Preface

Biophysics is the branch of biology that applies the methods of physics to the study of biological structures and processes.

Biophysicists study life at every level, from atoms and molecules to cells, organisms, and environments. In the 20th century, great progress was made in treating disease and biophysics provided both the tools and the understanding for treating of diseases. Advanced biophysical research instruments are the daily workhorses of drug development in the world's pharmaceutical and biotechnology industries. Biophysics gives us medical imaging technologies including MRI, CAT scans, PET scans, and sonograms for diagnosing diseases. It provides the life-saving treatment methods of kidney dialysis, radiation therapy, cardiac defibrillators, and pacemakers. Biophysicists invented instruments for detecting, purifying, imaging, and manipulating chemicals and materials. Biophysics discovers the biological cycles of heat, light, water, carbon, nitrogen, oxygen, heat, and organisms throughout our planet. Biophysics harnesses microorganisms to clean our water and to produce lifesaving drugs.

From the above it is obvious the importance of Biophysics as a interdisciplinary science. In the course of Medical Biophysics is studied a range of problems, absolutely necessary in the professional training of those who study at the faculties of General Medicine, Dentistry and Pharmacy. Conventionally, these problems can be divided into three groups of problems:

- the physical analysis of the basic mechanisms that refers to composition, structure and development of living matter;
- study of physical methods used in biomedical exploration as well as the construction and operating principles of apparatus used in medicine;
- analysis of the biological effects of the environmental physical factors and fundamental level of interaction of these factors with biological-system.

Therefore, the purpose of this book is to train some specific practical skills of the students in the first year of study at State University of Medicine and Pharmacy "Nicolae Testemitanu". Thus, we have selected a set of 10 practical work that will demonstrate some physical phenomena and principles that govern living systems. The practical works contains both theoretical concepts necessary to understand the essence of the studied phenomena and practical instructions necessary for performing the experiments and measurements.

Educational devices used for this purpose were developed by collaborators of the department of Human Physiology and Biophysics.

Student's guide
to laboratory work on the discipline of Medical Biophysics

General rules

- Laboratory work is carried during a semester as one hour per week;
- A laboratory session lasts 3 hours = 2h 15min (general medicine and farmacy) and 2 hours = 1h 30min (dentistry and farmacy second semester);
- Students are advised to be punctual, because otherwise they will not be admitted in class;
- Students must present themselves in class dressed in white coat and have prepared the practical work manual, notebook and pen;
- Warm clothes must be left in the locker room, which is on the first floor. It is strictly forbidden storing them on tables or chairs;
- Students are asked to put their bags on the table behind the class;
- Inside the lab is forbidden
  - to eat or drink;
  - to smoke;
  - to use mobile phones;

Performing laboratory sessions

- The professor will check the attendance, the last week laboratory work performing and sign the finishing work;
- The professor checks student's theoretical knowledge of the theme of the current laboratory;
- According to the procedure of the laboratory work, students, under the guidance of professor, do the practical part of the work;
- At the end of the laboratory students are required to present the laboratory sheet to the professor, which includes experimental measurements values;
- At the end of the lesson students must bring in order the working instruments and the service student must clean the blackboard and to check the general order in the laboratory room.

The practical works recovering

- Students will not be allowed in the exam if they do not have all the laboratory works;
- More than 2 unrecovered absences are not acceptable; all practical works scheduled for the dates of the absences must be recovered;
- Recoveries are performed once a week, according to professor’s predetermined timetable;
- Per session students can recover maximum two topics.

Performing of the exam

- The biophysics exam has a written form;

THEORETICAL BASIS OF ERROR ANALYSIS

- The examination test includes two practical questions and one theoretical question;
- At the exam only students who meet the following requirements may present:
  - all their laboratory works were signed by teacher;
  - all their absences were recovered;
  - have an average grade of 5.00 or more;
- Students collaboration is strictly forbidden during the exam;
- The final mark is calculated as:

$$FM = \frac{PM + EM}{2}$$

where FM- the final mark
PM- the mark from practical works
EM- the mark from exam

- The practical mark is calculated as:

$$PM = \frac{AM1 + TM1 + AM2 + TM2}{4}$$

Where AM1, AM2- the current average of the marks from two cycles of practical works
TM1, TM2- the marks from tests.

THEORETICAL BASIS OF ERROR ANALYSIS

Sources of Experimental Uncertainties (Experimental Errors):

The terms "***uncertainty***" and "***error***" have a number of possible meanings. We use "uncertainty" in the straightforward sense. That is, the uncertainty in a measured value represents the extent to which we are uncertain that the value quoted indeed represents the value of the quantity which we have set out to measure. "Error" has two common meanings:

1. Error is the difference between a measured or calculated value of some quantity and the "true" value. In most cases of interest, the "true" value is not known and the magnitude of the error in this sense is somewhat hypothetical;
2. More commonly, the word "error" is used to mean our best guess as to how far the value of the measured value may differ from the true value. Frequently, error in this sense means simply the estimated uncertainty in the measured value.

All measurements are subject to some uncertainty as a wide range of errors and inaccuracies can and do happen. Measurements should be made with great care and with careful thought about what you are doing to reduce the possibility of error as much as possible.

There are three main sources of ***experimental uncertainties*** (***experimental errors***):

1. Limited accuracy of the measuring apparatus – some apparatus cannot determine an experimental measure better than with a limited accuracy.
2. Limitations and simplifications of the experimental procedure – for example: there is no air friction if objects are not moving fast. Strictly speaking, that friction is small but not equal to zero.
3. Uncontrolled changes to the environment – for example: small changes of the temperature and the humidity in the laboratory.

Do not list *your mistakes* as experimental errors. These mistakes should have already been detected and eliminated during the preparation of the laboratory report. A calculator should not be listed as a source of experimental error. You may always use more significant figures during calculations to reduce round-off error. In other words, you may always make all calculations with a better accuracy then you can do lab measurements.

Random Errors

Random errors arise from the fluctuations that are most easily observed by making multiple trials of a given measurement. Let us consider an example of the measurement of the density of a liquid. If you were to measure the volume V of the liquid many times using a graduated cylinder, you would probably find that your measurements were not always exactly the same. In this case, the main source of these fluctuations would probably be the difficulty of judging exactly where the level of the meniscus was on the cylinder. Since you would not get the same value of the volume each time that you tried to measure

it, the volume would be uncertain, at least to the degree that your measurements disagreed with one another. There are several common sources of such random uncertainties:

1. Uncontrollable disturbances and fluctuations in initial conditions in a set of measurements (the room temperature may fluctuate causing the fluid volume to change).
2. Limitations imposed by the least count of your measuring apparatus, and the uncertainty in interpolating between the smallest divisions.
3. Lack of precise definition in the quantity being measured. (The length of a table in the laboratory is not well defined after it has suffered years of use and abuse. You would find different "lengths" if you measured at different points on the table.)

No matter what the source of the uncertainty, to be labeled "random" an uncertainty must have the property that the fluctuations from some "true" value are equally likely to be positive and negative. This fact gives us a key for understanding what to do about random errors.

We could make a large number of measurements, and average the results. If the uncertainties are really equally likely to be positive and negative, we would expect that the average of a large number of measurements would be very near to the correct value of the quantity measured, since positive and negative fluctuations would tend to cancel each other.

Systematic Errors

An uncertainty arising from random errors is unavoidable. By contrast, systematic errors should, in principle, never be present. Systematic errors arise from a flaw in the measurement scheme which is repeated each time a measurement is made. If you do the same thing wrong each time you make the measurement, your measurements will differ systematically (that is, in the same direction each time) from the correct result. Some sources of systematic error are:

1. Errors in the calibration of the measuring instruments.
2. Incorrect measuring technique: For example, while trying to measure the density, a substantial amount of a liquid might evaporate between measuring the volume and measuring the mass. Or one might make an incorrect scale reading because of parallax error.
3. Bias of the experimenter. The experimenter might consistently read an instrument incorrectly, or he might let his knowledge of the "true" value of a result influence his measurements.

It is clear that systematic errors do not average to zero, if we average many measurements.If a systematic error is discovered, sometimes a correction can be made to the data for this error. One must simply sit down and think about all of the possible sources of error in a given measurement, and then do small experiments to see if these sources are active.

Absolute and Relative Errors:

If we do not know the accepted value of the measured quantity, but the measurements have been repeated several times for the same conditions, one can use the spread of the results themselves to estimate the experimental error.

Average Values, Absolute and Relative Errors:

The general formula for calculation of the ***average value*** $\bar{X}$ or X_{AV} (sometimes also called ***mean value***) is as follows:

$$\bar{X} = \frac{x_1 + x_2 + \cdots + x_n}{n} \quad (1)$$

where n is the number of repeated measurements (for this example $n = 6$).
The general formula for calculation of the ***deviations*** from the average value(also called ***absolute error***) is as follows:

$$\Delta X_n = |\bar{X} - X_n| \quad (2)$$

The values of the deviation from the average value are used to calculate the experimental error. The quantity that is used to estimate these deviations is known as the ***relative error*** and is defined as:

$$\varepsilon = \frac{\overline{\Delta X}}{\bar{X}} \quad (3)$$

where $\overline{\Delta X}$ is the mean value of the absolute error.
The relative error is dimensionless, but is often expressed in percentage, as:

$$\varepsilon = \frac{\overline{\Delta X}}{\bar{X}} 100\% \quad (4)$$

The general format for presenting experimental results with experimental error is given by one of the following expressions:

"***final result***" = "***average value***" ± "***relative error***"

$$X = \bar{X} \pm \overline{\Delta X} \text{ (units)} \quad (5)$$

Obviously, the average value and the relative error must have the same units.

Example:

Consider the following results of velocity measurements:

$v_1 = 0{,}38 m/s,\ v_2 = 0{,}38 m/s, v_3 = 0{,}35 m/s, v_4 = 0{,}44 m/s, v_5 = 0{,}43 m/s, v_6 = 0{,}42 m/s.$

The average value of these six velocity measurements is equal to:

$$\bar{v} = (0.38 + 0.38 + 0.35 + 0.44 + 0.43 + 0.42) m/s \,/\, 6 = 0.40\, m/s.$$

Next, one needs to calculate the ***deviations*** from the average velocity:

$\Delta v_1 = |0.40 - 0.38| = 0.02 m/s;$ $\Delta v_4 = |0.40 - 0.44| = |-0.04 m/s| = 0.04 m/s;$

$\Delta v_2 = |0.40 - 0.38| = 0.02\, m/s;$ $\Delta v_5 = |0.40 - 0.43| = |-0.03 m/s| = 0.03 m/s;$

$\Delta v_3 = |0.40 - 0.35| = 0.05 m/s;$ $\Delta v_6 = |0.40 - 0.42| = |-0.02 m/s| = 0.02 m/s.$

In our example the mean value of the absolute error (deviation) is:

THEORETICAL BASIS OF ERROR ANALYSIS

$$\overline{\Delta v} = \frac{\Delta v_1 + \Delta v_2 + \cdots + \Delta v_6}{6} = \frac{(0.02 + 0.02 + 0.05 + 0.04 + 0.03 + 0.02)m/s}{6} = 0.03m/s$$

and the relative error:

$$\varepsilon = \frac{\overline{\Delta v}}{\bar{v}} = \frac{0.03m/s}{0.4m/s} = 0.075 \quad \text{or} \quad \varepsilon = 0.075 \cdot 100\% = 7.5\%$$

The final result of measurements and error analysis should be written as:

$$v = \bar{v} \pm \overline{\Delta v} = 0.40 \pm 0.075\ (m/s)$$

(do not forget to write the appropriate units!)

FIRST CYCLE

1. VISCOSITY MEASUREMENT OF BIOLOGICAL LIQUIDS

Purposes:

- Theoretical notions of viscosity of biological liquids;
- Determination of coefficient of viscosity by direct method (demonstrative experiment);
- Determination of coefficient of viscosity by relative method (Ostwald viscometer);
- Importance of viscosity in medicine.

Theoretical notions

The ***viscosity*** of a fluid is a measure of its resistance to gradual deformation by shear stress or tensile stress. For liquids, it corresponds to the informal concept of "thickness". For example, honey has a much higher viscosity than water.

A ***viscous fluid*** is one which resists movement or the movement of an object through the fluid. All fluids, liquid, gas, or plasma, have some measure of viscosity which can be compared using mathematical formulas or direct measurements of movement. Though all fluids have viscosity, a viscous fluid, in the everyday sense of the term, is one that has a high level of viscosity. These types of fluid may move slowly or not at all, depending on how viscous they are.

Viscosity is a property arising from friction between neighboring particles (Fig.1.1.) in a fluid that are moving at different velocities. When the fluid is forced through a tube, the particles which comprise the fluid generally move faster near the tube's axis and more slowly near its walls: therefore some stress, (such as a pressure difference between the two ends of the tube), is needed to overcome the friction between particle layers and keep the fluid moving. For the same velocity pattern, the stress required is proportional to the fluid's viscosity.

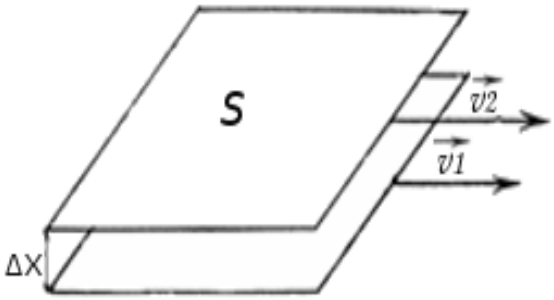

Fig.1.1. Friction between surfaces of particles

A fluid that has no resistance to shear stress is known as an ***ideal fluid***. Zero viscosity is observed only at very low temperatures. Otherwise, all fluids have positive viscosity, and are technically said to be ***viscous***. With other words, a liquid is said to be viscous if its viscosity is substantially greater than water's, and may be described as mobile if the viscosity is noticeably less than water's. If the viscosity is very high, for instance in pitch, the fluid will appear to be a solid in the short term.

The difference between high and low viscosity is the thickness of the material being measured. Low viscosity refers to substances that are thin, such as water, while high viscosity substances are thick. An example of a high viscosity liquid is syrup.

In addition to measuring thickness, viscosity measures resistance to motion. For example, a

substance with a thick viscosity resists movement while a low viscosity substance moves quickly. Therefore, viscosity measures the internal friction of a substance's molecular makeup during movement (Fig.1.1). Liquids are the most common substances measured in terms of viscosity; however, some gases have measurable viscosity that cannot be seen by the naked eye.

For a formal definition of viscosity, consider the friction force, F, between two adjacent layers of fluid in laminar flow (Fig.1.2a) or turbulent flow (Fig.1.2b), with different speeds. According to ***Newton's formula***:

$$F = \eta \cdot S \cdot \frac{\Delta v}{\Delta x}. \qquad (1)$$

Thus, the force of interaction between the layers, F, is proportional to their area of contact (S), to the relative speed of the layers ($\Delta v = v_2 - v_1$), and inversely proportional to the distance between the layers (Δx). The coefficient of proportionality, η, is called ***absolute viscosity*** of the fluid. If $S = 1$ and $\frac{\Delta v}{\Delta x} = 1$, we find $F = \eta$, so dynamical viscosity coefficient is numerical equal to friction force from the inside of the liquid, which is exercised by a monomolecular layer on another monomolecular layer.

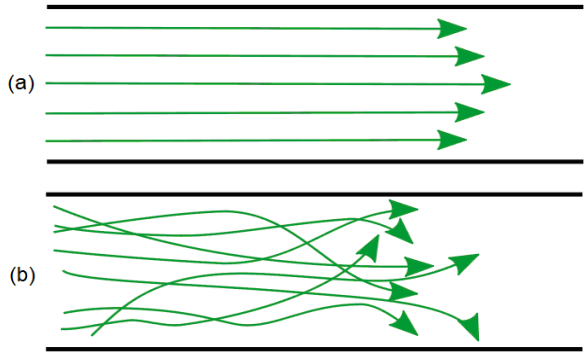

Fig.1.2. Laminar flowing (a) and turbulent flowing (b)

From equation (1) the formula for coefficient of absolute viscosity is

$$\eta = \frac{F}{S \cdot \frac{\Delta v}{\Delta x}}, \qquad (2)$$

and we can infers the unit of measurement of the absolute viscosity in the International System (S.I.):

$$[\eta]_{S.I.} = \frac{N \cdot m}{m^2 \cdot \frac{m}{s}} = \frac{N \cdot s}{m^2} = Pa \cdot s.$$

In the C.G.S. system of units, the unit of measurement of the absolute viscosity is *Poise* (P), named after the French physicist and physiologist Jean Louis Marie Poiseulle.

$$[\eta]_{C.G.S.} = \frac{dyn \cdot s}{cm^2} = 1\text{P}.$$

Considering that

$$1dyn = g \cdot \frac{cm}{s^2} = 10^{-3} Kg \cdot \frac{10^{-2} m}{s_2} = 10^{-5} N,$$

we obtain:

$$1\text{P} = \frac{10^{-5} N \cdot s}{\left(10^{-2} m\right)^2} = 10^{-1} \frac{N \cdot s}{m^2} = 10^{-1} Pa \cdot s.$$

In medical practice η is express in: $1\text{cP} = 10^{-2} P = 10^{-3} Pa \cdot s = mPa \cdot s$.

Water's viscosity at room temperature happens to be roughly 1cP, making this unit especially handy. The absolute viscosity of a liquid depends on its nature and temperature, getting smaller as the temperature rises.

In the laboratory practice are also used:

- the ***kinematic coefficient ofviscosity***equals to the rapport between dynamic coefficient of viscosity and its density;
- the ***relative coefficient of viscosity***equals to the rapport between the viscosity of researched solution and viscosity of standard solution.

Determination of coefficient of viscosity using direct method (Stokes method)

Instead, a *Stokes method* is used, in which small metal ball falls in a glass tube filled with liquid. When an object (like a metal ball) falls gravitationally in viscous liquid it drags certain amount of the liquid with itself due to the molecular interactions between surface of the object and the molecules of the liquid. These layers situated close to the moving object drag farther layers.

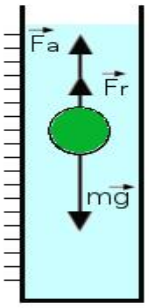

Fig.1.3. The forces which act on the ball

Thus viscosity of the fluid slows down the falling object and creates a velocity gradient in the fluid perpendicular to the direction of motion of the object and the layers. The friction force (Fr) that appear between liquid and spherical body is given by:

$$Fr = 6\pi\eta r v, \tag{6}$$

Where r stands for the radius of the ball, v –velocity, η - viscosity. There are two more forces that act on the ball (Fig.1.3). The first one is obviously the gravity (G).

$$G = mg = \frac{4}{3}\pi r^3 \rho_m g\,, \tag{7}$$

where ρ_m is the density of the ball and the volume of the sphere is $V = \frac{4}{3}\pi r^3$. The second force is the Arhimede force (Fa). It occurs because the pressure in the fluid increases with depth. In case of a metal ball submerged in a liquid the floating force is equal to:

$$F_a = \frac{4}{3}\pi r^3 \rho_f g \tag{8}$$

Thus calculating the velocity we can determine the viscosity using equations (6, 7 and 8).

$$\eta = \frac{2}{9}\frac{r^2 g\left(\rho_m - \rho_f\right)}{v} \tag{9}$$

Determination of coefficient of viscosity using relative method

The liquid flow through a rigid tube of circular cross-section is given by Poiseuille's formula:

$$Q = \frac{\pi r^4 \Delta p}{8\eta l}\,, \tag{3}$$

where Q is the liquid flow, r is the radius of the tube, l is its length, η is the liquid's absolute viscosity, $\Delta p = p_2 - p_1$ is the pressure difference between the two ends of the tube. The volume of the liquid that was elapsed through a capillary from **a** to **b** mark is given by:

$$V = \frac{\pi r^4 \Delta p}{8\eta l} t\,. \tag{4a}$$

If the volume of the standard sample (distilled water) and the volume of the studied solution are the same, we can also write that:

$$V_0 = \frac{\pi r^4 \Delta p_0}{8\eta_0 l} t_0\,. \tag{4b}$$

In the context of the Ostwald viscometer the pressure difference is caused by different liquid levels in the two branches of the *U*-shaped tube, so it is a hydrostatic pressure. If the level difference has the same value, h, in the two cases:

$$\Delta p = \rho g h\,, \qquad \Delta p_0 = \rho_0 g h\,,$$

where ρ is the density of the solution under study and ρ_0 refers to distilled water.

VISCOSITY MEASUREMENT OF BIOLOGICAL LIQUIDS

Ostwald viscometer (Fig.1.4.), also known as *U*-tube viscometer or capillary viscometer is a device used to measure the viscosity of the liquid with a known density. The method of determining viscosity with this instrument consists of measuring the time for a known volume of the liquid (the volume contained between the **a** and **b** marks). Ostwald viscometers named after the German chemist Wilhelm Ostwald

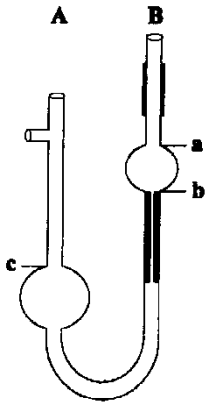

Fig.1.4. Ostwald viscometer

(1853-1932).The instrument must first be calibrated with materials of known viscosity such as distilled water. Knowing the value of viscosity of one liquid, one can calculate the viscosity of other liquid.

$$\eta = \eta_0 \frac{\rho \cdot t}{\rho_0 \cdot t_0}, \tag{5}$$

whereη and η_0 are viscosity coefficients of the liquid and water, and ρ and ρ_0 are the densities of liquid and water, respectively.

Working mode:

1. Clean the viscometer with distilled *water for cleaning*;
2. Measure the time of flowing the distilled water (*for experiment*), from level **a** to level **b** by chronometer (5 time). Present the results in the table 1, section $t_{0,}s$;
3. Clean the viscometer with *alcohol for cleaning*;
4. Repeat the experiment (described in item 2) with *alcohol for experiment* and present the results in the table bellow, in section t,s;
5. Calculate the errors;
6. Write conclusions.

Table

No. of experiment	η_0, cP	$\rho_0, g/cm^3$	$\rho, g/cm^3$	t_0, s	t, s	η, cP
1						
2						
3						
4						
5						

The equation (1) is valid only for small velocities (low values of Reynolds number, $Re < 1160$). Fluids, which obey this equation, are called *Newtonian fluids*.It would be rather difficult to calculate viscosity of the liquids directly from the above equation. Especially it would be difficult to measure the velocity gradient and make sure that the area of contact between the plates is kept constant.

Medical importance:

Blood viscosity is a measure of the thickness of blood. The thinner the blood, the less it resists flow, moving smoothly throughout the body. Some studies have linked moderate to high blood viscosity with cardiovascular problems and sometimes people can develop a medical condition known as hyper-viscosity syndrome. In such cases, thickened blood leads to health problems ranging from visual anomalies to coma.

Several factors are involved in blood viscosity. The composition of the blood is one factor. The more fluid in the blood, the thinner it will be. High counts of red blood cells and particles lead to an increase in blood thickness. Fats that circulate in the blood can also play a role in making it thicker or thinner, with high concentrations of fats increasing viscosity.

Temperature is another contributing factor. As with many other fluids, in low temperatures, the blood becomes thicker and moves more sluggishly. This is a concern with frostbite, when chilling of the extremities can make the blood so viscous that it does not circulate and the tissue dies as a result of lack of oxygen and nutrients. Hypothermia can also lead to concerns about blood viscosity.When the flow of blood is slow, cellular reactions that lead to adhesions can take place. Cells in the blood will start to stick together, forming clumps that thicken the blood. Viscosity also tends to increase in narrow blood vessels. People with conditions that lead to a slowing in flow rates or a narrowing of the vessels can be at risk for higher blood viscosity.

Unusually thick blood can potentially clot in the patient's veins, leading to health problems. High blood viscosity also forces the heart to work harder to pump the blood, increasing the risk that a patient's heart will give out.If a patient has high blood viscosity, there are treatment options available. Medications can be prescribed to reduce the viscosity and break up any clots that may have formed. Patients with thickened blood due to exposure to extreme cold can be slowly warmed up to allow the blood to thin and blood-flow to normalize. It's important to be aware that the return of blood to areas with poor circulation can be painful. Although a patient may want to stop because of the unpleasant sensations, slow and steady warming should be continued until the blood flow normalizes and the patient is stable.

High blood viscosity can be a secondary symptom of many different diseases. Treatment for this condition depends on how thick the blood is and can include the administration of fluids, plasmapheresis, or phlebotomy. It may be possible to wait and see whether treatment is actually needed, though severe

cases of high blood viscosity require immediate treatment.

The first step in the treatment of high blood viscosity is positive identification of the disorder. Symptoms may include sleepiness, headaches, redness of the skin and seizures. These symptoms can be indicative of other conditions, including conditions that involve a low red blood cell count. In order to make sure that a patient is receiving proper treatment, a doctor will need to do a test that measures the level of red blood cells in the body. This will ensure that treatment is appropriate and will be beneficial for the patient. Once a doctor determines that the red blood cell count is too high, treatment can begin. Patients are often given fluids that are used to treat dehydration. These fluids, added to the bloodstream, can thin out the ratio of blood cells to blood plasma.

Knowledge evaluation exercises:

1. The viscosity of a liquid results from:

a) the friction between the liquid molecules and the walls of the vessel it is in;
b) the attraction forces between liquid molecules and the vessel walls;
c) the attraction forces between liquid molecules, which oppose the relative displacement of the neighboring molecules;
d) the slowing dawn of the liquid layer that has a higher flowing speed by the layer with a lower flowing speed;
e) the modification of the density with temperature.

2. Which of the following relations represent Newton's formula, with the correct interpretation of the physical quantities?

a) $F = \eta \cdot S \cdot \frac{\Delta v}{\Delta x}$, where $\frac{\Delta v}{\Delta x}$ is the velocity gradient, η is the absolute viscosity of the fluid, and *S* is the area of contact between the layers;
b) $F = \eta \cdot S \cdot \frac{\Delta v}{\Delta x}$, where $\frac{\Delta v}{\Delta x}$ is the velocity gradient, η is the relative viscosity coefficient, and *S* is the total surface area of the liquid;
c) $F = \eta \cdot S \cdot \frac{\Delta v}{\Delta x}$, where $\frac{\Delta v}{\Delta x}$ is the velocity gradient, η is the relative viscosity coefficient, and *S* is the relative surface of the liquid;
d) $F = \sigma \cdot l$, where σ is the surface tension of the liquid, and l is the length of the surface contour;
e) Newton's formula refers to the rate of flow a liquid through a capillary tube.

3. Which of the following is Poiseuille's equation?

a) $Q = \frac{pR^4}{8\pi\eta l}$;
b) $Q = \frac{pR^2}{8\pi\eta l}$;
c) $Q = \frac{\pi pR^4}{8\eta l}$;
d) $Q = \frac{\pi p^4 R}{8\eta l}$;

e) $Q = \frac{\pi R^4}{8\eta l}$.

4. The absolute viscosity of a liquid:
a) is measured inN/m in the S.I.;
b) is a physical quantity that characterizes the liquid during flow;
c) varies inversely proportional with the flow rate of the liquid;
d) depends on the nature and on the temperature of the liquid;
e) can be modified by dissolving tensioactive agents in the liquid.

5. Identify the unit of the absolute viscosity in S.I.?
a) $N \cdot s \cdot m^2$;
b) $N \cdot s^{-1} \cdot m^{-2}$;
c) $N \cdot s \cdot m^{-2}$;
d) $N \cdot m^2 \cdot s^{-1}$;
e) P (Poise).

6. Identify the relation of the relative viscosity coefficient of a liquid with respect to distilled water.Hereη_{rel} is the relative viscosity coefficient of the liquid with respect to distilled water; t_0, t are the flowing times corresponding to the distilled water and the studied liquid, respectively; ρ_0 and ρ represent the density of distilled water and of the studied liquid, respectively.
a) $\eta_{rel} = \frac{\rho_0 t}{\rho t_0}$;
b) $\eta_{rel} = \frac{\rho t}{\rho_0 t_0}$;
c) $\eta_{rel} = \frac{\rho_0 t_0}{\rho t}$;
d) $\eta_{rel} = \frac{\rho t_0}{\rho_0 t}$;
e) $\eta_{rel} = \frac{\rho_0 \rho}{t_0 t}$.

7. Which of the following formulas gives the specific viscosity coefficient? Here η_0, η is the absolute viscosity coefficient of distilled water and of the studied liquid, respectively;
a) $\eta_{sp} = \frac{\eta}{\eta_0} - 1$;
b) $\eta_{sp} = \frac{\eta_0}{\eta} - 1$;
c) $\eta_{sp} = \eta_{rel} - 1$;
d) $\eta_{sp} = \frac{\eta}{\eta_0} + 1$;
e) $\eta_{sp} = \eta_{rel} + 1$.

8. Which are the normal values of the absolute viscosity of blood and plasma?
a) blood: 3.2 P; plasma: 1.5 cP;
b) blood: 1.5 cP; plasma: 3.2 cP;

c) blood: 2.2 cP; plasma: 1.0 cP;
d) blood: 3.2 P; plasma: 1.5 P;
e) bothblood and plasma have the same viscosity value – 3.2 cP.

9. Identify the true statements:
a) the viscosity of a liquid depends on the size of its molecules;
b) the viscosity of a liquid depends on the intermolecular attraction forces;
c) the viscosity of a liquid is independent of the temperature;
d) the viscosity of a liquid decreases with the rise in temperature;
e) the unit of measurement of the absolute viscosity in S.I. is N/m.

2. ULTRASOUND EFFCTS

Purposes:

1. Presentation of theoretical notions about ultrasound effects;
2. Study of the producing and receiving of ultrasound;
3. Familiarization with physical and biological effects of ultrasound;
4. Applications of ultrasound in practical medicine.

Theoretical notions:

Sound is a vibration that propagates as an audible mechanical wave through a medium such as solid, liquid and gas. The sound waves are generated by a sound source, such as the vibrating diaphragm of a stereo speaker. The sound source creates vibrations in the surrounding medium. As the source continues to vibrate the medium, the vibrations propagate away from the source at the speed of sound, thus forming the sound wave. At a fixed distance from the source, the pressure, velocity, and displacement of the medium vary in time. Note that the particles of the medium do not travel with the sound wave. Due to the physical properties the sound can be classified in *infrasound, acoustic sound* and *ultrasound* (see Fig.2.1).

Ultrasound is an oscillating sound pressure wave with a frequency greater than the upper limit of

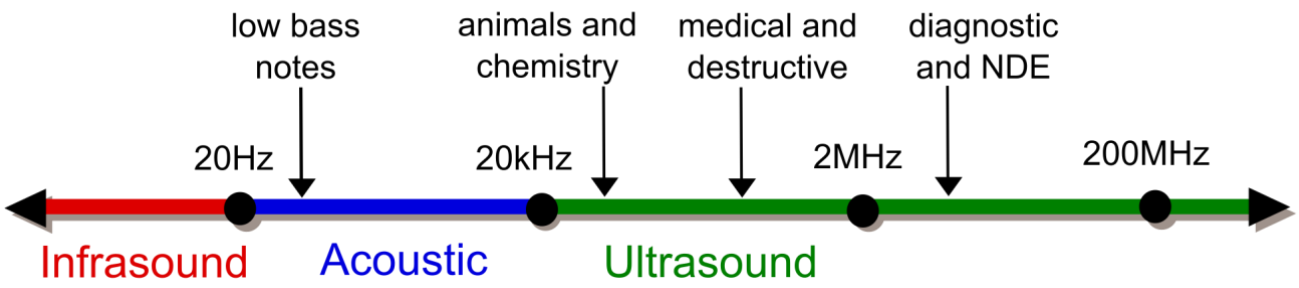

Fig.2.1. Sound diagram

the humanhearing range. Ultrasound is not different from 'normal' (audible) sound in its physical properties, only in that humans cannot hear it. This limit varies from person to person and is approximately from 16Hz to 20kHz in healthy, young adults. Ultrasound devices operate with frequencies from 20 kHz up to several GHz.

The first technological application of ultrasound was an attempt to detect submarines by Paul Langevin in 1917. Ultrasound emitters called ultrasound transducers generate ultrasound. The main components of these emitters are the *generator* that produces an alternative current at a required frequency and a vibrator, which transforms electrical pulse into mechanical vibrations.

The sound is propagated in the surrounding medium as longitudinal waves and in order to indentify a sound wave we need to have three components: *a sound source*, *medium of propagation* of the sound and *detector or receiver of the sound*.

Ultrasound transducer

ULTRASOUND EFFCTS

The working principle of transducer is based on the *piezoelectriceffect*, discovered by Pierre Curie to generate and detect ultrasonic waves in air and water, and *magneto-strictivephenomenon*.

Piezoelectric effect is the ability of certain materials to generate an electric charge in response to applied mechanical stress. The word Piezoelectric is derived from the Greek “piezein”, which means to squeeze or press, and “piezo”, which is from Greek for “push”.

One of the unique characteristics of the piezoelectric effect is that it is reversible, meaning that materials exhibiting the *direct piezoelectric effect* (the generation of electricity when stress is applied) also exhibit the *converse (inverse) piezoelectric effect* (the generation of stress when an electric field is applied) see fig.2.2.

1

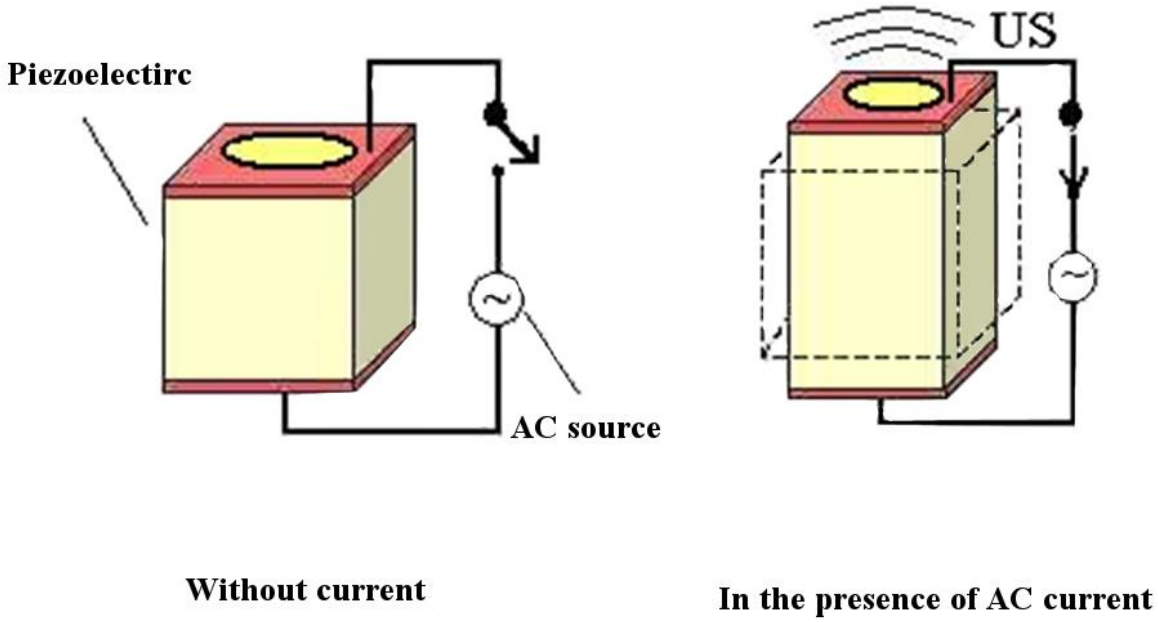

Fig.2.2. Direct and convert piezoelectric effect

When piezoelectric material is placed under mechanical stress, a shifting of the positive and negative charge centers in the material takes place, which then results in an external electrical field. When reversed, an outer electrical field either stretches or compresses the piezoelectric material.

