous range are used. The antimony-cesium photo cell with a window from quartz glass is applied to measurements in the field of a range from 186 to 650 nanometers, an oxygen-cesium photo cell - to measurements in the field of a range from 600 to 1100 nanometers. Wavelength at which it is necessary to pass from measurements with one photo cell to measurements with other photo cell, is specified in the passport of the spectrophotometer. The deuterium lamp intends for work in the field of a range from 186 to 350 nanometers, the glow lamp - for work in the field of a range from 340 to 1100 nanometers. For check of graduation the mercury-helium lamp is used.

Spectrophotometer device.

The spectrophotometer (fig. 5.15.1) consists of the monochromatic 1 with the measuring device 2, cuvette office 3, a chamber 4 with photo detectors and the amplifier, and the lighter 5 with sources of radiation and the stabilizer.

In the spectrophotometer two sources of a continuous range are used: a deuterium lamp for work in the field of a range from 186 to 350 nanometers and the glow

lamp - for work in the field of a range from 340 to 1100 nanometers. Change of sources of radiation is made in the range from 340 to 350 nanometers by switching of the handle 6 at switching off of the device.

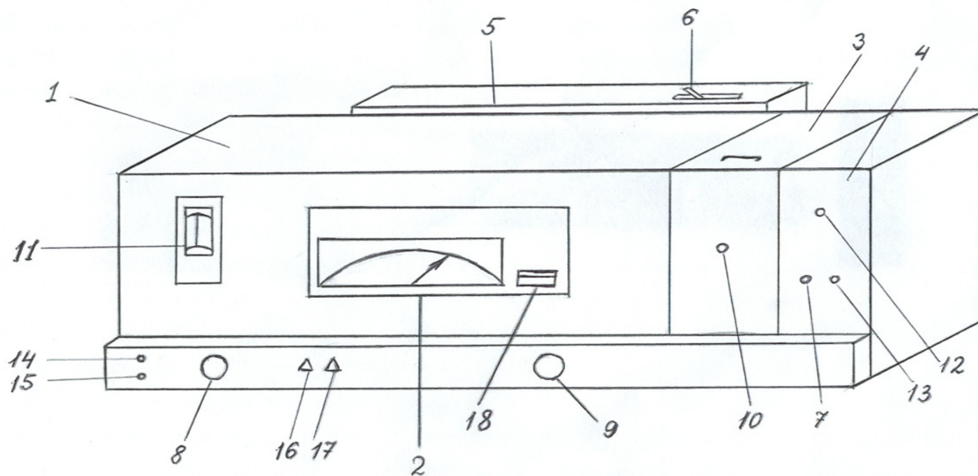


Figure 5.15.1.

Preparation for work

Turning on the spectrophotometer.

1. Establish into position a photo cell and a source of radiation, corresponding to the chosen spectral range of measurements.
2. Close a photo cell, having put the blind handle 7 in situation ZAKR.
3. Turn on the NETWORK toggle-switch then the NETWORK and an alarm lamp D or N according to the chosen radiation source have to light up an alarm lamp.
4. Stable functioning of the spectrophotometer is ensured in 1 hour after its inclusion.
5. You make switching off of the spectrophotometer the NETWORK toggle-switch.

Procedure:

Measurement of coefficients of a transmission is performed at densely closed cover of cuvette office.

1. Turn on the spectrophotometer as it is specified earlier.
2. The compensation handle has to be installed in situation "C".
3. Establish the demanded wavelength, rotating the handle 8 towards increase in lengths of waves. If thus the scale turns at a big size, return back it on 3-5 nanometers and again bring it to the demanded division.
4. Not to touch the "sensitivity" and "compensation" handle.

Task 1.

To measure a range of absorption of solution of a tyrosine. Initial solution (10mg tyrosine + 3 ml N2odist. + 7 ml the GAME of 0,01 N) to part 1:10. To measure absorption ranges in 3 analytical frequency with the following lengths of waves: 220, 240, 260, 280, 300 nanometers. By results of measurements to construct absorption ranges.

Task 2.

Definition of a range of absorption of phenylalanine. From initial solution (10 mg of phenylalanine + 3 ml N2odist. + 3 ml of HCl of 0,1 N + 3 ml of NAON of 0,1 N) to prepare cultivation 1:10. To measure optical density with the following lengths of waves: 220, 240, 260, 280, 300 nanometers. According to the obtained data to build an absorption range curve.

Task 3.

Definition of a range of absorption of proteins.

Egg white. To take a protein hinge plate (10 mg) on the torsion scales, to dissolve in 5 ml of H2O a dist. From this solution to prepare cultivation 1:10.To measure an absorption range at 220, 240, 260, 280, 300 nanometers. Halve initial solution, sustain in a water bath (40°C) within 10-15 minutes. Cool one test tube gradually at the room temperature, and cool another quickly, having placed in ice. To measure ranges of absorption of solutions in both test tubes. To construct absorption ranges.

Conclusions: to characterize ranges of absorption of all studied substances.

Report design: To measure ranges of absorption of amino acids and chicken protein, enter values in the table. On the basis of the obtained data to construct schedules. Define adequate light filters for each of amino acids. Make conclusions.

Chapter 5 "PHOTOMETRIC METHODS of RESEARCH of BIOLOGICAL SYSTEMS" questions

1. What kind of values could be measure by CPC-2MP?
2. What is the transmission coefficient?
3. What is the optical density?
4. What method of coloring is called vital?
5. Lifetime dyes interact with what components of a cage?
6. What occurs at a protein denaturation?
7. How external factors influence properties of protoplasm?
8. For what are the painted objects filled by alcohol?
9. Explain distinctions between coloring of living and dead tissue of onions?
10. In what condition does be water in living tissues?
11. Why under the influence of external factors sorption activity of seeds changes?

12. Describe the possible mechanism of change of sorption activity?
13. What role of water in living tissues?
14. Define Bouguer-Lambert -Beer's law.
15. How to choose light filters?
16. How to choose ditches?
17. What is the absorption range?
18. What is the lifetime dyes and why living tissues occlude them more?
19. What impact has the method of lifetime coloring of living tissues?

Chapter 6. OPTICAL PROPERTIES OF BLOOD

Hemoglobin is the main component of red blood cells. One erythrocyte contains about 280 million hemoglobin molecules, each of which consists of about ten thousand atoms of hydrogen, carbon, nitrogen, oxygen, sulfur and iron.

The main function of hemoglobin is reversible binding of molecular oxygen and its delivery to all cells of the body. The molecular weight of it, by various methods and found to be 66,000 - 68,000.

Hemoglobin - haemoproteins representative, ie the group of complex proteins. Its molecule consists of four polypeptide chains, which are divided into two groups. 2-alpha and 2-beta polypeptide chains. All four hemoglobin chains very similar to each other in shape. Form and function of hemoglobin of different animals are similar, but different amino acid composition of the stronger, the more they are evolutionarily distant from each other.

In the absorption of oxygen alpha-chain of hemoglobin get closer together, while giving it – locate far from each other. Position of beta-chain is not changed. Therefore, joining and giving oxygen molecule hemoglobin accompanied by a change in its structure, which leads to a change in the absorption spectrum of hemoglobin. Adding oxygen molecule hemoglobin passes into oxygenated form.

Among the abundant hemoglobin derivatives there are also methemoglobin and carboxyhemoglobin. Methemoglobin iron is in the ferric state, while in dioxygenerated and oxygenated hemoglobin - in the divalent. A significant amount of methemoglobin appears in the blood of irradiated animals, it is dangerous for their lives.

It was also found that if methemoglobin is formed by storing on air oxyhemoglobin solutions. Hemoglobin has a greater affinity for carbon monoxide (CO), passing by the reaction in carboxyhemoglobin, not having the ability to reversibly attach oxygen. The basis of carbon monoxide poisoning in humans lies in the formation of significant amounts of blood carboxyhemoglobin, which leads to hypoxia of an organism.

Each hemoglobin particle is characterized by a specific absorption spectrum, which depends on the optical density of the solutions on the wavelength of light.

Therefore, according to the spectral absorption bands we can discuss the structural transformations of hemoglobin molecules. For the study of the absorption spectra of substances special Equipment such as spectrophotometers are used. In real study for the development of the optical properties of hemoglobin photoelectrocolorimeter is used. It is choosen due to the fact that photoelectrocolorimeter is the most affordable device for small laboratories, and can also be used as a turbidity meter. It is known that during the storage of hemoglobin solution, their transparency decreases, which lead to an increase

spectrophotometry at optical density due to an increase in its light-scattering solutions. Light scattering is a function of the size and number of particles in solution, and gives an opportunity to judge the process changes in the structure of substances, in particular proteins, under the influence of certain factors. The most intense band in the absorption spectrum of hemoglobin is a Soret band belonging to the porphyrin part of its molecules. According to the position and intensity of absorption of this band, the structural changes of different forms of hemoglobin molecules can be distinguished.

Photometric measurements can be performed on photoelectrocolorimeter and also on spectrophotometer.

Laboratory work № 16
Effects of different chemicals on the light scattering and light absorption of hemoglobin solution

Objective: To investigate the effect of various chemicals on the light scattering and light absorption of hemoglobin solution

Tasks:

1. Determine the optical density of the blood by the action of various chemicals
2. Create a table and make it in the results.
3. Construct a timetable for action on hemoglobin test substances

Equipment and materials:

Photoelectrocolorimeter, hemoglobin solutions of 0.85% solution of NaCl, heparin, a centrifuge at 9000 rev / min, centrifuge tubes, vials, Pasteur pipette, potassium dichromate, potassium ferricyanide, sodium dithionite, mice.

Procedure:

Blood is taken from decapitated white mice. To reduce the scatter of the data is desirable that hemoglobin is isolated from the blood of linear mice.

The method of obtaining hemoglobin is the phenomenon of hemolysis of red blood cells under the influence of a number of compounds (toluene, water, etc.). Whole blood, stabilized with heparin, dissolved in a 0.85% solution of NaCl, centrifuged to separate plasma for 20 minutes at 6000 rev / min. Blood plasma is taken with a Pasteur pipette. To the erythrocytes was added three times the volume of 0.85% strength NaCl solution, while carefully stirring the slurry with a glass rod, and centrifuge it for 5 minutes at 6000 rev / min.

The red cells are washed three to four times. Then, washed erythrocytes are subjected to hemolysis with distilled water for 20 minutes at 9000 rev / min to remove stroma. The resulting solution of oxyhemoglobin is diluted with distilled water to the desired concentration. Methemoglobin from oxyhemoglobin obtained

by adding to it a solution of 1-2 drops of a saturated solution of potassium ferricyanide. Methemoglobin solution has a brown color. For obtaining the reduced (dioxygenerated) hemoglobin a few drops of concentrated solution of freshly prepared sodium dithionite was added to oxyhemoglobin aqueous solution. When you add latest one, the bright scarlet stain (color oxyhemoglobin) becomes bluish-red characteristic of hemoglobin. In the study of the optical properties of hemoglobin λ = 400 nm filter is the most appropriate, as the band of its light transmission coincides with the hemoprotein absorption band (most of the light energy falls on the Soret band).

The task of the students included:

1) Choose the right concentration of oxyhemoglobin. It is necessary to prepare the protein solution, the optical density is at least equal to a predetermined color filter 0,40-0,45;
2) To study the kinetics of the change in the optical density of the solution of oxyhemoglobin for 3-4 hours of incubation with free access of air. It may be recommended intervals absorbance measurement: 20, 40, 60, 80, 100, 120, 140, 160, 180, etc. minutes after the first determination of the light absorption of oxyhemoglobin solution. A kinetic curve of oxyhemoglobin absorption of the solution, that is, the dependence of the optical density of the solution incubation and time of oxyhemoglobin in the air;
3) Measure the absorbance of the solution obtained by adding to the oxyhemoglobin sodium dithionite and potassium ferricyanide. To compensate for light absorption by potassium ferricyanide to solvent is necessary to add the same volume of solution of the substance, which was added to a solution of oxyhemoglobin;
4) Follow the change of light scattering in solutions of oxyhemoglobin, aged for 3-4 days with free access of air (use a filter with λ= 540 nm).

Determination of light scattering in solutions of oxyhemoglobin will be carried out by an indirect method - by the value of light transmission control (freshly prepared solutions) and experienced (aged in air) solutions of oxyhemoglobin.

It should be remembered that the concentration of a freshly prepared solution must be equal to the concentration of oxyhemoglobin solution to store it for a specified period of time. To compensate for light absorption by colored pigment necessary to add a few drops of a concentrated solution of potassium dichromate to the solvent (water), that is necessary to equalize the light transmission of the solution of oxyhemoglobin and solvent. The difference in light

transmission values of test and control solutions are judged to change the light scattering in solutions of oxyhemoglobin during storage in the free access of air. If the light scattering oxyhemoglobin solutions upon storage increases, the light transmittance of its solutions must thus decrease and vice versa.

On the basis of the absorbance values (2 and 3) of oxyhemoglobin solution may be concluded on the nature of the structural changes in its molecules.

Procedure:

Measure the absorbance of hemoglobin in the cell by the action of various chemicals. Tabulate and plot the values. Make conclusions.

Laboratory work № 17

The study of photodynamical hemolysis of erythrocytes

Objective: To study photodynamical hemolysis of erythrocytes due to the action of different dyes.

Tasks:

1) To determine the optical density of initial solutions and prepared dilutions of different wavelenghts.
2) To construct the graph of investigated substances.

Visible light usually does not affect cells and tissues of an animal in noticeable manner. But in the presence of some colourants such as erythrosine, eosine, methylene blue, and some natural pigments like porphyrine, hyperycine, and others, the illumination causes the destruction, damage, and consequental death of a cell. This phenomenon is called photodynamic activity. In the basis of photodynamic activity lies the photooxidation reaction of proteins due to the light energy consumed by the dye.

Task 1. The study of hemolysis using photoelectrocolorimeter.

When the destruction of erythrocytes occurs (hemolysis), the decrease of suspension's turbidity, and consequentally. the increase of light transmission also happen. The solution becomes more transparent. The change in erythrocytes suspension's transmission can serve as the hemolysis measurement. The erytrocyte transmission can be measured by the photocolorimeter CPC-2MP. The hemoglobine absorbtion should not disturb the light dissipation, that is why the hemolysis measurement is carried out using red light filter, in the spectral field

where hemoglobin is not absorbed. All light transmission measurements are exclusively conducted with the turbidity changes.

Equipment and materials: photoelectrocolorimeter, water, blood, NaCl.

The process of work.

To prepare 1% g of washed rat erythrocytes in physiological solution (the description is in lab work #4). Two samples of initial suspension is diluted hundred times: one with distilled water, another one with physiological solution. The transmission of both solutions is performed on the photoelecntocolorimeter CPC-2MP with green and red light filters. The solution for comparision is the distilled water or physiological solution. The repeated measurements are performed in 10, 20, and 30 min intervals after dilution of solution. It is important to make sure that the suspension's transmission, diluted by the water (hypotonic hemolysis), significantly exceeds the erythrocytes' suspension's transmission in physiological solution (control).

Task 2. To study the hemolysis of erythrocytes under the illumination in the presence of colorants.

As the source of erythrocytes' suspension radiation the incandescence lamp with the power of 500 V is used. The flasks with radiating suspension are located at a lenght of 25 cm long from the lamp. To exclude the heating of the object by the infrared emitting, between the light source and flasks with suspension the glass vessel with flat parallel walls filled with 0.5%NaCl should be put.

Equipment and materials: photoelectrocolorimeter, water, blood, NaCl. incandescence lamp (500 V), erythrosin.

The process of work:

To prepare 1% suspension of washed erythrocytes, 0.04% erythrosine solution on the physiological solution (8 mg of erythrosine and 20 ml of physiological solution). 10 ml erythrosine solution is poured into two flasks and precisely 0.1 ml of 1% erythrocyte solution is added into both of them (flask 1 an flask 2). 10 ml of physiological solution and 0.1 ml erythrocyte suspension should be poured into two other flasks serving as controls (flask 3 and flask 4). After rigorous mixing of solutions, flasks 1 and 3 are radiated during 10 min interval. Flasks 2 and 4, which serve as the dark controls, should be located in the dark place at the same room temperature.

Then all flasks are left in the darkness, and their transmission is measured per 15 minutes during 3 hours. The measurements are performed in the same way

as before. The 0.04% erythrosine solution prepared on the physiological solution serves as the comparative mixture. In the utmost case, the distilled water can serve as well.

The results of measurement should be summitted in the form of a graph as the relation of transmission (T) to the radiation time for all four flasks.
erythrocytes+colorant+illumination;
erythrocytes+colorant, without illumination;
erythrocytes+illumination, without colorant;
erythrocytes, without colorant, without illumination.

Report design: Measure the optical density in the cuvette with erythrocytes subjected to different chemical substances.
Write results to the table, make the graph on the basis of the resulted data, draw conclusions.

Chapter 6 "Optical characteristics of blood" questions

1. What is the absorbtion spectrum of erythrocyte?
2. What does mean the term "animal pigments'?
3. What is the Soret band?
4. Why different colorants are able to cause erythrocytes' hemolysis?
5. What is the photodynamic hemolysis?

Chapter 7. POLARIMETRY

Light is the electromagnetic waves. Chemical and biological activity of light is tightly connected with electrochemical factor of an electromagnetic wave. That is why the vector (E) of electromagnetic field's tension is called the light. A natural light is the set of waves, radiating from dozens of atoms and molecules from the light source. The fluctuations of light vectors happen in all directions, that is why the planes of their fluctuations are constantly changing their position in the space. If the directions of fluctuations are somehow organised, the light is called polarized. In particular conditions the light, in which the plane of the vector (E) fluctuations takes a certain positionin the space, can be established (flat polarized light). The plane, where the vector (E) fluctuations are promoted, is called the polarization plane.

The polarized light can be obtained from natural with the help of polarizator (Nickole's prism, polariod, etc). It skips he fluctuations being parallel to only one (the main) plane, and arrests fluctuations being parallel to this plane. To determine whether the light is flat-polarized after the passage through polarizator, the additional polarizator is put on the light passage way, which is called as analyzator, indicating that it serves to analyze the polarized light.

Let the oscillations of the vector E of the polarized light wave to make the plane at an angle$\varphi$from the main plane of the analyzer. The amplitude of these oscillations E can be decomposed into two mutually perpendicular components: E1 - coinciding with the main plane of the analyzer and E2 - perpendicular to it.

$$E_1 = E\cdot\cos\varphi,\ E_2 = E\cdot\sin\varphi.$$

The first component vibrations pass through the analyzer, the second will be delayed them. The light intensity is proportional to the square of the amplitude, hence the intensity of the light passing through the analyzer is proportional $E^2\cdot\cos^2\varphi$ (Malus's law).

$$I = I_0\cdot\cos^2\varphi,$$

where, I_0- intensity polarized light falling on the analyzer; φ - the angle between the plane of polarization of the falling light and the plane of the analyzer.

If the plane of the polarizer and analyzer are parallel, $\varphi = 0$, ie, $\cos\varphi=\pm1$, the screen is placed behind the analyzer will be of maximum illumination. If $\varphi = \pi/2$, $3\pi/2$, ie, $\cos\varphi = 0$(polarizer and analyzer crossed), the screen will be dark. When

passing polarized light through some substances observed rotation of the polarization plane. Such substances are called optically active (quartz, cinnabar, turpentine, nicotine, aqueous solutions of sugar, tartaric acid, etc.).

In solutions αrotation angle of the polarization plane is proportional to the beam path l in the solution and the solution concentration C:

$$\alpha = [\alpha_0]cl,$$

where $[\alpha_0]$- specific rotation. It is inversely proportional to the square of the wavelength depends on the nature of the substance and temperature.

The method used in the qualitative and quantitative analysis of various substances with a polarimeter, called polarimetry. Polarimeter used for the qualitative determination of sugar in solution, called Brix.

The device saccharometer SU-4 used the international sugar scale. 100°SS this scale correspond 34,62° corner. Saccharometer shows 100°Sat ambient temperature +20°C, if it is measured in the cuvette 200 mm aqueous solution of sucrose containing at +20°C of 100 cm^3 of 26 g of chemically pure sucrose, suspended in air with brass weights.

Laboratory work №18

Sugar concentration measurements by the polarimeter SU-4

Objective: the study working principle of polarimeter. The determination of sugar solution's specific rotation, the determination of sugar concentration in the solution.

Tasks:

1) To understand the working principle of sugarometer SU-4;
2) To determine the sugar's specific rotation in different concentrations;
3) To construct the calibration curve.

Equipment and materials: polarimeter, cuvettes with sugar, glucose, and sucrose solutions.

The device and principle of work. During the work the sugarimeter SU-4 is used, which functions to determine the concentration of sucrose in the solution according to the rotation degree of polarization plane. The optical scheme of the device is on the picture 7.18.1.

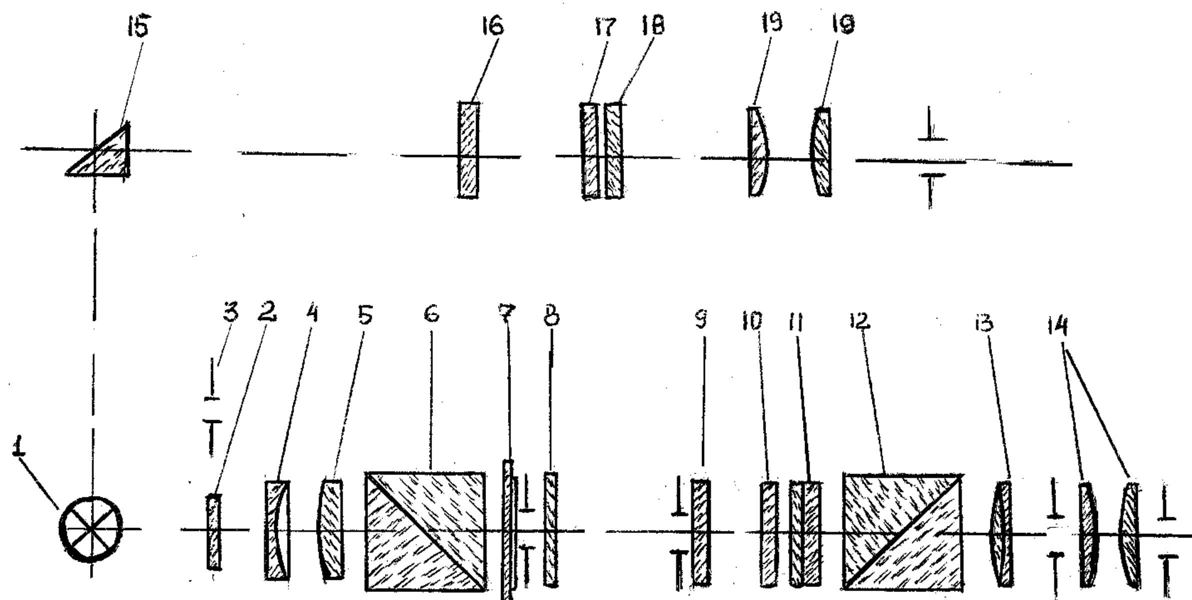


Figure 7.18.1.