The piezoelectric effect is very useful within many applications that involve the production and detection of sound, generation of high voltages, electronic frequency generation, microbalances, and ultrafine focusing of optical assemblies. It is also the basis of a number of scientific instrumental techniques with atomic resolution, such as scanning probe microscopes. The piezoelectric effect also has its use in many applications as well, such as acting as the ignition source for cigarette lighters or shoe generator power from walking.

However, the ultrasound piezoelectrical emitter works due to the *inverse piezoelectric effect*, which causes a change in length of materials when an electrical voltage is applied. This actuator effect converts electrical energy into mechanical energy. This mechanical energy causes vibrations in the surrounding medium. As the source continues to vibrate the medium, the vibrations propagate away from the source at the speed of sound, thus forming the sound wave.

The ***magnetostrictive emitter*** is based on the *magnetostrictive effect* and consists in the modification of dimensions of a ferromagnetic body placed in a solenoid connected at alternative current. Magnetostrictive materials transduce or convert magnetic energy to mechanical energy and vice versa. As a magnetostrictive material is magnetized, it strains, that is it exhibits a change in length per unit of length. Conversely, if an external force produces a strain in a magnetostrictive material, the material's magnetic state will change. In many devices, conversion between electrical and magnetic energies facilitates device use. Hence, most magnetostrictive devices are in fact electro-magneto-mechanical transducers.

The magnetostrictive emitter shown in the fig.2.3 is composed from a ferromagnetic bar fixed in a solenoid supplied by an alternative electric current. The bar is compressed when the electrical current reaches the maximal values and returns to the initial dimensions when the current tends to be minimal. The alteration of the ferromagnetic bar produces vibrations followed by an ultrasound. In the case when the solenoid is connected to a continue current, the ferromagnetic bar do not return to the initial form, that means the absence of ultrasound.

Some of the earliest uses of magnetostrictive materials during the first half of this century include

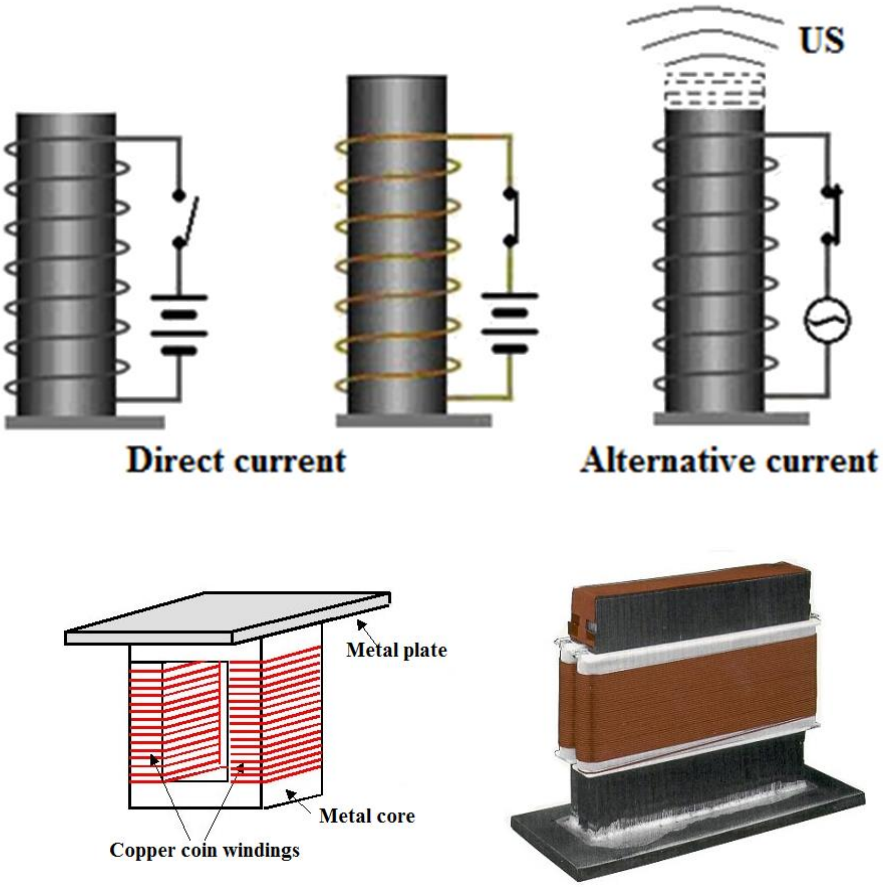

Fig.2.3. Scheme and an example of a magnetostrictive transducer

telephone receivers, hydrophones, magnetostrictive oscillators, torque-meters and scanning sonar.

Current applications for magnetostrictive devices include ultrasonic cleaners, high force linear motors, positioners for adaptive optics, active vibration or noise control systems, medical and industrial ultrasonics, pumps, and sonar. In addition, magnetostrictive linear motors, reaction mass actuators, and

tuned vibration absorbers have been designed, while less obvious applications include high cycle accelerated fatigue test stands, mine detection, hearing aids, razor blade sharpeners, and seismic sources. ***Ultrasonic magnetostrictive transducers*** have been developed for surgical tools, underwater sonar, and chemical and material processing.

Interaction of ultrasound with matter

In order to use ultrasound for either diagnostic or therapeutic purposes, a beam of ultrasound must be directed into the tissues of the subject over a selected area of interest. The ultrasonic energy will interact with the tissues along its path. The interaction processes are influenced by the characteristics of the US wave, as well as the physical proprieties of the tissues through which the beam passes.

Reflection can be categorized as either *specular* or *diffuse* (see fig.2.4). *Specular reflectors* are large, smooth surfaces, such as bone, where the sound wave is reflected back in a singular direction. The greater the acoustic impedance between the two tissue surfaces is, the greater the reflection and the brighter the echo will appear on ultrasound. Conversely, soft tissue is classified as a *diffuse reflector*, where adjoining cells create an uneven surface causing the reflections to return in various directions in relation to the transmitted beam.

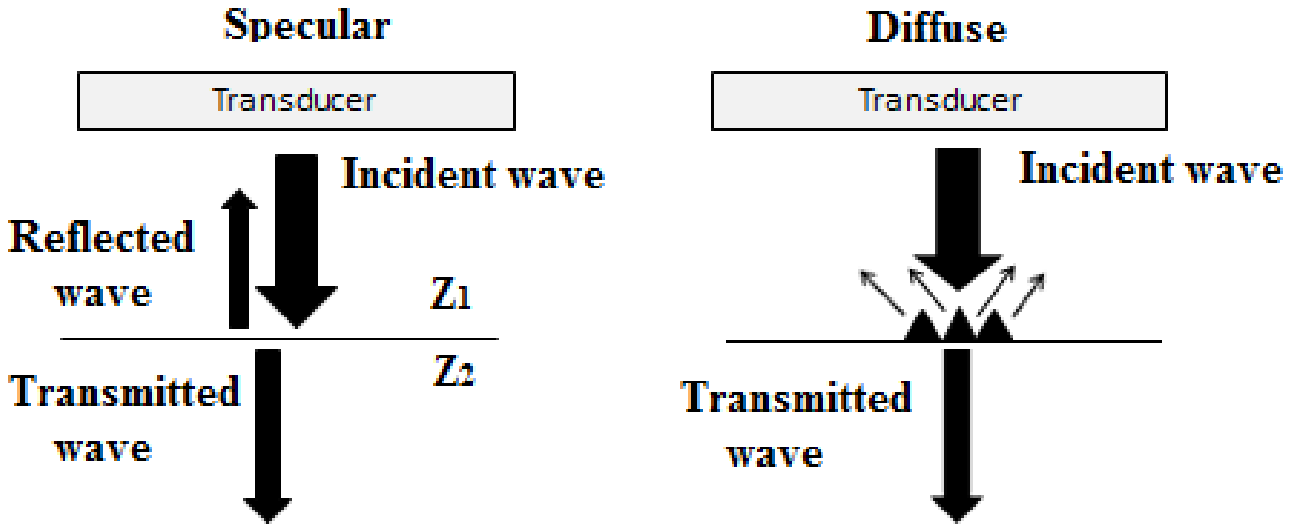

Fig.2.4. Specular vs diffuse reflection

Refraction is a change of beam direction at a boundary between two media in wich ultrasound travels at different velocities (see fig.2.5). It is caused by a change of wavelength as the ultrasound crosses from the first medium to the second while the beam frequency remains unchanged.

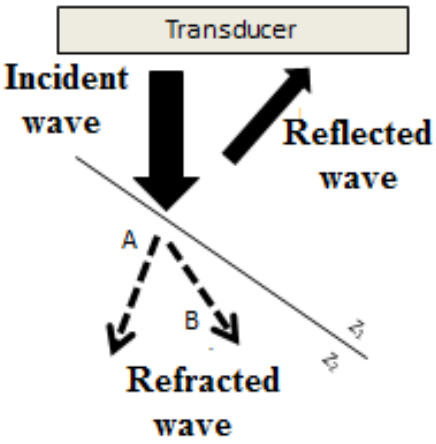

Fig.2.5. Refraction of the waves

Rayleigh ***scattering*** occurs at interfaces involving structures of small dimensions (see fig.2.6). This is common with red blood cells, where the average diameter of an red blood cells is 7μm, and an ultrasound wavelength may be 300μm (5 MHz). When the sound wave is greater than the structure it comes in contact with, it creates uniform amplitude in all directions with little or no reflection returning to the transducer.

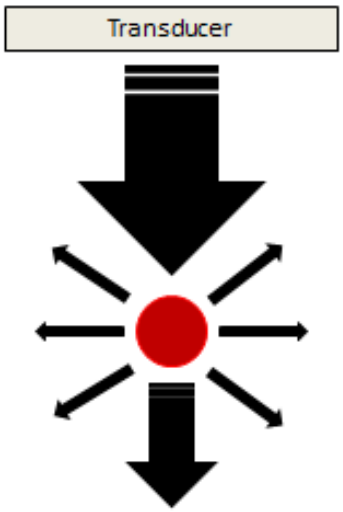

Fig.2.6. Scattering

The culminating effect of tissue on sound as it travels through the body is ***attenuation***. Attenuation is the decreasing intensity of a sound wave as it passes through a medium. It is the result of energy absorption of tissue, as well as reflection and scattering that occurs between the boundaries of tissue with different densities. The attenuation coefficient of tissues is the relation of attenuation to distance, and depends on the tissues traversed and the frequency of the ultrasound wave. In general, a reduction of 3dB equals diminuation of the original wave intensity by half. To compensate for attenuation, returning signals can be amplified by the ultrasound system, known as gain. Attenuation is high in muscle and skin, and low in fluid-filled structures. Higher frequency waves are subject to greater attenuation than lower frequency ones (see table below).

ULTRASOUND EFFCTS

Body Tissue	Attenuation Coefficient (db/cm at 1MHz)
Water	0,002
Blood	0,18
Fat	0,63
Liver	0,5-0,94
Kidney	1,0
Muscle	1,3-3,3
Bone	5,0

1. ***Physical effects*** of ultrasound are: primary effects and secondary effects.
   Primary effects:
   - radiation pressure;
   - ultrasound absorption;
   - cavitation- is the fast production and possible subsequent collapse of millions of microscopic bubbles (or cavities) in a liquid. Ultrasounds, as all sound waves, are an alternation of high and low frequency waves. This alternation may cause cavitation. During the low frequency phase, these bubbles grow, whereas in the high frequency phase they are compressed and therefore implode. Imploding, they cause a temperature rise with pressures and heat exchange which take place in microsecond time fractions. In the fat tissue, cavitation can contribute, together with the other typical effects of cavitation (thermal, mechanical and chemical) to modify cell membranes with the formation of micro-pores which helps the lipids to pour out.

   Secondary effects:
   - mechanical effects: degassing the liquid, dispersion, precipitation and coagulation.
   - thermal effects are those that are due to heating and may include increased blood flow, reduction in muscle spasm and increased extensibility of collagen fibers;
   - electrical effects: in colloidal systems ultrasound determine displacement of electric double layer on the surface of separation of the phases;
   - optical effects: due to the crossing of US through a medium, the refractive index may change;
   - chimical effects: depending on medium temperature and concentration of the substance, ultrasounds can accelerate or slow some chemical reaction, destroy the chemical bounds, change the distribution of the atoms or molecules in the substance, oxidation processes, reduction, depolymerization.

2. ***Biological effects of ultrasound*** are the potential biological consequences due to the interaction between the ultrasound wave and the scanned tissues. The use of ultrasound for cardiac imaging has not known significant adverse biological effects. Concern about the safety of ultrasound prompted several agencies

to devise regulatory limits on the machine output intensities. The visual display of thermal and mechanical indices during ultrasound imaging provides an aid to limit the output of the machine.

Biological effects of ultrasound depend on its characteristics: *intensity, amplitude* and *frequency* of ultrasound waves. At small intensities the tissues do not suffer morphological changes producing only functional modifications. In the cytoplasm's a stimulation of physiological processes can be realized. In the middle range of ultrasound intensity, the cytoplasm current is stronger and stops the normal development of cell mechanism. At high intensities, the irreversible structural modification of the cell can be realized.

High intensity, high frequency ultrasound has been shown to result in adverse effects including:

- Tissue necrosis,
- Chromosomal damage,
- Genetic mutations,
- Teratogenic changes.

So far these adverse effects have not been reported following diagnostic ultrasonography but safety limits to minimise potential risks have been set as follows:

- Intensities over 100mW/cm^2 should only be applied for a few seconds.
- For human use 10mW/cm^2 is approved for commercial 2-D ultrasound.

Sonographic evaluation of the human body, including potentially sensitive tissues, such as developing fetus and the eye, have been performed on millions of patients without documentation of serious adverse events. However, ultrasound waves have the potential to cause significant biological effects, depending on ultrasound wave characteristics and scanned tissues sensitivity. Physicians and sonographers must be aware of these potential biological effects in assessing the overall safety of the procedure (see fig.2.7).

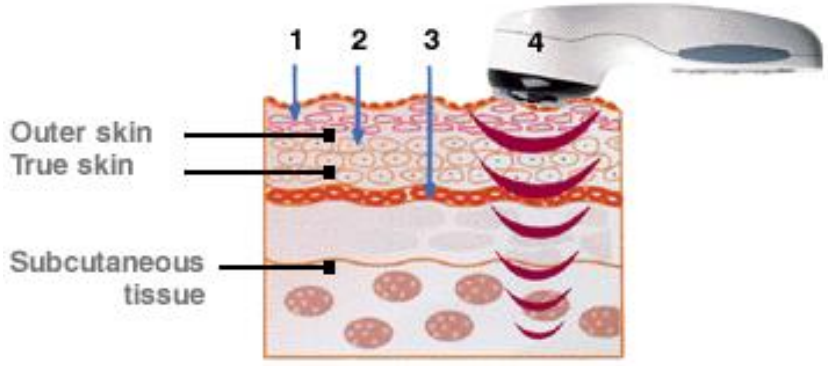

Fig.2.7. Interaction of US with tissues

The ***biophysical effects*** of Therapeutic US have been examined mainly *in vitro* studies. Therapeutic results obtained by ultrasonic energy are thought to be due to:

- Increased vascular and fluid circulation,
- Increase in cell permeability,
- Increase in pain threshold and a break in pain cycle.

Doppler ultrasound

ULTRASOUND EFFCTS

The Doppler effect is observed whenever the source of waves is moving with respect to an observer. The Doppler effect can be described as the effect produced by a moving source of waves in which there is an apparent upward shift in frequency for observers towards whom the source is approaching and an apparent downward shift in frequency for observers from whom the source is receding. It is important to note that the effect does not result because of an actual change in the frequency of the source.

The Doppler effect can be observed for any type of wave - water wave, sound wave, light wave, etc. We are most familiar with the Doppler effect because of our experiences with sound waves. Perhaps you recall an instance in which a police car or emergency vehicle was traveling towards you on the highway. As the car approached with its siren blasting, the pitch of the siren sound (a measure of the siren's frequency) was high; and then suddenly after the car passed by, the pitch of the siren sound was low. That was the Doppler effect - an apparent shift in frequency for a sound wave produced by a moving source (Fig.2.8).

A ***Doppler ultrasound*** is a noninvasive test that can be used to estimate your blood flow through blood vessels by bouncing high-frequency sound waves (ultrasound) off circulating red blood cells. A regular ultrasound uses sound waves to produce images, but can't show blood flow.

A Doppler ultrasound may help diagnose many conditions, including:

- Blood clots;
- Poorly functioning valves in your leg veins, which can cause blood or other fluids to pool in your legs (venous insufficiency);
- Heart valve defects and congenital heart disease;
- A blocked artery (arterial occlusion);
- Decreased blood circulation into your legs (peripheral artery disease);
- Bulging arteries (aneurysms);
- Narrowing of an artery, such as in your neck (carotid artery stenosis).

A Doppler ultrasound can estimate how fast blood flows by measuring the rate of change in its

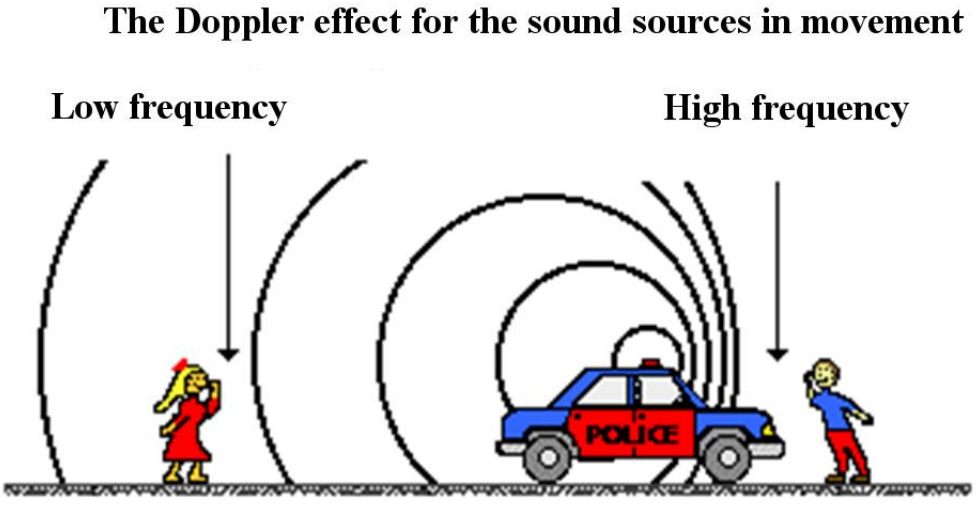

Fig.2.8. Doppler effect

pitch (frequency). During a Doppler ultrasound, a technician trained in ultrasound imaging (sonographer) presses a small hand-held device (transducer), about the size of a bar of soap, against your skin over the area of your body being examined, moving from one area to another as necessary.

This test may be done as an alternative to more invasive procedures, such as arteriography and venography, which involve injecting dye into the blood vessels so that they show up clearly on X-ray images. A Doppler ultrasound test may also help your doctor check for injuries to your arteries or to monitor certain treatments to your veins and arteries.

Working principle

The equation that describe the Doppler effect is as follows:

$$\Delta f = \frac{2\, v\, cos\theta}{c} f_0, \tag{1}$$

where:

- c is the velocity of waves in the medium
- v is the velocity of the blood
- $\Delta f = f\ - f_0$ is the change in frequency between observed frequency (the Doppler shift frequency) f and emitted (transmitted) frequency f_0
- $cos\theta$ is the angle of insonation (see fig.2.9.).

The Doppler shift increases with transmitted frequency, increasing velocity of blood cells and with a decreased angle of approach. The goal is to determine the velocity of blood within the vessel so the equation is rewritten as:

$$v = \frac{\Delta f\, c}{2\, f_0\, cos\theta} \tag{2}$$

In practical applications, direction of blood flow is relative to the transducer, that is, either towards or away from the transducer. The angle of insonation is very important. $Cos\ 0° = 1$with the $cos\ 90° =$

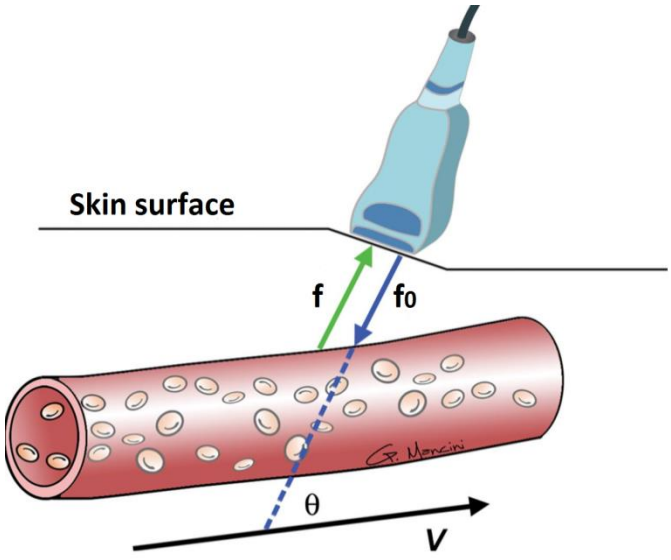

Fig.2.9. Doppler effect

0. Angles between 0° and 60° are acceptable. The velocity measurements become unreliable with angles more than 60°. If a normal non-occluded blood vessel is scanned with an angle of insonation of 90°, no trace will register. This will give the impression that the blood vessel is occluded.

Ultrasonic colour Doppler is an imaging technique that combines anatomical information derived using ultrasonic pulse-echo techniques with velocity information derived using ultrasonic Doppler techniques to generate colour-coded maps of tissue velocity superimposed on grey-scale images of tissue anatomy. The most common use of the technique is to image the movement of blood through the heart, arteries and veins, but it may also be used to image the motion of solid tissues such as the heart walls

Once the information is received by the ultrasound machine, colour is assigned on the basis of the direction of flow. Bloodflow that is moving toward the transducer is displayed in one colour (for example red) and blood flow away from the transducer is displayed as another colour (for example blue). This contextual information is graphically represented on the ultrasound image as a colour bar.

Some colour maps use a range of colours to represent the frequency shifts. As the frequency increases the colours can range from deep red to orange and yellow. In the reverse channel or negative spectrum which represents flow away from the transducer, the colours might range from blue to green.

Ultrasound applications in medicine

Ultrasonic cleanersare used to clean many different types of objects, including jewellery, lenses and otheroptical parts, watches, medical instruments tools, coins, musical instruments, industrial parts and electronic equipment.

An ultrasonic cleaning is a process that uses ultrasound (usually from 20–400 kHz) and an appropriate cleaning solvent (sometimes ordinary tap water) to clean items. The ultrasound can be used with just water, but use of a solvent appropriate for the item to be cleaned along with the soiling enhances the effect. Ultrasonic cleaning uses cavitation bubbles induced by high frequency pressure (sound) waves to agitate a liquid. The agitation produces high forces on contaminants adhering to substrates like metals, plastics, glass, rubber, and ceramics. This action also penetrates blind holes, cracks, and recesses. The intention is to thoroughly remove all traces of contamination tightly adhering or embedded onto solid surfaces. Water or other solvent can be used, depending on the type of contamination and the workpiece. Contaminants can include dust, dirt, oil, pigments, rust, grease, algae, fungus, bacteria, lime scale, polishing compounds, flux agents, fingerprints, soot wax and mold release agents, biological soil like blood, and so on. Ultrasonic cleaning can be used for a wide range of workpiece shapes, sizes and materials, and may not require the part to be disassembled prior to cleaning. Objects must not be allowed to rest on the bottom of the device during the cleaning process, because that will prevent cavitation from taking place on the part of the object not in contact with water.

Sonochemistry: Power ultrasound in the 20–100 kHz range is used in chemistry. The ultrasound does notinteract directly with molecules to induce the chemical change, as its typical wavelength (in the millimeter range) is too long compared to the molecules. Instead, the energy causes cavitation which generates extremes of temperature and pressure in the liquid where the reaction happens. Ultrasound also breaks up solids and removes passivating layers of inert material to give a larger surface area for the reaction to occur over.

Ultrasonic disintegration. Similar to ultrasonic cleaning, biological cells including bacteria can

be disintegrated. High power ultrasound produces cavitation that facilitates particle disintegration or reactions. This has uses in biological science for analytical or chemical purposes and in killing bacteria in sewage. High power ultrasound can disintegrate corn slurry and enhance liquefaction and saccharification for higher ethanol yield in dry corn milling plants.

Ultrasonic wave nebulizer (inhaler) presented in the fig.2.10, is a drug delivery device used to administer medication in the form of a mist inhaled into the lungs. The technology inside an ultrasonic

Fig.2.10. Picture of an ultrasonic nebulizer

wave nebulizer is to have an electronic oscillator generate a high frequency ultrasonic wave, which causes the mechanical vibration of a piezoelectric element. This vibrating element is in contact with a liquid reservoir and its high frequency vibration is sufficient to produce a vapor mist. As they create aerosols from ultrasonic vibration instead of using a heavy air compressor, they only have a weight around 170 grams (6.0 oz). Another advantage is that the ultrasonic vibration is almost silent (see fig.2.10).

Ultrasound imaging (sonography or ultrasonography) is an ultrasound diagnostic technique used for visualizinginternal body structures including tendons, muscles, vessels and internal organs for possible pathology or lesions. The practice of examining pregnant women using ultrasound is called *obstetric sonography*, and is widely used.

Because ultrasound images are captured in real-time, they can also show movement of the body's internal organs as well as blood flowing through the blood vessels. Unlike X-ray imaging, there is no ionizing radiation exposure associated with ultrasound imaging.

In an ultrasound exam, a transducer (probe) is placed directly on the skin or inside a body opening (see fig.2.11.). A thin layer of gel is applied to the skin so that the ultrasound waves are transmitted from the transducer through the gel into the body.

Fig.2.11. Picture of a transducer (probe) used during an ultrasound exam

The ultrasound image is produced based on the reflection of the waves off of the body structures. The strength (amplitude) of the sound signal and the time it takes for the wave to travel through the body provide the information necessary to produce an image.

Typical diagnostic sonographic scanners operate in the frequency range of 2 to 18 megahertz, though frequencies up to 50–100 megahertz have been used experimentally in a technique known as biomicroscopy in special regions, such as the anterior chamber of the eye. The choice of frequency is a trade-off between spatial resolution of the image and imaging depth: lower frequencies produce less resolution but image deeper into the body. Higher frequency sound waves have a smaller wavelength and thus are capable of reflecting or scattering from smaller structures. Higher frequency sound waves also have a larger attenuation coefficient and thus are more readily absorbed in tissue, limiting the depth of penetration of the sound wave into the body.

Ultrasound imaging is a medical tool that can help a physician evaluate, diagnose and treat medical conditions. Common ultrasound imaging procedures include:

- Abdominal ultrasound (to visualize abdominal tissues and organs),
- Bone sonometry (to assess bone fragility),
- Breast ultrasound (to visualize breast tissue),
- Doppler fetal heart rate monitors (to listen to the fetal heart beat),
- Doppler ultrasound (to visualize blood flow through a blood vessel, organs, or other structures),
- Echocardiogram (to view the heart),
- Fetal ultrasound (to view the fetus in pregnancy),
- Ultrasound-guided biopsies (to collect a sample of tissue),
- Ophthalmic ultrasound (to visualize ocular structures),
- Ultrasound-guided needle placement (in blood vessels or other tissues of interest).

Some systems where the medical sonography is used:

- **Anesthesiology**: Ultrasound is commonly used by anesthesiologists to guide injecting needles when placinglocal anaesthetic solutions near nerves.
- **Cardiology:** Echocardiography is an essential tool in cardiology, to diagnose e.g. dilatation of parts of theheart and function of heart ventricles and valves.
- **Neurology** for assessing blood flow and stenoses in the carotid arteries and the big intracerebral

arteries.

- **Ophthalmology**: Ultrasound images of the eyes, also known as ocular ultrasonography
- **Urology**: to determine, for example, the amount of fluid retained in a patient's bladder. In a pelvicsonogram, organs of the pelvic region are imaged.
- **Pulmonology:** Endobronchial ultrasound probes are applied to standard flexible endoscopic probes and usedby pulmonologists to allow for direct visualization of endobronchial lesions and lymph nodes prior to transbronchial needle aspiration. Among its many uses, endobronchial ultrasound aids in lung cancer staging by allowing for lymph node sampling without the need for major surgery.
- **Dentistry**

Ultrasound in dentistry

Dentistry in the Modern era is emerging with the use of advanced imaging techniques such as computed tomography(CT), Magnetic Resonance Imaging (MRI), Nuclear Medicine (NM), and Ultrasound (US), of which MRI and Ultrasound are the only imaging technique, which operate without causing radiation hazards to the patients. And ultrasound is one of the advanced imaging technique which uses sound waves for viewing the normal and Pathological conditions involving bone and soft tissue of the oral and Maxillofacial region. In Dentistry for detecting a bony (or) Soft tissue Mass, patients are exposed to multiple radiographs which might cause radiation effects at the tissue and organ level. So this valuable technique can be used prior to use of X-ray radiation.

Ultrasonic echography has been used as an instant, non-invasive method for the observation of relatively deep areas, recently, however high frequency echography has been developed that can provide detail investigation of more superficial regions.

Ultrasound in dentistry is used for detection of fractures of the Maxillo facial region, Nasal bone fractures, Orbital rim fractures, Maxillary fractures, Mandibular fractures, Zygomatic arch fractures and for locating the position of Mandibular condyles. And post operative view can be done instantly to view the reduction aand healing of fractures. Ultrasound can be used to detect parotid lesions, where solid and cystic lesions are reliably differentiated and diffuse enlargement of the parotid gland (or) focal disease is readily shown by Ultrasound.

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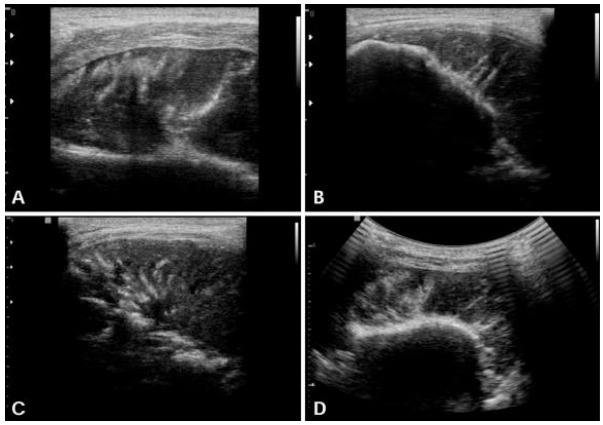
Sonographically, benign lesions usually look well defined, homogeneous and hypoechoic, while Malignant lesions tend to be ill defined and hypoechoic with heterogeneous internal architecture and enlarged cervical lymph node may be visible and reactive intra parotid lymph nodes may also be readily assessed (see fig.2.12).

a.



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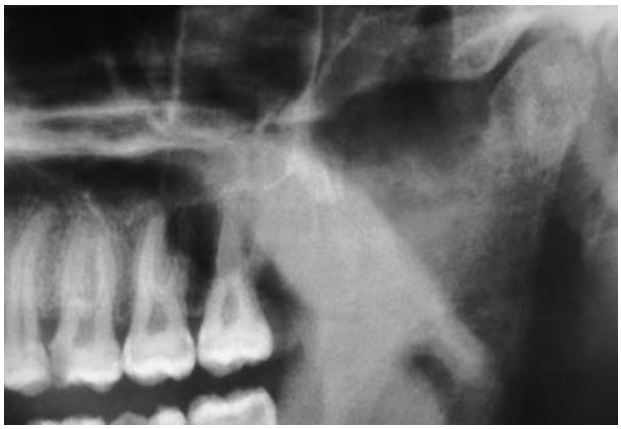


Fig.2.12.a. Ultrasonographic image shows soft tissue component of the lesion with well-defined hypo-echogenic pattern and hard tissue component of the lesion with ill-defined hyper-echoic pattern. B, C, and D. **b**. Cropped panoramic radiograph reveals destruction of cortical bone in the region of left sigmoid notch and ascending ramus. Irregularity of cortical border in the region is observed.

The value of Ultrasonography is well recognized in inflammatory soft tissue conditions of the head and neck region and superficial tissue disorders of the maxillofacial region. Ultrasound can provide the content of the lesion before any surgical procedures, both solid and cystic contents could be identified in ultrasound. The mixed lesions should be considered neoplastic and should be biopsied before surgical procedure. Modality for measuring the thickness of Muscles, data regarding thickness may provide information useful in diagnosis and treatment.

In Ultrasound, color Doppler sonography has been developed to identify vasculatures and to enable evaluation of the blood flow, velocity and vessel resistance together with surrounding Morphology. It can be used for detecting the coarses of the facial artery and for detecting hemangioma.

Ultrasonic cleaners are the first stage in the decontamination process of dental instruments. Due to the gold standard for instrument decontamination, all instruments should be processed in an ultrasonic cleaner before being disinfected and sterilised.

Ultrasonic tooth scalers use transducers that convert electricity into mechanical energy which generate up to 50,000 vibrations per second. These vibrations produce microscopic high and low pressure areas. This results in cavitation, which might sound bad for cavity prone teeth, but it means the formation of bubbles filled with water vapour rather than air. The vibration stretches and compresses these tiny bubbles until they implode and release a burst of heat and pressure. You can find it more pleasant to have your teeth cleaned this way and less with the scraping, but ultrasound may add extra wear on metal implants, so it might be worth discussing the pros and cons of this technology with your dentist or hygienist.

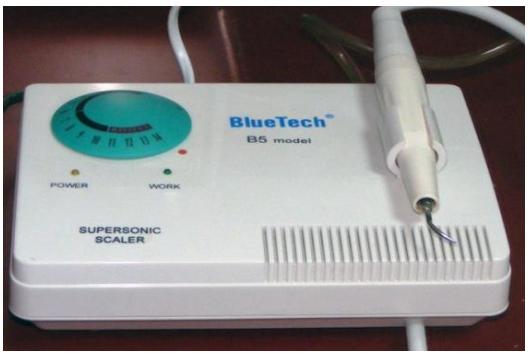


Fig.2.13. Ultrasonic scaler

An ***ultrasonic toothbrush*** is an electric toothbrush designed for daily home use that operates by generating ultrasound in order to aid in removing plaque and rendering plaque bacteria harmless.

Electric toothbrushes (Fig.2.14.) have been used by the public since the early 1950s. Today, they

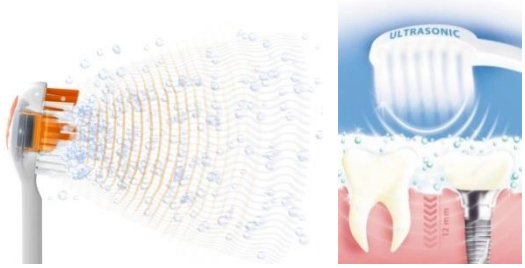


Fig.2.14. Ultrasonic toothbrush

have evolved and based on the speed of their vibration, can be divided into three categories: *electric, sonic* and *ultrasonic*.