The light source in the sugarimeter is the incandescence lamp. The light flow coming from the light source through the lightfilter 2 or diaphragm 3 and condenser 4, 5 goes through the polarizer's prism 6, which converts it into polarized light flow. Then the light goes through semishadowing slab 7, dividing it into two halves by the division line. The slab is oriented in such a way, that the polarization plates of both halves of light flow arrange identical edges with the polarization plate pattern of analyzer 12.

That is the reason why the analyzer omits identical light powered halves of the flow, and in the vision field of the tube, composed of lenses 13 and oculars 14, located right after the analyzer, two identical bright fields can be observed, separated only by the thin line, which are called as comparison fields.

In the installation of cuvette with the solution between the polarizer and analyzer the equilibrium of brightness fields' comparison factors is disturbed. That happens because of the studying solution turns the polarization plane on a degree proportional to the solution's concentration.

The compensator is installed to equalise the brightness of comparision fields in the sugarimeter, which is composed of motile quartz line of the left

rotation10 and non-motile cortcline of the right rotation 11. The transportation of the moving line relative to the contrcline is compensated by the turn-over degree of the polarized plane solution, and at the same time the brightness of the comparison field is also equalised. With the moving line the scale 17 is also transported. According to the null division of nonius 18 the value suiting the identical brightness condition of comparison fields is measured. The scale and the nonius can be observed by the loupe 19 and are illuminated by the electro lamp through the reflective prism 15 and light filter 16.

The setup of sugarimeter.

The main units of sugarimeter are the unit of measuring head 2 and illuminating unit 9, connected with each other by the traverse 6. The traverse is restrained by the rack 17 to the base 18. On the traverse the cuvette restricting units 5 for polarimetrics, and rim 7 with polarizer and semishadowing slab are located (fig. 7.18.2)

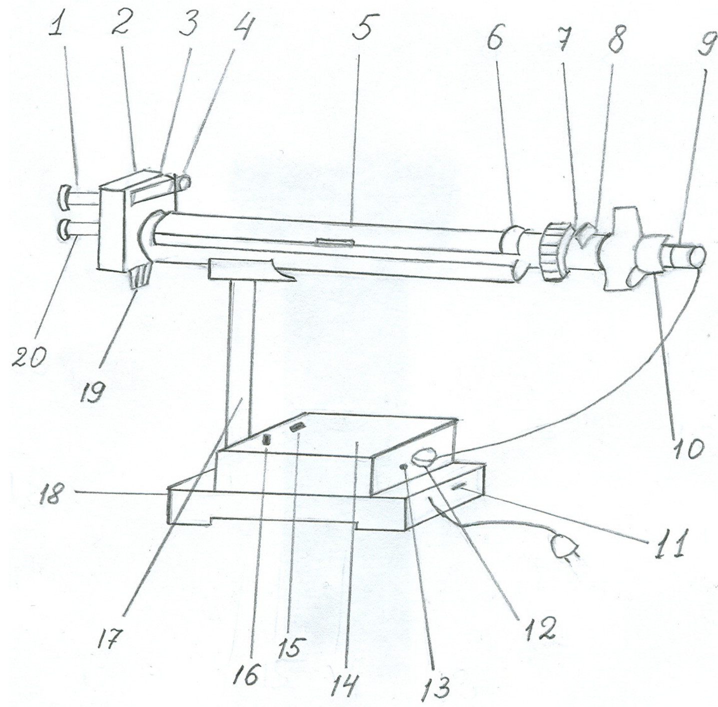


Figure 7.18.2.

The loupe 1 is located at the front side of the measuring head to measure the data from the scale, and also the vision pipe 20 is located. On the dorsal side of the measuring head the screw of nonius 3 installation mechanism is located to combine the null divisions of nonius with null divisions of the scale with the help of key 4.

In the lower part of the measuring head the handle of cline compensator 19 is located, by the rotation of which the moving quartz cline and the scale can be transported.

Into the illuminating unit content enters the following equipment: patron with a lamp, installation of which is obtained due to the action of three screws 10, and turning holder 8 with the light filter and a diaphragm.

On the basis of the device are located the colon 15 to turn on the illumination and a bundle of resistor 16 to regulate the brightness of the vision field.

In the inside of the base the decreasing transformator is inserted. In the dorsal side of the base are located the screw of grounding 11, the fork of a rosette 12 to connect the illumination unit of sugarimeter ti the transformator and the cutout 13.

The safety rules:

While working, the sugarimeter should be grounded. It is forbidden to open the cap of the base 14 when the device is connected to the electric network.

The preparation of the device to work:

The preparation of the device to the work should be promoted in the following manner:

- To conduct the external inspection of the sugarimeter;
- to check the grounding;
- to establish the proper location of the hand of the resistor 18 by its counter-clockwise rotation until it meets the stop;
- to insert the electrical fork of the sugarimeter into the network;
- to turn on the illumination by the button 15;
- to install the lens of a the vision pipe into the loupe of scale on the maximal picture tartness;
- to install the least fatigable (for eyes) regime brightness by the hand of the resistor 16.

Procedure:

1. The determination of specific rotation of sugars.

a. to put into the device the empty cuvette, then to close the cap of a cuvette unit;
b. to achieve complete division of comparative fields by rotating the handle of cline compensator 19, and then achieve the equal blackout of both halves of comparative fields;
c. to measure n_0. Repeat the measurements three times and find the average.
d. to put the cuvette with known concentration C1 of cugar into the cuvette unit of sugarimeter, by rotating the 19 handle once again achieve the equal blackout of both halves of the field;
e. to measure the *n*. The measurements should be repeated three times, then find the average $\bar{n}$;
f. To find the degree of plane polarization $\alpha = \bar{n} - \overline{n_0}$;;
g. to measure the specific rotation of sugar solutions by the following formula: $[\alpha_0] = \frac{\alpha}{l \cdot C_1}$;
h. the measurements should be put into the following table. All data should be performed using SI units.

Table 7.18.1.

	$\overline{n_0}$	*C, %*	n'	n''	n'''	$\bar{n}$	α, °S	α, *grad*	$[\alpha_0]$, $\frac{grad \cdot m^2}{kg}$
Sugar									
Glucose									
Saccharose									

2. The construction of the calibration curve.

a. to prepare solutions with following concentrations: 5%, 10%, 20%, 26%, 30%.
b. to put into the sugarimeter the cuvette with the length of 200 mm with one of the earlier prepared sucrose solution;
c. to conduct the measurements by the steps d-f

d. to fill out the table with the measurements;
e. to repeat the measurements for each concentration, the results should be written in the table, according to the table to design the graph of the relationship between the rotation degree of sucrose solution polymerization and its concentration.

Table 7.18.2.

substance		C, %	$\overline{n_0}$	n	n'	n''	n'''	$\overline{n}$	α, °S
Saccharose	5%								
	10%								
	20%								
	26%								
	30%								

3. The determination of the unknown concentration of the sucrose solution.
a. to put the cuvette with the unknown sucrose concentration into the cuvette unit of sugarimeter, ti determine the rotation degree of polymerization plane for this particular solution;
b. to measure the unknown sucrose concentration c by two different ways 1) by the calibration curve and 2) by the following formula

$$C_x = \frac{\alpha_x}{[\alpha_0] \cdot l}$$

c. to compare the measurements and draw the conclusions out of all three stages of the lab work.

After the end of the work the followings should proceed.

1) the turn-off of the sugarimeter from the electric network;
2) to clean the cuvette from the left residues within the unit;
3) to wash the cuvettes with the tap water, and then with distilled water, to dry them with the use of filter paper;
4) to wipe the protective glasses with the cotton very accurately, and prevent the disturbance of the surface;
5) it is not allowed to leave the handle of the resistor 16 in the position of the turnover clockwise.

Report design: To measure the specific rotations of different sugar concentrated solutions. To construct the calibration curve. To fill out the table with the

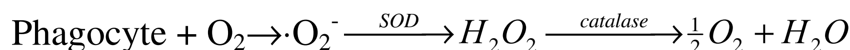
laboratory measurements. According to the collected data to construct the graphs. To draw cocnlusions.

Chapter 7 “Polarimetry” questions

1) What kind of light is polarized?
2) What is used to obtain the polarized light?
3) Which optically active substances do you know?
4) Malus’s law.
5) The arrangement of sugarimeter.

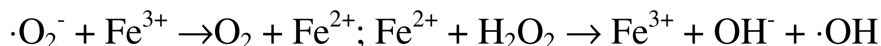
CHAPTER 8. LASERS IN BIOLOGY AND MEDICINE

With the advent of lasers has opened up new possibilities of using intense monochromatic light in surgery and therapy. Laser scalpel having a high radiation power and based on the high-beam heating of tissue used in surgery. In recent years, more and more lasers are widely used for therapeutic purposes. The most famous example - acceleration of healing of wounds at radiation of a wound surface with helium - neon lasers light.. The mechanism of action of such radiation is probably based on the reactivating effect of laser light (λ = 632 nm) to the protective enzymes catalase and superoxide dismutase (SOD), prosthetic group of this enzymes have absorption bands in the red region of the spectrum. The enzyme superoxide dismutase (SOD) catalyzes the reaction for generating hydrogen peroxide from superoxide radicals (O_2-), which are produced by phagocytes in inflammatory sites:



$$\text{Phagocyte} + O_2 \rightarrow \cdot O_2^- \xrightarrow{SOD} H_2O_2 \xrightarrow{catalase} \tfrac{1}{2} O_2 + H_2O$$

At insufficient activity of SOD and catalase with the superoxidic radical react iron ions; The process ends with the formation of highly toxic hydroxyl radicals $\cdot OH$:



$$\cdot O_2^- + Fe^{3+} \rightarrow O_2 + Fe^{2+};\ Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$$

In inflammation occurs inactivation of SOD by hydrogen peroxide, and the resulting low pH, may also decrease the activity of catalase and a transition of catalase reactions to the way of decomposition of H_2O_2 with forming a hydroxyl radical. Reactivation of these enzymes happens under the influence of laser radiation.

The word "laser" is made up of the initial letters of five English words: Light Amplification by Stimulated Emission of Radiation. The laser or optical maser, was established in 1954, almost at the same time by N.G Basov and A.M Prokhorov in the USSR (Lebedev Physical Institute) and Ch. Townes with co-workers in the United States (Columbia University). As working substance of the first quantum generator ammonia was used. Ammonia molecules brought into an excited state, and then created the conditions for their simultaneously to the baseline, resulting in a powerful radio pulse emitted.

From 1954 to 1960 there was a rapid development of quantum radio physics. Have been created various designs of lasers and developed their theory.

An important role in the development of quantum electronics played the works of the French scientist A. Kastler. Already in 1949 Kastler concluded that

atoms especially strongly absorb light in the case where their own frequency resonate with the frequency of the exciting radiation. In 1952, he developed a method of optical pumping: the atoms of the working medium are excited by an external source of light or microwaves. The work of Kastler largely accelerated the creation of the laser.

In 1958, Ch. Townes and A. Shavlov offered the principle of the laser. To implement the idea Shavlov suggested using ruby rods, having in its composition microinclusions of chromium, which atoms emit light. In 1960, American physicist, T. Meyman, was created the first ruby laser. At the same year, another American physicist Ali Javan created a gas laser.

Some types of lasers

Lasers are classified, as a rule, or under the name of classes of the substances applied in them as the active environment or on a method of achievement of population inversion in these environments.

In accordance with a first classification distinguishes solid, gas, semiconductor, liquid lasers, and other organic dyes.

On a method of obtaining population inversion distinguish chemical, gas dynamic, molecular, etc.

The solid-state laser on a ruby was the first laser. The crystal of a pink ruby was the active environment in this laser. For ions of a ruby the three-level scheme of an arrangement of power states is characteristic. Population inversion in the first laser was reached by an optical method of excitement by means of a powerful pulse xenon lamp. In later designs were applied other schemes of optical excitement of a crystal, allowing to improve conditions of illumination of a ruby, for example, elliptic reflectors. In one focus of such reflector the ruby crystal was located, and in other - a cylindrical lamp of a rating.

After the ruby laser in the fall of 1960 it was created gas laser, working at mix of gases of helium and neon. In the first gas laser tube length L = 100 cm, and 1.5 cm in diameter, filled with helium at a partial pressure (~ 130 Pa) and neon at a pressure (~ 13 Pa), placed between the resonator mirrors fixed to the bellows. Population inversion is achieved electrically by excitation of molecules. Fastening the cavity mirrors directly on the gas laser discharge tube was uncomfortable, since the output of the tube system made unusable and the optical resonator. It was suggested to have the end walls of the discharge tube at the Brewster angle. It is possible to eliminate the reflection on the end walls of the tubes and separate the resonator mirror of the tube.

Unlike solid-state lasers gas lasers work in the continuous mode. The efficiency of such systems is small, on the order of 0.1 to 2%, due to the in efficient method of pumping. The advantages of gas lasers should include a high

degree of coherence of the radiation. Gas lasers with appropriate stabilization generate line width of about 10^3 Hz, which is inaccessible to other types of lasers. Because of these characteristics gas lasers have found wide spread use.

In 1962, the semiconductor lasers have been developed, which are characterized by high efficiency, since they directly converts electric energy into light. Semiconductors for which direct transitions from a conductivity zone to a valent zone are characteristic are used to semiconductor lasers. For performance of a condition of generation initial material is strongly alloyed, so that Fermi's level for the semiconductor with p-type of conductivity was in a conductivity zone, and Fermi's level for the semiconductor with conductivity n-type – in a valent zone. On the basis of these materials creates a diode structure in pn-transition. When giving the potential barrier between p-and n-areas decreases by such diode of direct shift to zero and through transition rather big current in the forward direction starts flowing. Directly in the p- n- transition is a process of recombination of electrons and holes. As thus in area where recombine charges, conditions of inverse density of population are satisfied, that, having placed active area of a crystal in the resonator, it is possible to receive generation of light. Actually, the sides of the crystal diode applies as resonator which turn out as a result of the corresponding processing serve. The main difficulty at realization of semiconductor lasers consists in obtaining small force of threshold currents for generation of light in such lasers. The first semiconductor lasers at the room temperature had density of threshold current equal about 10^9 A/m ^{2} and could work only in the pulse mode. That these lasers could work in the continuous mode, they needed to be cooled up to the temperature of liquid nitrogen.

In 1964 semiconductor lasers on the basis of difficult structures of p-n-of the transitions consisting of various semiconductor materials were created (such transitions unlike diodes where as initial material the same semiconductor was used, (homojunction), received the name of heterojunctions). In such structures it was succeeded to reduce force of threshold currents at 10-20 times that allowed to carry out the continuous mode of generation at the room temperature.

Currently, the most powerful lasers operating in continuous mode, are molecular lasers. Although the nature of the medium, which is used in molecular lasers, they relate to gas, but they are isolated in a separate class of Equipment, as molecular lasers, unlike gas not used electronic and vibrational excitations.

In the first molecular CO2 laser population inversion between vibrational levels achieved under the conditions of the electrical discharge. In such lasers use the digit tubes reaching tens meters. In the largest lasers of this kind in the continuous mode the power of the radiation of 1-9 kW is reached.

It is possible to carry to molecular lasers also gas dynamic lasers though their way of obtaining inverse density of population is connected with use of thermal

methods of excitement of molecules. Gas dynamic lasers are the most powerful of known generators of optical range. With their help capacities of the radiation of ≈ 60 kW in the continuous mode are received.

Are available also chemical lasers in which energy of radiation directly is defined by energy of chemical reactions. The chemical laser in which the active environment is CO_2, generates, as well as other molecular OQG, on the wavelength of λ =10.6 microns.

Absolutely unique properties have liquid lasers in which the active medium is a solution of an organic dye in water or alcohol. The oscillation frequency of the dye lasers can be turned over a fairly wide range. Currently, we created tunable lasers, the frequency of the radiation that covers the entire visible range.

Source of pumping lasers with transverse pumping is a pulsed ruby laser. Light of this laser falls on a ditch with dye solution, placed in the resonator formed by mirrors between which there is a dispersing prism. In such resonator of a wave, being reflected from two mirrors, multiple passes through the active environment, providing under the corresponding conditions possibility of generation of light on this wavelength. By the prism turn is possible to change smoothly the wavelength or frequency of the generated light. In other resonators providing smooth reorganization of frequency of light, apply instead of a prism and one of mirrors a reflective diffraction lattice. The widespread dye applied in liquid lasers is rhodamine (6Zh). In the lasers on colorants dyes reorganization of wavelength of radiation in the range of 260-1200 nanometers is provided, and in the range from 260-360 nanometers it is possible will receive the power of the radiation of 1 MW, power in the range from 360-720 nanometers - 10 MW, in a near Infrared range - 100 MW. Duration of an impulse of a rating and the generated radiation about 20 nanoseconds.

The principle of operation of all lasers is the same. By means of the laser it is possible to receive a high-intensity bunch of the electromagnetic waves possessing such obligatory properties as monochromaticity, coherence and polarization.

The schematic diagram of any laser includes the active agent (firm, liquid, gaseous) which is a radiation source, the source of excitement (rating) supplying active agent with energy and the resonant device allowing to concentrate an energy stream in a certain direction.

It is known that atoms and molecules consist of nuclei and electrons. Energy of the relative movement of the particles making atoms can accept only strictly certain values. These E1, E2 values … Are called as EC as energy levels. The system of power levels (E1, E2,…, EC) makes a power range of atom. "Bottom" level – with the minimum energy - is called the basic, by the others - excited. The power range of atom depends on its structure (fig. 8.1).

Figure 8.1. Power range of atoms

Under normal conditions in the material levels with lower energy settled greater than the levels above. Consider the matter in which there is a sufficient number of excited atoms with the energy E2. The number of atoms is called settle level E2. If the settle of the level E2 is larger than the settle of level E1, then the substance is called active. In the process of electromagnetic radiation passing through the active substance, its` reinforcement will occur with help of the fact that the number of displaced atoms transition from level E1 to level E2 will exceed the number of absorption. Thus, the quantum gain is due to the internal energy of atoms, "flight" of the photons through matter cause the birth of new photons. There is an avalanche increase in the number of photons in matter. To wave energy is not weakened, increased when it passes through matter, it is necessary to artificially increase the population of the upper level and reduce the population of the lower level, ie transfer matter in a state with a population inversion (from the Latin. Inversion - turning, permutation). This is the first necessary condition for the functioning of the lazer as a laser-generating system.

The part of remained avalanche of electrons must always be in matter to cause the stimulated emission of new proton portions. This is achieved by mirrors. Passing through the working substance of the light wave is emitted into the environment, and the other part is reflected by the mirror, gives an impetus to the emergence of a new avalanche of photons. This is the second necessary condition.

A third suggests that the gain in the active substance should be sufficiently strong, ie, the number of excited atoms in the active substance should be greater than a certain threshold value.

Thus, to create a coherent light source must comply with the following conditions:

1. The substance must have an inverted settle. Only then the light can be amplified by stimulated transitions.

2. The substance is to be placed between the mirrors, which provide feedback. Through the mirrors the part of the radiated light energy that is

constantly remained within the working substance, causing stimulated emission can be achieved.

3. The amplification produced by the working substance, and hence the number of excited atoms or molecules in the working substance must be greater than a threshold value, which depends on the reflectivity of a semitransparent mirror.

Only under these three conditions we get a system that can amplify light by stimulated emission, called a laser, or lasers.

Helium-neon laser - is a laser where working substance is inert neon gas. The admixture of helium increases the inversion of settle levels of neon atoms (Figure 8.2).

For helium-neon lasers high stability of radiation frequency, simple structure, high directivity of radiation and long service life are characterized. In practice a helium-neon laser with a wavelength of 632.8 nm is traditionally used (0.64 microns).

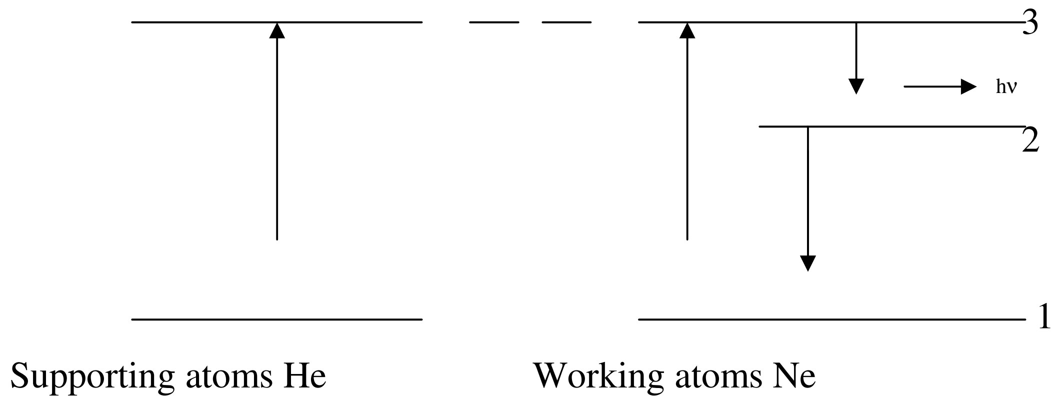
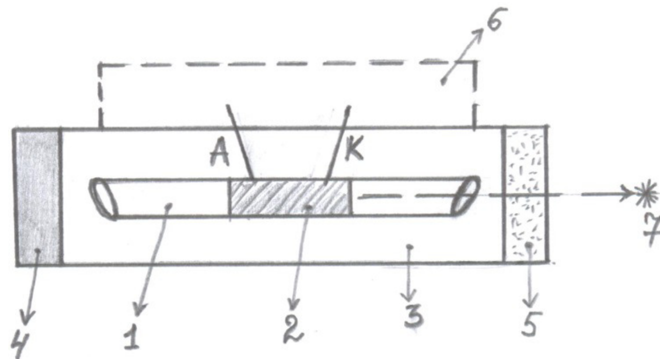


Figure 8.2 – Energetic spectrum of helium-neon laser

However, helium-neon lasers are with a different wavelength. For example, "green" laser with a wavelength of 543.3 nm and a power of 0.5 - 1 mW and infrared laser with a wavelength of 1523.1 nm. Generally helium-neon lasers, typically have a small output power (10 to 100 mW).

The main elements of the gas laser (fig.8.3) are: discharge tube containing a mixture of gases (helium and neon), an optical resonator (mirror), providing

feedback between the light and the excited atoms; power supply that provides ignition and maintaining a discharge in the tube.



1 discharge tube, 2 - active medium (helium-neon), 3 - hermetic case 4 - transparent mirror, 5 – semi-transparent mirror, 6 - power supply, 7 - the laser beam, A - anode,
K - cathode

Figure 8.3 – Scheme of gas laser arrangement

The ends of the discharge tube are closed by a plane-parallel glass plate arranged at fixed angle to the horizontal axis of the tube. Through the glass "window" the radiation passes without lossless and hits the mirror. They have a high reflectivity (up to 98 - 99%). At the same time, part of the radiation passes through the semi-transparent mirror in the form of intense red light beam.

Laboratory work № 19
Working principle of laser

Purpose of work: to study the principles of gas laser action
Task: check such properties of laser as monochrome, coherence, polarization.
Equipment: He-Ne gas laser, semiconductor lasers (Pointers), optical table, a monochromator, polaroids, lightfilters.

Laser radiation differs in a number of great features not distinctive for other radiation sources including sun. Laser light has next specific properties:
1 - Temporary and spatial coherence;
2 - Strictly monochromatic;

3 - Great power of radiation;
4 - Low divergence of light beam.