Electric toothbrushes vibrate in either an up/down direction, or in a circular motion, and sometimes in a combination of the two. Typically, the speed of their vibration is measured in movements per minute, where common electric toothbrushes vibrate at a speed of between a few thousand times a minute to approximately 10000 to 12000 times per minute.

Sonic toothbrushes are called sonic because the speed or frequency of their vibration, as opposed to the sound of the motor, falls within the average range that is used by people in communication. The voiced speech of a typical adult male will have a fundamental frequency from 85 to 180 Hz (10200 to 21000 movements per minute), and that of a typical adult female from 165 to 255 Hz (19800 to 30600 movements per minute).

Ultrasonic toothbrushes work by generating an ultrasonic wave usually from an implanted piezo crystal, the frequency of which technically could begin at 20000 Hz (2400000 movements per minute). The most common frequency however, around which many scientific studies have been conducted, is in the area of approximately 1.6MHz, which translates to 96000000 waves or 192000000 movements per minute.

Knowledge evaluation exercises:

1. Defines the notion of sound?

2. Human ear can receive frequencies between:
   a) 0<20Hz;
   b) 20Hz<20000Hz;
   c) 0<200000000Hz;
   d) 20000Hz<20GHz.

3. To identify a sound wave it needs to have:
   a) a sound source;
   b) medium of propagation;
   c) ultrasound transducer;
   d) piezoelectric effect;
   e) detector.

4. Production of ultrasound takes place:
   a) in the presence of alternating current;
   b) only due to magnetostrictive effect;
   c) only due to inverse piezoelectric effect;
   d) due to magnetostrictive effect and due to piezoelectric inverse effect.

5. Biological effects of ultrasound depend on:
   a) thermal effect;
   b) intensity;
   c) non thermal effect;
   d) amplitude;
   e) frequency.

6. The equation that describe Doppler effect is:
   a) $\Delta f = \frac{2v\cos\theta}{c}$;
   b) $\Delta f \cdot c = 2v\cos\theta f_0$;
   c) $v = \frac{\Delta f c}{2f_0\cos\theta}$;
   d) $no\ one\ is\ true.$

7. A Doppler ultrasound is a test that can be used to determine:
   a) heart valve defects and congenital heart disease;
   b) ultrasound frequency;
   c) blood flow;
   d) blood viscosity.

8. Inverse piezoelectric effect consists:
   a) piezo mechanical deformation under the action of the electric field;

b) the occurrence of potential difference in deformation piezoelectric;
c) destruction of the piezoelectric;
d) piezo volume variation under the influence of the magnetic field.

9. A *4MHz* Doppler probe is inclined relative to the direction of flow with 30°. Are known ultrasound velocity $c = 1500\frac{m}{s}$ and laminar flow velocity arterial is$v = 25\frac{m}{s}$. calculate frequency variationΔf in *Hz*.

3. DETERMINATION OF SURFACE TENSION OF BIOLOGICAL LIQUIDS

Purposes:

1. Presentation of theoretical notions about surface tension;
2. Study of the relative method of surface tension determination;
3. Determination of surface tension using the direct method;
4. Study of the importance of surface tension for medical practice.

Theoretical notions:

Surface tension is caused by the inter-molecular attraction between molecules resulting into small distances between the molecules (on the order of 0.1 nm). Attractive cohesive forces are short range forces which are based on the electronic interactions. They affect molecules in their close vicinity only (zone of molecular interaction).

In the bulk of the liquid, each molecule is attracted equally in all directions by the neighboring molecules, hence zero net force. However, the molecules at the surface do not have other like molecules on all sides around them and they are pulled inwards the liquid core by non-zero net force (Fig.3.1). Consequently, they cohere more strongly to those associated with them directly on the surface and form a surface "film". Nevertheless, these surface molecules are in the energetically unfavorable state, which forces liquid to minimize the surface area. The geometrical requirement of smallest surface area at the fixed volume is satisfied by the sphere. It is the reason why the free drops of water form spherical droplets.

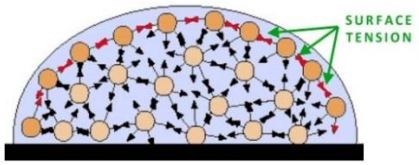


Fig.3.1. Cohesive forces in a case of the molecule at the liquid surface and in the bulk.

Surface tension is the property of matter by virtue of which the free surface of the liquid at rest behaves like a stretched elastic membrane having contractive tendency. On the ather hand ***surface tension*** is a contractive tendency of the surface of a liquid that allows it to resist an external force.

The ***surface tension***σ is the magnitude F of the force exerted parallel to the surface of a liquid divided by the length l of the free surface contour over which the force acts:

$$\sigma = \frac{F}{l}. \qquad (1)$$

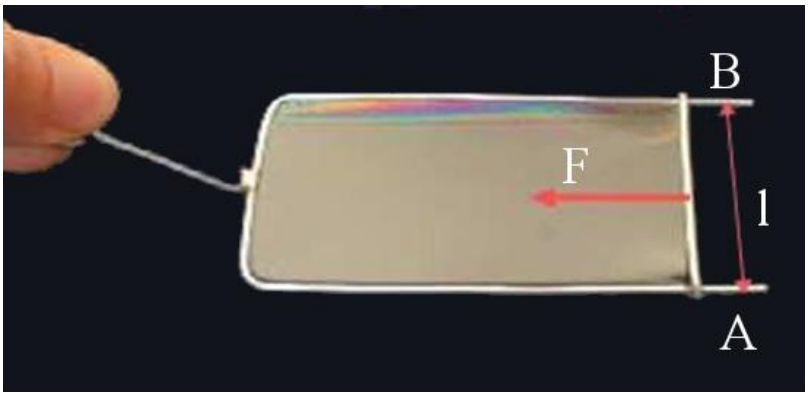


Fig.3.2. Soap bubble spanning the space inside the wire rim with a movable side AB

Surface tension, usually represented by the symbol σ, is measured in force per unit length.

$$[\sigma]_{SI} = \frac{J}{m^2} = \frac{Nm}{m^2} = \frac{N}{m}$$

$$[\sigma]_{CGS} = \frac{dyn}{cm} = 0.001\frac{J}{m^2} = 0.001\frac{N}{m}$$

$$1\ dyn\ =\ 1\ g\cdot cm/s^2\ =\ 10^{-5}\ kg\cdot m/s^2\ =\ 10^{-5}\ N,$$

$$1\ Newton\ =\ 1\ kg\cdot m/s^2\ =\ 10^{5}\ \ g\cdot cm/s^2\ =\ 10^{5}\ dyne.$$

For example, the surface tension of distilled water is $72\ dyn/cm$ at $25\ °C$ $(77\ °F)$; in SI units this is $72\cdot 10^{-3}\ N/m$ or $72\ mN/m$.

The surface tension is a constant which value is influenced by the nature of states being in contact, their concentration, temperature, and also the possible action of various chemical and physical factors. The most common methods for determining the surface tension coefficient are:

1. **Stalagmometric method** – drop weight method which is a relative method;
2. **Wilhelmy plate** or **du Noüy ring method** - direct method.

Determination of the surface tension coefficient using the relative method

The ***stalagmometric method*** is one of the most common methods for measuring surface tension. The principle is to measure the weight of the drops of the fluid falling from the capillary glass tube, and then calculate the surface tension of the specific fluid which we are interested in. We know the weight of each drop of the liquid by counting the number of the drops falling out.

Stalagmometer is a glass tube, widened in the middle part (Fig.3.3). Its volume is calibrated by the scale shown on the tube, or by the top and bottom lines. The bottom part of stalagmometer is modified such that the liquid flowing through its smaller diameter forms the drops.

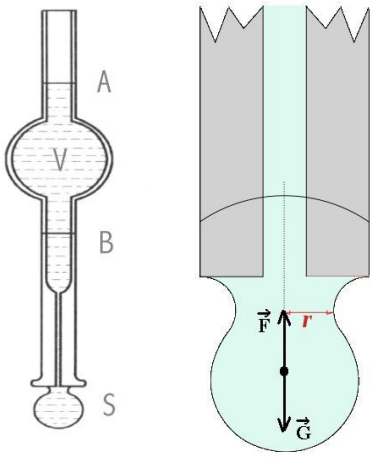


Fig.3.3. Stalagmo-meter Traube

The drop of a mass m gets released when its weight $G = mg$ is equal to the surface force at the end of tube

$$mg = 2\pi r\sigma. \quad (2)$$

Equation (2) suggests that the surface tension σ can be calculated from the known water mass m and the radius of stalagmometer tube r. On the other hand, the ratio of the mass and surface tension is constant for all the liquids. Alternatively, since the surface tension is proportional to the weight of the drop, the fluid of interest may be compared to a reference fluid of known surface tension (typically water).

The working formula for the relative method of surface tension determination is:

$$\sigma = \sigma_0 \frac{n_0 \cdot \rho}{n \cdot \rho_0}, \quad (3)$$

where n_0 is number of distilled water drops and n- number of studied liquid drops, ρ_0-density of distilled water and ρ-density of studied liquid, σ_0-surface tension of distilled water and σ-surface tension of studied liquid.

Determination of the surface tension coefficient using the direct method

The ***du Noüy ring method*** is one technique by which the surface tension of a liquid can be measured. The method involves slowly lifting a ring, often made of platinum, from the surface of a liquid. The ring should be very clean without blemishes or scratches because they can greatly alter the accuracy of the results. The force, F, required to raise the ring from the liquid's surface is measured and related to the liquid's surface tension, σ:

$$F = \pi(d_1 + d_2) \cdot \sigma \quad (4)$$

or

$$\sigma = \frac{F}{\pi(d_1 + d_2)} = \frac{F}{2\pi d_{average}} \quad (5)$$

where d_1 is the diameter of the inner ring of the liquid film pulled and d_2 is the diameter of the outer ring of the liquid film. The system used in this laboatory is the torsion balance (see fig.3.4).

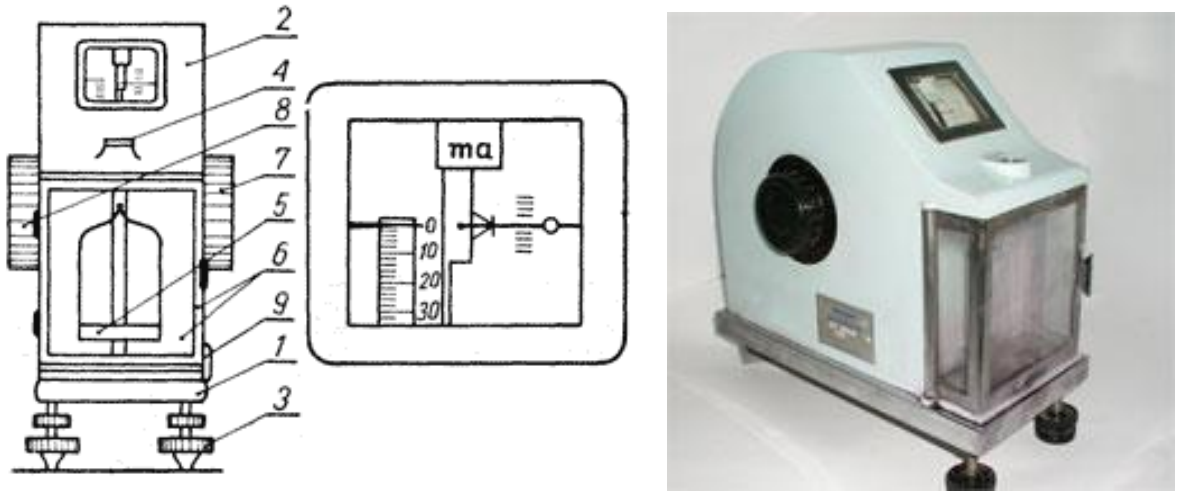


Fig.3.4. Torsion balance

1. metal support
2. metal case
3. adjustment screws
4. air bubble
5. ring or metal plate
6. the glass door
7. handle for equilibration
8. handle for drum rotation
9. handle that bloks the lever

The ***Wilhelmy plate*** consists of a thin plate usually on the order of a few square centimeters in area. The plate is often made from filter paper, glass or platinum which may be roughened to ensure complete wetting. In fact, the results of the experiment are irrelevant of the material used, as long as the material is wetted by the liquid. The plate is cleaned thoroughly and attached to a scale or balance via a thin metal wire (see fig.3.5). The force on the plate due to wetting is measured via a microbalance and used to calculate the surface tension using the Wilhelmy equation:

$$\sigma = \frac{F}{l\,cos\theta} = \frac{F}{2(l_1 + l_2)} = \frac{F}{l} \qquad (6)$$

where l is the wetted perimeter ($l = 2l_1 + 2l_2$; l_1 is plate width and l_2 is plate thickness) of the Wilhelmy plate and θis the contact angle between the liquid phase and the plate. In practice the contact angle is rarely measured, instead either literature values are used, or complete wetting ($\theta = 0$) is assumed.

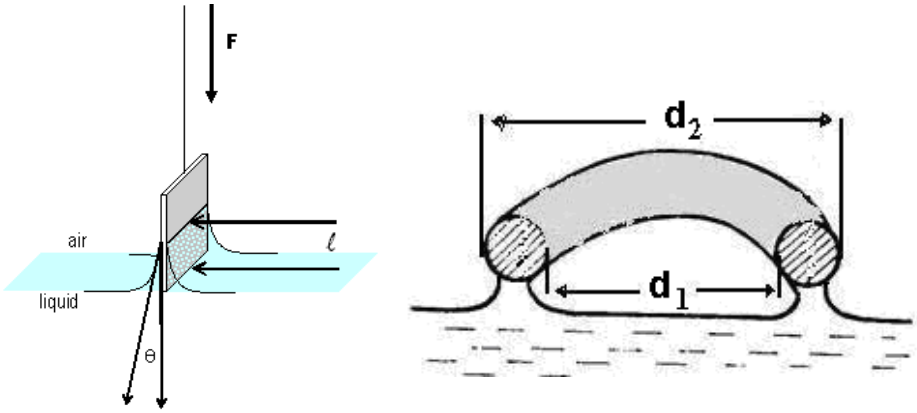


Fig.3.5. The Wilhelmy plate method and ring

Work procedure

- We determine the value of the surface tension force formed at the moment when the ring is taken off from the surface of liquid by using a torsion balance. In order to perform it, the metal ring is hung instead of the scale. The diameter of the ring and metal plate are indicated on the worktable.
- Solutions of ethyl alcohol (100%, 50%, 25% and 0%) are used as the searched liquids.
- Three measurements are performed for each concentration and the average value of the surface tension force is determined.
- The obtained results are written in table 2.

Table 2

Concentration	#	d, m	F, N	$\sigma, N/m$
0%	1			
	2			
	3			
	Average			
25%				
50%				
100%				
X%				

- Finally, a graph is drawn, where Ox corresponds to the concentration of solutions $n, \%$ and Oy- to the surface tension coefficient $\sigma, N/m$.
- The $X\%$ for unknown solution is determined from the graphic dependence.

Use a millimeter paper!!

- The necessary observations and conclusions needs to be formulated.

The importance of surface tension for medical practice

Blood surface tension, as one of the crucial blood parameters, affects many vital functions of human body. Over the time human body undergoes different natural thermal conditions. Therefore the knowledge about temperature dependence of blood surface tension is important.

Biological liquids usually represent the saline water solutions and have surface tension coefficient smaller than distilled water. Proteins are the substances that decrease the surface tension coefficient according to the Gibbs law and they are concentrated to the surface of these liquids in the monomolecular layers playing a great role in the exchange of substances at the cell level.

The surface tension coefficient of the blood serum at the body temperature is about $67\ dyn/cm$.The normal value is modified by the presence of some substances or by the existence of some pathological processes as:

- Jaundice- a common condition in newborns, jaundice refers to the yellow color of the skin and whites of the eyes caused by excess bilirubin in the blood. Bilirubin is produced by the normal breakdown of red blood cells;
- Neoplasia- new, uncontrolled growth of cells that is not under physiologic control**.** A "tumor" or "mass lesion" is simply a "growth" or "enlargement" which may not be neoplastic (such as a granuloma). The term "cancer" implies malignancy, but neoplasms can be subclassified as either benign or malignant.

The existence of biliary acids, and the salt of these, decreases the value of urine surface tension coefficient that allows the easy detection of their presence.

The phenomenon of surface tension is the base of different living and pathological process taking place in the human organism such as:

- Chemotaxis (from chemo- + taxis) is movement of an organism in response to a chemical stimulus. Somatic cells, bacteria, and other single-cell or multicellular organisms direct their movements according to certain chemicals in their environment. Ex. Movement of bacteria to find food (glucose); movement of sperm towards the egg during fertilization; migration of neurons or lymphocytes.
- Venous air embolism (VAE), a subset of gas embolism, is an entity with the potential for severe morbidity and mortality. Venous air embolism occurs when atmospheric gas is introduced into the venous system.

The dropper is build using the surface tension phenomenon used for dosing some medicinal substances.

Examples of surface tension:

- *Walking on water:* small insects such as the water strider can walk on water because their weight is not enough to penetrate the surface.
- *Floating a needle:* A carefully placed small needle can be made to float on the surface of water even though it is several times as dense as water. If the surface is agitated to break up the surface tension, then needle will quickly sink.

- *Don't touch the tent!:* Common tent materials are somewhat rainproof in that the surface tension of water will bridge the pores in the finely woven material. But if you touch the tent material with your finger, you break the surface tension and the rain will drip through.
- *Clinical test for jaundice:* Normal urine has a surface tension of about 66 dynes/centimeter but if bile is present (a test for jaundice), it drops to about 55. In the Hay test, powdered sulfur is sprinkled on the urine surface. It will float on normal urine, but will sink if the surface tension is lowered by the bile.
- *Surface tension disinfectants:* Disinfectants are usually solutions of low surface tension. This allow them to spread out on the cell walls of bacteria and disrupt them.
- *Soaps and detergents:* These help the cleaning of clothes by lowering the surface tension of the water so that it more readily soaks into pores and soiled areas.
- *Washing with cold water:* The major reason for using hot water for washing is that its surface tension is lower and it is a better wetting agent. But if the detergent lowers the surface tension, the heating may be unneccessary.
- *Why bubbles are round:* The surface tension of water provides the necessary wall tension for the formation of bubbles with water. The tendency to minimize that wall tension pulls the bubbles into spherical shapes.
- *Surface Tension and Droplets:* Surface tension is responsible for the shape of liquid droplets. Although easily deformed, droplets of water tend to be pulled into a spherical shape by the cohesive forces of the surface layer.

Knowledge evaluation exercises:

1. How is the superficial layer defined?

a) it is the contact zone between two molecule layer from the interior of the liquid;
b) it is the contact zone of two molecule layers belonging to two different phases (for example liquid - gas), the resulting forces being always oriented towards the interior of the liquid;
c) is the zone in which the superficial tension forces are oriented towards the exterior of the liquid;
d) is the contact zone between two molecules layers belonging to two different phases (for example liquid - gas), in which the superficial forces are zero;
e) is the liquid layer the vicinity of the vessel walls.

2. What is superficial tension force?

a) it is the force that appear between two miscible liquids and favors their mixing;
b) it is the force from the superficial layer, which tends to reduce its surface;
c) it represents the force with which the liquid molecules are attracted towards the vessel walls;
d) it is given by the relation: $F = \sigma/l$;
e) it is the force that is exerted between molecules from the internal layer of the liquid.

3. The superficial tension force F, is given by the relation:

a) $F = \sigma \cdot S$;

b) $F = \sigma/S$;
c) $F = \sigma/l$;
d) $F = l/\sigma$;
e) $F = \sigma \cdot l$.

4. The superficial tension force that sustains a water drop at the end of a capillary tube of radius r is:
a) $F = \sigma \cdot \pi \cdot r^2$;
b) $F = \frac{\sigma}{\pi r^2}$;
c) $F = \sigma \cdot 4\pi r^2$;
d) $F = \sigma \cdot 2\pi r$;
e) $F = \sigma \frac{4\pi r^3}{3}$.

5. The coefficient of surface tension depends
a) on the nature and concentration of the dissolved substance;
b) on the nature of the solvent, on temperature and on the nature of the separation surface;
c) on the nature of the solvent and on temperature;
d) on the nature of the solvent and on the liquid surface area;
e) on the shape of the vessel that contains the liquid.

6. What is the unit of measurement of the coefficient of surface tension in the S.I. and C.G.S. system?
a) N/m^2; dyn/cm^2;
b) $N \cdot m$; $dyn \cdot cm$;
c) J/m^3; dyn/cm;
d) N/m; dyn/cm;
e) N/cm; dyn/m.

7. What is the formula used to calculate the coefficient of surface tension in the Traube stalagmometer experiment?
a) $\sigma = \sigma_0 \frac{n\rho_0}{n_0\rho}$;
b) $\sigma = \sigma_0 \frac{n_0\rho_0}{n\rho}$;
c) $\sigma = \sigma_0 \frac{n\rho}{n_0\rho_0}$;
d) $\sigma = \sigma_0 \frac{n_0\rho}{n\rho_0}$;
e) $\sigma = \frac{n_0\rho}{n\rho_0}$.

8. The mechanism that lowers the surface tension in aqueous solutions is due to:
a) the chemical reactions between the molecules of the solvent and of the dissolved substance;
b) the positioning of polar molecules between water dipoles, weakening the intermolecular forces of the superficial layer;

c) the positioning of molecules between water dipoles, weakening the intermolecular forces from the profound layer;
d) the increase in the viscosity of the solution;
e) the interpenetration of the tensioactive molecules and the ones from the internal liquid layers.

9. A tensioactive agent:
a) is also called surfactant;
b) is a substance that increases the value of the surface tension of a liquid;
c) is a substance that, when dissolved in a liquid, decreases its superficial tension and has the tendency to lay down on the bottom of the vessel;
d) is a substance that, when dissolved in a liquid, self-assembles at the interface, thus reducing the surface tension of the liquid;
e) is a substance that modifies the surface tension of a liquid, by either increasing or decreasing its value.

10. Which of the following assertions are true?
a) the surface tension of a liquid increases with the rise in temperature;
b) the surface tension of water is greater than of most liquids;
c) the interface behaves like an elastic membrane due to the action of the surface tension forces;
d) the surface tension of a liquid decreases with rise in the temperature;
e) the interface behaves like an elastic membrane under the action of gravitational forces.

4. CELL OSMOTIC PHENOMENA

Purposes:

- Presentation of theoretical notions of osmotic phenomena;
- Study of devices applied for pointing out and measurement of osmotic pressure;
- Familiarization with turgescent and plasmolysis phenomena;
- Evaluation of the cell dimension by method of two micrometers;
- Familiarization with biological and medical aspects of osmotic pressure.

Theoretical Notions:

Diffusion refers to the process by which molecules intermingle as a result of their kinetic energy of random motion.

Osmosis is a special kind of diffusion involving water molecules. It occurs when two solutions are separated by a partially permeable membrane. If two solutions of different concentration are separated by a ***semipermeable membrane*** which is permeable to the smaller solvent molecules but not to the larger solute molecules, then the solvent will tend to diffuse across the membrane from the less concentrated to the more concentrated solution. The definition of osmosis is as follows:

Osmosis is the movement of water molecules from an area of high water concentration (weak/dilute solution) to an area of low water concentration (strong/concentrated solution) through a semipermiable membrane (Fig.4.1.a,b).

A ***semipermeable membrane*** is a membrane that allows certain types of molecules to pass through but blocks others. Body cells are surrounded by this type of membrane, which helps to control what substances can and cannot pass into the cells. By serving as a barrier between the interior and the exterior of the cell, it protects the cell from foreign bodies that could be harmful. Outside of the body,

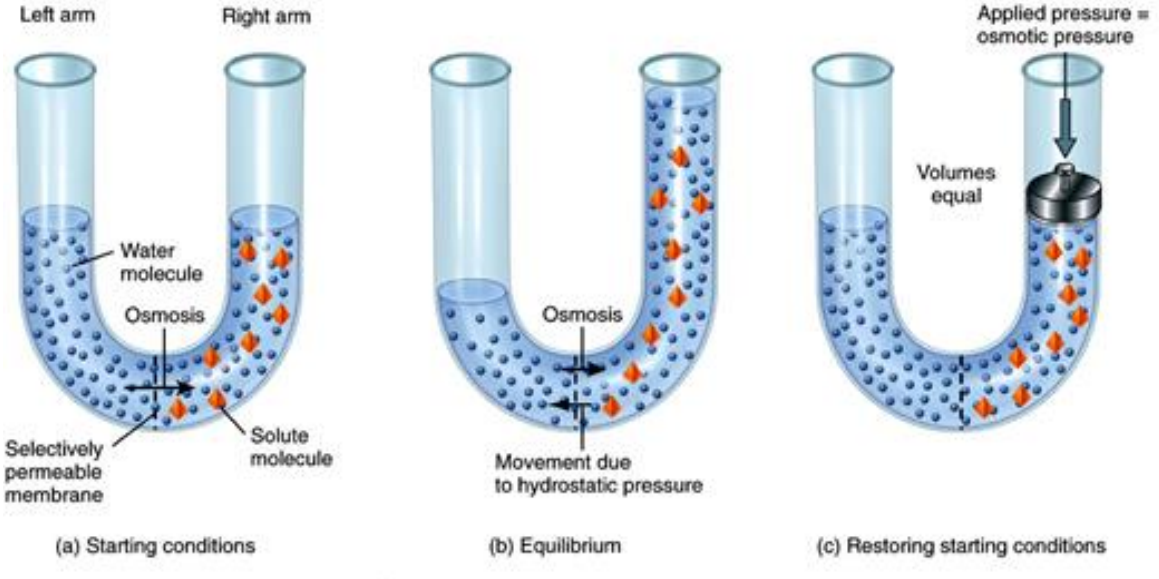


Fig.4.1. Osmotic pressure

these membranes, usually artificially created, are used for specific functions such as

waterdesalinationandpurification.

Take a vase divided in two parts by dividing semi-permeable membrane. In one part there is water (dilute solution), but in the other part there is substance solution, their molecules do not pass through the separable wall (Fig.4.1.a).

Practice shows that the water passes through the semi-permeable membrane and after a while the level difference is established in the divisions of the vase and, the difference of pressures, named the ***osmotic pressure*** of solution. Thus the ***osmotic pressure*** is the pressure which must be exercised on the solution to bring it in the equilibrium with the pure solvent separated by it through the semipermeable membrane (Fig.4.1.c).

$$P_{osm} = \frac{F}{S} \tag{1}$$

$$[P_{osm}]_{SI} = \frac{N}{m^2}$$

Osmosis is of great importance in biological processes where the solvent is water. The transport of water and other molecules across biological membranes is essential to many processes in living organisms.

Living organisms contain water in the proportions 50 – 90 % of their total weight. Different substances are dissolved in the water making a solution. The membranes of living cells being semipermeable and selective let pass water and different substances as a function of metabolic necessities. Just in case of small concentrations, the osmotic pressure can obtain the essential values. In different cells of vegetable nature its values can be of 5 – 20 atmospheres, assuring the penetration of water from ground to the high heights (for example, up to the top of trees). The actions of osmotic pressure is felt by swimmers when they open the eyes in the water (especially in the lake water); the intraocular pressure is increased due to the penetration of water through the cornea.

Experiments confirm that the osmotic pressure is similar to the pressure of ideal gases having the same laws.

1. The first law of osmoses is the ***law of concentrations*** according to which the osmotic pressure of dilute solutions at the constant temperature is in the proportion to the molar concentration of the solvent.

   $P_{osm} = K_T \cdot C^M$, $\quad K_T = constant\ of\ temperature$, $\quad [K_T]_{SI} = \frac{N \cdot m}{mol}$.

2. The ***law of temperature***: for the examined solution, the osmotic pressure increases in proportion to the coefficient $\mathbf{1} + \boldsymbol{\alpha t}$, the value $\boldsymbol{\alpha}$being equal to $1/273$ (at high temperatures this law is not respected).

   $P_{osm} = K_C \cdot T$, $\quad K_C = constant\ of\ concentration$, $\quad [K_T]_{SI} = \frac{N \cdot m^{-2}}{^0K}$.

3. The ***law of Vant Hoff***: the osmotic pressure does not depend on either the solvent nature or the nature of the dissolved substances, it depending only on the number of particles from the volume of solutions. It follows from the laws of osmoses that the osmotic pressure P_{osm}can be calculated after the formula of Mendeleev – Clapeyron:

$$P_{osm}V = nRT, \tag{2}$$

where$V$ is the volume occupied by the solution, R – gas universal constant, T – absolute temperature, n

– number of moles. The following equation results from the equation (2):

$$P_{osm} = \frac{nRT}{V} = C^M RT, \quad (3)$$

where $C^M = n/V$ is the molar concentration of solution.

4. The ***law of Dalton*** is confirmed for a mixture of solutions. According to this total osmotic pressure is equal to the sum of osmotic pressure of each solution, is taking in consideration that each dissolved substance has its own osmotic pressure, as though it were single in the whole quantity of solvent.

The osmotic pressure of two different solutions, which have the common solvent, is the same as the equal temperatures and molar concentrations. These solutions are named ***isotonic***.

The medium with the less or greater osmotic pressure with respect to the other medium is named ***hypotonic*** or ***hypertonic*** respectively. The solution being in contact with the semipermeable membrane bear a flux of solvent always oriented to the hypertensive medium.

Noilet observed the phenomenon of osmoses in 1748 for the first time. The detailed study belongs to Dutrochet. The devices used for revealing and measurement of osmotic pressure are named ***osmometers***. The osmometer of Dutrochet is represented in Fig.4.2; it is made of glass vase, in which the bottom is replaced by a semipermeable membrane (a pig's urinary bladder or cellophane).

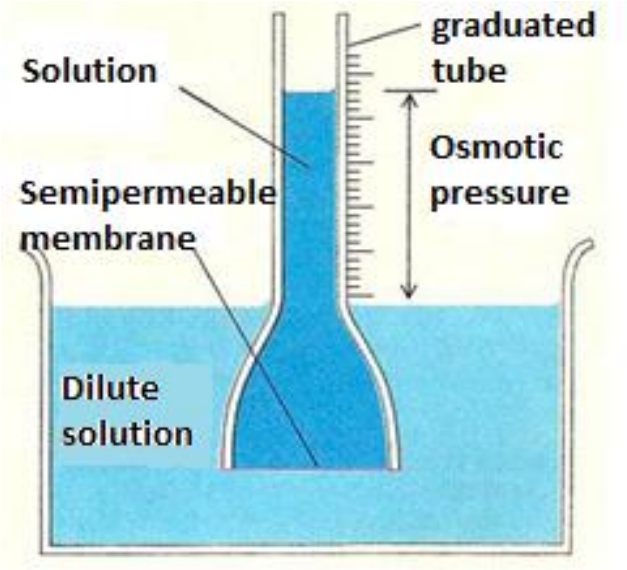


Fig.4.2. Osmometer of Dutrochet

The vase is prolonged of the superior part with a capillary tube placed on graded millimeter plane. The solution of sugar is introduced in the osmometer up to the inferior level of the vertical tube and is sunk into the vase with distilled water that must be at the same level with the solution from the interior. The liquid from the internal vase will rise slowly in the capillary tube, the maximal height of the column determines the osmotic pressure of solution.

There is a series of indirect methods of osmotic pressure measurements based upon the procedures of determination of solution molar concentration after which the osmotic pressure is calculated. In biology this method is called the ***cryoscopy*** (the molar concentration is determined after the solidification

point of solution).

The fall of solidification point of solution is direct proportional to molar concentration C_m:

$$\Delta t = t_0 - t_1 = K \cdot C_m \quad (4)$$

where t_0 is the freezing temperature of pure solvent; t_1 – the freezing temperature of solution.

The determination of cryoscopic point of solution-7 is made by the Beckman's cryoscope (Fig.4.3). It is supplied with a vase made of glass-3 in witch cooling mixture is introduced (ice and salt). In the mixture two coaxial test tubes of glass-2 are introduced. The interior test tube-1 is supplied with two lateral branches and on the axes with a punched cork through which the thermometer Beckman is introduced inside. The air stratum between two test tubes allows a slow cooling solution.

The state of extra fusion is performed by stirring the magnetic bar-5, being in rotation moving by the magnet-6 in the form of a horseshoe. The magnet is fixed on the axes of electrical motor supplied with the electrical set.

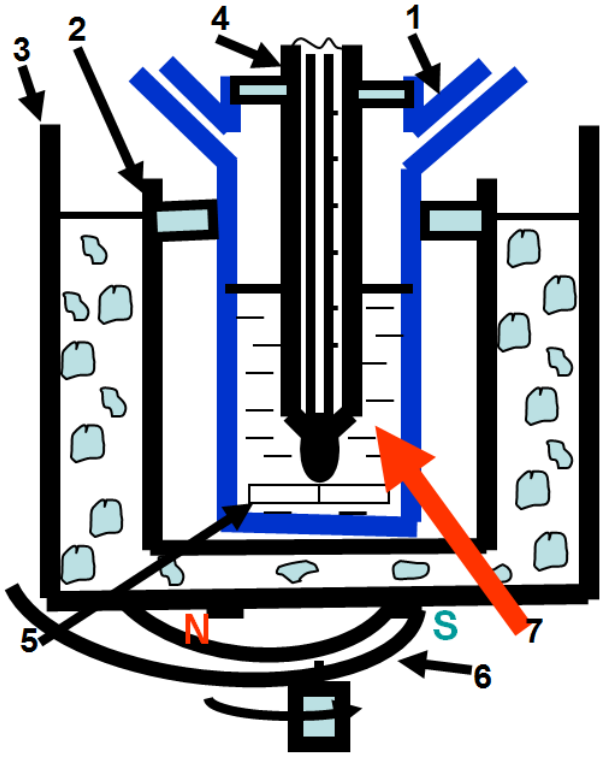


Fig.4.3. Beckman's cryoscope

The used thermometer named the Beckman's thermometer-4 has the reduced scale graduated in the hundredths of degree. In the upper part it is supplied with a supplementary reservoir with mercury, which communicates with the inferior reservoir. The mercury form this reservoir is used for adjusting mercury quantity from the inferior reservoir, that is necessary to make possible the measurements of temperature variation in the needed interval. Dividing formula (4), which is called the ***Raoult's law*** to formula (3) we will obtain:

$$P_{osm} = \frac{RT}{K}\Delta. \quad (5)$$

Using the R and K values, for liquids we will obtain $P_{osm} = 12.2 \cdot 10^5 \Delta t \; N/m^2$.

Turgescent and plasmolysis phenomena in the animal and vegetable cells

When an animal cell or a plant cell is placed in a medium, which is a water solution, the possible consequences are listed below:

1. If the water concentration inside the cell is the same as that in the surrounding medium (the medium is a **isotonic solution**) there will exist a dynamic equilibrium between the number of molecules of water entering and leaving the cell and so the cell will retain its original size.For example, the red blood cell in the blood plasma retains its shape because of the isotonic nature of the plasma.
2. If the water concentration of the cells cytoplasm is lower then that of the medium (the medium is a **hypotonic solution**) surrounding the cell then osmosis will result in the cell **gaining** water. The water molecules are free to pass across the cell membrane in both directions, but more water molecules will enter the cell than will diffuse out with the result that water enters the cell, which will then **swell** up and could possibly **burst**.
3. If the water concentration inside the cell is higher then that of the medium (the medium is a **hypertonic solution**) the number of water molecules diffusing out will be more than that entering and the cell will **shrink** and **shrivel** due to osmosis (see fig.4.4).