Procedure: With a help of monochromator observe light from the He-Ne gas lasers. Describe the observed spectra, pay attention on the presence of lines on varying color. Explain their presence.

With the help of polaroids make sure of the polarization of laser light, and evaluate the angular divergence of the beam by measuring its diameter at different distances from 10 cm to 2 meters.

Work design. Put in a table values of the axis of the drum and show the color line. Make graphics based on the received data. Explain the presence of lines of different color from the He-Ne gas lasers and methods of their elimination. Make conclusions.

Laboratory work №20
Study of the properties of natural light and artificial light sources

Purpose of work: study the differences of properties of light flow from different type of sources (incandescent lamp, neon and mercury vapour lamps, lasers), research the polarization and monochromaticity of laser light.

Tasks:

1. Analyze the monochromaticity of light from light sources that we have (incandescent, neon and mercury vapor lamps, lasers)
2. The angular divergence of light streams from the different light sources.
3. Use the polaroids to check the polarization of the light fluxes.

Equipment and materials: monochromator, optical table, light sources: incandescent lamp, mercury lamp, neon lamp, a helium-neon laser, polaroids, a diffraction bars, variable slits.

Procedure.

Task 1. Study of monochromatic light.

Send a light of monochromator from different radiation sources on slit. The work is best to start by considering the natural sunlight, then the light bulb, neon and mercury lamp, and then laser. The laser light must be seen only through a gray filter. By rotating a monochromator drum see all the spectra in the visible range. Pay attention to the intensity of the individual parts of the spectrum, especially the transition from one color to another, depending on the source of radiation, ie, the

presence of a smooth transition, or the presence of boundaries. Note the difference between linear and continuous spectrum. Determine the diapason where we perceive light. Make a graph postponing on ordinate the light (color) in nanometers, and the abscissa – indications of drum monochromator. Mark the red line of a helium-neon laser.

Check the perception of light and its passage through the goggles, first visual, wear them and then putting glasses in front of the slit of the monochromator to verify how the observed spectrum of the laser is changed.

Task 2. Monitoring the angular divergence of the laser radiation.

Placing a workbook in a light flux of the laser, mark the diameter at a distance of 10 cm, 50 cm, 100 cm, 200 cm. Determine the angle of divergence and compare with published data. Tangent of angle is equal to the relation of distance between dot and radius, find the angle according to Bradis. Make calculations for each measured distance and determine the average value. Make conclusions why the divergence of the laser light is different from different radiation sources.

Task 3. The observation of the linear polarization of the laser light.

Polaroid is placed Perpendicular to the luminous flux, rotate it around its axis and observe changes in the intensity of light passing through light analyzer. The experiment is performed with all light sources, noting the observed features. Make conclusions about the presence or absence of light polarization. Repeat experiments with two polaroids, explain changes.

Work design. The study of natural and artificial light source`s properties (incandescent lamp, neon and mercury vapor lamps, lasers). Fill the table. Draw graphics based on the data. Make conclusions.

Chapter 8 «Lasers in biology and medicine» questions

1. Which of radiation source gives the polarized light?
2. Which of the radiation sources provides monochromatic light?
3. Which of the radiation sources does not give monochromatic light?
4. Which of the radiation sources does not give polarized light?
5. What is the working fluid in a helium-neon laser?
6. Why spectrum of neon lights and helium-neon laser different from each other?

CHAPTER 9.
ELECTROCONDUCTIVITY OF LIVING SYSTEMS

The presence of animal electricity has been shown in certain experiments Galvani in the 18th century. Subsequent study of bioelectric phenomena showed special significance for the living presence of potential differences in the organs, tissues, cells. For example, without the potential difference on the membranes of organelles (mitochondria and chloroplasts) possible processes of respiration and photosynthesis. Of excitation, movement of animals, diffusion and transport of many substances as determined by the difference of potentials.

Living tissue composed of cells washed by tissue fluids. The cytoplasm of cells and tissue fluid electrolytes are separated by a poorly conducting cell membrane. Such a system has the statistical capacity and the polarization. Polarization capacity - the result of an electrochemical polarization that occurs when passing a constant electric current through an electrolyte. It depends on the current strength and time of its occurrence. According to modern concepts, the living tissues do not have the inductance and resistance is their only active and capacitive components.

When passing an alternating current through a living tissue observed dispersion of conductivity: tissue impedance increases with decreasing frequency current to a maximum value of Z max and tends to a minimum value Z min with increasing frequency. Fig. 9.1 shows a graph of the impedance of the muscle frequency AC (curve 1).

To estimate the variance conductivity B.N. Tarussov proposed polarization dispersion coefficient:

$$K = \frac{R_4}{R_6} \qquad (9.1)$$

where R_4- tissue resistance to the current frequency of 10 000 Hz;

R_6- tissue resistance to the current rate of 1 000 000 Hz.

The dispersion of the electrical conductivity of the living tissue is the result of capacitance depending on the frequency of the AC, as well as the effect of the polarization capacity, which at low frequencies affects stronger and decreases with increasing frequency. Living tissues only could show dispersion of tissues' conductivity. Slope of the curve (Fig. 9.1) decreases with the damage of tissue.

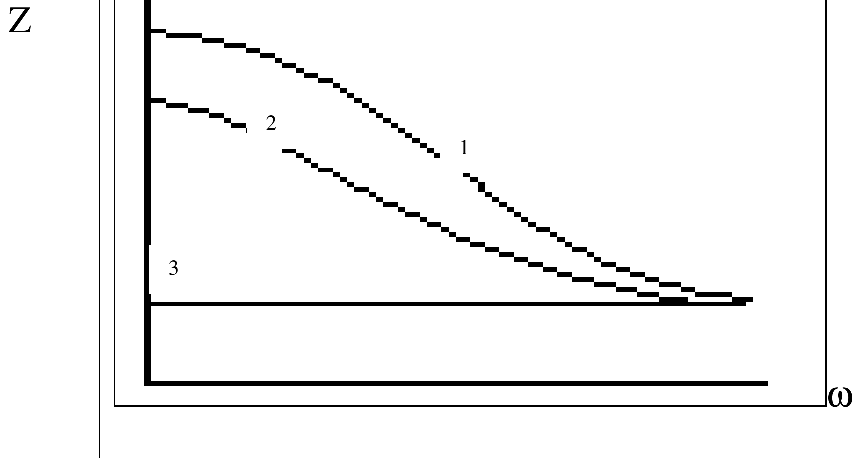


Figure 9.1. The dependence of the resistance area of the living tissue in the withering away of the frequency: 1 - living tissue, 2 - damaged tissue, 3 - dead tissue.

In connection with the transplantation of tissues and organs method for determining the electrical conductivity is used as one of the tests assessing the viability of preserved skin, cornea, bone etc.

Another manifestation of resistance to the reactive properties of living tissue is the presence of the phase shift between the voltage and ampeRAGe. For biological characteristic of a large phase shift between the power voltages, indicating that a significant proportion of the capacitance. For human skin, for example, 1 kHz the phase angle is equal to 55.

Impedance of tissue depends on the physiological condition and its value may be used for diagnosis. Diagnostic method based on measuring the impedance of tissue, called rheography.

The impedance of the living tissue can be modeled by equivalent circuits. Fig. 9.2 shows such schemes are shown graphs of Z (f) for these schemes.

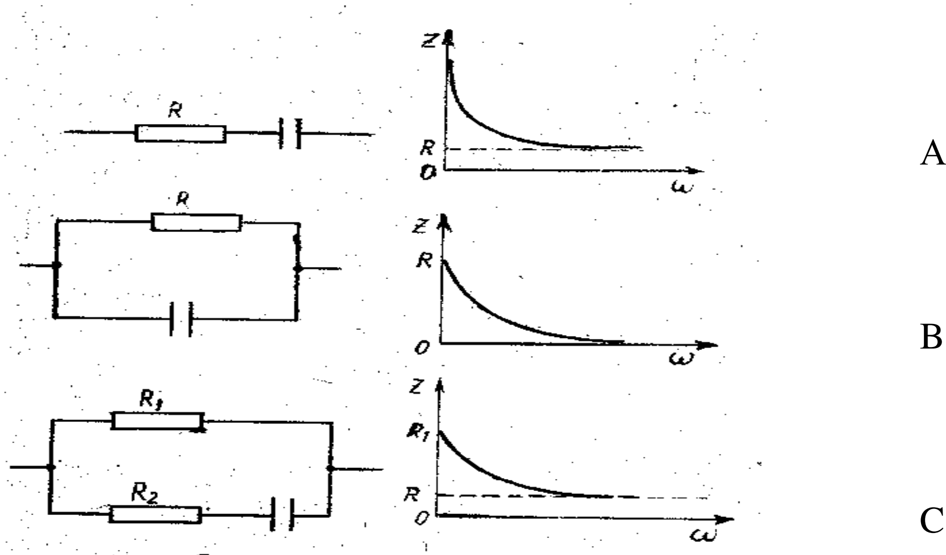


Figure 9.2 - Equivalent circuit diagrams

They show that the closest relationship to the living tissue is obtained for the circuit B.

Equipment for measuring the impedance is shown in Figure 9.3. You can turn the switch to turn on the circuit one of the three equivalent circuits. Consistently them included additional $R_D$resistor.

Since the equivalent circuit and includes an additional resistor in series, the current in them is the same. Legend: U_z- voltage in the equivalent circuit; U_R - the voltage across the resistor; Z - impedance of the equivalent circuit, I - current in the circuit. According to Ohm's law, $U_Z = IZ$, $U_R = IR_D$, where Z = UZ / I, I = UR / Rd; Consequently,

$$Z = \frac{U_Z R_Д}{U_R}. \quad (9.2)$$

The voltage at the input of the circuit is supplied with a sound generator, with which you can change the frequency of the applied signal. To measure the amplitude values of the voltages used channel oscilloscope that allows simultaneously monitor the voltage on one of the equivalent circuits (channel A) and the voltage on the additional resistor (channel B). When voltage is applied to the input setting on the oscilloscope screen there are two sine wave (Figure 9.4).

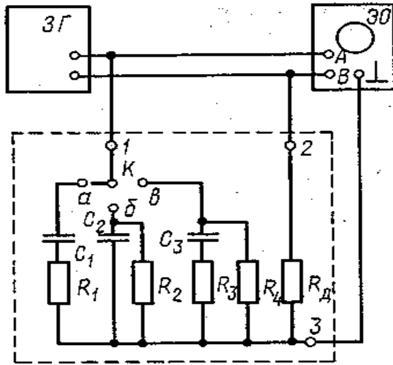


Figure 9.3.

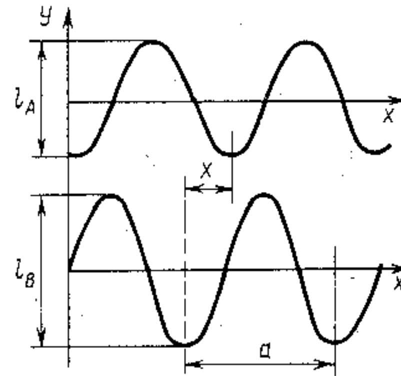


Figure 9.4.

By measuring the amplitude of the double l_A and l_B signals and knowing the sensitivity of the S_A and S_B of the oscilloscope respective channels, it is possible to determine the voltage

$U_Z = l_A/(2S_A)$, $U_R = L_B/(2S_B)$.

Substituting these relations in (9.2), we obtain

$$Z = \frac{l_A S_B R_Д}{S_A l_B}.$$

If $S_A = S_B$

$$Z = \frac{R_Д l_A}{l_B}. (9.3)$$

The obtained curve on the oscilloscope screen (Figure 9.4) can determine the phase shift between the voltage and ampeRAGe. Such as the voltage across the R_d resistor is in phase with current intensity, the phase difference φbetween the current intensity and voltage in the circuit is equal to the phase difference between the curves obtained:

$$\varphi = \frac{2\pi x}{d}, \quad (9.4)$$

Where, d - distance between two adjacent points of the curve with the same phase; x - distance between the different curves with the same phases.

Laboratory work № 21
The study of the laws of the passage of an electric current through living tissue

Objective: To investigate the changes in the dispersion and polarization resistance of healthy and damaged skin of the animal.

Tasks:

1. Measure the resistance of healthy and damaged skin of the animal
2. Calculate the coefficient of dispersion of polarization for a healthy and damaged skin of a frog.
3. Record the data in the table and plot
4. To draw conclusions about the observed phenomena and to prepare a report.

Equipment and materials: Oscilloscope, Equipment for measuring the impedance of the frog.

Procedure:

1. 2 immobilized frog, remove skin bags. The skin of a frog hurt (by any means, mechanical, to make electrical or thermal or chemical burn).
2. Put the switch to position A, including a sound generator and oscilloscope.
3. Set on the audio oscillator frequency f, to obtain a stable picture on the oscilloscope screen, provided $S_A=S_B$.
4. Measure l_A and l_B, then calculate the impedance Z and the equivalent circuit (Figure 9.2). R_d value indicated on the unit.
5. Measure the distance x and d, and calculate the phase shift φ between amperage and voltage in the equivalent circuit as well (Figure 9.2).
6. Repeat steps 3-6 for 10 different frequencies.
7. The results of measurements and calculations, store in the Table. 9.21.1.

Table 9.21.1.

Tissue	F, Hz	l_A, mm	l_B, mm	Z, Ohms	d, mm	X, mm	φ, mm
Healthy							
damaged							

Report design. Add these results to the table. Calculate the coefficient of dispersion of polarization for a healthy and damaged skin of a frog. Draw conclusions.

Laboratory work №22
Galvanizing. Electrophoresis of drugs

Objective: To study the effect of electric current on the movement of large macromolecules.

Tasks:

1. Introduce to experimental animals various organic substances by electrophoresis method.
2. To observe changes in the behavior of animals
3. Write the data to the table.
4. To draw conclusions about the observed phenomena and to prepare a report.

Equipment and materials: device for galvanizing, NaCl, water, pancuronium bromide (pipecuronium, vecuronium, atracurium) in ampoules, flannel or gauze pads, rabbits.

Continuous DC voltage 60-80 in use as a therapeutic method of physiotherapy (galvanization). Direct current is used in medical practice and administration of drugs through the skin or mucous membranes. This method is called electrophoresis drugs.

Procedure:

Two rabbits were shaved skin on both sides. Split ampoule with anycurariform substance and prepared saline. By shaven places attached flannel layer; some of them are wetted with a solution of curare, other saline. In flannel superimposed electrodes and current is passed through the chain force 50 mA. Trace the reaction of the animals.

Report design. Add results to the table. Study the influence of an electric current to the movement of large macromolecules. Draw conclusions.

Chapter 9 "Electrical Conductivity of living systems" questions

1. What is a carrier of electric current in living organisms?
2. What is the electrical conductivity?
3. What are the resistances of living organisms?
4. What is the variance of the electrical conductivity?
5. What is impedance?
6. Draw the equivalent circuit diagrams.
7. What is the galvanization and electrophoresis?

CHAPTER 10. BIOLOGICALLY ACTIVE POINTS OF HUMAN AND ANIMAL SKIN

In the early 20th century, I.R.Tarkhanov found on the body reduce the potential for local areas and, later, A.K.Podshibyakin showed that most of the points with a change in the electrical characteristics (reducing the potential resistance decrease dramatically increased conductivity) correspond to the points used in acupuncture in the south-eastern countries (China, Tibet, Japan, etc.).
Method of Zhen-Chui therapy originated in the territory of ancient China in the late Stone Age (Neolithic) and has about 4-5 thousand years. It is believed that these points, known as biologically active points (BAP), have been found empirically for both human and animal (horses, cows, dogs, etc.). Methods of Zhen-Chui therapy is very effective and widely used in many countries around the world, now acupuncture had a serious theoretical basis, which enabled her to rank the empirical Chapter of the medicine go to the rank of scientific.
A significant difficulty in studying the peripheral mechanisms of acupuncture is the lack of a clear common definition of the concept of "acupuncture point". In Chinese medicine, the point of impact, or acupuncture points are defined by the term Kung-sue, which literally means kun - hole Sue - burrow, cave, or hole spirit. Through these "holes" is an exchange of energy of the body and the environment, and through them the "CE" - the beginning of the disease-causing - penetrate into the human body or leave. According to traditional Chinese concepts, acupuncture points are 3 levels: superficial - Tian (heaven), the average Zhen (people) and lower - di (earth). The least effect occurs during insertion of the needle into the surface zone, and the maximum - by immersing it in a particular canonical point for each depth, the lower bound is achieved when the acupuncture point and the impact takes place at all levels. Hieroglyphic image of acupuncture points - KungSue - it determines the depth of the so incompetent identify skin projections of the acupuncture points with the proper acupuncture points. In most cases (except for the face and scalp) acupuncture points are located at a depth of 0.5 to 10-12 cm (Juan-tiao), the size of the points is very limited (a few millimeters).
So far, there is no consensus on the number of points, different authors are from 693 to 10,000, while others believe that it is enough to know 120 points. There are 12 paired and 2 unpaired channels. The channel name is usually associated with the body, but in terms of its influence on other organs. There are channels of Yin and Yang, based on the concepts of vital energy Chi, while Yang (masculine, day) - the positive pole of Chi, while yin (feminine, night) - Chi negative pole. Channel name: light (P), colon (GI), stomach (E), spleen and pancreas (RP), heart (C), small intestine (IG), the bladder (V), the kidneys (R), pericardial (MC), triple

heater (TP), gallbladder (VB), the liver (F) and wonderful channels (median): Yan - Du Mai Yin - Shen Men.

Modern medicine in the diagnosis, etiology, pathogenesis and treatment of diseases prefer to use specific categories (morphological, physiological, biochemical, and others.). In this regard, most of the studies of Western scholars on the mechanism of acupuncture directed to the study of individual aspects of acupuncture effects on the human body. It is no accident there is a significant number of theories of the mechanism of action of acupuncture.

The theory of tissue therapy. The main factors are the impact of neurohormones and protein decomposition products are formed during the traumatizing tissue at the injection needle.

The theory of capillary blood flow normalization. According to her under the influence of acupuncture normalizes capillary blood flow, followed by a secondary elimination of the pathology of an organ.

Theory of histamine alignment. When acupuncture reflex through the corresponding segments of the spinal cord and the sympathetic part of the autonomic nervous system in the affected tissues of the patient's body to normal histidine content and images from histamine; as a result there is an influence on the blood flow in the capillaries and normalization of metabolism.

Electrical theory: a) arising from acupuncture bioelectric currents exert a therapeutic effect due to the resonance phenomenon, i.e., the coincidence of the wavelength and frequency fluctuations arising bio currents with similar indications the patient's body tissue; b) local change of electric charge with the introduction of the needle into the acupuncture point (AT) affects the electric charge of the whole organism. The potential effects arising at the site of the needle and spreads along the canal, is an additional irritant primary impact points and points, which are located, downstream of the channel.

Thermoelectric concept. Drawing attention to the thermoelectric primary mechanism of action of acupuncture needles, the authors of this theory point out that the needle is introduced is a kind of core temperature and thus can influence the thermal homeostasis of the organism as a whole. Since the needle is immersed in an electrolyte medium, in relation to different temperature gradient therein an electric potential energetically most adequate to affect the nervous system. This Romodanov A.P. et al. biphasic changes in temperature and respectively the functional state of "points of impact" on the introduction of acupuncture needles: the first phase - the excitement, accompanied by a local increase in temperature caused by increased blood flow; The second phase - inhibition, accompanied by a local decrease in temperature.

Bioelectric energy and information theory. A living organism, interaction with the environment, forced to constantly adapt to the conditions and requirements of this

environment, providing homeostasis. Biophysical standpoint organism is a power system, the existence of which are of great importance electrical and electromagnetic processes occurring in the environment, and within the body, as well as their junction. It is assumed (E.S.Velhover, V.G. Vogralik).That the information and energy exchange between macro- and microcosm is carried out mainly through the AT. Y.P.Limansky (1990) puts forward the hypothesis that the AT is a specific system, capable to perceive and transmit signals to the brain about the changes the electromagnetic fields of the Earth and meteorological factors, calling it "eco-ceptical sensitivity." Y. P.Limansky said that system of eco-ceptical sensitivity, is a special afferent input, through which the body continuously monitors the quality and quantity of environmental factors, which in cases of significant deviation can alter the activity of vital functional systems. This information is integrated in the brain with similar information obtained through viscerosensory sensitivity of the internal organs, and is used to run the adaptive mechanisms aimed at weakening or full compensation for the negative changes in functional systems of the body.

From the standpoint of modern biophysical concepts of integrity of the living human body, having a specific aura, and possibly bio plasma is an integrated energy system of a living organism exists and develops in a bulk three-dimensional space in which the energy-connection is made by holographic feature: any part of the whole gives Introduction of the whole and the whole contains information about any part thereof. Thus, the matching points and channels reflect different organs embryological communication are scanned genetic information encoded in time and space.

Reflex mechanisms of acupuncture. The reflex principle of acupuncture, cauterization, and other methods of influence on AP is common. Many scientists held autonomic reflex theory of acupuncture and cauterization action developed A.E.Shcherbak (1936) for physiotherapy. According to this theory the main role in the mechanism of action of acupuncture belongs to the autonomic nervous system, skin and visceral relationship and others. There is now evidence that the response to acupuncture and other methods of influence on AT realized through the nervous system to include neurohormonal mechanisms. Stimulation of AP is the most pronounced reflex reaction within the metamere or spinal segment of the relevant internal organs, which is most closely connected stimulated point. This principle, known as "metameric reflexology," has a clear neuroanatomical study because individual spinal segments are not only appropriate skin (dermatomes), and the corresponding muscle (myotomes), bones and ligaments (sclerotomes), blood vessels and internal organs (enterotomes). These facts have been known from the work of M.I.Astvatsaturov (1929) and confirmed in relation to acupuncture, A.K.Podshibyakin (1960), Y.P.Sudakov et al. (1986). They are based on a

mechanism of convergence of different modalities of afferent impulses on the same neural elements. This explainsviscerosomatic influences, most clearly manifested at the level of the spinal cord. Similar viscerosomatic overlaps occur in the overlying formations of the central nervous system, for example, at the level of the thalamus. Within the brain viscerosomatic relationships are more complex, and yet they are objectively established in animal models and confirmed by clinical observations.
Thus, in response to the organism acupuncture involves all parts of the nervous system, beginning with the receptor unit and segmental spinal cord, including the central parts of the brain, including reticular formation, subcortical-stem structures, the limbic system and cortical formation.

Laboratory work №23
The study of biophysical parameters of biologically active points of human skin

Objective: To investigate the localization of biologically active points on the skin of the hands of man and the influence of time measurement, pressure and humidity on the electrical conductivity.

Tasks:

1. Sketch the topography of the points on the back and palm of the hand.
2. Find the point Ho - Gu, Wang - Gu Shen - Men.
3. Determine the electrical conductivity changes depending on humidity, pressure, measuring time, bring to the table.
4. Draw conclusions.

Equipment and materials: device for measuring the electrical conductivity of the skin "Search", alcohol, glycerin, water, cotton, topographic maps acupuncture hands.

Procedure.