For animal cells the absence of the rigid wall limits the increase of volume in the hypotonic medium leading to breakage of the cell membrane. In the particular case of red blood cells the phenomenon is called ***hemolysis***.

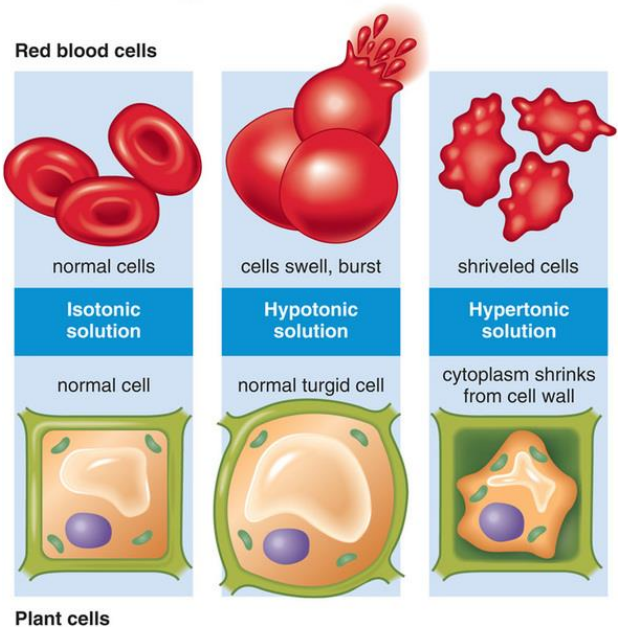


Fig.4.4. Osmosis in animal and plant cells

Effects of osmosis in plant cells

When a plant cell is placed in an ***isotonic solution***, a phenomenon called ***incipient plasmolysis*** is

said to occur. "Incipient" means "about to be". Although the cell is not plasmolsysed, it is not turgid (see fig.4.4.a). When this happens the green parts of the plant droop and are unable to hold the leaves up into the sunlight.

Plant cells are enclosed by a rigid cell wall. When the plant cell is placed in a ***hypotonic solution***, it takes up water by osmosis and starts to swell, but the cell wall prevents it from bursting. The plant cell is said to have become ***"turgid"*** , swollen and hard (see fig.4.4.b). The pressure inside the cell rises until this internal pressure is equal to the pressure outside. Turgidity is very important to plants as it helps in the maintenance of rigidity and stability of plant tissue and as each cell exerts a ***turgor pressure*** on its neighbor adding up to plant tissue tension which allows the green parts of the plant to "stand up" into the sunlight.

When a plant cell is placed in a ***hypertonic solution***, the water from inside the cells cytoplasm diffuses out and the plant cell is said to have become ***"flaccid"*** (see fig.4.4.c). If the plant cell is then observed under the microscopic, it will be noticed that the cytoplasm has shrunk and pulled away from the cell wall.This phenomenon is called ***plasmolysis***. The process is reversed as soon as the cells are transferred into a hypotonic solution (deplasmolysis).

Effects of osmosis in animal cells

Animal cells do not have cell walls. In *hypotonic solutions*, animal cells swell up and explode as they cannot become turgid because there is no cell wall to prevent the cell from bursting. When the cell is in danger of bursting, organelles called contractile vacuoles will pump water out of the cell to prevent this. In *hypertonic solutions*, water diffuses out of the cell due to osmosis and the cell shrinks. Thus, the animal cell has always to be surrounded by an isotonic solution.

The process of regulating the concentration of water and mineral salts in the blood is called ***osmoregulation***. Animals which live on dry land must conserve water and so must animals which live in the salty sea water, but animals which live in freshwater have the opposite problem; they must get rid of excess water as fast as it gets into their bodies by osmosis.

In the human body, the ***kidneys*** provide the necessary regulatory mechanism for the blood plasma and the concentration of water and salt removed from the blood by the kidneys is controlled by a part of the brain called the hypothalamus.

Dialysis and artificial kidney

In a multi-component system, ***dialysis*** is a process by which only certain compounds including both the solvent molecules and small solute molecules are able to pass through the selectively permeable dialysis membrane but other larger components such as large colloidal molecules like proteins cannot pass through pores in the dialysis membranes. Dialysis can therefore be used for separation of proteins from small ions and molecules and hence is used for purification of proteins required for laboratory experiments. Examples of membranes through which dialysis occurs are animal bladders, parchment and cellophane (cellulose acetate).

Artificial kidney refers to renal replacement therapies (with exclusion of kidney transplantation) that are in use and/or in development (see fig.4.5).

Hemodialysis is a method for removing waste products such as creatinine and urea,as well as free

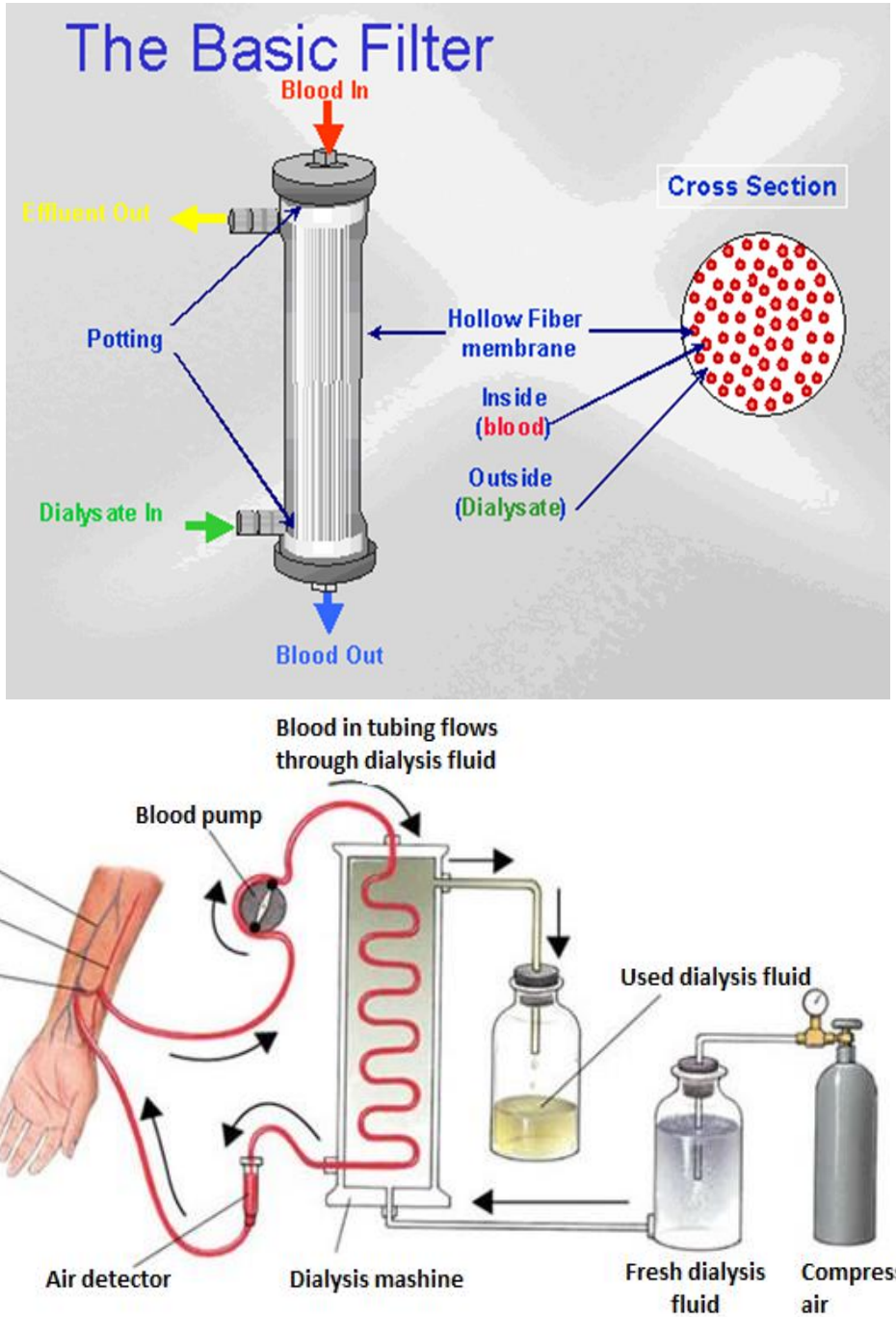


Fig.4.5. Process of hemodialysis

water from the blood when the kidneys are in kidney failure. The mechanical device used to clean the patients blood is called a *dialyser*, also known as an *artificial kidney*. Modern dialysers typically consist of a cylindrical rigid casing enclosing hollow fibers cast or extruded from a polymer or copolymer, which is usually a proprietary formulation. Blood from the patient is circulated through a long cellophane dialysis tube suspended in an isotonic solution called the dialysate which is an electrolyte solution

containing the normal constituents of blood plasma. The toxic end products of nitrogen metabolism such as urea from the blood pass through the dialysis membrane where they are removed while cells, proteins and other blood components are prevented by their size from passing through the membrane. Also, the dialysate concentration can be controlled so that salt, water and acid-base imbalances in electrolytes are corrected. Purified blood is then returned to the body.

The importance of osmosis phenomenon study for medical practice

The osmotic pressure is the important factor that assures the maintenance of volume and consequently – the cell structure, the morphological and functional integrity cells. The osmosis takes place in exchange of substances between organisms and an environmental medium between cells and an extracellular medium.The determination of a cryoscopic point can give information of the molecular concentration of blood serum or other biological liquids. The calculations of the osmotic phenomena allow the study for the functions of various physiological systems. Comparing the osmotic pressures of different liquids in the normal and pathological state, some aspects of hydroelectric metabolism of renal function can be followed.The passage of some substances between the vascular and intercellular compartments takes place under the influence of pressure difference on both parts of the capillary wall. The cell volume varies as it is dependant upon of extra cellular medium concentration. This variation offers the model for the study of permeability phenomena of cell membranes.

We notice that in order to introduce various solutions in the blood circulation, they must be isotonic, and not to make any modifications of the cell volume.

Equipment and Materials

The binocular microscope with the ocular micrometer and object lens micrometer; 4 glass blades; 4 mounts; 3 Petri vessels; 20 ml of 20% NaCl isotonic solution (the hypertonic solution); 20 ml of distilled water (the hypotonic medium); 3 Pasteur pipettes; lint; xilol, razor blade; 6 – 8 leaves pf *Elodea canadensis* or of *Vallisneria spiralis*.

Work procedure

Only 20 ml of distilled water, NaCl isotonic solution and hypertensive solutions are poured in those 3 petri vessels, respectively. Some leaves of alga are put in each Petri vase. In 30 minutes each leaf from each medium is examined under the microscope.

In order to examine the leaf of *Vallisneria spiralis* under the microscope the leaf is sectioned thickly by the razor blade and then a more transparent fragment is maintained. The leaf of *Elodea canadensis* does not need any sectioning because it is transparent.

For this item of the paper we shall follow only the morphologic aspect of vegetable cell from those three media.

Take a fragment of the leaf of Vallisneria spiralis from the hypotonic solution and place it accurately on a glass blade. Cover it with another glass blade and tap it gently then introduce it under microscope and using focusing buttons search for the image of the vegetable cell.

The same procedure is performed for the leaves fragments from hypertonic media. Formulate the observations and the necessary conclusions.

Knowledge evaluation exercises:

1. What is a semipermeable membrane?

2. Defines the phenomenon of osmotic pressure?

3. The solutions are called isotonic if:
   a) they have the same temperature and common solvent;
   b) at the same temperature they have equal molar concentrations;
   c) have the same molar concentration and the same solvent;
   d) at the same temperature have the same solvent and the same molar concentration.

4. The osmotic pressure increases:
   a) proportional to the temperature;
   b) proportional to the $(1+\alpha t)$ only up to a certain temperature;
   c) inversely proportional to $(1+\alpha t)$;
   d) inversely proportional to the temperature.

5. Osmosis is:
   a) the movement of water molecules from an area of low water concentration to an area of low water concentration;
   b) the movement of water molecules from an area of high water concentration to an area of low water concentration;
   c) the movement of water molecules from an area of low water concentration to an area of high water concentration;
   d) is a membrane that allows certain types of molecules to pass through but blocks others.

6. Which of the following assertions are true:
   a) the law of concentrations is determined by $P_{osm} = K_C \cdot T$;
   b) the law of concentrations is determined by $P_{osm} = K_C \cdot C$;
   c) the law of Van't Hoff is determined by $P_{osm} = C^M RT$;
   d) the law of Dalton is determined by $P_{osm} = C^M RT$.

7. Which devices are used for measuring of osmotic pressure?
   a) Osmometer of Dutrochet;
   b) artificial kidney;
   c) hemodialysis method;
   d) Beckman's cryoscope.

8. Plasmolysis phenomenon occurs only:

a) in plant cells;
b) in animal cells;
c) in plant cells and animal cells;
d) neither in plant cells and neither animal cells.

9. To execute the dialysis process we need:
   a) artificial kidney;
   b) Osmometer Dutrochet;
   c) fresh dialysis fluid;
   d) microscope.

5. DETERMINATION OF ION MOBILITY BY THE ELECTROPHORESIS METHOD

Purposes

- Study the phenomena which are on the base of electrophoresis method;
- Familiarization with application of electrophoresis method in biology and medicine;
- Study the electrophoresis equipment construction and electrophoresis separation of protein fractions of blood serum;
- Study of electrophoresis separation of inorganic ions (Cu^{2+} and Fe^{3+}) and visualization of colored ions moving into electrical field.

Theoretical notions

Electrophoresis is a method of separation of charged molecules in an electric field so as to make them migrate towards opposite charged electrodes. The migration is due to charge on the molecules and potential applied across the electrodes. These molecules migrate at different speed and to different lengths based on their charge, mass and shape. It is ultimately caused by the presence of a charged interface between the particle surface and the surrounding fluid. It is the basis for a number of analytical techniques used in biochemistry for separating molecules by size, charge, or binding affinity.

Electrophoresis is based on the electrokinetic phenomena, by which the movement processes of electrical charged particles, irrespectively of their nature (ions, colloidal particles, bubbles of gas suspended in liquid, etc.) are understood.

Electrophoresis of positively charged particles (cations) is called ***cataphoresis***, while electrophoresis of negatively charged particles (anions) is called ***anaphoresis***. Electrophoresis is a technique used in laboratories in order to separate macromolecules based on size. The technique applies a negative charge so proteins move towards a positive charge. This is used for both DNA and RNA analysis. Polyacrylamide gel electrophoresis (PAGE) has a clearer resolution than agarose and is more suitable for quantitative analysis. In this technique DNA foot-printing can identify how proteins bind to DNA. It can be used to separate proteins by size, density and purity. It can also be used for plasmid analysis, which develops our understanding of bacteria becoming resistant to antibiotics.

The **ionic mobility** (*M*) is defined as the velocity attained by an ion moving through a gas under unit electric field. Its expression is:

$$M = \frac{v}{E} \tag{1}$$

A suitable unit is therefore $[M]_{SI} = \frac{m^2}{V \cdot s}$.

In medical practice the unit $[M]_{pract} = \frac{\frac{cm}{s}}{\frac{V}{cm}} = \frac{cm^2}{V\,s}$ is used.

The migration of any colloidal particles carrying charge in the external electrical field is called ***electrophoresis***. Force $\boldsymbol{F_e}$ is exercised on the particle mass $\boldsymbol{m}$and charged by $\boldsymbol{q = Ze}$ in the electrical homogenous field by intensity $\boldsymbol{E}$, given by the equation:

$$F_e = q \cdot E = Z \cdot e \cdot E, \quad (2)$$

where Z represents the number of charged particles.

The directions of the force corresponds to the directions of the electrical field, when the particle has the positive charge; in the opposite case the direction of the field has the contrary sense, according to the fundamental law of dynamics:

$$F_e = ma. \quad (3)$$

The friction force from the environment that influences particles increases concomitantly. The value of friction force is determined by *Stokes law*, which supposes the spherical form of the particle:

$$F_s = 6\,\pi\eta r v, \quad (4)$$

here r is the radius of the particle, v- the speed of participle, η- viscosity medium coefficient in which particle moves.

In a while the friction force (F_s) is compensated by electrical force (F_e). From this moment the accelerated movement is conversed into the uniform movement. In this case the following equation is true:

$$F_e = F_s \text{ or } Z\,e\,E = 6\,\pi\eta r v. \quad (5)$$

From this:

$$v = \frac{Z\,e\,E}{6\,\pi\eta r} = \frac{Z\,e}{6\,\pi\eta r}E, \quad (6)$$

it should be noted that

$$\frac{Z\,e}{6\,\pi\eta r v} = M, \quad (7)$$

because from (1) $v = M\,E$.

The value M is called the mobility of the charged electrical particle.Concluding from the equation (7), the mobility of a particle is a value numerically equal to the speed of its uniform motion under the influence of electrical field by the intensity $E = 1\ V/m$ (Volt/meter).

It results from (6) that the mobility of the charged particle depends on

- the nature of the particle (Ze and r)
- the nature of medium and its temperature (because η depends on the temperature).

Types of electrophoresis and their techniques

Electrophoresis can be broadly divided into 2 types: zone electrophoresis and immune-electrophoresis.

1. ***Zone electrophoresis:*** Here the charged particles are separated into different zones or bands. This is of two types as paper electrophoresisand gel electrophoresis.

Paper electrophoresis is a technique which employs a Whattman filter paper which is moistened by a buffer and then connected at two ends to two opposite charged electrodes. Then sample is applied on to one end and let for separation of components under electric field. After separation, the paper is dried and stained to get colored bands. These colored bands are recognized for the nature of sample by comparing with the standard. For a sample of serum, 5 bands of proteins can be separated by paper electrophoresis.

Gel electrophoresis is a similar technique wherein instead of paper, a gel made of agarose or SDS (sodium dodecyl sulphate). The separation is more efficient than paper type as the rate of movement is slow and area of separation is larger by thickness. The sample is applied and subjected to electric field

which can lead to separation of molecules. These molecules form bands and can be recognized by staining and comparing with standard sample bands (see fig.5.1). The method is more effective than

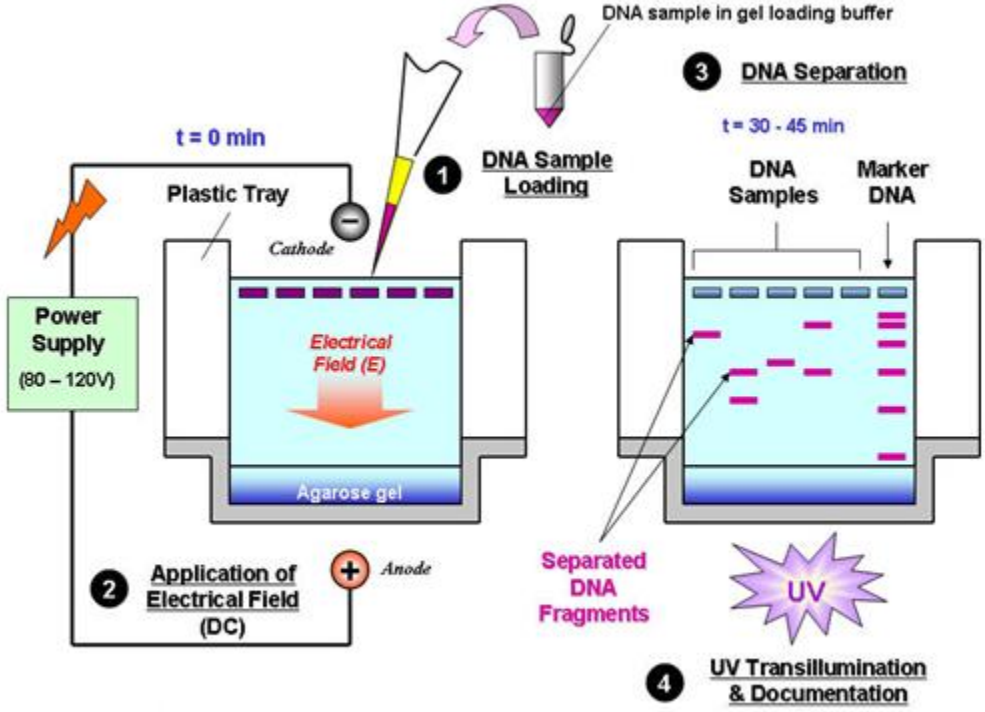


Fig.5.1. Gel electrophoresis

paper and for instance from serum sample, 15 proteins bands can be isolated.

2. ***Immuno electrophoresis:*** This is the method where the proteins are separated on to the electrophoresis paper. Then the antibodies are allowed to diffuse through the paper and react with separated protein molecules in bands

The importance of electrophoresis method for medical practice

Electrophoresis is frequently used in medical practice for examinations of biological liquids as proteins – an important factor for the diagnostics of different diseases. Electrophoresis is a major method for separation of different fractions of serum proteins based upon physical-chemical proteins, it being a qualitative and quantitative determination.

DNA Analysis

- Electrophoresis is one way of analyzing DNA, deoxyribonucleic acid, which is the code that contains all the traits you inherited from your parents. DNA is arranged in sequences, for instance, one sequence represents the color of your eyes and another sequence represents the color of your skin. Through electrophoresis, specific DNA sequences can be analyzed, isolated and cloned. The analyzed DNA may be used in forensic investigations and paternity tests.

Protein Analysis

- Electrophoresis has advanced our understanding on the structure and function of proteins. These molecules are needed by our body cells and may be analyzed, for instance, by getting blood and urine samples. Then through electrophoresis, the amount of proteins in your blood or in your urine is measured and compared to established normal values---lower or higher than the normal levels usually indicates a disease.

Antibiotics Analysis

- The application of electrophoresis in antibiotic studies dates back to the 1950s. Further studies led to improved electrophoretic techniques and new antibiotics. These drugs, such as penicillin, are among the widely prescribed drugs against bacterial infections. With electrophoresis, experts are not only able to synthesize new antibiotics but are also able to analyze which types of bacteria are antibiotic-resistant.

Vaccine Analysis

- Vaccine analysis is one of the many important applications of electrophoresis. There are several vaccines that have been purified, processed and analyzed through electrophoresis, such as the influenza vaccine, hepatitis vaccine and polio vaccine. The exact steps done in the vaccine analysis, however, cannot be determined due to confidentiality reasons of the pharmaceutical companies.

The principle of method

The electrophoresis methods use the equipment in which the migration is made in the tube of liquid, and equipment in which the migration is made in a porous body (filter paper, synthetic resin, gelose etc.). We shall use the on the filter paper.

The electrophoresis method is based upon the principles of the charged principles migrating into the electrical field due to their sizes and their charges, have are separated among them in space, thus, obtaining the electrophoresis.

Description of electrophoresis equipment

The electrophoresis device with which we work in our laboratory is made of electrophoresis cavity and a continuum current supply source. The electrophoresis cavity (Fig.5.2) is made of plastic material and contains two organic glass tubes 1 for the tampon solution. Each tub has two compartments: one with the penetrated electrode of Pt 5, and the other with the penetrated end of paper band 2. These two compartments are connected with each other by diaphragms. The lid is of organic grass 4 covers the electrophoresis cavity.

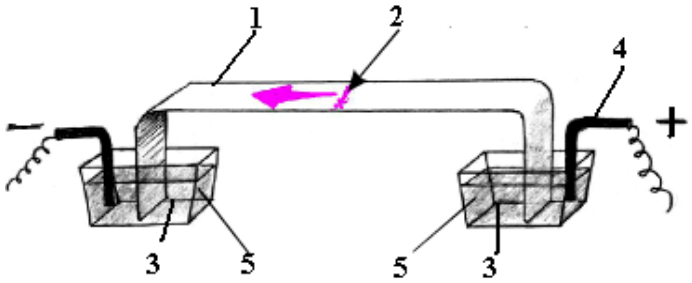


Fig.5.2. Paper electrophoresis apparatus

The supply source (Fig.5.3)represents a changeable redress (0-500 V, 0-40 mA) endowed with the tools of sensible measurements (voltmeter, millimeter).

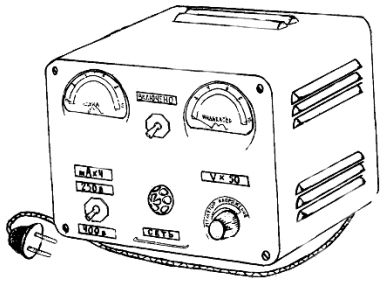


Fig.5.3. The supply source

At the back part of source there are sockets with the respective inscriptions "+" and "-"for the connection of electrophoresis cavity.

Devices and materials

The electrophoresis cavity, the continuum current supply source, filter paper bands 2/26 cm and 2/20 mm, the researched product a mixture of $FeCl_3$, micropipette of 0.01 ml, glass stick, three plastic boards, cotton, tampon solution (solution of 2% hydrochloric acid poured in the distilled water), colored solution (potassium Ferro cyanide), filter paper band, tweezers, chronometer, scale rule.

Work Procedure

1. The current source is connected to the device and then to the electrical circuit, the tension of work of 300 V is established in accordance with the indication of voltmeter. After 30 seconds the source is disconnected from the circuit and the lid of electrophoresis cavity is removed.
2. Three bands of the filter paper are soaked successively into the tampon solution. These bands are placed horizontally one by one on the plastic boards, then placed carefully into the electrophoresis cavity with the free ends of the papers in the tubs with the tampon solution.
3. The current source is connected to the electrical circuit, adjusting the time on the chronometer. During experiment the tension on the electrodes must be maintained constantly. Within 15 – 20 min the device is disconnected from electricity and chronometer stops.
4. We remove the papers on the side, then with a glass stick that has cotton on its end, we process it with 5% ferro cyanide potassium solution that colors the Fe+++ ions to blue and the Cu++ ions to orange.
5. Measuring the length d of the bands from one level of the tampon solution to another and knowing the value of the applied tension at the electrodes U, the intensity of electric field is determined by the equation:

$$E = \frac{U}{d} \tag{7}$$

6. Subsequently, the average distance l is measured, moved by the respective ions (the distance trough the point where the ions arrived from the middle of each colored band). Knowing the distance of migration, the interval of time and intensity of electric field the work equation is obtained by the determination of ion mobility:

$$M = \frac{v}{E} = \frac{\frac{l}{t}}{\frac{U}{d}} = \frac{l\,d}{U\,E} \tag{8}$$

7. The experiment is preformed 3 times for three different pieces of filtered paper.
8. The table below needs to be filled in with the experimentally obtained data.

Nr of Experiment	***ions***	$\boldsymbol{U, V}$	$\boldsymbol{t, s}$	$\boldsymbol{d, m}$	$\boldsymbol{E, V/m}$	$\boldsymbol{l, m}$	$\boldsymbol{M, \frac{m^2}{Vs}}$
1	Cu^{2+}						
	Fe^{3+}						
2	Cu^{2+}						
	Fe^{3+}						
3	Cu^{2+}						
	Fe^{3+}						

9. The necessary observations and conclusions needs to be formulated.

Knowledge evaluation exercises:

1. The rate of migration (ion mobility) during electrophoresis will depend on the following factors:
   a) the Sample (charge, mass, shape);
   b) the Electric Field;
   c) the intensity of the light;
   d) the humidity.

2. In gel electrophoresis, how do we make the DNA migrate through the gel?
   a) large fragments drift to the end of the gel;
   b) gravity;
   c) we place a negative electrode away from the wells;
   d) we place a positive electrode away from the wells.

3. In gel electrophoresis, what does the gel act like?
   a) a gate;
   b) a barrier;
   c) an open window;

d) a filter.

4. Proteins are separated in an SDS-PAGE experiment on the basis of their
   a) positively charged side chains;
   b) molecular weight;
   c) negatively charged side chains;
   d) different isoelectric points.

5. In an SDS-PAGE
   a) proteins are denatured by the SDS;
   b) proteins have the same charge-to-mass ratio;
   c) smaller proteins migrate more rapidly through the gel;
   d) all of the above.

6. Proteins can be visualized directly in gels by
   a) staining them with the dye;
   b) using electron microscope only;
   c) measuring their molecular weight;
   d) none of these.

7. Paper electrophoresis is a techniques which use
   a) Gel;
   b) whattman filter paper;
   c) both of them;
   d) none of them.

8. Which is NOT a reason for using gel electrophoresis?
   a) organizing DNA by the shape of the backbone;
   b) comparing two sets of DNA;
   c) organizing DNA fragments from largest to smallest;
   d) organizing DNA in an order that we can see.

9. If we have a baby, and we know who the mother is but we want to determine the father using gel electrophoresis, how will we know we have found the father?
   a) the father will not match any DNA bands;
   b) the father will match the baby at any DNA band where the baby matches the mother;
   c) the father will match the baby at any band where the baby does not match the mother;
   d) the father will match all of the DNA bands of the baby.

10. The unit measurement for ion mobility is:
    a) $\frac{m^2}{V\,s}$;

b) $\frac{cm^2}{V\,s}$;
c) $\frac{m}{V\,s}$;
d) $\frac{m^2}{V}$.

11. Explain the difference between DNA Analysis and Protein Analysis.

12. Describe the paper electrophoresis equipment.

13. Using the ion mobility formula, explain which factors influence its value.

14. After performing an experiment the following data were obtained: the average distance $l = 2.5\ cm$moved by the respective ions, the distance of migration $d = 21\ cm$, the time interval equal to $10\ min$ and the intensity of electric field $400V$. Calculate the ion mobility, using the SI and CGS systems of units.

15. What value of the electrical voltage was used for molecules to migrate *3 cm*, during *15 min*, where the distance of migration was *25 cm*?

SECOND CYCLE

6. SPECTRAL ANALYSIS

Purposes:

- Presentation of theoretical notions about spectral analysis;
- Familiarization with the dispersion phenomena;
- Study of the structure and work principle of the spectroscope with two tubes;
- Study of the applications of spectral analysis in medical-biological research.

Theoretical notions:

Spectroscopy is the study of the interaction of electromagnetic radiation in all its forms with matter. Spectroscopy originated through the study of visible light dispersed according to its wavelength, by a prism, then the concept was expanded greatly to comprise any interaction with radiative energy as a function of its wavelength or frequency.

The ***spectral analysis*** is a method of physical analysis used for chemical composition determination of different substances by studying their electromagnetic radiation spectrum.

There are two different types of spectral analysis: ***qualitative*** and ***quantitative*** spectral analysis. The qualitative method is one of the most sensitive and rapid methods for the detection of many elements in solids, liquids and gases. The quantitative spectral analysis offers the opportunity to detect the quantity of these elements in materials.The spectrum emitted by an atom is characteristic of that atom and of its energy condition.

The***emission spectrum*** is the pattern of dark lines and colors made when electromagnetic energy such as light, passes through a substance and excites its atoms. The excited atoms give off energy in the form of light. Because each type of atom gives off light having a unique range of colors, the emission spectrum can be used to determine the substance's chemical composition.

The***absorption spectrum*** is the pattern of dark lines and colors made when light passes through an absorbing medium, such as a gas or liquid. The dark lines represent the colors that are absorbed. Because each type of atom absorbs a unique range of colors, the absorption spectrum can be used to identify the composition of different substances, such as the gaseous outer layers of stars.

Kirchhoff's three laws of spectroscopy:

1. A hot solid object produces light with a continuous spectrum (Fig.6.1a). Kirchhoff coined the term black-body radiation.
2. A hot tenuous gas produces light with spectral lines at discrete wavelengths (specific colors) which depend on the energy levels of the atoms in the gas (Fig.6.1b).
3. A hot solid object surrounded by a cool tenuous gas (cooler than the hot object) produces light with an almost continuous spectrum which has gaps at discrete wavelengths depending on the energy levels of the atoms in the gas (Fig.6.1c).

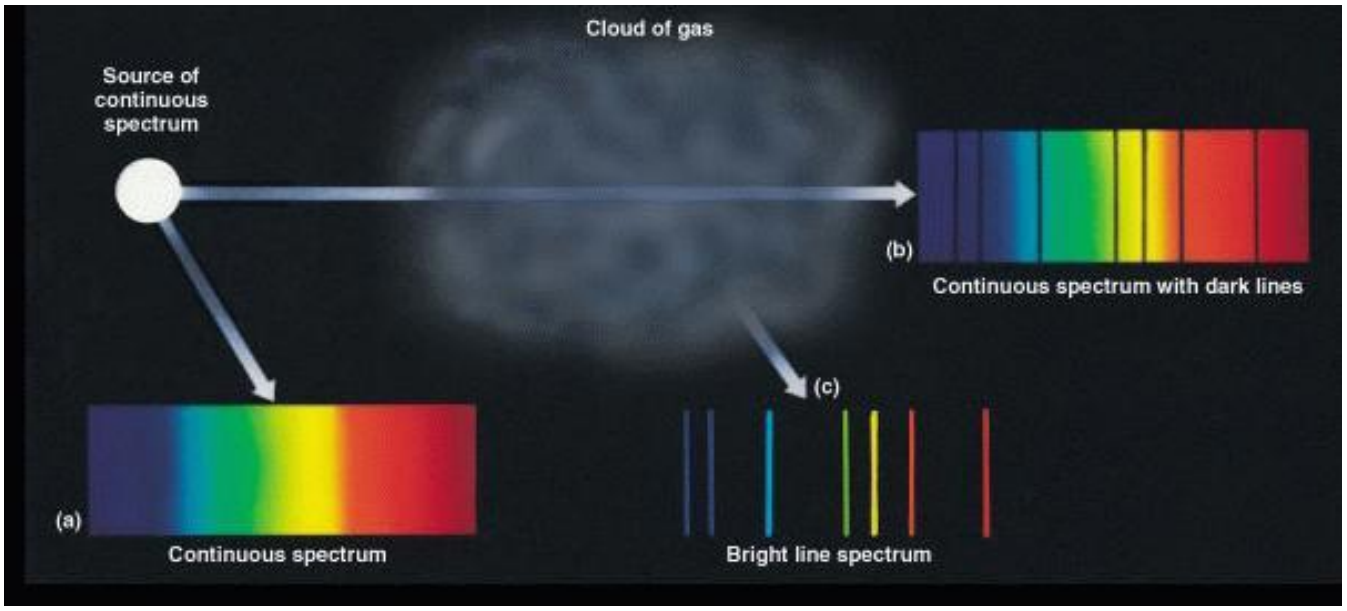


Fig.6.1. Producing of continuous, emission and absorption spectrum

Kirchhoff did not know about the existence of energy levels in atoms. The existence of discrete spectral lines was later explained by the Bohr model of the atom, which helped lead to quantum mechanics.

When solids are heated, they emit waves of radiation (think of the red color from a hot stove).Einstein proposed that radiant energy acts like streams of 'packets' (or quantize-able amounts) called ***photons.*** These photons could be considered 'particles' of energy.

It gave an equation that gave the energy E of a single photon:

$$E = hv, \quad (1)$$

where E is energy (of one quantum in joules), h=Planck's constant ($6.626\ x\ 10^{-34}\ Js$) and v is frequency (in s^{-1} or Hz)

When a source of radient energy is emits a single wavelength (like a laser), it is considered ***monochromatic.*** When there are multiple wavelengths it is considered ***polychromatic.*** A ***spectrum*** is produced when radiation is separated into their components (Fig.6.2).

When we look at light through a prism, we observe the top line spectrum, called the ***continuous spectrum***, named because there are no breaks. We observe this in nature when individual water molecules act as a prism for sunlight; we call it a rainbow. However, as we see with the lower charts, we see that when we take a tube, create a vacuum (meaning that no external elements exist within the tube), and pump it full of X element (shown here are Hydrogen, Helium, Mercury and Uranium) we observe different spectra. A spectrum containing only certain radiation of only specific wavelengths is called ***line spectrum (discrete spectrum)***.

In optics, a prism is a transparent optical element with flat, polished surfaces that refract light. At least two of the flat surfaces must have an angle between them.A dispersive prism can be used to break light up into its constituent spectral colors (the colors of the rainbow). Light changes speed as it moves from one medium to another (for example, from air into the glass of the prism). This speed change causes the light to be refracted and to enter the new medium at a different angle. The refractive index of many materials (such as glass) varies with the wavelength or color of the light used, a phenomenon known

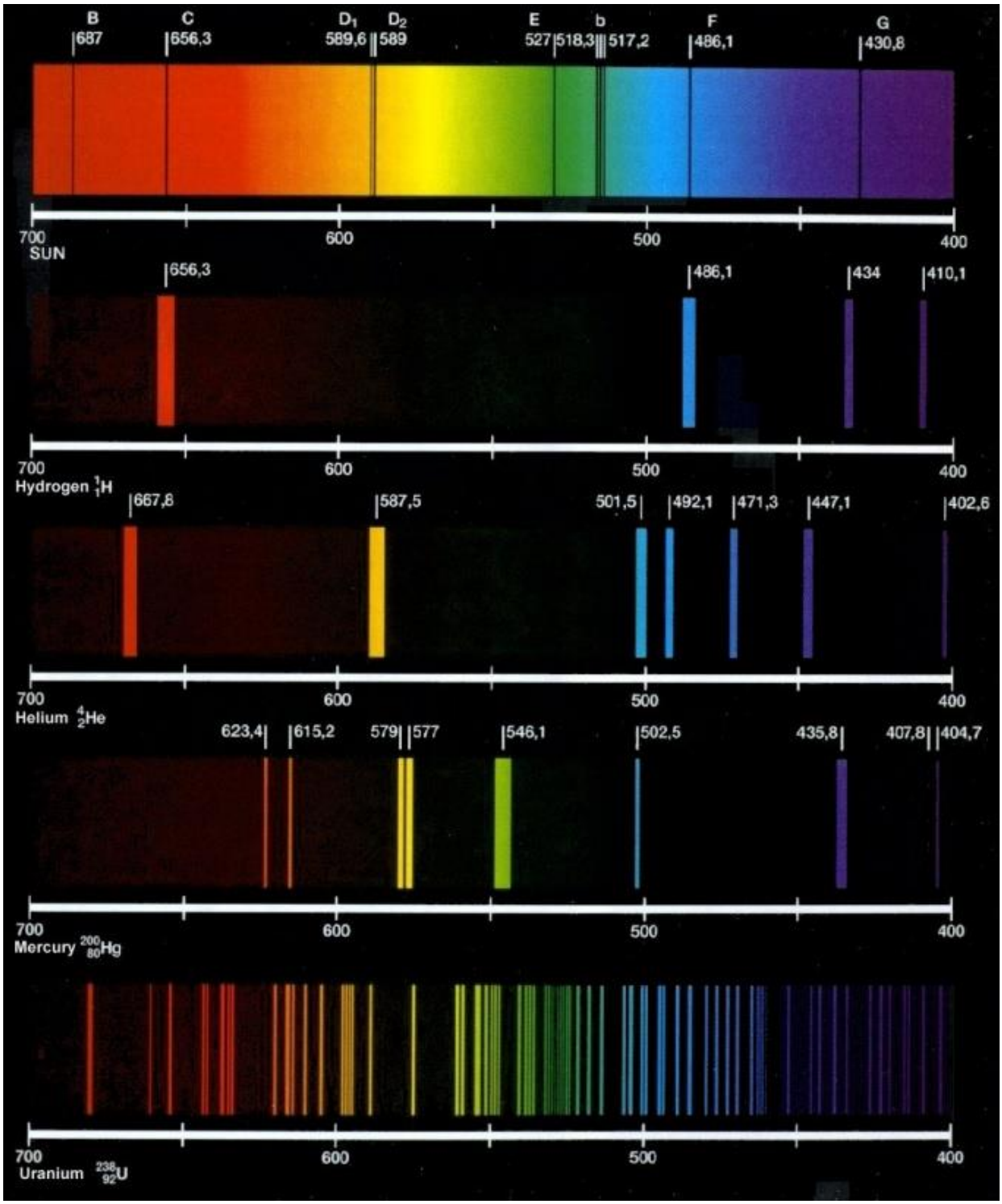


Fig.6.2. Samples of emission spectra

as ***dispersion*.** This causes light of different colors to be refracted differently and to leave the prism at different angles (Fig.6.3), creating an effect similar to a rainbow.

The amount of overall refraction caused by the passage of a light ray through a prism is often expressed in terms of the angle of deviation (D).

$$D = (n_2 - n_1)A, \tag{2}$$

where*A* is a prism angle, n_2 and n_1 is refracted index of prism and refracted index of medium.

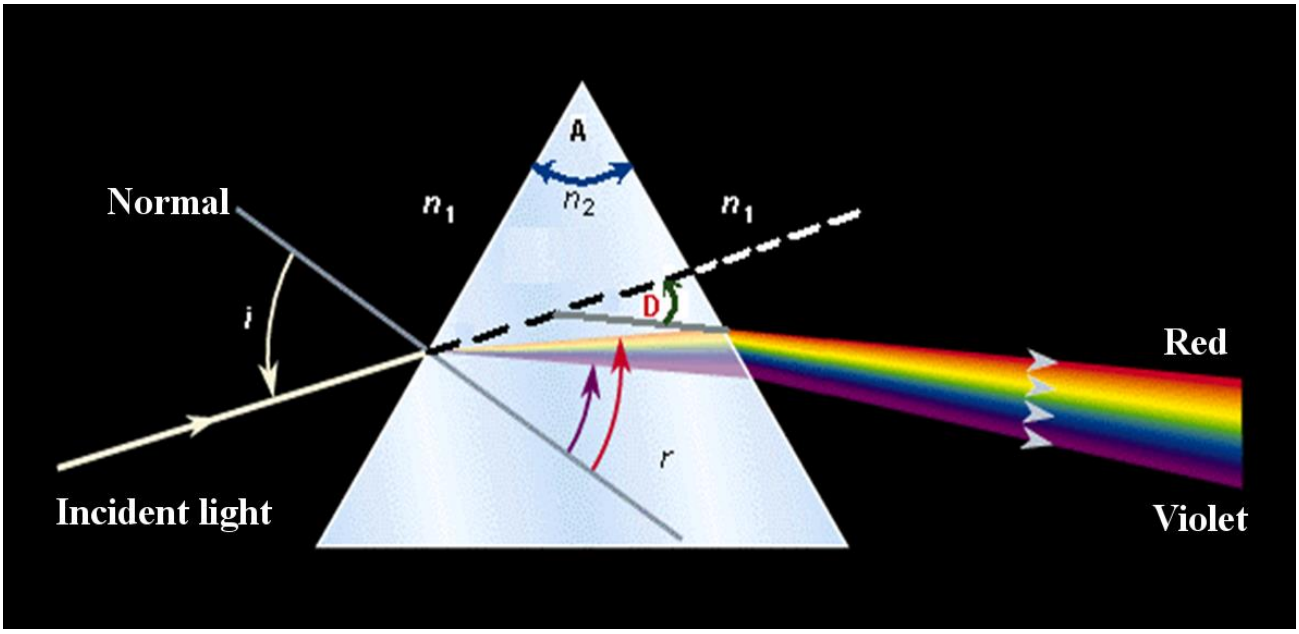


Fig.6.3. Dispersion in a triangular prism

The angle of deviation is the angle made between the incident ray of light entering the first face of the prism and the refracted ray that emerges from the second face of the prism. Because of the different indices of refraction (*n*) for the different wavelengths of visible light, the angle of deviation varies with wavelength. Colors of the visible light spectrum that have shorter wavelengths will deviated more from their original path than the colors with longer wavelengths. The emergence of different colors of light from a triangular prism at different angles leads an observer to see the component colors of visible light separated from each other.

The visible light spectrum is the very narrow band of wavelengths located to the right of the infrared region and to the left of the ultraviolet region. Though electromagnetic waves exist in a vast range of wavelengths, our eyes are sensitive to only a very narrow band. Since this narrow band of wavelengths is the means by which humans see, we refer to it as the visible light spectrum. This "visible light" corresponds to a wavelength range of 400 – 700*nm* (nanometers) and a color range of violet through red (Fig.6.4).

The human eye is not capable of "seeing" radiation with wavelengths outside the visible spectrum. The visible colors from shortest to longest wavelength are: violet, blue, green, yellow, orange, and red. Ultraviolet radiation has a shorter wavelength than the visible violet light. Infrared radiation has a longer wavelength than visible red light. The white light is a mixture of the colors of the visible spectrum. Black is a total absence of light.

Prism Spectroscope: When light passes through a prism, it is refracted or bent from its original path. Light of different wavelengths is bent by different amounts. It exits the prism in different directions producing the rainbow. A prism spectroscope will be used to separate the wavelengths of light given off by gas discharge tubes filled with hydrogen or mercury (Fig.6.5). There are four basic parts to a prism

spectroscope: (1) the slit and collimator, (2) the prism, (3) the telescope and (4) the scale. These are

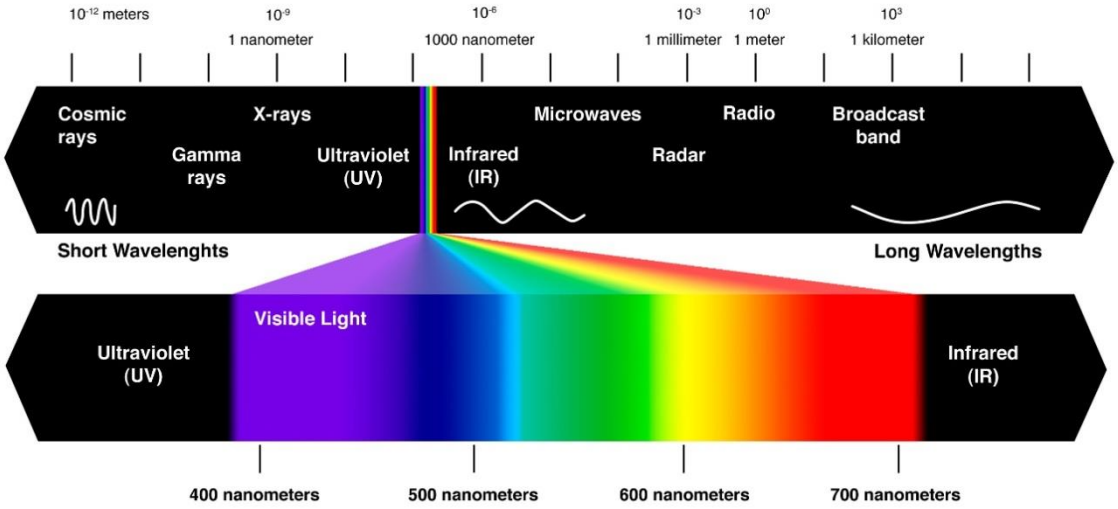


Fig.6.4. Visible spectrum of light

shown schematically in the Fig.6.5 at the top of the next page. The dotted line illustrates the path of a single ray of light from the gas discharge tube to the observer's eye.

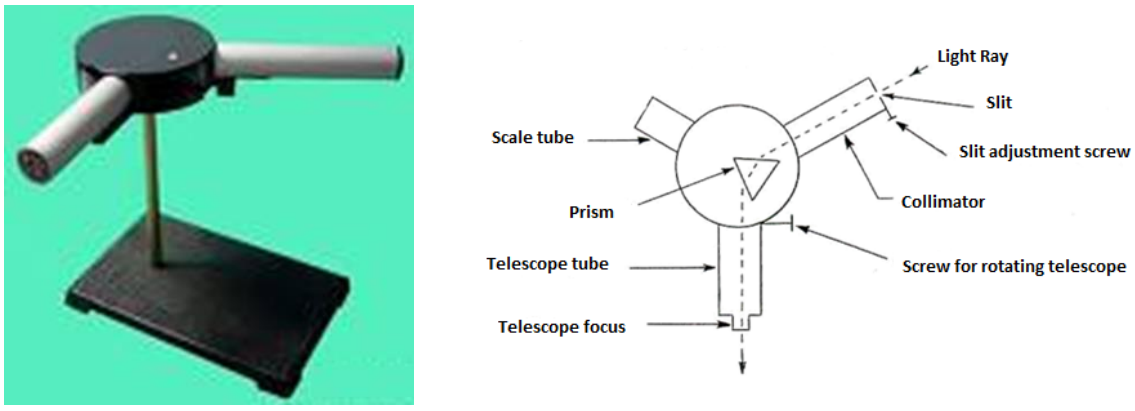


Fig.6.5. Prism spectroscope

Work procedure:

In this Activity, you will use a spectrum whose wavelengths are accurately known. You will use the know wavelengths of the mercury spectral lines to plot a calibration curve that gives scale readings versus wavelength. Once the calibration curve is obtained, unknown wavelengths (sodium emission spectrum) can be read directly off the curve.

Become familiar with the spectrometer:

1. Identify each component: the black table, the prism table, the collimator, and the telescope. Note how to adjust the telescope focus and the eyepiece. Note how to adjust the slit focusing in the collimator tube. Note how the slit width can be adjusted and how the slit orientation can be rotated.

SPECTRAL ANALYSIS

2. The spectroscope on your lab table has already been set up for you. The telescope points towards you. The short scale tube will be to the left and the slit and collimator on the right. The light source containing a glass tube filled with mercury vapor is in front of the scale tube.
3. Switch on the light source to the *ON* position. Adjust the position the light source in front of the spectroscope slit so that the slit is illuminated. Look through the telescope and adjust the slit opening until the spectral lines are very narrow but clearly visible.
4. After obtaining a sharp, clear spectrum, record the scale reading for each of the lines indicated on the table below. ***The value for a horizontal scale is 1^0 and for a vertical scale $0{,}02^0$***. There may be other lines present, however, these are the brightest and should be easy to identify.

No	Color	Wavelength, λ, nm	Scale Reading
Standart Hg			
1	Red	690	
2	Yellow	579	
3	Yellow	577	
4	Green	546	
5	Blue/Green	491	
6	Blue	436	
7	Violet	407	
	Sodium- studied substance		
	Yellow		

5. Plot the wavelength (in nanometers) versus the scale reading for each data point. ***Use a millimeter paper!!!***

6. Draw a smooth curve that comes closest to all the data points. The curve is *not* a straight line. If all points except one fall very near the curve, recheck that point to insure that the correct spectral line was used. Draw the curve carefully because it will be used to determine the wavelengths for a studied substance- Sodium.
7. Call the teacher to replace the mercury tube with an alcohol lamp. The teacher will drip a few drops of substance which contains sodium. Adjust the lamp's position, the slit opening and the telescope focus to obtain a sharp clear spectrum.
8. Record the scale readings of the yellow line on the data sheet. Draw straight a vertical line (use a ruler) at this position on your graph.
9. Draw straight a horizontal line (use a ruler) at this position where your vertical line intersect the curve you drew in part 6. Estimate the corresponding wavelengths for this sodium spectral line by reading (to the nearest nanometer) the values where your horizontal line intersects the y-axis. Record the results for this wavelength on the data sheet.

Applications of spectral analysis in medical-biological research

- *Applications of spectral analysis in medical ultrasonography:*

Spectral analysis of ultrasonic reflections from biological tissues can be used to determine basic tissue parameters for use in differential diagnosis.

The Doppler effect provides an ultrasonic method for the detection of echoes from moving structures, particularly flowing blood. In its most simple form, the continuous wave Doppler offers velocity information without depth resolution and is therefore used mainly for the examination of superficial structures (see Subject 2). Spectral analysis permits features of the Doppler signal to be identified which are associated with hemodynamic phenomena, such as flow disturbance and wave reflection. In addition, it allows the quantitative application of Doppler to the estimation of such physiological variables as velocity, flow rate, and pressure difference.

- *Spectral Factor Analysis for Multi–isotope Imaging in Nuclear Medicine;*
- *Spectral analysis of blood and melanin contents in skin sites:*

Many factors can change skin pigmentation, including aging, exposure to UV light, certain drugs, as well as certain diseases. A simple technique for measuring skin pigmentation could be a helpful tool for research and diagnostics. The same goes for measuring the skin blood content. Alteration of blood flow in the skin can, for example, be linked to skin irritations, inflammatory disorders, or diseases, such as psoriasis and rosacea. In addition, some systemic diseases, such as rheumatoid arthritis, atherosclerosis, and asthma, have shown to be associated with peripheral microvascular modifications.

There are practical approaches for assessing the melanin and blood content of the skin from total diffuse reflectance spectra and the method offers a quick spectral analysis using just three wavelengths, namely 585 nm, 700 nm, and 800 nm.

- *Magnetic resonance spectroscopy (MRS*): also called nuclear magnetic resonance spectroscopy, diagnostic imaging technique based on the detection of metabolites in tissues. Magnetic resonance spectroscopy (MRS) is related to magnetic resonance imaging (MRI) in that it uses the same machinery; however, instead of measuring blood flow, MRS measures the concentration of specific chemicals, such as neurotransmitters. MRS holds great promise in the diagnosis of diseases of the brain and of other parts of the body, including cancers of the cervix, pancreas, and prostate.

By measuring the molecular and metabolic changes that occur in the brain, this technique has provided valuable information on brain development and aging, Alzheimer disease, schizophrenia, autism, and stroke. Because it is noninvasive, MRS is ideal for studying the natural course of a disease or its response to treatment. See also nuclear magnetic resonance and magnetic resonance.

- *Near infrared spectroscopy in sports medicine:*in the last 15 years the study of the human muscle energetics in sports medicine underwent a radical change thanks to the progressive introduction of non-invasive techniques, including near infrared (NIR) spectroscopy (NIRS). NIR light (700-1000 nm) penetrates skin, subcutaneous fat and underlying muscle, and is either absorbed (by oxy- and deoxy-haemoglobin) or scattered within the tissue. NIRS is a non-invasive and relatively low cost optical technique that is becoming a widely used instrument for measuring muscle O_2saturation and changes in haemoglobin volume. Muscle O_2saturation represents a dynamic balance between O_2supply and O_2consumption in the small vessels such as the capillary, arteriolar and venular bed.

  The results of several studies suggest that NIRS is a powerful tool for being applied successfully

in sports medicine. NIRS can objectively evaluate muscle oxidative metabolism in athletes and its modifications following potential therapeutic strategies and specific training programs.

- *Fluorescence spectroscopy:*there are many diagnostic techniques and methods available for diagnosis of medically important microorganisms like bacteria, viruses, fungi and parasites. But, almost all these techniques and methods have some limitations or inconvenience. Most of these techniques are laborious, time consuming and with chances of false positive or false negative results. Infectious diseases are caused by microorganisms such as bacteria, viruses, fungi and parasites. Infectious diseases are major killer around the world especially in developing countries.

Fluorescence spectroscopy seems to be promising diagnostic technique with fast and rapid diagnosis ability. Studies indicate high sensitivity and specificity rate which makes Fluorescence spectroscopy an ideal diagnostic tool for medical microbiology field. But, there is need for further studies and clinical trials to validate this new diagnostic technique. At present, Fluorescence spectroscopy is being applied in medical microbiology field for various purposes. Fluorescence spectroscopy is a type of electromagnetic spectroscopy which analyzes fluorescence from a sample. The sample is excited by using a beam of light which results in emission of light of a lower energy resulting in an emission spectrum which is used to interpret results

- *Breast Cancer Diagnosis using Spectroscopy:*the analysis of small deposits of calcium in breast tissue can help differentiate cancerous and benign tumors, but it is sometimes not easy to make such a diagnosis. There is a new method that uses a special type of spectroscopy to locate calcium deposits during a biopsy and this method could greatly improve the accuracy of diagnosis.

Microcalcifications, or small deposits of calcium, form when calcium from the bloodstream deposits onto degraded proteins and fats left behind by injured and dying cells. They can be a telltale sign of breast cancer, but most tumors that contain them are benign. Microcalcifications are most often seen in breast tumors, but they can also occur, albeit rarely, in other types of cancer. Calcification also plays a major role in atherosclerosis, or hardening of the arteries.

Diffuse reflectance spectroscopy collects and analyzes light after it has interacted with the sample. This gives a unique "spectrographic signature". The authors of this method describe how they examined 203 tissue samples within minutes of their removal from 23 patients. Each sample could be one of three types, each with its own spectrographic signature. It could be healthy, it could contain lesions with no microcalcifications, or it could contain lesions with microcalcifications. By analyzing these patterns, the team produced a computer algorithm that showed a success rate of 97% in identifying tissue with microcalcifications. This technology can be integrated into the system that is already used to take biopsies. It's a very simple technology that can get the same amount of accuracy as more complicated systems.

Knowledge evaluation exercises:

1. There is two types of spectral analysis:
   a) quantitative and numerical,
   b) analytic and quantitative,
   c) quantitative and qualitative,
   d) none of those.

2. There is two types of spectra:
   a) the emission and the absorption spectra,
   b) the linear and the continuous spectra,
   c) the emission and the continuous spectra,
   d) the linear and the emission spectra.

3. The spectral analysis is:
   a) a method of analyzing the properties of matter from their electromagnetic interactions,
   b) the study of human activity in the past, primarily through the recovery and analysis of the material culture and environmental data that has been left behind by past human populations,
   c) a method of physical analysis used for chemical composition determination of different substances,
   d) the study of celestial objects, the physics, chemistry, and evolution of such objects, and phenomena that originate outside the atmosphere of Earth.

4. The emission spectrum
   a) is the pattern of dark lines and colors made when electromagnetic energy, such as light, passes through a substance and excites its atoms,
   b) is the pattern of dark lines and colors made when light passes through an absorbing medium, such as a gas or liquid,
   c) can be used to determine the substance's chemical composition,
   d) can be used to identify the composition of different substances, such as the gaseous outer layers of stars.

5. The third Kirchhoff's law of spectroscopy says:
   a) a hot solid object produces light with a continuous spectrum,
   b) a hot solid object surrounded by a cool tenuous gas (cooler than the hot object) produces light with an almost continuous spectrum which has gaps at discrete wavelengths depending on the energy levels of the atoms in the gas,
   c) a hot tenuous gas produces light with spectral lines at discrete wavelengths (specific colors) which depend on the energy levels of the atoms in the gas,
   d) none of those.

6. The continuous spectrum is:
   a) energy at all wavelengths,
   b) energy at only certain wavelengths,
   c) bright lines,
   d) dark lines.

7. The phenomena that causes light of different colors to be refracted differently and to leave the prism at different angles, creating an effect similar to a rainbow is called:
   a) diffraction,

b) dispersion,
c) reflection,
d) refraction.

8. The visible light spectrum is the very narrow band of wavelengths located/ coresponding to:
   a) the right of the ultraviolet region and to the left of the infrared region,
   b) the right of the infrared region and to the left of the ultraviolet region,
   c) a wavelength range of 400 - 700 nanometers (nm),
   d) a wavelength range of 400 - 700 milimeters (mm),

9. The Prism Spectroscope doesn't not include as a part of it
   a) the slit and collimator,
   b) the mirror,
   c) the telescope,
   d) the scale.

10. If the energy of a photon is *2.4eV*, what's its frequency? Estimate *h* as $4.14 \times 10^{-15} eVs$.
    a) 2.9×10^{14} Hz,
    b) 4.3×10^{14} Hz,
    c) 5.8×10^{14} Hz,
    d) 5.8×10^{15} Hz.

7. DETERMINATION OF WAVELENGTH AND ENERGY OF LASER RADIATION QUANTUM

Purposes:

- Presentation of theoretical notions about basic laser principles;
- Study of the structure and work principle of the He-Ne Laser;
- Determination of the wavelength and energy of quantum of laser radiation by means of the diffraction grating;
- Study of the applications of lasers in medicine and medical-biological research.

Theoretical notions:

A LASER is a device that emits light through a process of optical amplification based on the stimulated emission of electromagnetic radiation. The term "LASER" originated as an acronym for "*Light Amplification by Stimulated Emission of Radiation".*

Components of laser:

As shown in Fig.7.1, the three basic components of a laser are:

- *Laser material* or *active media* (crystal, gas, semiconductor, dye, etc.)
- *Pump source* (adds energy to the lasing material, e.g. flash lamp, electrical current to cause electron collisions, radiation from a laser, etc.)

A helium–neon (HeNe) laser uses an electrical discharge in the helium-neon gas mixture, a

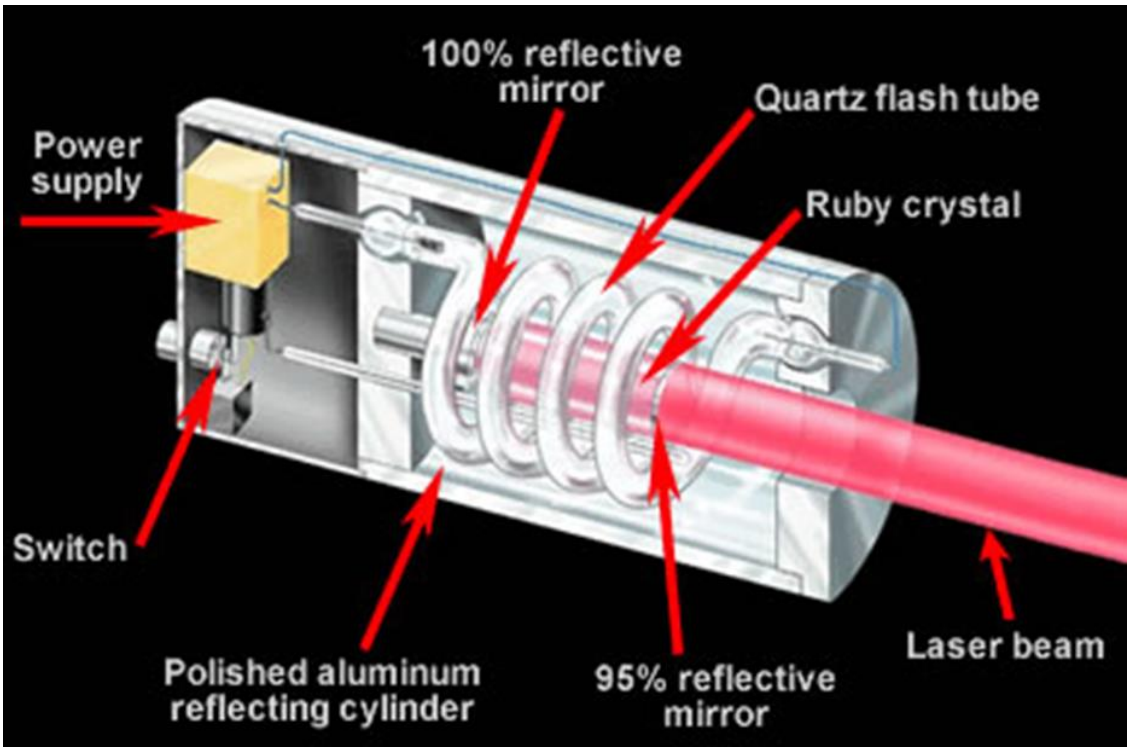


Fig.7.1. Solid state laser construction

Nd:YAG laser uses either light focused from a xenon flash lamp or diode lasers, and excimer lasers use a chemical reaction.

- *Optical cavity* (resonator) consisting of reflectors to act as the feedback mechanism for light amplification (two mirrors, one total reflective and one partially-reflective).

In Bohr's model, shown in Fig.7.2, electrons orbit the nucleus of an atom. The Bohr atom has a limited number of fixed orbits that are available to the electrons. Under the right circumstances an electron can go from its ground state (lowest-energy orbit) to a higher (excited) state, or it can decay from a higher state to a lower state, but it cannot remain between these states. The allowed energy states are called "quantum" states and are referred to by the principal "quantum numbers" 1, 2, 3, etc. The quantum states are represented by an energy-level diagram.

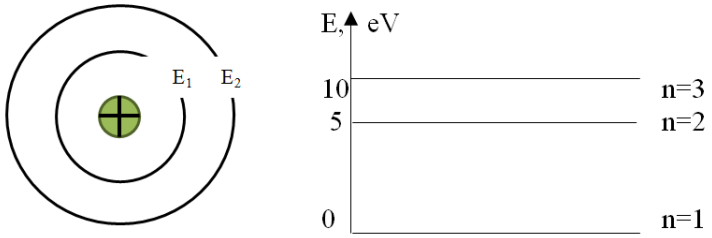


Fig.7.2. The Bohr atom and a simple energy-level diagram

For an electron to jump to a higher quantum state, the atom must receive energy from the outside world. This can happen through a variety of mechanisms such as absorption of energy in the form of electromagnetic radiation. Likewise, when an electron drops from a higher state to a lower state, the atom must give off energy. Light is made up of particles called "photons" which exhibit both particle-like and wave-like properties. Each photon has an intrinsic energy determined by the equation

$$E = h\nu, \tag{1}$$

where$E$ is energy (of one quantum in joules), h is Planck's constant ($6.626\,x\,10^{-34}\,Js$) and ν is wavelength frequency (in s^{-1} or Hz). Since, for a wave, the frequency and wavelength are related by the equation

$$\lambda\nu = c, \tag{2}$$

whereλ is the wavelength of the light and c is the speed of light in a vacuum ($c = 3 \cdot 10^8 m/s$), and equation (1) can be rewritten as

$$E = \frac{hc}{\lambda} \tag{3}$$

It is evident from this equation that the longer the wavelength of the light, the lower the energy of the photon; consequently, ultraviolet light is much more "energetic" than infrared light.

Returning to the Bohr atom: for an atom to absorb light (i.e., for the light energy to cause an electron to move from a lower energy state E_1 to a higher energy state E_2), the energy of a single photon must equal, almost exactly, the energy difference between the two states. Too much energy or too little energy and the photon will not be absorbed. Consequently, the wavelength of that photon must be

$$\lambda = \frac{hc}{\Delta E}, \tag{4}$$

where$\Delta E = E_2 - E_1$.

So,a transition to the higher state is called "***absorption***", and it destroys an incident photon (the photon's energy goes into powering the increased energy of the higher state (see fig.7.3).

Likewise, when an electron decays to a lower energy level in a radiative transition, the photon of light given off by the atom must also have the energy equal to the energy difference between the two states.

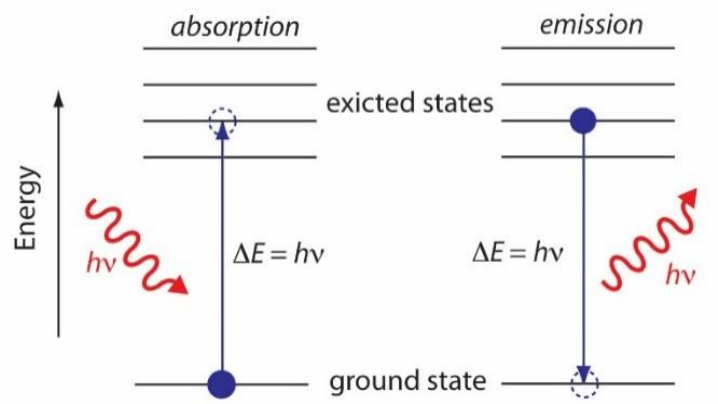


Fig.7.3. Absorbtion and spontaneous emission

In general, when an electron is in an excited energy state, it must eventually decay to a lower level, giving off a photon of radiation. This event is called "***spontaneous emission***," and the photon is emitted in a random direction and a random phase (see fig.7.4). The average time it takes for the electron to decay is called the time constant for spontaneous emission, and is represented by $\tau \approx 10^{-8}s$.

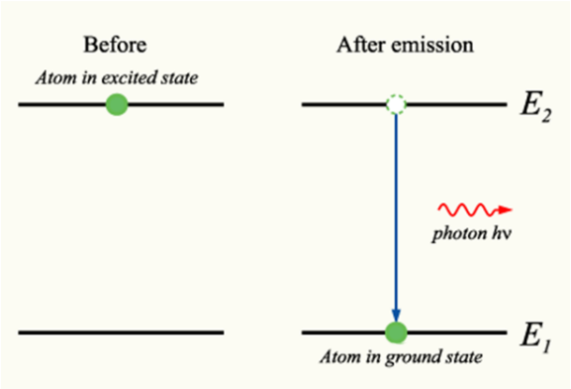


Fig.7.4. Spontaneous emission

On the other hand, if an electron is in energy state E_2, and its decay path is to E_1, but, before it has a chance to spontaneously decay, a photon happens to pass by whose energy is approximately $E_2 - E_1$, there is a probability that the passing photon will cause the electron to decay in such a manner that a photon is emitted at exactly the same wavelength, in exactly the same direction, and with exactly the same phase as the passing photon. This process is called "***stimulated emission***"(see fig.7.5).

Of course, in any real population of atoms, the probability for stimulated emission is quite small. Furthermore, not all of the atoms are usually in an excited state; in fact, the opposite is true. For a normal population of atoms, there will always be more atoms in the lower energy levels than in the upper ones. Since the probability for an individual atom to absorb a photon is the same as the probability for an excited atom to emit a photon via stimulated emission, the collection of real atoms will be a net absorber,

not a net emitter, and amplification will not be possible. Consequently, to make a laser, we have to create a "***population inversion***".

When the number of particles in one excited state exceeds the number of particles in some lower-

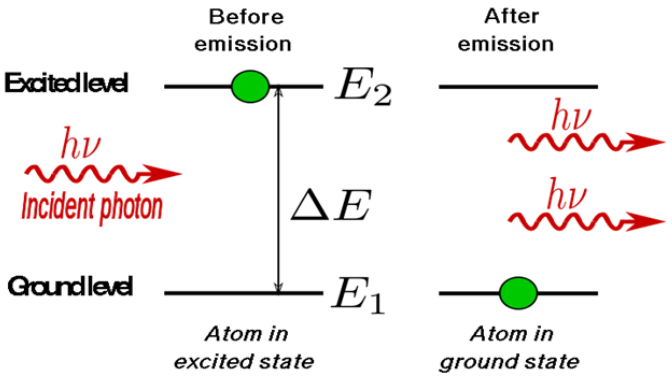


Fig.7.5. Stimulated emission

energy state, *population inversion* is achieved and the amount of stimulated emission due to light that passes through is larger than the amount of absorption. Hence, the light is amplified.

Atomic energy states are much more complex than indicated by the description above. There are many more energy levels, and each one has its own time constants for decay. The three and four-level energy diagram shown in Fig.7.6 is representative of some real lasers.