Find certain points (Hae-Gu-Gu Wang, Shen-Men) using the acupuncture topography map. During the measuring vary the time of electrode' contact with the skin surface, recording the measurements in the table. Determine the value of the electrical conductivity after treatment with hand alcohol. To investigate changes in the values of conductivity depending on the moisture of the skin to increase its arm can be moisten with plain water. After application of glycerin (or Vaseline) to the skin investigate changes in the values of electrical conductivity.

Report design. Add results to the table. Draw the topography of the points on the back and palm of the hands. Compare the data obtained in different conditions. Draw conclusions.

Chapter 10 "Biologically active points of human and animal skin" questions

1. What is the biologically active point?
2. What theories explain mechanism action of acupuncture?
3. Where use knowledge of biologically active points?
4. What is the meridian, vital energy Chi?
5. How can affect the body for acupuncture points?

CHAPTER 11. ELECTROCARDIOGRAPHY

Electrocardiography is called the method of recording the potential difference on the surface of the human body, which occurs during the contraction of the heart and reflects the electrical processes occurring in it and its physiological condition.

According to the Einthoven theory the heart treated as a generator of electric currents, which arise in the time of contraction of the heart muscle and gradually cover it completely. The excitation of the heart muscle reflect three positive wave directed upwards (P, R, T), and two negative, downward (Q, S), relaxation of the heart muscle is recorded as straight between the teeth T and P. Barb P, or atrial complex is the algebraic sum of the potentials arising in the right and left atrium (right are positive, and the left - negative. QRST - ventricular complex, reflecting the excitation and contraction of the ventricles. Features of each of the teeth, their amplitude and duration characterize the two main physiological properties of cardiac muscle: conductivity. The heart is located in a conductive medium, therefore believe that the potential difference on the surface of the body reflects the processes taking place in the heart muscle. Figure 11.1 shows a diagram of a typical ECG.

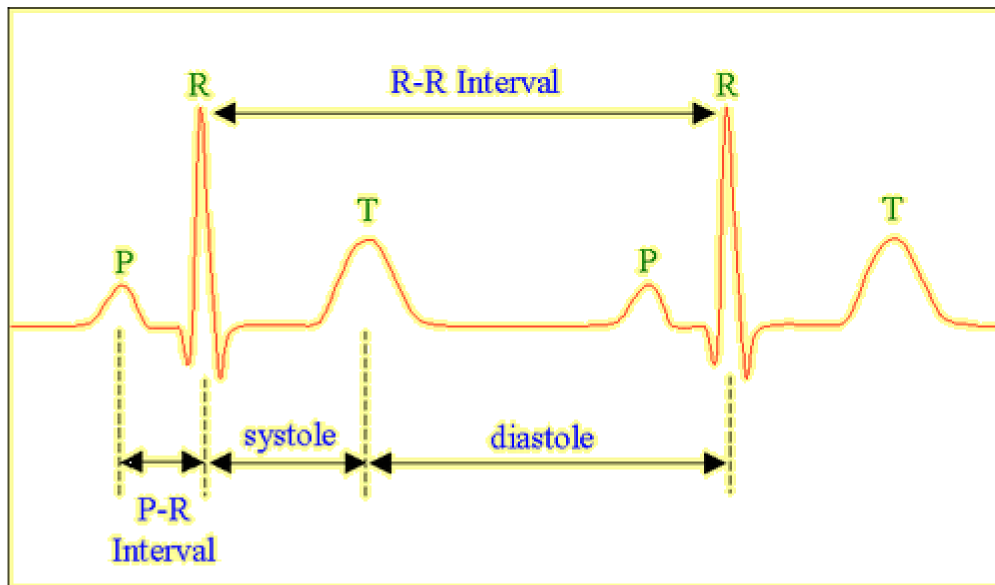


Fig. 11.1 – Human' electrocardiogram.

The figure below shows the direction of the electrical axis of the heart and the possible pathology.

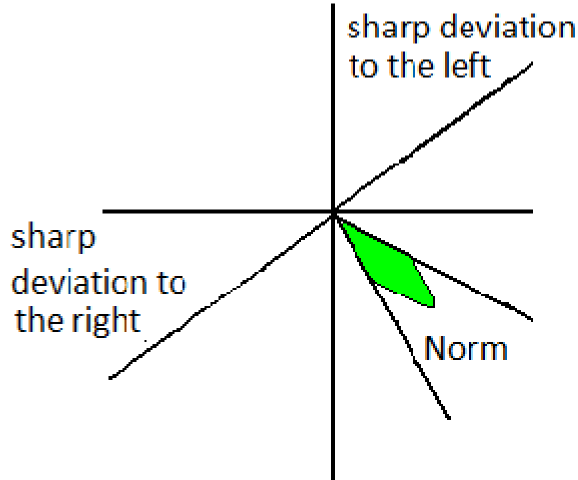


Fig. 11.2 - Electrical axis of the heart of man

The heart in the chest is an electric dipole, which generates currents propagating along the cardiac muscle. Measurement of body surface potentials to determine the direction of propagation of the currents during each cardiac cycle. In other words, to determine the electrical axis of the heart. In a healthy person, and electrical anatomical axis of the heart are the same. Diseases of left ventricular hypertrophy, especially his cause sharp deviation of the electrical axis to the left, and the defeat of the right stomach- axis deviation to the right.

Laboratory work №24
The study of human electrocardiogram during exercise

Objective: To investigate the changes in the ECG caused by human physical activity.

Tasks:

1. Record 5-7 cardiac cycles with each sample ECG in the standard Einthoven' leads before and after exercise.

2 Calculate the amplitude and duration of the teeth P, Q, R, S and interval of the cardiac cycle.

3. Compared with the values of the norms conclude state on the cardiovascular system.

Equipment and materials: ECG electrodes, saline, gauze, medical couch. The work carried out on the students themselves.

Figures 11.1 and 11.2 shows a diagram of a typical ECG and Table 11.24.1 are the amplitude and duration of the teeth typical for a healthy heart at rest and finding the body in a horizontal position (lying on the couch).
Any exercise, even a simple change in the position of the body, ie, when a person stands up, leading to changes in the heart, which is reflected in the changing characteristics of ECG peaks.

Table 11.24.1 - normal ECG indicators

ECG peaks, A – amplitude, mV; D – duration, s														
P		Q		R		S		T		Intervals, s				
A	D	A	D	A	D	A	D	A	D	P Q	Q R S	Q R S T	S T	R R
0.05-0.25	0-0.1	0-0.2	max. 0,03	0,3-1,6	max. 0,03	0-0,03	max. 0,03	0,25-0,6	max. 0,25	0,12-0,2	0,06-0,09	0,30-0,49	0,-0,15	0,7-1 (It depends on the heart rate)

Procedure:
To determine the use of special pathology tests - ECG recorded before and after exercise.
1. orthostatic test. ECG is recorded in the position of the subject lying down and as soon as he gets up. Normally only allowed a small increase rate, no other changes.
2. Exercise testing. Dosed physical load, most often use a) 20 sit-ups, b) a quick 15 or 20 second run on the spot.
With a good functional state of the heart after exercise can have the following ECG changes:
- Increase in the contraction frequency by 50-60% compared to the original and is stored in sinus rhythm;
- Electrical axis varies significantly;
- PQ interval does not change or slightly shorter;
- QRS duration is not changed or slightly shorter;
- Displacement of the isoelectric line is not greater than 1 mm;
- Change of P wave is not more than 3 mm;
- Recovery of all baseline after 5 minutes of rest.

Check your ECG using standard limb leads. Under the electrodes required to apply wet gauze pads. Electrodes: Red - right hand, yellow - left hand, black - right foot,

green - left foot. Electrodes are placed on the wrists and legs, check the grounding of the device. Set the speed of 25 mm / s, calibrate the instrument.

1. Record 5-7 cardiac cycle.
2. Determine the amplitude and duration of the teeth P, Q, R, S; find the length of the cardiac cycle and heart rate. Measure interval P-Q; QRS; Q, T. Analyze the condition of the heart, comparing with those typical of the standards (see.Table). ECG recorded at rest, lying down, just as the subject will rise sharply, and then after exercise. After loading the first ECG recording made immediately and then after 5-7 minutes rest. In a healthy human EEG is restored completely.

Report design. Electrocardiogram recorded at each sample glued into the notebook, refer to the teeth and intervals. The obtained values are compared with the physiological norm. Comparing data obtained in different conditions. Draw conclusions about the state of the cardiovascular system of the subject.

Laboratory work №25
Construction of the electrical axis of the heart

Objective: To determine the electrical axis of the heart and evaluate the state of cardio - vascular system of the subject.

Tasks:

1. Record 5-7 cardiac cycles during orthostatic test before and after exercise in the standard leads for Eythoven.
2. From the values of I and II standard leads calculate electric systole, and build an electric axis of the heart, determine the angle α.
3. Compared with the values of the norms conclude on the state of the cardiovascular system.

Equipment and materials: ECG electrodes, saline, gauze, medical couch. The work carried out on the students themselves.

Procedure: the examinee is laid on the couch. Electrodes are placed on the wrists and lower legs, putting them under a wet gauze pad. Left Hand - yellow, left leg - green, right hand - red, right foot - black. The electrode on the right lower leg is grounded and indifferent. Registration is carried out in standard leads I, II and III. (I abduction- from the right and left hands, II abduction- on the right hand and left foot, III abduction- left hand and left foot). Before registering recorded calibration signal 1 mV = 1 cm. Each test write at least 3-7 cycles of contraction in each lead. Recorded in the supine position (-7 5 minutes of adaptation to the situation) and the upright right after lifting. Calculate the electrical systole, and build an electric axis of the heart, determine the angle α (Fig.11.25.1).

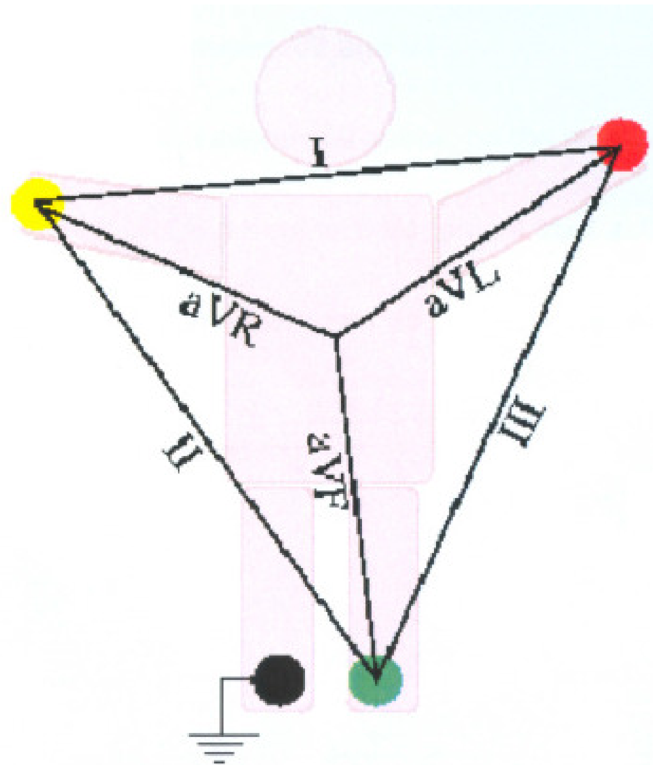


Figure 11.25.1 - Diagram of the electrical axis of the heart

For the construction of the electrical axis arbitrarily build an equilateral triangle, which then from the middle of the sides is reduced perpendiculars on intervals equal to the sum of modules R-wave amplitude, and Q waves and S with respect to the direction of the vector (ie, up or down from the center line for each lead ECG, respectively).
R wave upwards - is the direction of the vector plus the lower axis - negative direction. Values of Q wave and S vary widely and can be any value, as zero, so the positive and negative. To construct the averaged values for axis 5-7 cardiac cycles. For a healthy heart a change in body position in space does not lead to abrupt shifts the electric axis.
Report design. Electrocardiogram recorded at orthostatic test, glued into the notebook, refer to the teeth and intervals. Build electrical axis of the heart. The obtained values are compared with the physiological norm. Draw conclusions about the state of the cardiovascular system of the subject.