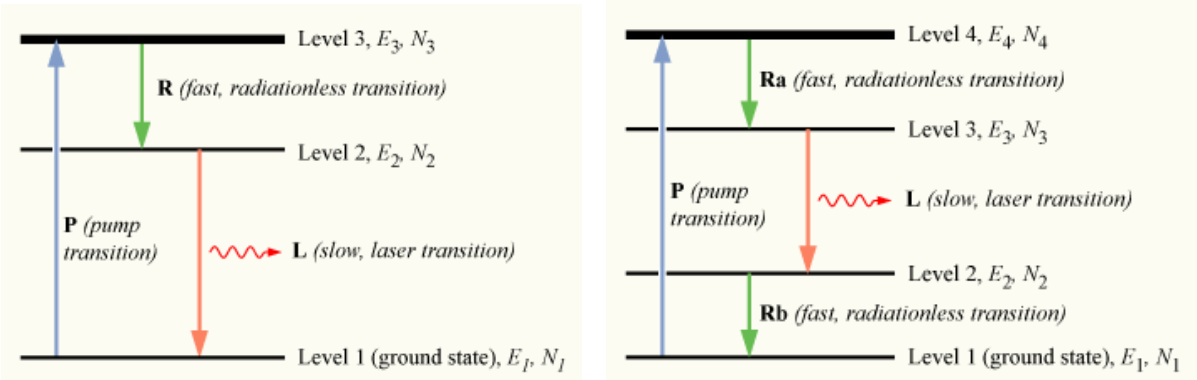


Fig.7.6. The three and four-level energy diagram

Let's consider a three-level energy sistem. The electron is pumped (excited) into an upper level E_3 by some mechanism (for example, a collision with another atom or absorption of high-energy radiation). This process is caled „***pumping***". So pumping is the process of achieving the „***population inversion***". Some of the electrons may loose very less energy $E_3 - E_2$, called *thermal energy,* and jump to the state E_2. This transition is called non-radiative transition or invisible transition. The electrons in this state E_2 remains for longer period of time compare to state E_3,in order of $\tau \approx 10^{-6}s$. This electronic state is called as „***metastable state***".

Metastable state, in physics and chemistry, particular excited state of an atom, nucleus, or other system that has a longer lifetime than the ordinary excited states and that generally has a shorter lifetime than the lowest, often stable, energy state, called the ground state. Auroras, fluorescent lights, and

lightning are all examples of plasmas whose atoms and molecules are excited when energetic particles slam into them. The atoms and molecules emit light when they return to their ground states, but often they hesitate for some time in "metastable" states before relaxing.

In result, the number of electrons in this state will be increasing rapidly. At some point, number of the electrons in metastable state will exceed the number of electrons in the excited state, thus the *population inversion* will be achieved.

Types of lasers

There are many types of lasers available for research, medical, industrial, and commercial uses. Lasers are often described by the kind of lasing medium they use - solid state, gas, excimer, dye, or semiconductor.

***Solid state** lasers* have lasing material distributed in a solid matrix, e.g., the ruby laser. Theese lasers emits infrared light at 1.064 micrometers.

***Gas** lasers* (helium and helium-neon, HeNe, are the most common gas lasers) have a primary output of a visible red light. CO_2 lasers emit energy in the far-infrared, 10.6 micrometers, and are used for cutting hard materials.

***Semiconductor** lasers*, sometimes called diode lasers, are not solid-state lasers. These electronic devices are generally very small and use low power. They may be built into larger arrays, e.g., the writing source in some laser printers or compact disk players.

The properties and main characteristics of laser radiation

1. *Coherent.* The light from a laser is said to be coherent, which means the wavelengths of the laser light are in phase in space and time. This coherence property is what makes holograms possible.
2. *Monochromatic.* Laser light consists of essentially one wavelength, having its origin in stimulated emission from one set of atomic energy levels.
3. C*ollimated or Directional.* Lasers emit light that is highly directional, because of bouncing back between mirrored ends of a laser cavity. Laser light is emitted as a relatively narrow beam in a specific direction. Ordinary light, such as coming from the sun, a light bulb, or a candle, is emitted in many directions away from the source.

The construction and working principle of a Helium-Neon Laser

The most common and inexpensive gas laser, is a type of small gas laser, a four-level laser, usually constructed to operate in the red at $632.8\ nm$.

One of the excited levels of helium at $20.61\ eV$ is very close to a level in neon at $20.66\ eV$, so close in fact that upon collision of a helium and a neon atom, the energy can be transferred from the helium to the neon atom. Helium-neon lasers are common in the introductory physics laboratories, but they can still be dangerous! An unfocused $1mW$ HeNe laser has a brightness equal to sunshine on a clear day ($0.1\ watt/cm^2$) and is just as dangerous to stare at directly.

Construction

1. The setup consists of a long, narrow cylindrical tube made up of fused quartz. The diameter of the tube will vary from 5 to $15\ mm$ and the length will vary from 50 to $100cm$ (see fig.7.7).

2. The gain medium (active media) of the laser is a mixture of helium and neon gases, in a 10: 1 ratio, contained at low pressure in a glass envelope.
3. The energy or pump source of the laser is provided by an electrical discharge of around 1000V through an anode and cathode at ach end of the glass tube.
4. The optical cavity of the laser typically consists of a plan, high-reflecting mirror at one end of the laser tube, and a concave output coupler mirror of approximately 1% transmission at the other end.

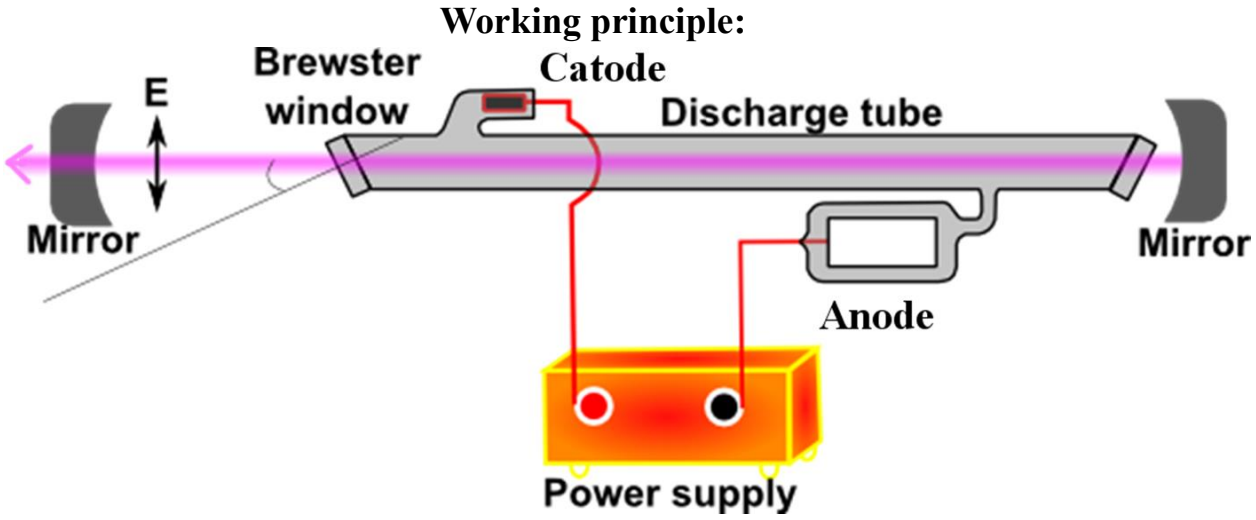


Fig.7.7. Schematic diagram of He-Ne laser construction

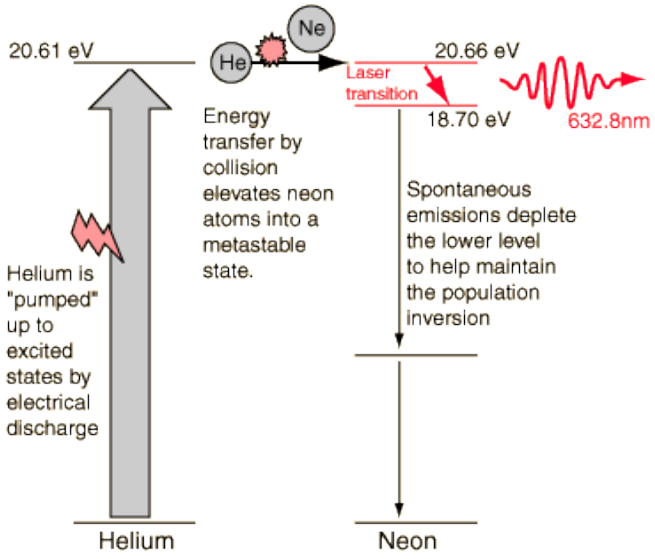


Fig.7.8. Schematic energy diagram of He-Ne laser

1. When the power is switch on, an energetic electron collisionally excites a He atoms.
2. The excited He atoms collides with an unexcited Ne atom and the atoms exchange internal energy, resulting in an unexcited He atom and excited Ne atom.
3. This energy exchange process occurs with high probability because the two excitation energy levels of these atoms are nearly equal. Thus, the purpose of population inversion is fulfilled.

4. When the excited Ne atom passes from metastable state to lower level, it emits photon of wavelength $632nm$.
5. This photon travel through the gas mixture parallel to the axis of tube, it is reflected back an forth by the mirror ends until it stimulates an excited Ne atom and causes it to emit a photon of $632nm$ along with the stimulating photon.
6. This process is continued and when a beam of coherent radiation becomes sufficiently strong, a portion of it escapes through partially silvered end.
7. The Ne atom passes to lower level $1s$ by spontaneous emission and finally the Ne atom comes to ground state through collision with tube wall and undergoes radiationless transition.

Work procedure:

The ***diffraction*** of classical waves refers to the phenomenon wherein the waves encounter an obstacle that fragments the wave into components that interfere with one another.

Mathematically, the relation is simple:

$$d\ sin\ \theta\ =\ n\lambda \tag{5}$$

where d is the distance between adjacent slits,

θ is the angle the re-created image makes with the normal to the grating surface,

λ is the wavelength of the light, and n = 0, 1, 2, . . . is an integer of the maximum

$$\lambda = \frac{d}{n} sin\theta \approx \frac{d}{n} tg\theta \approx \frac{d}{n}\frac{S}{L} \tag{6}$$

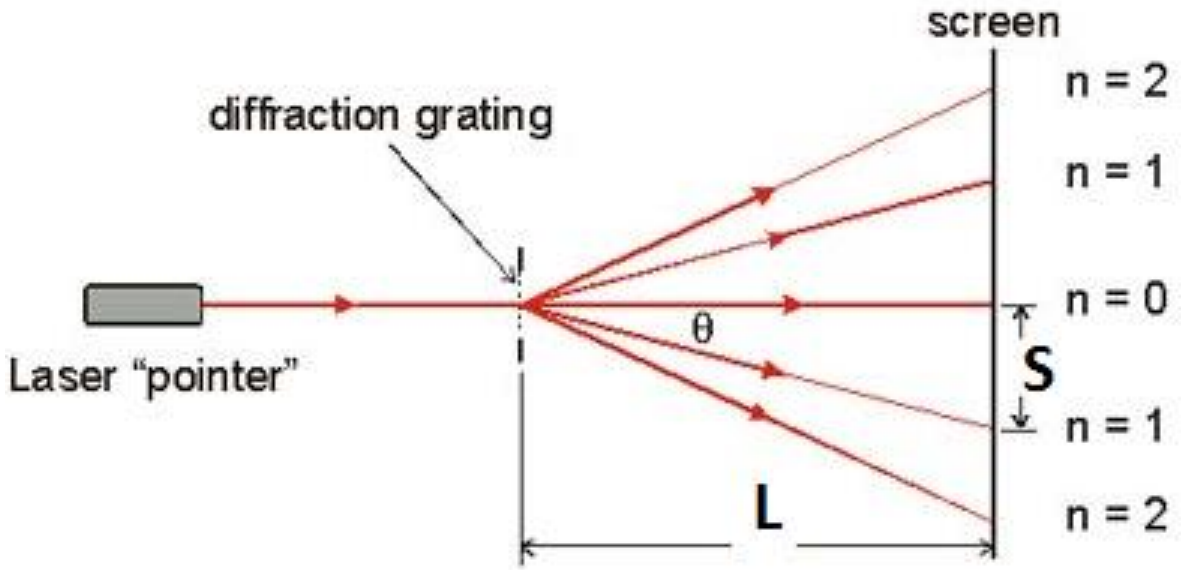
$$\lambda = \frac{d}{n}\frac{S}{L} \tag{7}$$

where L is the distance from the grating to the screenand S is the distance from the central maximum ($n = 0$) and an onother maximum $n = 1,2,$ ...

Do not look into the laser!

Procedure:

1. Set up the laser and grating as shown in Fig.7.8.



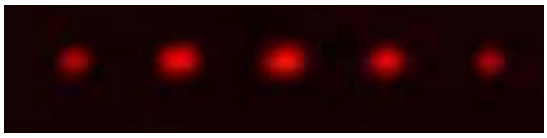


Fig.7.9. Monochromatic light diffraction

2. Turn on the laser and center the meter stick at the 0th order image.
3. Define the position of the diffraction grating so that on-screen interference maxima occur in the order 1, 2 and 3.
4. Measure the distance L between the screen and the diffraction grating.
5. Measure the distance S_1 between the 1st order images appearing on the left or right sides of the center line.
6. Measure the distance S_2 between the 2nd order images appearing on the left and right sides of the center line.
7. Do the same measurement for S_3.
8. Repeat the 4-7 steps for 2 others random positions of the diffraction grating.
9. Calculate laser light wavelength using formula No (7).
10. Calculate laser light energy using formula No (4).
11. Introduce the obtained results in the table bellow:

No	n	L, cm	S, cm	λ, nm	E, J
1					
Average No1					
2					
Average No2					
3					
Average No3					
Average					

12. Write necessary observations and conclusions.

Applications of LASERs in medicine

Over the past half century, lasers have found their way into ophthalmology, oncology, cosmetic surgery,angioplasty, cancer diagnosis, cancer treatment, cosmetic dermatology such as scar revision, skin resurfacing, laser hair removal, tattoo removal, dermatology to treat melanoma, lithotripsy, laser

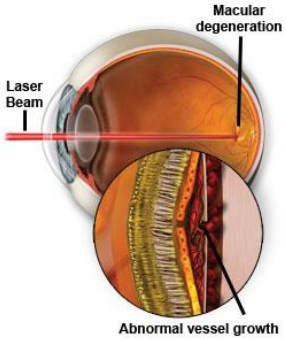
mammography,medical imaging, microscopy, ophthalmology, optical coherence tomography, prostatectomy, plastic surgery, in laser liposuction, surgery to ablate and cauterize tissue and many areas of medicine and biomedical research.

The possibility of using light in treating illness has been known for thousands of years. The ancient Greeks and Egyptians used sunlight as a therapy and the two ideas were even tied together in mythology, with the Greek god Apollo taking responsibility for both light and healing.

However, it has only been since the invention of the laser 50 years ago, that the potential of light in medicine has really been revealed.

The special properties of lasers make them much better than sunlight or other light sources at targeting medical applications. Each laser operates within a very narrow wavelength range and the light emitted is coherent. They can also be very powerful. The beams can be focused to a very small point, giving them a high power density. These properties have led to lasers being used in many areas of medical diagnosis and treatment.

- ***Lasers repair skin and eyes***



The earliest medical applications for lasers were in ophthalmology and dermatology. Just a year after the invention of the laser in 1960, Leon Goldman demonstrated how a ruby laser, which emits red light, could be used to remove port wine stains, a type of birthmark, and melanomas from the skin.This application relies on the ability of lasers to operate at a specific wavelength. Lasers are now widely used in dermatology for things like tumor, tattoo, hair, and birthmark removal.

Later, ophthalmologists used argon lasers (which emit green-wavelength light) to treat detached retinas. This application uses the properties of the eye itself–specifically the lens–to focus the laser beam onto the area where the retina has become detached. The highly-localized power from the laser causes the retina to reattach.

Another medical approach, also with argon lasers, is used to stop internal bleeding in patients. Green light is selectively absorbed by hemoglobin, the pigment in red blood cells, in order to seal off bleeding blood vessels. This can also be used in cancer treatment to destroy blood vessels entering a tumor and deprive it of nutrients.

- ***Laser imaging and diagnosis***

Lasers have a major role to play in the early detection of cancer as well as many other diseases. For example, in Tel Aviv, Katzir's group is looking at infrared spectroscopy using IR lasers. This is interesting, according to Katzir, because cancer and healthy tissue may have different transmissions in the IR range. One promising application of the technique is to measure melanomas. With skin cancers, early detection is very important for the patients' survival rates. Currently melanoma detection is done by eye, so relies on the skill of the physician.

Laser-based systems are also starting to replace the x-rays traditionally used in mammography. Using x-rays poses a challenge: high intensities are needed to be able to detect cancers well, but as the intensity of the x-ray is raised, so is the risk of the x-ray itself causing cancer. The alternative being studied is to use very fast laser pulses to image breasts as well as other parts of the body such as the brain.

- ***OCT for eyes and beyond***

There is much enthusiasm about the potential of optical coherence tomography (OCT) in many areas of medicine. This imaging technique can give high-resolution (on the order of microns), cross-sectional, and three-dimensional images of biological tissue in real time, using the coherence properties of laser light. OCT is already used in ophthalmology and can, for example, enable ophthalmologists to see a cross section of the cornea to diagnose retinal disease and glaucoma. It is now beginning to be used in other areas of medicine too.

- ***In vivo microscopy***

Lasers also play a key role in many different types of microscopy. There have been many medical developments in this area and the aim is to be able to see what is going on inside the body without cutting the patient open.

Right now the most sophisticated way to remove a cancer is to have the surgeon run back and forth to the microscope to see if he's got it all.One example of an emerging area in medical applications is scanning near-field optical microscopy, which can produce images with a resolution much greater than that obtained from standard optical microscopes. This technique is based on optical fibers that have been etched at their tips at a smaller scale than the wavelength of the laser. This enables sub-wavelength imaging and paves the way for imaging biological cells.

- ***PDT and other treatments***

Developments in optical fibers are helping extend the potential uses of lasers in other ways too. The same optical fiber used in diagnosis could also be used in treatment.

In one type of photodynamic therapy, patients are injected with a light-activated drug called a photosensitizer which is selectively retained by cancer cells. Doctors then use fiber-optic probes to expose the cancer to laser light. This activates the photosensitizer and produces a toxic reaction that destroys the tumor without irreparably damaging the surrounding normal cells.

The area of photomedicine, using light-sensitive chemicals that act with the body in particular ways, also enables lasers to be used in both diagnosis and treatment. In photodynamic therapy (PDT), for example, a laser and a photo-sensitive drug can restore vision for patients with the "wet" form of age-related macular degeneration (AMD), the leading cause of legal blindness in people over the age of 50.

In oncology, some porphyrins will accumulate in cancers and fluoresce if illuminated with a particular wavelength of light to show where the cancer is. If these same compounds are then illuminated with a different wavelength they become toxic and kill the cancer cells.

- ***Personalized medicine***

Another future area of medicine for lasers is genetics and epigenetics.

In the future things will go to the nanoscale and this will enable us to do medicine at a cell level. Lasers, which can operate at femtosecond pulses and be tuned to exact wavelengths, are perfect partners for this.

Many other laser uses in medicine

- Urologists can treat urethral strictures, benign warts, urinary stones, bladder obstructions, and enlarged prostates with lasers.
- Neurosurgeons use lasers for precision cutting and endoscopic guidance into the brain and spinal cord.
- Veterinarians make use of lasers for endoscopic procedures, photocoagulation of tumors, excision, and photodynamic therapy.
- Dentists use lasers for drilling cavities, gum surgery, antibacterial treatments, tooth desensitization, and orofacial diagnostics.

Knowledge evaluation exercises:

1. Why is it necessary to use a laser in this experiment? Why is sunlight or a light from a lightbulb insufficient?

2. What are the characteristic of laser radiation?

3. In a diffraction experiment using a straight edge, the diffraction pattern is obtained _____ the edge of geometric shadow
   a) above,
   b) below ,
   c) parallel to,
   d) close to.

4. Population inversion in an active medium means:
   a) more atoms in the upper state,
   b) more atoms in the ground state,
   c) atoms are uniformly distributed,
   d) atoms are unstable.

5. In Ruby Laser the excitation of active medium is done by
   a) flashlight,
   b) electric discharge,
   c) heating,
   d) rubbing.

6. Explain spontaneous emission and stimulated emission in Laser.

7. Explain the working of Ruby Laser.

8. Using the Bohr's model, explain the absorption and emission processes in Laser theory.

9. Explain the working of He-Ne Laser. Give two uses of Laser.

10. In He-Ne laser the laser emission takes place from:

a) He-atoms only,
b) Ne-atoms only ,
c) both He and Ne atoms,
d) 50% from Helium and 50% from Neon.

11. Which of the following leads coherent light?
    a) absorption,
    b) spontaneous emission,
    c) stimulated emission,
    d) none of these.

12. The pumping method used in He-Ne laser is:
    a) optical pumping,
    b) electric discharge,
    c) chemical reactions,
    d) none of these.

13. The life time of metastable state is about
    a) 10^{-3} s,
    b) 10^{-13} s,
    c) 10^{2} s,
    d) 10^{-9} s.

14. The transition of an atom between two energy levels in which two coherent photons are emitted is called:
    a) absorption,
    b) spontaneous emission,
    c) stimulated emission,
    d) population inversion.

15. Brewster's windows are used in He-Ne laser to obtain:
    a) coherent light,
    b) monochromatic light,
    c) powerful light,
    d) polarized light.

16. A mode-locked laser emits an average power P equal to 1 W. The rate of repetition of the pulses from this laser is equal to 100 MHz. Calculate the energy of each pulse.

17. What is the kinetic energy of an electron with a frequency of $8.3 \times 10^{14} Hz$? Estimate h as 4.14×10^{-15} eVs.
    a) 1.2 eV,
    b) 3.4 eV,
    c) 1.2 J,

d) 34 J.

18. If the energy of a photon is *2.4eV*, what's its frequency? Estimate *h* as *4.14* × 10^{-15}*eVs*.
    a) 2.9 × 10^{14} Hz,
    b) 4.3 × 10^{14} Hz,
    c) 5.8 × 10^{14} Hz,
    d) 5.8 × 10^{15} Hz.

19. If the frequency of a bunch of lights waves is *5.4* × 10^{15} *Hz*, what's the energy of each photon? Estimate *h* as *4.14* × *10-15 eVs*.
    a) 18.7 eV,
    b) 21.4 eV,
    c) 22.4 eV,
    d) 23.5 eV.

20. Which of the following will increase the energy of a photon?
    a) increase the frequency of the photon,
    b) decrease the frequency of the photon,
    c) increase the intensity of light,
    d) decrease the intensity of light.

8. DETECTION OF NUCLEAR RADIATION

Purposes:

- Presentation of theoretical notions about radioactivity, types of radiation and their interaction with living matter;
- Familiarisation with the radioactive decay types, activity and half-life time notions;
- Analysis of operations and working model of the nuclear radiation detectors;
- Detection of nuclear radiation by means of installation of B-4 type;
- Study of the applications of radioactive isotopes in medicine and medical-biological research.

Theoretical notions:

Radiation is energy traveling in the form of particles or waves in bundles of energy called photons. Some everyday examples are microwaves used to cook food, radio waves for radio and television, light, and X-rays used in medicine.

Ionization is a particular characteristic of the radiation produced when radioactive elements decay. These radiations are of such high energy that when they interact with materials, they can remove electrons from the atoms in the material. This effect is the reason why ionizing radiation is hazardous to health, and provides the means by which radiation can be detected.

There are two main types of radiation which includes **non-ionising** and **ionising radiation**.

1. ***Non-ionising radiation*** has less energy than ionising radiation but can still excite molecules and atoms causing them to vibrate faster. Near ultraviolet, visible light, infrared, microwave, radio waves are all examples of non-ionising radiation.
2. ***Ionising radiation*** has enough energy to change the chemical composition of matter by forcing an atom or molecule to give up an electron, therefore ionising it. These electrically-charged particles are called ions. Ionising radiation sources include alpha and beta particles, gamma rays, neutrons and cosmic rays.

Radioactivity is a natural and spontaneous process by which the unstable atoms of an element emit or radiate excess energy in the form of particles or waves. These emissions are collectively called ionizing radiations. Depending on how the nucleus loses this excess energy either a lower energy atom of the same form will result, or a completely different nucleus and atom can be formed. This process is referred to as a transformation, a decay or a disintegrations of an atom.

A material that spontaneously emits such radiation which includes ***alpha*** particles, ***beta*** particles and ***gamma rays*** is considered ***radioactive***.

In order to understand the nature of these radiation types, one needs to understand the component parts of an atom and some characteristics of it. Atoms cannot be divided using chemicals. They do consist of parts, which include protons, neutrons, and electrons, but an atom is a basic chemical building block of matter. Each electron has a negative electrical charge. Each proton has a positive electrical charge. The charge of a proton and an electron are equal in magnitude, yet opposite in sign. Electrons and protons

are electrically attracted to each other. Each neutron is electrically neutral. In other words, neutrons do not have a charge and are not electrically attracted to either electrons or protons. Protons and neutrons are about the same size as each other and are much larger than electrons. The mass of a proton is essentially the same as that of a neutron. The mass of a proton is 1840 times greater than the mass of an electron.The nucleus of an atom contains protons and neutrons. The nucleus carries a positive electrical charge.

Electrons move around outside the nucleus. Almost all of the mass of an atom is in its nucleus; almost all of the volume of an atom is occupied by electrons. The particles within an atom are bound together by powerful forces. In general, electrons are easier to add or remove from an atom than a proton or neutron. Chemical reactions largely involve atoms or groups of atoms and the interactions between their electrons.

Symbols for Nuclear Quantities		
Symbol	**Name**	**Explanation**
A	Mass Number	the number of nucleons (protons and neutrons) in the nucleus
Z	Atomic Number	the number of protons in the nucleus
N	neutron Number	the number of neutrons in the nucleus

$^{A}_{Z}X$ → a random nucleus
$^{27}_{13}Al$ → Aluminium nucleus

A typical aluminum atom has a mass numberof 27 and an atomic number of 13. Therefore it has 13 protons (atomic number) and 14 neutrons ($27 - 13 = 14$).
So the relationship is $\boldsymbol{A = Z + N}$.

The number of protons determines what the atom is, and some atoms have different numbers of neutrons. These called **isotopes.** For example, hydrogen has three isotopes:

$^{1}_{1}H$ →hydrogen$^{2}_{1}H$ → deuterium $^{3}_{1}H$ → tritium

Historically, the products of radioactivity were called ***alpha***, ***beta***, and ***gamma*** when it was found that they could be analyzed into three distinct species by either a magnetic field (see fig.8.1) or an electric field.

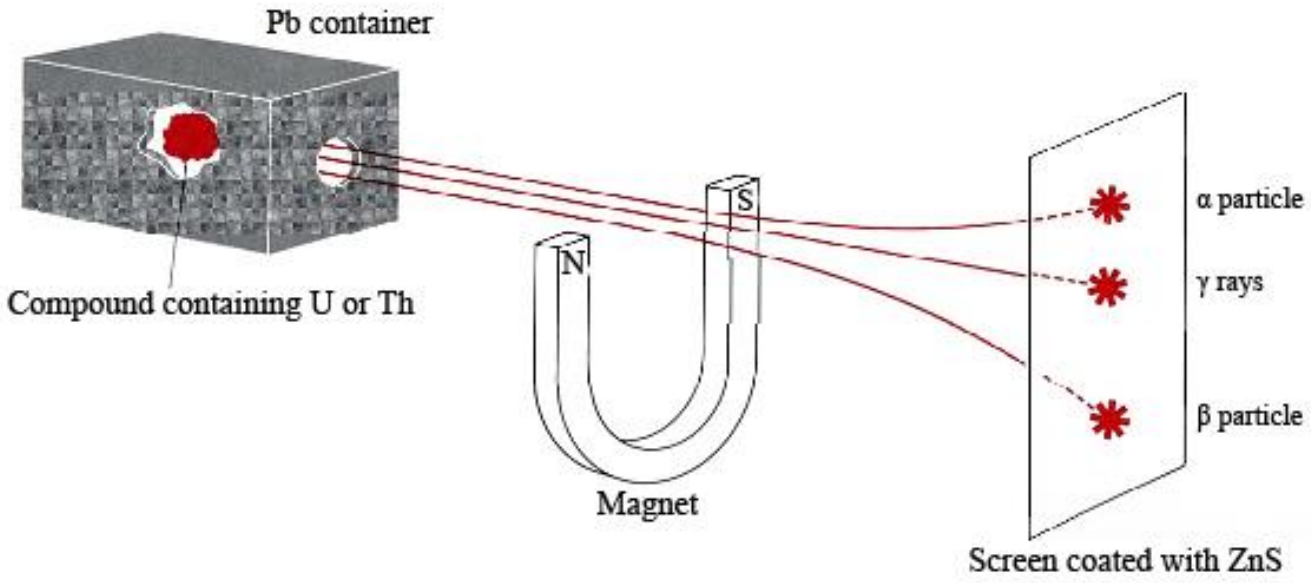


Fig.8.1. The action of the magnetic field on nuclear radiations

Alpha: $\boldsymbol{\alpha \rightarrow {}^{4}_{2}He}$

Composed of two protons and two neutrons, the alpha particle is a nucleus of the element ***helium*** (see fig.8.2.a). These particles are also very dense which, with their strong **positive charge**, precludes them from penetrating more than an inch of air or a sheet of paper. Because of this, Alpha particles are not a serious health hazard, except when they are emitted from within the body as a result of ingestion, for instance, when their high energy poses an extreme hazard to sensitive living tissue. Its main radiation hazard comes when it is ingested into the body; it has great destructive power within its short range. In contact with fast-growing membranes and living cells, it is positioned for maximum damage.

A weak form of ionizing radiation detectable on some models of Geiger counters, typically those that incorporate a thin mica window at one end of the Geiger -Mueller tube.

Beta: $\boldsymbol{\beta \rightarrow {}^{0}_{-1}e}$

Beta radiation consists of **negatively charged** particles (electrons) emitted from an atom in the process of decay (see fig.8.2.b). These particles are relatively light and can penetrate somewhat better than an Alpha particle, though still only through a few millimetres of aluminium at best. If ingested, Beta radiation can be hazardous to living tissue. A relatively weak form of ionizing radiation detectable on many Geiger counters, generally dependent on the thickness of the Geiger-Mueller tube wall or the existence of a window at the end of the tube.

Gamma:γ

Gamma radiation represents one extreme of the electromagnetic spectrum, particularly that radiation with the highest frequency and shortest wavelength (see fig.8.2.c). (That same spectrum also includes the more familiar X-rays, ultraviolet light, visible light, infrared rays, microwaves, and radio waves, listed in order of decreasing frequency and increasing wavelength from Gamma rays.) Gamma rays can pass through virtually anything, and are effectively shielded or absorbed only by materials of high atomic weight such as lead. Gamma rays are produced naturally by the sun and other bodies in outer space, their transmission to earth being known as "cosmic radiation". A very powerful and potentially very dangerous type of ionizing radiation detectable on virtually all Geiger counters.

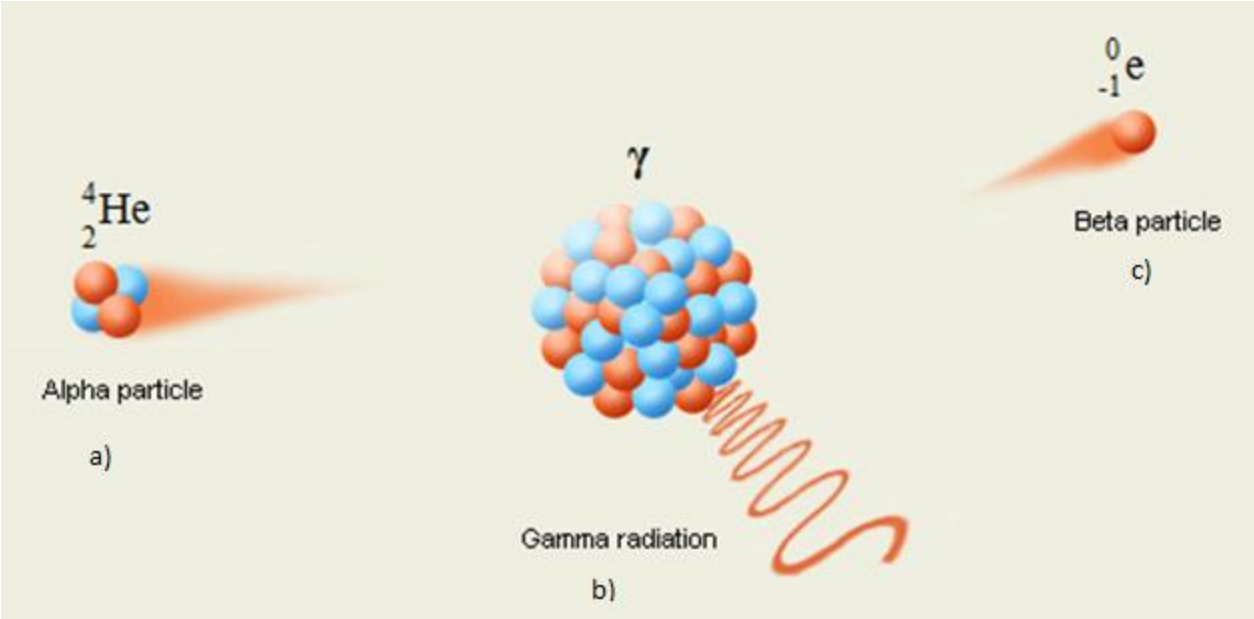


Fig.8.2. Nuclear radiations classification

The discovery of radioactivity has delivered many benefits, but it must be handled with care. A sheet of paper, or even the skin of our bodies, will stop alpha particles, while a thin sheet of perspex or glass will stop beta radiation. However, the energy of both can cause damage to cells if they enter the body through inhalation, swallowing or wounds. Thick barriers of lead, water and concrete are necessary to stop much more penetrating and damaging gamma radiation (see fig.8.3).

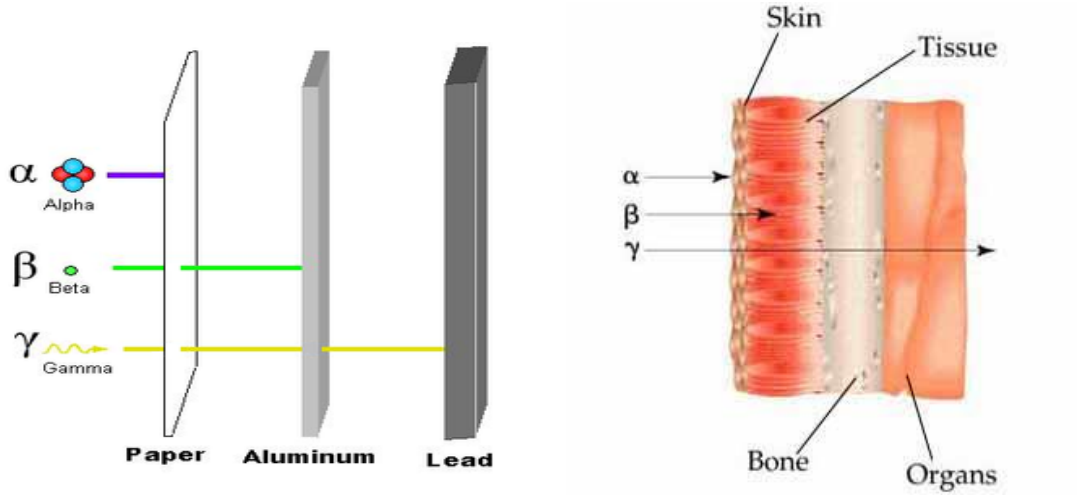


Fig.8.3. Illustration of the relative abilities of three different types of ionizing radiation to penetrate solid matter and biological tissue

Radioactive Decay Rates

Radioactive decay is the loss of elementary particles from an unstable nucleus, ultimately changing the unstable element into another more stable element. There are five types of radioactive decay: ***alpha emission, beta emission, positron emission, electron capture,*** and ***gamma emission.***Each type of decay emits a specific particle which changes the type of product produced. The number of

protons and neutrons found in the daughter nuclei (the nuclei produced from the decay) are determined by the type of decay or emission that the origional element goes through.