Chapter 11 "Electrocardiography" questions

1. Tell about the structure of the heart muscle and characteristics of cardiomyocytes.

2. Think of the structure of the conducting system of the heart rhythm, and pacemakers.
3. What processes occur during depolarization and repolarization of muscle fibers?
4. What is the electric dipole?
5. Why do I need grounding of the patient?

CHAPTER 12. GALVANIC SKIN RESPONSE

Galvanic skin response (syn .: galvanic reaction, galvanic skin reflex, reflex galvanic, GSR) - a change of the potential difference and a decrease in the electrical resistance between the two portions of the surface of the skin (eg, the back and the palm of the hand) irritations associated emotional reactions.

Research of GSR conducted to study psycho-physiological reactions human features of his personality and level of activity of the sympathetic nervous system, which is widely used in the psycho-physiological, physiological and clinical and physiological, forensic investigations as a highly sensitive, simple and technically easily defined indicators as well as to assess the neurodevelopmental voltage human. Register GSR is an important component of research on polygraph, the so-called "lie detector".

Bioelectrical activity on the skin caused by the activity of the sympathetic nervous system and the activity of the sweat glands. Is recorded everywhere from the surface, except for areas deprived of sweat glands (red border and al.), But generally used the fingers and hands or soles of the feet.

In the structure of GSR allocate the level of tonic activity, as background, a relatively long state, and reactions that occur in response to stimuli. In studies on the effectiveness of advertising, it was found that the performance of the GSR in the perception of advertising is not uniquely associated with behavioral responses.

There are two methods for registering GSR Tarkhanov, ie Check the potential difference between the two portions of the skin and a method Feret - the study of electrical conductivity of the skin current is passed through it. When registering skin potentials in the circuit there is no external power supply; the potentials recorded a sensitive galvanometer.

Skin - is complexly organized body of the human body and is not well understood. It complements the functions of a number of internal organs, taking part in the removal of products that are not allocated lungs and kidneys, it absorbs oxygen from the outside, being at the same time a good obstacle for all kinds of harmful substances, pathogens and others. An important role played by the skin in the heat exchange and metabolism .

Skin can perceive "radiosound" wave (e.g., within range of the RF transmitter), especially at the frequencies of 425, 1310 and 2982 MHz., is able to distinguish metals from nonmetals, feel radioactivity. A.K. Podshibyakin known researcher found that near-Earth magnetic storms to the potential of the skin increases, allowing weather-sensitive people to anticipate unseen eddies for 1-4 days before registration by physical Equipment.

According Tarkhanov' GSR changes associated with increased neural activity human is accompanied by increased secretion of sweat and manifested by the galvanic current on the skin surface.

The reaction of skin potential is in the form of one or two-phase oscillations. Negative phase bound, apparently with the release of adrenaline sympathetic endings in the skin, and the positive - to the activity of the sweat glands. Both these facts determine the value of the initial electric resistance and the impedance of the skin.

Skin cells have different permeability in the portion facing outwards and inwards, and can actively migrate into sodium ions, overcoming its concentration gradient. Therefore, along with simple physical and chemical factors in the dynamics of the potential leading role played by the vital processes of the skin.

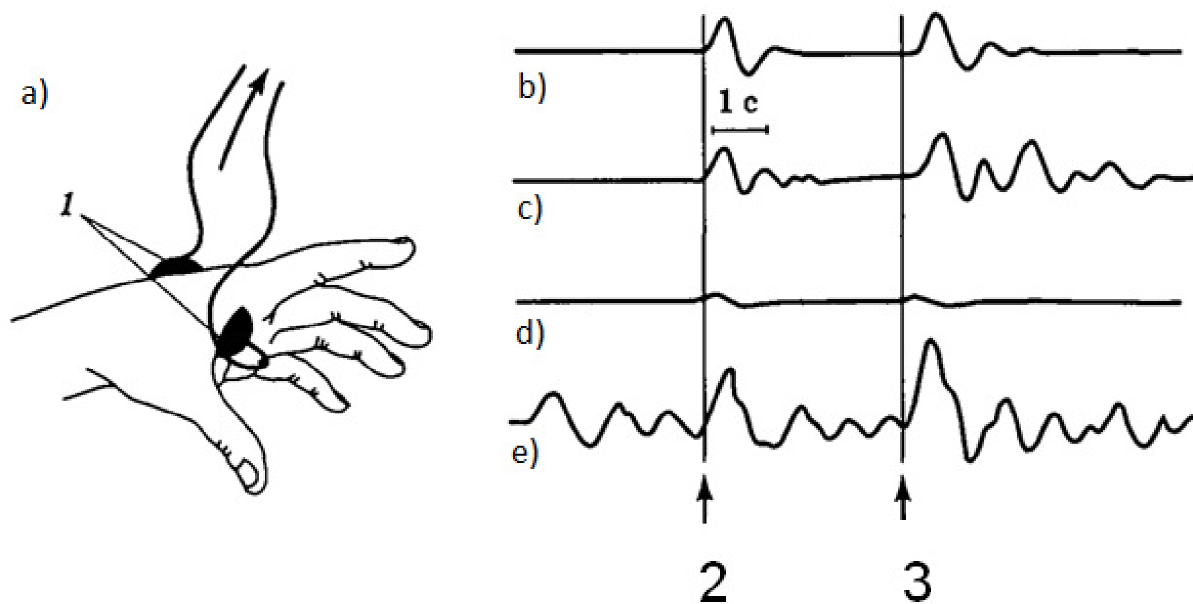
The origin of the GSR is essential as the distribution of active points of the skin formed by getting into the skin of the nerve fibers.

There are two methods of registering galvanic skin reactions: by Tarkhanov (check electrical potential of the skin) and Feret (Check the electrical resistance of the skin). Both methods give identical results, only the latent period of the change of resistance of the skin is slightly higher than the change in the potential of the skin.

Table 12.1 - Methods of study of the electrical activity of the skin

Ferre	Tarkhanov
active (with an external source of field)	passive
measurement of resistance and conductivity	measurement of the electrical potential of the skin
application of electrodes directly onto the skin test (contact)	

The appearance of the skin electrical potential in the world for the first time investigated the Russian physiologist, an expert on "animal electricity", the student Sechenov, Ivan Romanovich Tarkhanov. In the world literature, this method is called the phenomenon of Tarhanova is to strengthen and galvanic effects in human skin when irritated feelings and various forms of mental activity. Any irritation caused to a person through 1-10 seconds. latent period is slow and easy at first, then accelerates all deflection of the mirror galvanometer, often going beyond the scale. This deviation is sometimes lasts for several minutes after cessation of the stimulus. Already I.R.Tarkhanov noticed that electrical phenomena in human skin dramatically amplify the imaginary imagination feeling when an abstract mental activity when excited nervous system, fatigue.



a) - the location of the electrodes; b), c), d), e) - Entry to GR in different subjects:
1 - to an amplifier, 2 - a simple signal 3 - complex signal
Figure 12.1 - Typical GSR

Fixed potential difference skin is 10-20 mV at a distance of 1 cm between the electrodes. In case of irritation it may flicker to 100 mV or more.

Using the method of measuring the Feret requires two types of resistance: the resistance of the skin and the contact resistance of the electrode. It is found that the resistance of skin ranges from 10 kOhm to 2 MOhm. Therefore, in the studies of skin resistance is reduced, moisturizing it.

Laboratory work №26
The study of human' galvanic skin response

Objective: To investigate the development of the reaction of the GSR, in response to stimuli.

Tasks:

1. Register GSR in response to unexpected stimuli sound (say sharp knock) light. Determine the latent period of the reaction and its extinction.
2. Conduct a "lie detection" - to try to guess the unknown number or name. The test records on paper concealed number (usually 5-7 using sequence numbers, one of which becomes concealed) and displays it to the researchers.

Equipment and materials: electroencephalography, sound and photic stimulator.

Procedure. Register GSR using electroencephalograph, setting minimum paper speed and minimum gain. Electrodes should be placed by Tarkhanov on the back surface of the hands and incense. The skin under the electrode wipe cotton swab moistened with alcohol to degrease the surface. For better contact use a special gel or soap solution.

Recording is carried out monopolar, ie between the two electrodes.

The tested student should sit quietly in a relaxed state. Turn on the light and sound sudden, repeat signals 7-10 times in a row with 7-15 seconds interval watch fading GSR.

A series of numbers randomly offered for identification of at least 7-9 times so that the interval is called the number was certainly larger than the latent time of GSR, the reaction itself and its extinction. Typically, between is the number sufficient interval of 15-20 seconds. Bole often presenting numbers can cause reactions GSR layering one on another. The results of processing GSR bring to the table 12.24.1.

Table 12.24.1. An analysis of GSR student FI Registration Date

№p /p	Type of reaction	duration / amplitude response				
		1	2	3	4	5
1	sound					
2	light					
3	number 1					
3	number 2					
	. . .					

Find the maximum response with minimal fading GSR, it must be concealed number.

Report design. In notebook glued samples made records. The findings are recorded in a notebook.

Chapter 12 "Galvanic skin response" questions

1. What is difference between the Tarhanov method and Feret method?
2. Why GSR always observed as a component of the orientation reflex?
3. Is it correct to use the results of the GSR as evidence in court?
4. What is the role of the sweat glands in the GSR?

The International System of Units (SI)

SI base units

Value	Name of the unit	Identification unit
Length	Meter	m
Mass	Weight	kg
Time	Second	s
The strength of electric current	Ampere	A
Thermodynamic temperature	Calvin	C
The power of light	Candela	cd
Amount of substance	mol	mol

Additional units

Value	Name of the unit	Identification unit
Unit of plane angle	radian	rad
Unit of solid angle	steradian	sr

SI derived unit and special names

Value	Name of the unit	Identification unit	derived units
frequency	Cps	Cps	s^{-1}
Force	Newton	N	$m•kg•s^{-2}$
pressure	Pascal	Pa	N/m^2
Job; energy; heat	Joule	J.	N•m
Power, radiant flux	Watt	Wt	J/s
Electric charge; Quantity of electricity	coulomb	Cl	A•s
Electric potential; potential difference	Volt	V	Wt/A
Electrical capacity	Farad	F	Kl/V
Conductivity	Siemens	Sm	A/V
Electrical resistance	Ohm	Ohm	V/A
Light flow	lm	Lm	Kd•sr
Illumination	lux	Lx	$M^{-2}•kd•sr$
Induction magnetic flux	Weber	Wb	V•s
Magnetic induction	tesla	tl	Vb/m
Inductance	Henry	Hr	Vb/A

temperature Celsius	Degree Celsius	ºC	C

SI prefix

Console	Identification unit	Modifier	Console	Identification unit	Modifier
exa	E	10^{18}	deci	d	10^{-1}
Peta	P	10^{15}	centi	c	10^{-2}
Tera	T	10^{12}	milli	m	10^{-3}
Giga	G	10^{9}	micro	µ	10^{-6}
Mega	M	10^{6}	nano	n	10^{-9}
Kilo	k	10^{3}	pico	p	10^{-12}
Hekto	h	10^{2}	femto	f	10^{-15}
Deca	da	10^{1}	atto	c	10^{-18}

Units outside the SI

Several units outside the SI, are used so widely that it is difficult to do without them in their daily lives, so 8 of these units has been left to apply equally with SI units: minutes, hours, days - to measure time; degree, minute, second - to measure the angle of a plane; ton liter and - for measurement of volume and weight.

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