In terms of *entropy*, radioactive decay can be defined as the tendency for matter and energy to gain inert uniformity or stability. For elements, uniformity is produced by having an equal number of neutrons and protons which in turn dictates the desired nuclear forces to keep the nuclear particles inside the nucleus. The unstable nucleus then releases radiation in order to gain stability. For example, the unstable element Uranium-238 which has 54 more neutrons than its protons (Atomic umber =92). This element gains stability by passing through various types of decays (19 steps-- also known as the Uranium series) (see fig.8.4) and is converted into Pb-206 (atomic number 82).

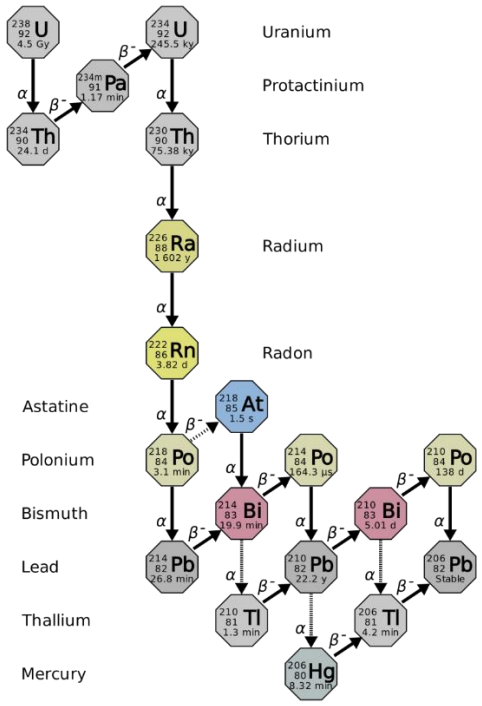


Fig.8.4. Decay Chain U238

In 1889, Ernest Rutherford recognized and named two modes of radioactive decay, showing the occurrence of both processes in a decaying sample of natural uranium and its daughters. Rutherford named these types of radiation based on their penetrating power: heavier ***alpha*** and lighter ***beta*** radiation. ***Gamma*** rays, a third type of radiation, were discovered by P. Villard in 1900 but weren't recognized as electromagnetic radiation until 1914. Since gamma radiation is only the discharge of a high-energy photon from an over-excited nucleus, it does not change the identity of the atom from which it originates.

Alpha Decay

When a radioactive element undergoes nuclear decay through the emission of alpha particle ($\mathbf{^4_2He}$) it termed as ***alpha decay***(see fig.8.5). Since one alpha particle contains two protons and two neutrons, hence the emission of one alpha particle involves the decreases of mass by 4 and the atomic number decreases by 2 in daughter nuclei. An example of alpha decay is conversion of Uranium-238 to Thorium-234 by the emission of alpha decay.

$$\mathbf{^{238}_{92}U \rightarrow {}^{234}_{90}Th + {}^{4}_{2}He}$$

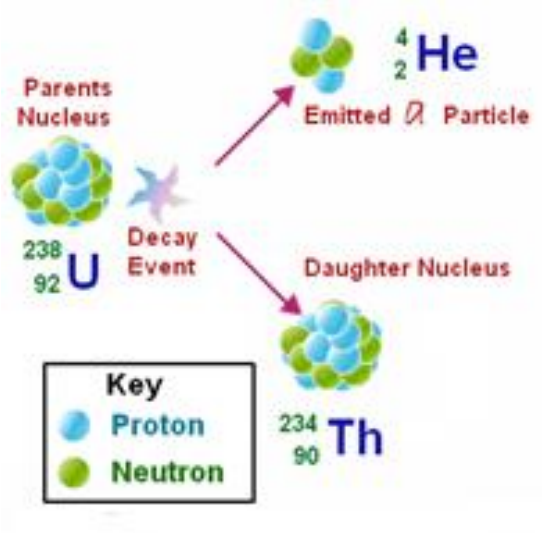


Fig.8.5. Alpha decay of an Uranium-238 nucleus

Beta Decay

Beta rays consist of beta particles or electrons with negative charge ($_{-1}^{0}e$). Because of the emission of beta particle with anti neutrino, there will be no change in atomic mass of daughter nuclei while atomic number increases by one(see fig.8.6). For example beta decay from Thorium-234 first forms excited nuclei of Protactinium-234 which further emitted some radiation to form stable Protactinium-234.

$$^{234}_{90}Th \rightarrow {}^{234}_{91}Pa + {}_{-1}^{0}e + anti\ neutrino$$

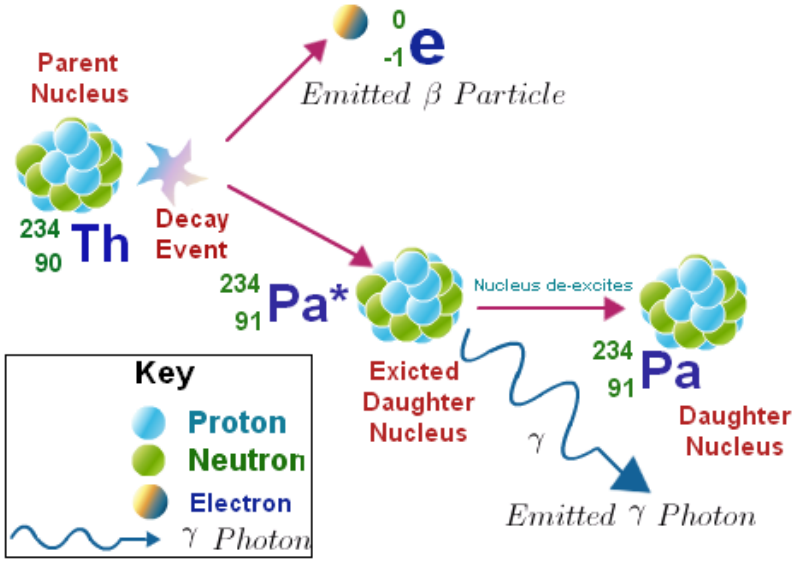


Fig.8.6. Beta decay of a Thorium-234 nucleus

Gamma Decay

A third type of radiation, gamma radiation, usually accompanies alpha or beta decay. Gamma rays are photons and are without rest mass or charge. Alpha or beta decay may simply proceed directly to the

ground (lowest energy) state of the daughter nucleus without gamma emission, but the decay may also proceed wholly or partly to higher energy states (excited states) of the daughter. In the latter case, gamma emission may occur as the excited states transform to lower energy states of the same nucleus. (Alternatively to gamma emission, an excited nucleus may transform to a lower energy state by ejecting an electron from the cloud surrounding the nucleus. This orbital electron ejection is known as internal conversion and gives rise to an energetic electron and often an X-ray as the atomic cloud fills in the empty orbital of the ejected electron.)

Half-life time of radioactivity

Half-life is the time period that is characterized by the time it takes for half of the substance to decay (both radioactive and non-radioactive elements). The rate of decay remains constant throughout the decay process.

An exponential decay process can be described by any of the following three equivalent formulas:

$$N(t) = N_0(\frac{1}{2})^{\frac{t}{t_{\frac{1}{2}}}}$$

$$N(t) = N_0 e^{-\frac{t}{\tau}}$$

$$N(t) = N_0 e^{-\lambda t}$$

Where

- N_0 is the initial quantity of the substance that will decay (this quantity may be measured in grams, moles, number of atoms, etc.),
- $N(t)$ is the quantity that still remains and has not yet decayed after a time t,
- $t_{\frac{1}{2}}$ is the half-life of the decaying quantity,
- τ is a positive number called the *mean lifetime* of the decaying quantity,
- λ is a positive number called the *decay constant* of the decaying quantity.
  The three parameters $t_{\frac{1}{2}}$, τ , and λ are all directly related in the following way:

$$t_{\frac{1}{2}} = \frac{\ln(2)}{\lambda} = \tau \ln(2)$$

  where$ln(2)$ is the natural logarithm of 2 (approximately 0.693).

Since the decay rate is constant, one can use the radioactive decay law and the half-life formula to find the age of organic material, which is known as radioactive dating. One of the forms of radioactive dating is radiocarbon dating. Carbon 14 $(C-14)$ is produced in the upper atmosphere through the collision of cosmic rays with atmospheric$14N$. This radioactive carbon is incorporated in plants and respiration and eventually with animals that feed upon plants. The ratio of $C-14$ to $C-12$ is $1:10^{12}$ within plants as well as in the atmosphere. This ratio, however, increases upon the death of an animal or when a plant decays because there is no new income of Carbon-14. By knowing the half-life of Carbon-14 (which is 5730 years) one can calculate the rate of disintegration of the nuclei within the organism or

substance and thereby determine its age. It is possible to use other radioactive elements in order to determine the age of nonliving substances as well.

Activity of radioactive substances and units of measurement

Activity, in radioactive-decay processes is the number of disintegrations per second or the number of unstable atomic nuclei that decay per second in a given sample. Activity is determined by counting, with the aid of radiation detectors and electronic circuits, the number of particles and photons (pulses of electromagnetic energy) ejected from a radioactive material during a convenient time interval. This experimental count, however, must be interpreted in the light of a thorough knowledge of the particular manner of radioactive decay in the sample material, because some sources emit more than one particle or photon per disintegration.

There are three measurement units for radioactivity: the Becquerel measures *radioactivity*, the Gray measures the *absorbed dose* and the Sievert measures the *biological effects*.

1. **The becquerel (Bq)** measures the ***activity of the radioactive source***, meaning the number of atoms which, within a particular time frame, transform and emit radiation.

1 Bq = 1 emission of radiation per second.

This is a very small unit, and multiples are often used:

- 1 MBq = 1 mega becquerel = 1,000,000 Bq
- 1 GBq = 1 giga becquerel = 1,000,000,000 Bq

The radioactivity of an environment, a material or a foodstuff is given in Becquerels per kilogram or per liter.

2. **The gray (Gy)** measures ***the absorbed dose***, meaning the energy transferred to the material by ionizing radiation upon encountering it.

1 Gy = 1 joule per kilogram

Sub-multiples are often used:

- 1 mGy = 1 milligray = 0.001 Gy
- 1 μGy = 1 microgray = 0.000001 Gy

3. **The sievert (Sv)** evaluates the ***effects of ionizing radiation*** on living material. At equal doses, the effects of radioactivity on living tissue depends on the type of radiation (alpha, beta, gamma, etc.), on the organ concerned and naturally on the length of exposure.

Contrary to the becquerel, the sievert is a very large unit, and we often use sub-multiples:

- 1 mSv = 1 millisievert = 0.001 Sv
- 1 μSv = 1 microsievert = 0.000001 Sv

Interaction of the ionized radiation with the living matter

Ionizing radiation is generated through nuclear reactions, nuclear decay, by very high temperature, or via acceleration of charged particles in electromagnetic fields. Natural sources include the sun, lightning and supernova explosions. Artificial sources include nuclear reactors, particle accelerators, and x-ray tubes.

DETECTION OF NUCLEAR RADIATION

Prolonged exposure to radiation often has detrimental effects on living matter. This is due to radiation's ionizing ability, which can damage the internal functioning of cells. Radiation either ionizes or excites atoms or molecules in living cells, leading to the dissociation of molecules within an organism. The most destructive effect radiation has on living matter is ionizing radiation on DNA. Damage to DNA can cause cellular death, mutagenesis (the process by which genetic information is modified by radiation or chemicals), and genetic transformation. Effects from exposure to radiation include leukemia, birth defects, and many forms of cancer.

Most external radiation is absorbed by the environment; for example, most ultraviolet radiation is absorbed by the ozone layer, preventing deadly levels of ultraviolet radiation to come in contact with the surface of the earth. Sunburn is an effect of UV radiation damaging skin cells, and prolonged exposure to UV radiation can cause genetic information in skin cells to mutate, leading to skin cancer.

Alpha, beta, and gamma rays also cause damage to living matter, in varying degrees. Alpha particles have a very small absorption range, and thus are usually not harmful to life, unless ingested, due to its high ionizing power. Beta particles are also damaging to DNA, and therefore are often used in radiation therapy to mutate and kill cancer cells. Gamma rays are often considered the most dangerous type of radiation to living matter. Unlike alpha and beta particles, which are charged particles, gamma rays are instead forms of energy. They have large penetrating range and can diffuse through many cells before dissipating, causing widespread damage such as radiation sickness. Because gamma rays have such high penetrating power and can damage living cells to a great extent, they are often used in irradiation, a process used to kill living organisms.

Radiation Effects on a Cell

When a cell absorbs radiation, there are four possible effects on the cell.

- The cell may suffer enough damage to cause loss of proper function, and the cell will die.
- The cell may lose its ability to reproduce itself.
- The cell's genetic code (i.e., the DNA) may be damaged such that future copies of the cell are altered, which may result in cancerous growth.
- The absorption of radiation by a cell may have no adverse effects.

Cells are made up of molecules. Cell damage may be caused by interaction of radiation with these molecules. If radiation strikes a molecule crucial to the cell's function, such as DNA, damage to the cell is likely to be greater than if the radiation strikes a less crucial molecule such as water. Cells that multiply rapidly are more likely to be affected by radiation than others. An example of rapidly dividing cells is fetal tissue. For this reason, a fetus is especially sensitive to radiation. Another example is a cancerous tumor, which can often be destroyed by radiation treatment. Cells can often repair radiation damage, but if the cell multiplies (splits into two identical cells) before it has had time to repair the most recent radiation damage, the new cells might not be accurate copies of the original one.

The radiation may interact directly with biologically significant molecules, like DNA and proteins. Radiation may also interact indirectly to cause damage, by interacting with chemicals in our bodies, such as water, and form very active chemicals like free radicals that may cause damage to the biologically significant molecules. The damage can be fixed, or the cell may die, or it may actually affect the tissue/organ if there is enough damage. It is felt that the damage to the DNA is of the most importance, and could lead to increase risk of cancer. The damage could be to a single base pair, could cause the DNA

to bind to itself or cause an actually break the DNA on one stand or more rarely, to both DNA strands. If the damage is not fixed or is fixed wrong and the cell escapes apoptosis (programmed cell death) it may be one of the several needed steps that results in the cell becoming a tumor. But the chain of events that leads from DNA damage to cancer is a long, multi-step process with many check points along the way where things must go wrong in order to cause cancer.

One of the reasons cancer is not more common is that every minute of the day for your whole life, your body's repair mechanisms are working to fix damage to your DNA. It is surprising how many times each hour, each cell's DNA is damaged:

Gamma-rays have many modes of interaction with matter. Those which are very important to nuclear imaging in medicine are the ***Photoelectric Effect*** and the ***Compton Effect***.

Photoelectric Effect-when a gamma-ray collides with an orbital electron of an atom of the material through which it is passing it can transfer all its energy to the electron and cease to exist (see fig.8.7). On the basis of the Principle of Conservation of Energy we can deduce that the electron will

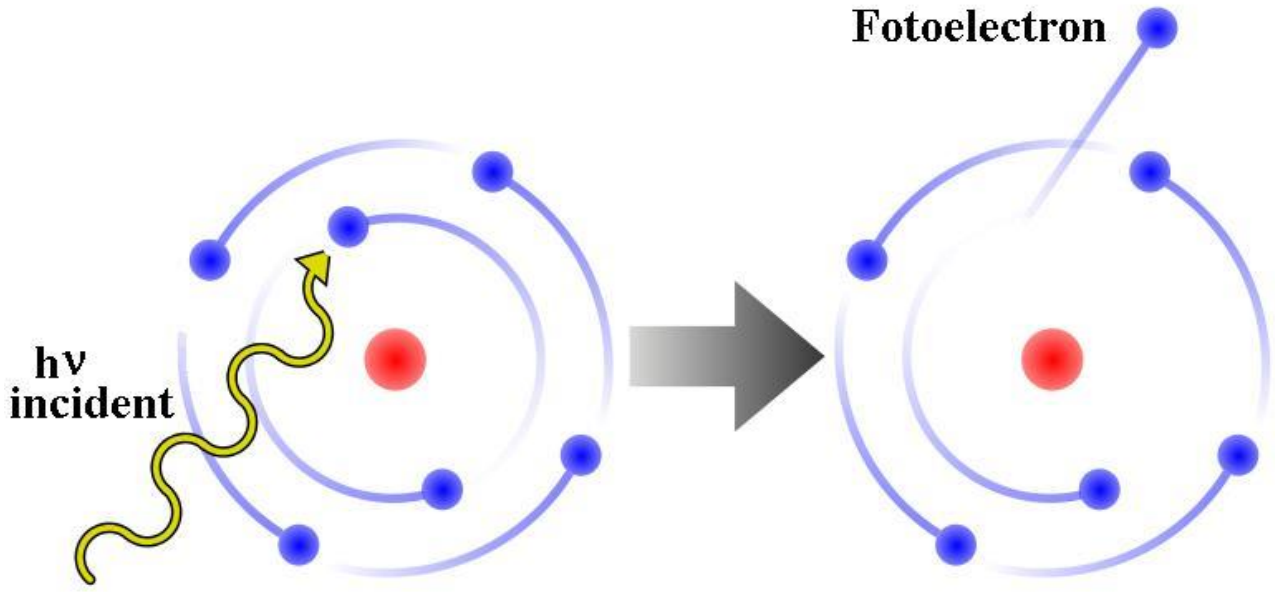


Fig.8.7. Photoelectric Effect illustration

leave the atom with a kinetic energy equal to the energy of the gamma-ray less that of the orbital binding energy. This electron is called a ***photoelectron***.

An ion results when the photoelectron leaves the atom. The gamma-ray energy is totally absorbed in the process. The photoelectron can cause ionisations along its track in a similar manner to a beta-particle. X-ray emission can occur when the vacancy left by the photoelectron is filled by an electron from an outer shell of the atom.

Compton Effect-This type of effect is somewhat akin to a cue ball hitting a coloured ball on a pool table. Here a gamma-ray transfers only part of its energy to a valance electron which is essentially free (see Fig.8.8). The electron leaves the atom and may act like a beta-particle and that the gamma-ray deflects off in a different direction to that with which it approached the atom. This deflected or scattered

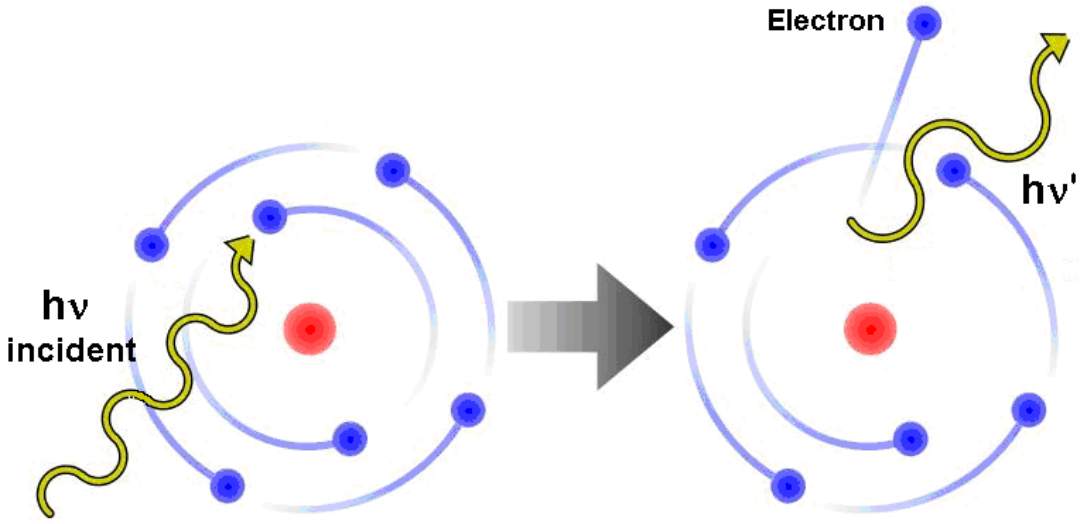


Fig.8.8. Compton Effect illustration

gamma-ray can undergo further Compton Effects within the material.This effect is sometimes called **Compton Scattering**.

Detectors of nuclear radiation. Geiger-Muller contour

Nuclear radiation detectors serve to determine the composition and measure the intensity of radiation, to measure the energy spectra of particles, to study the processes of interaction between fast particles and atomic nuclei, and to study the decay processes of unstable particles. The operation of all nuclear radiation detectors is based on the ionization or excitation by charged particles of the atoms of the substance that fills the effective volume of the detector. Thus, the passage of all nuclear particles through the medium is accompanied by the formation of free electrons and ions, the appearance of flashes of light (scintillations), and chemical and thermal effects. As a result, radiation can be registered by the appearance of electrical signals (current or potential pulses) at the output of the detector, by the darkening of a photoemulsion, or by other means.

Many of the detectors invented and used so far are:

- *ionization detectors* (of which gaseous ionization detectors and semiconductor detectors are most typical)
- *scintillation detectors* (phosphor detectors, scintillator crysals, liquid scintillators)
- *particle track devices* (cloud chambers, spark chambers, bubble chambers)

- **The Geiger–Müller counter**

The Geiger–Müller counter, also called a Geiger counter, is an instrument used for measuring *ionizing radiation* used widely in such applications as radiation dosimetry, radiological protection, experimental physics and the nuclear industry. It detects ionizing radiation such as alpha particles, beta

particles and gamma rays using the ionization effect produced in a Geiger–Müller tube; which gives its name to the instrument (see fig.8.9).

The tube is filled with Argon gas, and around +400 Volts is applied to the thin wire in the middle. When a particle enters the tube, it pulls an electron from an Argon atom. The electron is attracted to the central wire, and as it rushes towards the wire, the electron will knock other electrons from Argon atoms, causing an "avalanche". Thus one single incoming particle will cause many electrons to arrive at the wire, creating a pulse which can be amplified and counted. This gives us a very sensitive detector.

The operation of a ***scintillation counter*** is based on the phenomenon of fluorescence, which

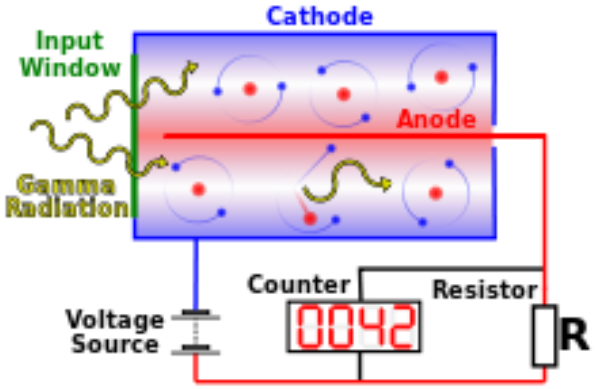


Fig.8.9. Construction principle of a Geiger–Müller counter

occurs through the interaction of nuclear particles and scintillators—special liquids, plastics, crystals, and noble gases (see fig.8.10). A light flash is registered by a photoelectric multiplier, which converts it into an electrical impulse. The amplitude of the output signal is proportional to the energy transferred to the scintillator by the particle; this fact makes it possible to use these detectors to measure the energy of nuclear particles. The high efficiency of scintillation detectors is a result of the fact that, unlike ionization chambers, proportional counters, and Geiger-Müller counters, the working medium of the detector is dense and its absorptivity is approximately 103 times greater than the absorptivity of a gas at a pressure of ≈ atmosphere.

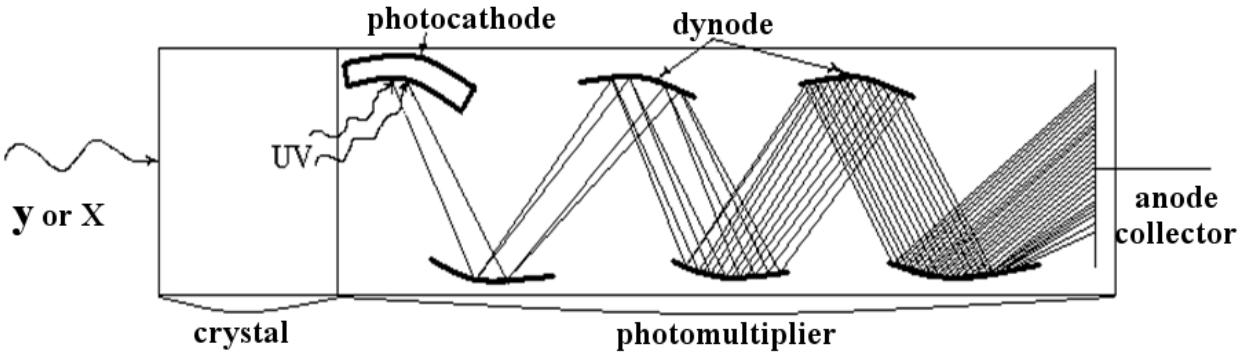


Fig.8.10. Construction principle of a scintillation counter

Work procedure:

The installation B-4 type consist of two blocks:

- The block of contours
- The main block wich contains the elecronic installation for suplying the contour with high voltage and the electronic circuits of counting and registering pulses.

On the heading panel (see fig.8.11) we have a series of command buttons with the respective instructions, the swich of polarity and also six decatrons (counting electronic devices) serving for registering pulses, their maximal number can be 1000000.

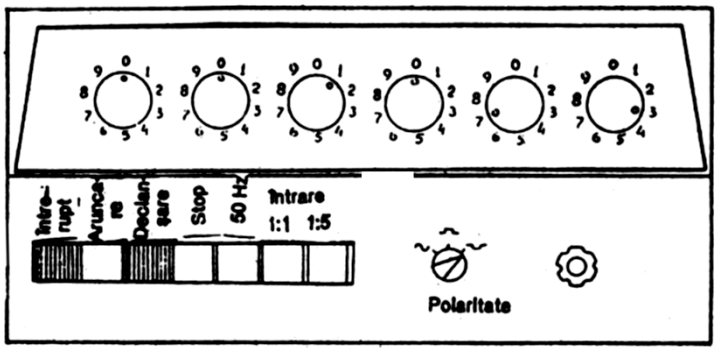


Fig.8.11. The appearance of the installation of B-4, which works in conjunction with a Geiger-Muller counter

Each dekatron represents a gas-discharge tube that recalculates at ten, so for each 10 registered pulses a single pulse transmitted to the next dekatron appears.

A. Determination of the counting speed of a Geiger-Muller counter

1. Connect the installation to the electrical circuit, then check the quality of functioning of the counting device. Press successively „≈" and „$50Hz$" buttons, then, in the same time, press the the „start" button and set the time marker (a regular chronometer). As a result of these manipulations, 50 pulses per second (the frequency of AC) are transmitted automatically to the device.
2. After 3 min, in the same time, press the „stop"button on the chronometer and counting device. If the speed of the pulses is about 300 pulses/min, the device works properly.
3. Disconnect „≈" and „$50Hz$" buttons , by making the second press on them. Then, also by pressing, connect the button „U" of the polarization signal.
4. Performing the above mentioned manipulations, register the number of pulses N arrived from the Geiger-Muller contour during 1 min. The measurements are repeated several times, counting each time the number of pulses per minute $n = \frac{N}{t}$.
5. The obtained results needs to be introduced in the table below:

No.	**Time** t, min	**Number of pulses** N	$n = \frac{N}{t}$

1			
2			
3			
4			
5			
Average			

B. Determination of the activity of a radioactive substance. The attenuation of radiation in different substances

In this part of the practical work we will determine the dependence of the counting speed in function of the contour-source distance and we will notice a decrease in value for the counting speed by placing of plates made by different metals (Al,Fe,Cu) of the same thickness, under the Geiger-Muller counter.

Althought the used radiation source in the laboratory has the activity smaller than $1\mu C_i$ ($1\mu C_i = 3.7 \cdot 10^{10} Bq$) and does not present any danger, during the work the protection rules must be strictly respected!!

1. With the rubber gloves and special tongs, the source need to be transferred from the box into the device for fixing the Geiger-Muller contour situated above.
2. Varying the contour-source distance with 5 cm each time, register the total number of pulses N.
3. The obtained results needs to be introduced in the table below:

No.	**Distance** d, cm	**Number of pulses** N	**Activity** $n = \frac{N}{t}$
0			
1	5		
2	10		
3	15		
4	20		
5	25		

4. Draw the graphic of the function $n = f(d)$, analogously to the one represented in fig.8.12. Use a millimetric paper.

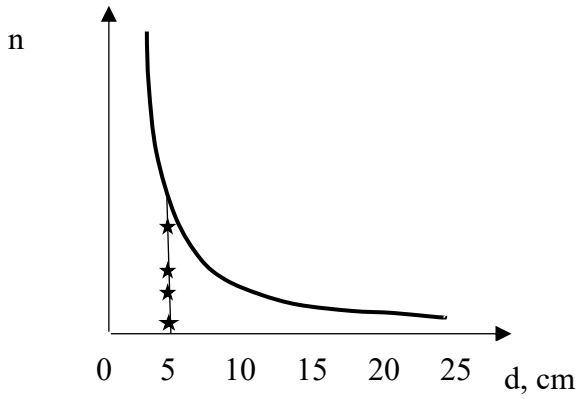


Fig.8.12. The dependence of counting speed on function of contour-source distance

Nuclear medicine

Nuclear medicine is a branch of medical imaging that uses small amounts of radioactive material to diagnose and determine the severity of or treat a variety of diseases, including many types of cancers, heart disease, gastrointestinal, endocrine, neurological disorders and other abnormalities within the body.

While radiology has been used for close to a century, "nuclear medicine" began approximately 50 years ago. Today, about one-third of all procedures used in modern hospitals involve radiation or radioactivity. These procedures are among the best and most effective life-saving tools available, they are safe and painless and don't require anesthesia, and they are helpful to a broad span of medical specialties, from pediatrics to cardiology to psychiatry.

While both nuclear medicine and radiology are used as a diagnostic procedure (to determine a patient's health, monitor the course of an illness or follow the progress of the treatment) and as a therapeutic procedure (to treat illnesses), they are different in that in nuclear medicine radioisotopes are introduced into the body internally, whereas in radiology X-rays penetrate the body from outside the body.

Diagnosis

Nuclear medicine imaging procedures are noninvasive and, with the exception of intravenous injections, are usually painless medical tests that help physicians diagnose and evaluate medical conditions. These imaging scans use radioactive materials called *radiopharmaceuticals* or *radiotracers*.

Depending on the type of nuclear medicine exam, the radiotracer is either injected into the body, swallowed or inhaled as a gas and eventually accumulates in the organ or area of the body being examined. Radioactive emissions from the radiotracer are detected by a special camera or imaging device that produces pictures and provides molecular information.

In many centers, nuclear medicine images can be superimposed with *computed tomography* (CT) or *magnetic resonance imaging* (MRI) to produce special views, a practice known as image fusion or co-registration. These views allow the information from two different exams to be correlated and interpreted on one image, leading to more precise information and accurate diagnoses. In addition, manufacturers are now making *single photon emission computed tomography/computed tomography* (SPECT/CT) and *positron emission tomography/computed tomography* (PET/CT) units that are able to perform both

imaging exams at the same time.

Therapy

Nuclear medicine also offers therapeutic procedures, such as *radioactive iodine* (I-131) *therapy* that use small amounts of radioactive material to treat cancer and other medical conditions affecting the thyroid gland, as well as treatments for other cancers and medical conditions.

Non-Hodgkin's lymphoma patients who do not respond to chemotherapy may undergo *radioimmunotherapy* (RIT).

Radioimmunotherapy (RIT) is a personalized cancer treatment that combines radiation therapy with the targeting ability of immunotherapy, a treatment that mimics cellular activity in the body's immune system.

Nuclear medicine is used to:

Heart

Visualize heart blood flow and function (such as a myocardial perfusion scan), assess damage to the heart following a heart attack, evaluate treatment options such as bypass heart surgery and angioplasty, evaluate heart function before and after chemotherapy (MUGA), etc;

Lungs

Scan lungs for respiratory and blood flow problems, assess differential lung function for lung reduction or transplant surgery, detect lung transplant rejection;

Bones

Evaluate bones for fractures, infection and arthritis, evaluate for metastatic bone disease, evaluate bone tumors, identify sites for biopsy;

Brain

Investigate abnormalities in the brain, such as seizures, memory loss and abnormalities in blood flow, detect the early onset of neurological disorders such as *Alzheimer disease*, evaluate for abnormalities in a chemical in the brain involved in controlling movement in patients with suspected Parkinson's disease, evaluation of brain tumor recurrence, surgical or radiation planning or localization for biopsy

Other Systems

Identify bleeding into the bowel, assess post-operative complications of gallbladder surgery, evaluate fever of unknown origin, locate the presence of infection, measure thyroid function to detect an overactive or underactive thyroid, help diagnose hyperthyroidism and blood cell disorders, evaluate stomach emptying, evaluate spinal fluid flow and potential spinal fluid leaks, stage cancer by determining the presence or spread of cancer in various parts of the body, localize sentinel lymph nodes before surgery in patients with breast cancer or skin and soft tissue tumors, detect the recurrence of cancer, detect rare tumors of the pancreas and adrenal glands

Personal health improves with radiation.

- It allows for quick, safe, early and more accurate medical diagnoses.
- It can be harnessed as a treatment for certain diseases.
- Employment of nuclear medicine technologists is projected to grow 20 percent from 2012 to 2022, faster than the average for all occupations.

- Tens of millions of patients are treated with nuclear medicine each year, and more than 10,000 hospitals worldwide use radioisotopes in medicine.

Knowledge evaluation exercises:

1. Ionizing radiation is
   a) mechanical waves with high potential energy;
   b) energy that is given off by unstable atoms as they decay;
   c) particles and waves that have sufficient energy to cause damage to DNA and cells if they are get into the environment and our bodies;
   d) thermal radiation emitted by objects near room temperature.

2. Non-ionising radiation include:
   a) gamma ray;
   b) infrared;
   c) microwave;
   d) X-ray.

3. Radioactivity is:
   a) an artificial and stimulated process by which the unstable atoms of an element emit or radiate excess energy in the form of particles or waves;
   b) a natural and induced process by which the unstable atoms of an element emit or radiate excess energy in the form of particles or waves;
   c) a natural and spontaneous process by which the unstable atoms of an element emit or radiate excess energy in the form of particles or waves;
   d) none of those.

4. Isotopes are any of two or more forms of a chemical element, having
   a) the same number of protons in the nucleus, but having different numbers of neutrons in the nucleus;
   b) the same atomic number and the same number of neutrons in the nucleus;
   c) different numbers of protons in the nucleus, but the same number of neutrons in the nucleus;
   d) none of those.

5. Alfa particles
   a) consists of negatively charged particles;
   b) are very dense, with strong positive charge;
   c) represents one extreme of the electromagnetic spectrum;
   d) are stopped by a sheet of paper, or even the skin of our bodies.

6. Beta radiation

a) is the radiation with the highest frequency and shortest wavelength;
b) can be hazardous to living tissue, if ingested;
c) consists of negatively charged particles;
d) are produced naturally by the sun and other bodies in outer space.

7. What are the biological effects of exposure to radiation?

8. What are some examples of applications of constructive uses of radioactive isotopes?

9. What methods are used to detect radiation? Describe the construction principle of a Geiger–Müller counter.

10. The Photoelectric Effect is
    a) a phenomenon in which electrically charged particles are released from or within a material when it absorbs electromagnetic radiation
    b) a phenomenon in which a gamma-ray collides with an orbital electron of an atom of the material through which it is passing it can transfer all its energy to the electron and cease to exist
    c) the ejection of electrons from a metal plate when light falls on it
    d) all of those

11. The Compton effect is
    a) the result of a high-energy photon colliding with a target, which releases loosely bound electrons from the outer shell of the atom or molecule;
    b) the loss of energy and concomitant increase in wavelength of a usually high-energy photon (as of X-rays or gamma rays) that occurs upon collision of the photon with an electron;
    c) a phenomenon in which electrically charged particles are released from or within a material when it absorbs electromagnetic radiation;
    d) none of those.

12. The number of atoms which, within a particular time frame, transform and emit radiation is measured in:
    a) Joule per kilogram (J/Kg);
    b) Sievert (Sv);
    c) Becquerel (Bq)**;**
    d) Gray (Gy)**.**

13. The energy transferred to the material by ionizing radiation upon encountering it is measured in
    a) Joule per kilogram (J/Kg);
    b) Sievert (Sv);
    c) Becquerel (Bq)**;**
    d) Gray (Gy)**.**

14. The effects of ionizing radiation on living material is measured in
    a) Joule per kilogram (J/Kg);
    b) Sievert (Sv);
    c) Becquerel (Bq);
    d) Gray (Gy)**.**

15. Half-life is the time period that
    a) is characterized by the time it takes for half of the substance to decay;
    b) helps to find the age of organic material;
    c) helps to calculate the rate of disintegration of the nuclei within the organism or substance and thereby determine its age;
    d) all of those.

9. DETERMINATION OF CONCENTRATION OF OPTICALLY ACTIVE SUBSTANCES BY POLARIMETRICMETHOD

Purposes:

- Study of physical phenomena that produce the polarized light;
- Study of some devices that produce the polarized light;
- Familiarisation with the possibilities of using the polarized light in medicine;
- Polarimeter construction and working principles.

Theoretical notions

Light is fluctuations of electric and magnetic fields, which can transport energy from one location to another. A light wave is an ***electromagnetic wave*** that travels through the vacuum of outer space. An electromagnetic wave is a ***transverse wave*** that has both an electric and a magnetic component (Fig.9.1).

Visible light is not inherently different from the other parts of the electromagnetic spectrum

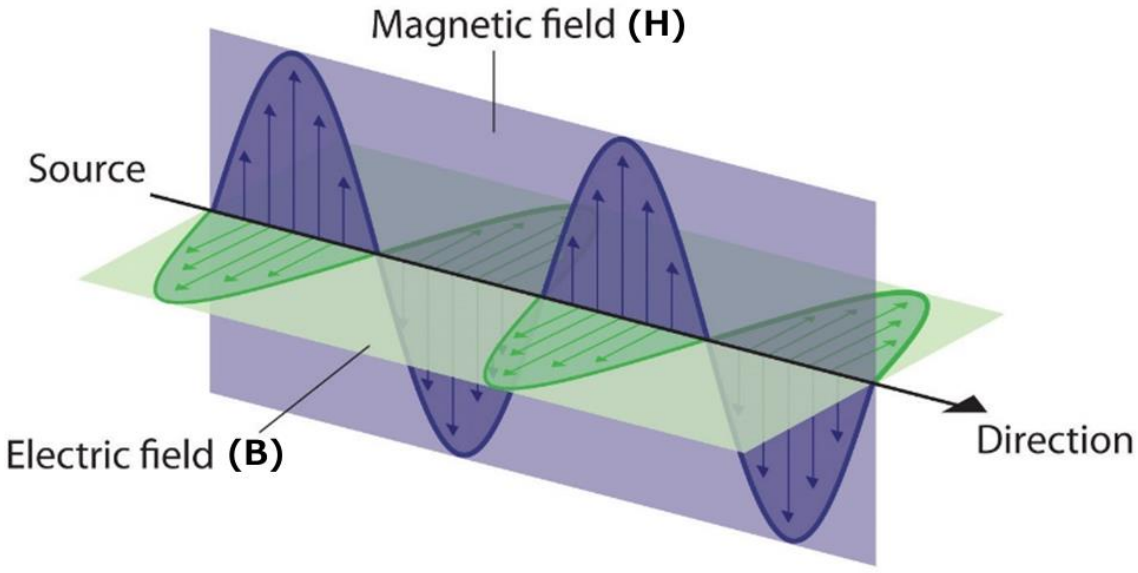


Fig.9.1. Electromagnetic wave

(Fig.9.2) with the exception that the human eye can detect visible waves. Electromagnetic radiation can also be described in terms of a stream of photons which are massless particles each travelling with wavelike properties at the speed of light.

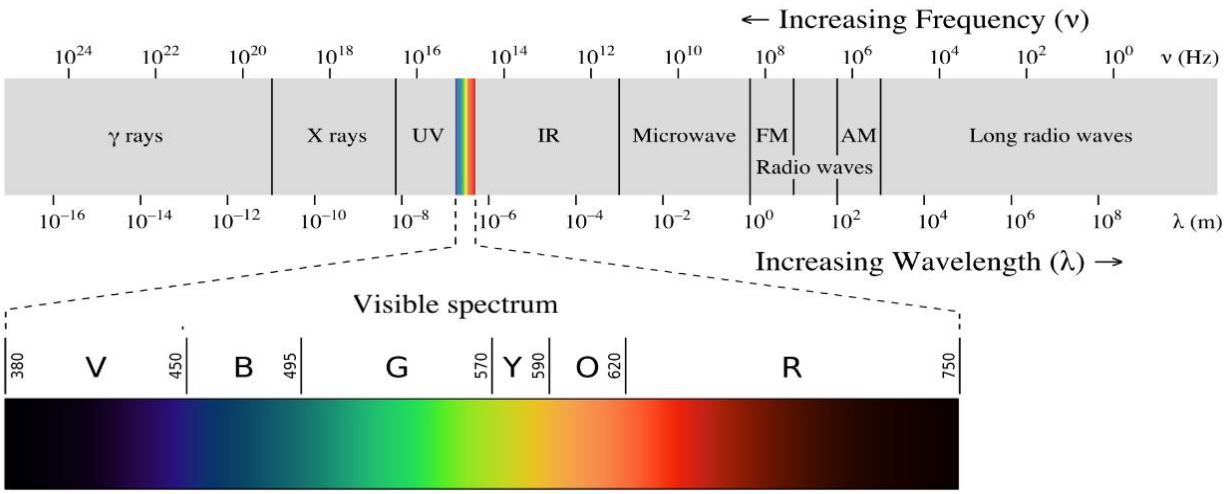


Fig.9.2. Electromagnetic spectrum

A light wave that is vibrating in more than one plane is referred to as ***unpolarized light***. Light emitted by the sun, by a lamp in the classroom, or by a candle flame is unpolarized light. Such light waves are created by electric charges that vibrate in a variety of directions, thus creating an electromagnetic wave that vibrates in a variety of directions (Fig.9.3a). This concept of unpolarized light is rather difficult to visualize. In general, it is helpful to picture unpolarized light as a wave that has an average of half its vibrations in a horizontal plane and half in a vertical plane (Fig.9.3b).

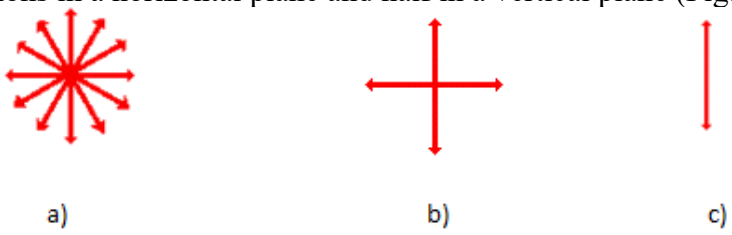


Fig.9.3. Unpolarized light a), b) and plan polarized light c)

It is possible to transform unpolarized light into ***polarized light***. Polarized light waves are light waves in which the vibrations occur in a single plane. The process of transforming unpolarized light into polarized light is known as ***polarization***. There are a variety of methods of polarizing light. The four methods discussed on this page are:

- Polarization by Transmission
- Polarization by Reflection and Refraction
- Polarization by Scattering

Polarization by use of a polaroid filter (by transmission)

The most common method of polarization involves the use of a ***polaroid filter***. Polaroid filters are made of a special material that is capable of blocking one of the two planes of vibration of an electromagnetic wave. (Remember, the notion of two planes or directions of vibration is merely a

simplification that helps us to visualize the wave like nature of the electromagnetic wave.) In this sense, a Polaroid serves as a device that filters out one-half of the vibrations upon transmission of the light through the filter. When unpolarized light is transmitted through a polaroid filter, it emerges with one-

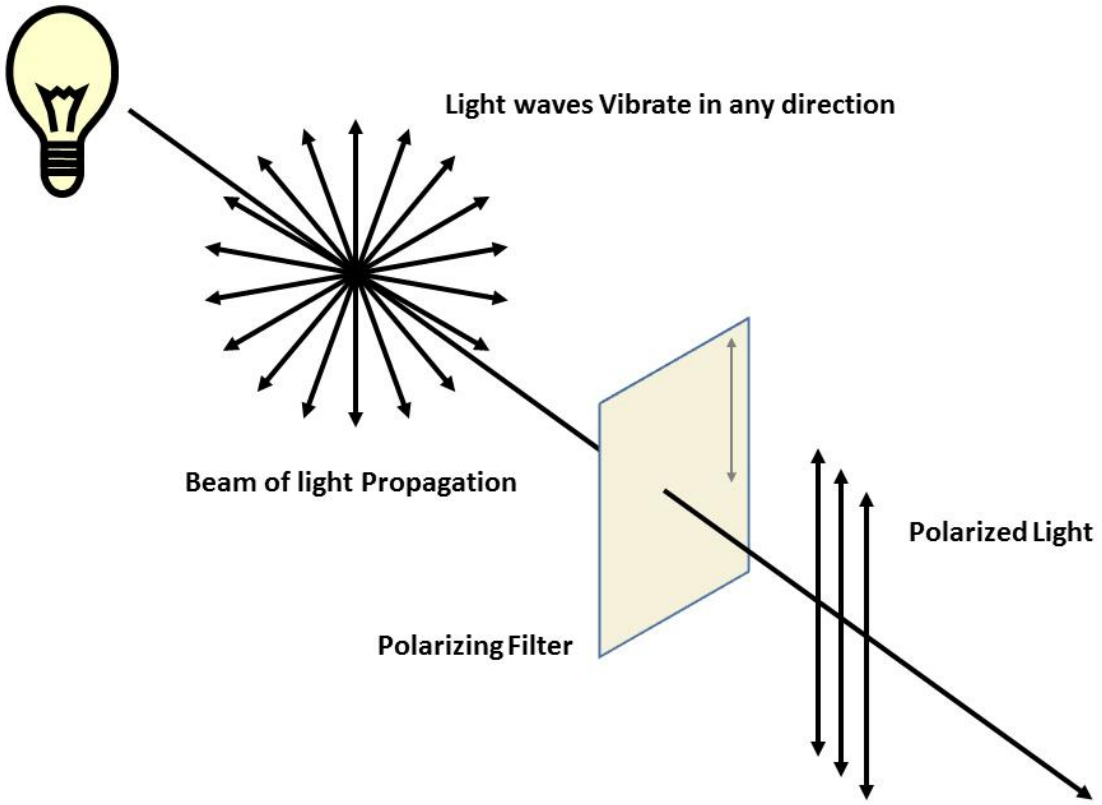


Fig.9.4 Polarisation by optical filter

half the intensity and with vibrations in a single plane (Fig.9.4), it emerges as ***polarized light***.

A polaroid filter is able to polarize light because of the chemical composition of the filter material. The filter can be thought of as having long-chain molecules that are aligned within the filter in the same direction. During the fabrication of the filter, the long-chain molecules are stretched across the filter so that each molecule is (as much as possible) aligned in say the vertical direction. As unpolarized light strikes the filter, the portion of the waves vibrating in the vertical direction are absorbed by the filter. The general rule is that the electromagnetic vibrations that are in a direction parallel to the alignment of the molecules are absorbed.

The alignment of these molecules gives the filter a ***polarization axis***. This polarization axis extends across the length of the filter and only allows vibrations of the electromagnetic wave that are parallel to the axis to pass through. Any vibrations that are perpendicular to the polarization axis are blocked by the filter. Thus, a polaroid filter with its long-chain molecules aligned horizontally will have a polarization axis aligned vertically. Such a filter will block all horizontal vibrations and allow the vertical vibrations to be transmitted (see diagram above). On the other hand, a Polaroid filter with its

long-chain molecules aligned vertically will have a polarization axis aligned horizontally; this filter will block all vertical vibrations and allow the horizontal vibrations to be transmitted.

Polarization of light by use of a polaroid filter is often demonstrated in a physics class through a variety of demonstrations. Filters are used to look through and view objects. The filter does not distort the shape or dimensions of the object; it merely serves to produce a dimmer image of the object since one-half of the light is blocked as it passed through the filter. A pair of filters is often placed back to back in order to view objects looking through two filters. By slowly rotating the second filter, an orientation can be found in which all the light from an object is blocked and the object can no longer be seen when viewed through two filters. The light is polarized upon passage through the first filter; perhaps only vertical vibrations were able to pass through. These vertical vibrations were then blocked by the second filter since its polarization filter is aligned in a horizontal direction. While you are unable to see the axes on the filter, you will know when the axes are aligned perpendicular to each other because with this orientation, all light is blocked. So by use of two filters, one can completely block all of the light that is incident upon the set; this will only occur if the polarization axes are rotated such that they are perpendicular to each other (see fig.9.5).

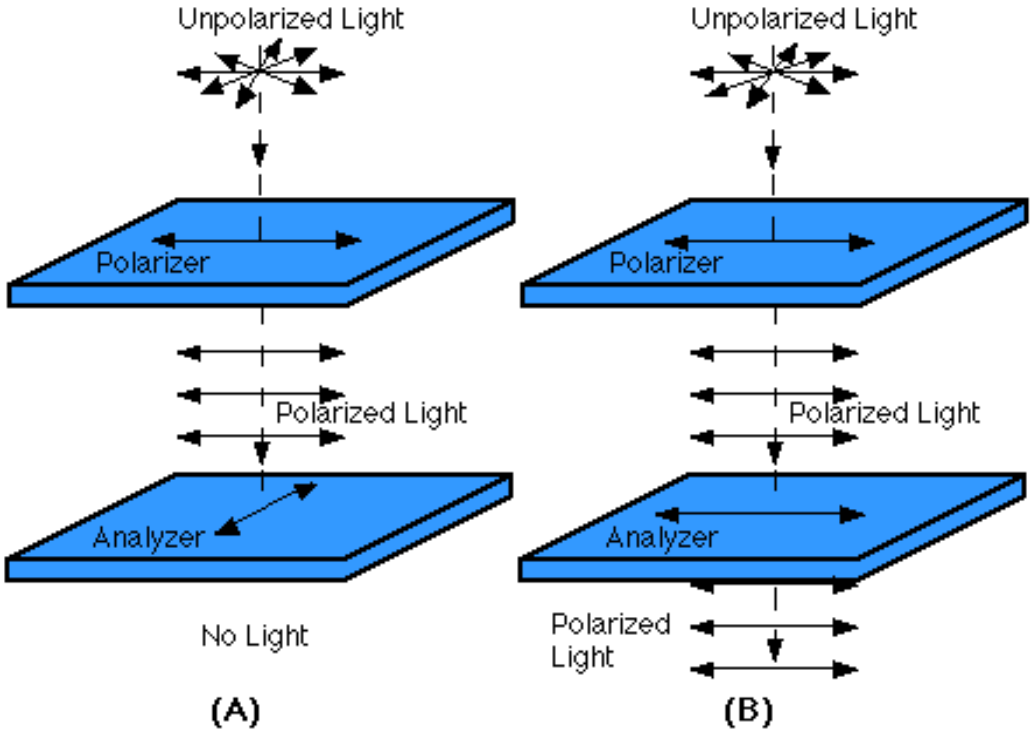


Fig.9.5. Dual-filter demonstration works

A picket-fence analogy is often used to explain how this dual-filter demonstration works (Fig.9.6). A picket fence can act as a polarizer by transforming an unpolarized wave in a rope into a wave that vibrates in a single plane.

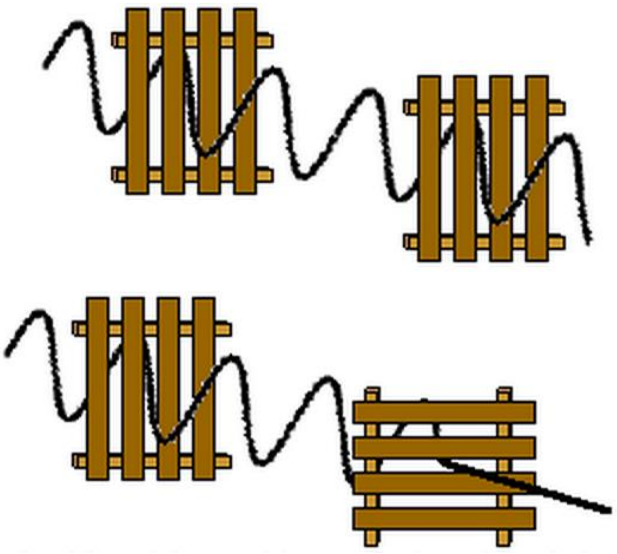


Fig.9.6. The picket-fence analogy

The spaces between the pickets of the fence will allow vibrations that are parallel to the spacings to pass through while blocking any vibrations that are perpendicular to the spacings. Obviously, a vertical vibration would not have the room to make it through a horizontal spacing. If two picket fences are oriented such that the pickets are both aligned vertically, then vertical vibrations will pass through both fences. On the other hand, if the pickets of the second fence are aligned horizontally, then the vertical vibrations that pass through the first fence will be blocked by the second fence.

Polarization by reflection and by refraction

Unpolarized light can also undergo polarization by ***reflection*** off of nonmetallic surfaces. The extent to which polarization occurs is dependent upon the angle at which the light approaches the surface and upon the material that the surface is made of. Metallic surfaces reflect light with a variety of vibrational directions; such reflected light is unpolarized. However, nonmetallic surfaces such as asphalt roadways, snowfields and water reflect light such that there is a large concentration of vibrations in a plane parallel to the reflecting surface. A person viewing objects by means of light reflected off of nonmetallic surfaces will often perceive a glare if the extent of polarization is large. Fishermen are familiar with this glare since it prevents them from seeing fish that lie below the water. Light reflected off a lake is partially polarized in a direction parallel to the water's surface. Fishermen know that the use of glare-reducing sunglasses with the proper polarization axis allows for the blocking of this partially polarized light. By blocking the plane-polarized light, the glare is reduced and the fisherman can more easily see fish located under the water.

Polarization can also occur by the ***refraction*** of light (Fig.9.7). Refraction occurs when a beam of light passes from one material into another material. At the surface of the two materials, the path of the beam changes its direction. The refracted beam acquires some degree of polarization. Most often, the polarization occurs in a plane perpendicular to the surface.

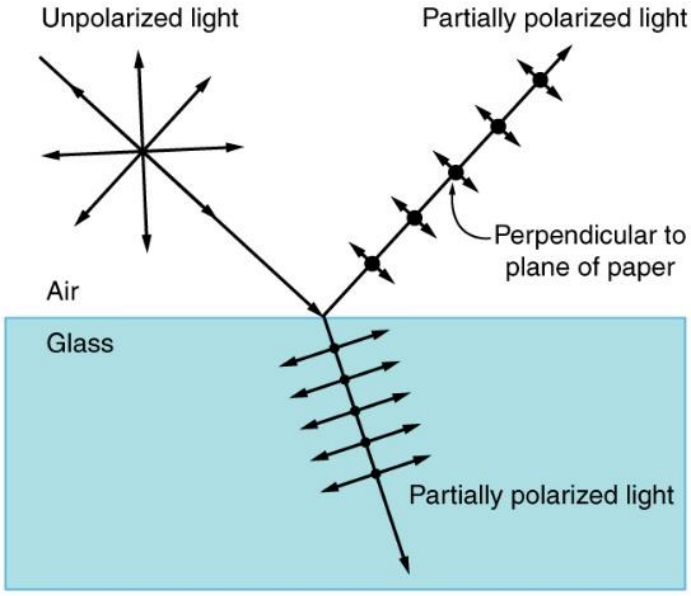


Fig.9.7. Reflection and refraction of light

Reflected and refracted lights are partially polarized; oscillations in the reflected ray take place in the incident plane, but the refracted ray-in the plane perpendicular to the first one. The polarization degree of the reflected light depends on the incident angle. If the incident angle satisfies the condition

$$tg\ i = n$$

where*n* is the refraction index of medium, then the total polarization of reflected light takes place (**law of Brewster**). It is demonstrated that angle between reflected and refracted light is 90°.

Polarization by scattering

The polarization of refracted light is often demonstrated in a physics class using a unique crystal that serves as a double-refracting crystal (Fig.9.8.). Iceland Spar, a rather rare form of the mineral calcite, refracts incident light into two different paths. The light is *split* into two beams upon entering the crystal. Subsequently, if an object is viewed by looking through an Iceland Spar crystal, two images will be seen.

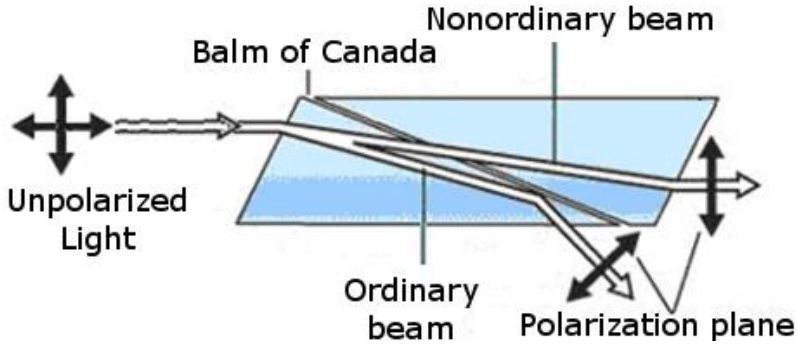


Fig.9.8. Crystal of Island

The two images are the result of the double refraction of light. Both refracted light beams are polarized - one in a direction parallel to the surface and the other in a direction perpendicular to the surface. Since these two refracted rays are polarized with a perpendicular orientation, a polarizing filter can be used to completely block one of the images. If the polarization axis of the filter is aligned perpendicular to the plane of polarized light, the light is completely blocked by the filter; mean while the second image is as bright as can be. And if the filter is then turned 90-degrees in either direction, the second image reappears and the first image disappears.

Determination of the sugar concentration in a solution by polarimeter

In 1813 Jean Baptiste Biot noticed that plane-polarized light was rotated either to the right or the left when it passed through single crystals of quartz or aqueous solutions of tartaric acid or sugar. Because they interact with light, substances that can rotate plane-polarized light are said to be ***optically active***. Those that rotate the plane clockwise (to the right) are said to be ***dextrorotatory*** (from the Latin *dexter*, "right"). Those that rotate the plane counterclockwise (to the left) are called ***levorotatory*** (from the Latin *laevus*, "left"). The instrument with which optically active compounds are studied is a polarimeter, shown in the figure below.

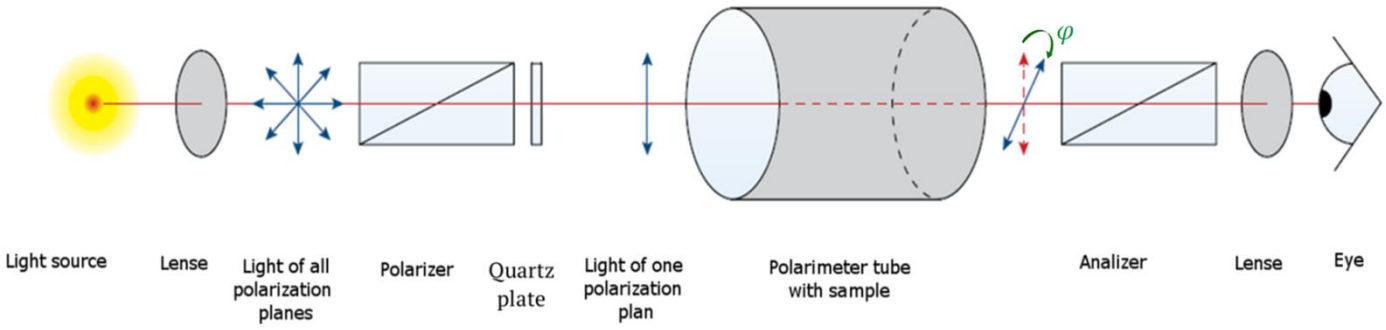


Fig.9.9. Schematic diagram of a polarimeter

When the sample tube is empty, the planes of polarization of the polarizing and the analyzing prisms are same and φ angle is 0°, but when the sample tube has a solution (optically active) substance, the plane of polarization of the emergent polarized light changes. One now needs to rotate the analyzer prism for its plane of polarization to coincide with the plane of the emergent light. This corresponds to the maximum intensity of the transmitted light. Theφdeviation angle is shown with a green arrow (see fig.9.9).

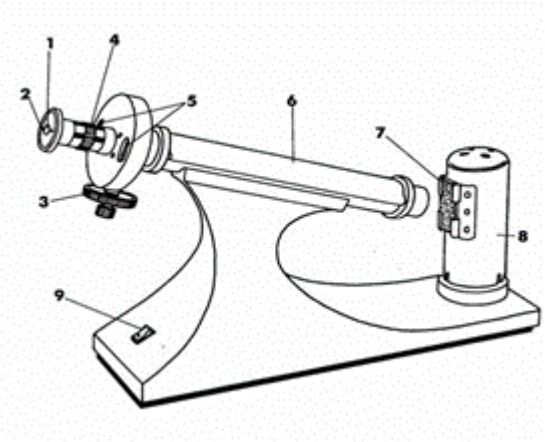
The magnitude of the angle through which an optically active substance rotates plane-polarized light depends on four quantities: (1) the wavelength of the light, (2) the length of the cell through which the light passes, (3) the concentration of the optically active compound in the solution through which the light passes, and (4) the ***specific rotation***of the compound, which reflects the relative ability of the compound to rotate plane-polarized light. ***Glucose*** is a carbohydrate, and is the most important simple sugar in human metabolism. Glucose is called a simple sugar or a monosaccharide because it is one of

the smallest units which has the characteristics of this class of carbohydrates. Glucose is also sometimes called dextrose. The specific rotation of the dextrorotatory isomer of glucose is written as follows:

$$[\alpha]_D^{20} = 52{,}75\frac{grad}{\%.m}.$$

Description of apparatus

The **Polarimeter** (Fig.9.10)is an instrument for measuring the optical rotation of a substance. By measuring the optical rotation, the Polarimeter can be used to analyse the concentration, content and



1. Reading lens
2. Eyepiece
3. Selection wheel
4. Scale and Verniers
5. Sample container
6. Polarizer
7. LED light
8. Polarimeter case

Fig.9.10. Polarimeter

purity of the substances. This instrument is suitable for use in laboratories in food, pharmaceutical and chemical industries, as well as in universities and research institutes.

The samples to be measured are placed in commercially avaible polarimeter tubes from 100 to 200 mm length. The light beam from the light source passes through the illuminating lens and the filter. The light beam is collimated and is polarized after passing through the polarizer. The light beam produces a triple shadow field on the $\pi/2$ wavelenght plate. The zero position can be ajusted by shifting the position of the analyser. The observation tube, filled with an optical active liquid, is placed between the polarazers. The light beam can then be seen on the wavelenght plate. By rotating one of the polarizers, the light beam is returned to the full shadow state and the angle of this rotation can be read from the scale.

The scale has 360º divisions of 1º each. The vernier has 20 divisions which are equivalent to 19 divisions on the scale. Two small reading magnifiers (1) are provided for easy scale reading and mounted at the side of the eyepiece. Without touching the selection wheel (3), read out the two opposite verniers.

Operating details:

1. Insert the power plug into the power source. Wait 3 minutes for the instrument to stabilize.
2. Open the sample compartment (5). Insert an empty polarimeter tube into the sample chamber.

3. Look through the eyepiece (2) and rotate it to the left or right until a clearly field will be observed. An equally illuminated yellow-orange field must be visible, like in the picture bellow (2).
4. The 0 division on the main scale and the 0 division on the Vernier must coincide. If this doesn't

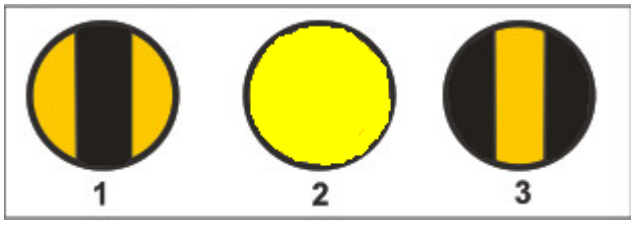
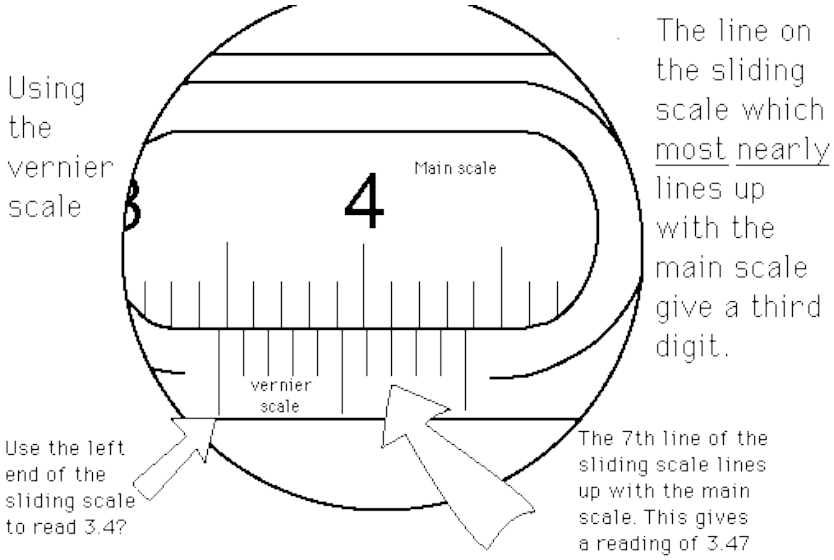


Fig.9.11. Field view

happen, then a calibration of the polarimeter should be done. Read the φ_0 angle from the scale, which describe the apparatus correction and insert the obtained value into the reserved column in the table below.The vernier caliper is used in length measurements to gain an additional digit of accuracy compared to a simple ruler. The details of the vernier principle are shown in the illustrations below.



5. Now, that the polarimeter is calibrated, take the tube out from it and fill it with the first solution. Introduce it back into the chamber.
6. Observe the field through the eyepiece and focus it until the same uniformly illuminated field (2 from Fig.9.11) is obtained.
7. Read the indication on the scale, which will be noted by φ'angle, that describes the analyzer position when the light passes through the first solution.
8. Repeat these manipulations three times for the same solution.
9. Repeat the 5, 6, 7, and 8th step for the second solution, which will be a glucose solution.
10. Determine the unknown concentration following the formula, which discribe a relative method of the concentration determination:

$$C_x = C\frac{\varphi_x}{\varphi}100\% \quad (1)$$

DETERMINATION OF CONCENTRATION OF OPTICALLY ACTIVE SUBSTANCES BY POLARIMETRICMETHOD

Note that here φ, C is the deviation angle and concentration for water, but φ_x, C_x is the deviation angle and concentration for the unknown solution, which is the glucose solution. The water concentration can be easily determined following the formula

$$C_x = \frac{10\varphi}{[\alpha]l} 100\% \quad (2)$$

where l is the length of tube with solution, $[\alpha]$ is specific rotation and $\varphi$is the angle with witch the solution rotates the polarization plan of the polarized light.

Table

The researched solution	Experiment No.	φ_0, degree	φ', degree	$\varphi = \varphi' - \varphi_0$	C ,%
1	1				
	2				
	3				
2	1				
	2				
	3				

11. Formulate the observations and the necessary conclusions.

Applications in medicine

The study of optical activity of liquids began in the early 19th century with Biot and others scientists. They found that solutions of sugar and certain other naturally occurring chemicals would rotate a beam of polarized light passing through the solution.

It may be noted that approximately 25% of all drugs are marketed as either racemates (mixtures of two enantiomers) or mixtures of diasteromers. The orientation around a chiral center can have a dramatic impact on the pharmacological response of that drug in the human body. Such recent observations brought about severe tightening in the laws surrounding the introduction of new drugs into the market. Thus, chiral synthesis and purification became a crucial aspect of all successful drug manufacturing procedures. This is just one of the several areas highlighting the importance of polarimetric studies.

In 1991 was used a method based on cross polarization to analyze some cancerous skin features. This method consisted on accenting or rejecting surface glare by viewing the skin through an analyzer oriented parallel or perpendicular to the incident polarized beam. The use of polarized light imaging can facilitate the determination of skin cancer borders before a Mohs surgery procedure. Linearly polarized light that illuminates the skin is backscattered by superficial layers where cancer often arises and is randomized by the collagen fibers. The superficially backscattered light can be distinguished from the diffused reflected light using a detector analyzer that is sequentially oriented parallel and perpendicular to the source polarization.

Some of the applications in the field of medicine include identification of gout crystals, amyloidal, muscle and nerve tissue as well as spindles and actomyosin fibers, and even identification of malarial pigments.

Polarized light microscopy and ***tooth polarized light microscopy*** has been widely used to study teeth, by making use of the differing birefringent properties of the components of the dental tissues. When compared to light microscope, polarized microscope helps in identification and distinguishing all the structures of the tooth, for example:

- Study of hard tissue structures and comparison of structures between deciduous and permanent teeth.
- Demineralization and remineralization studies using various materials on enamel.

DETERMINATION OF CONCENTRATION OF OPTICALLY ACTIVE SUBSTANCES BY POLARIMETRICMETHOD

- Studies on different aspects of caries.
- Identification of various structures in dentin (neo natal line).
- Study of cemental annulations for age estimation.
- Study of dental fluorosis.
- Study on enamel and dentin dysplasia
- Study on enamel hypo maturation.
- Effect of tooth bleaching.
- Collagen fiber identification in periodontal ligament (picrosirius red stain).
- Collagen analysis in human tooth germ papillae.

Light therapy or ***phototherapy*** consists of exposure to daylight or to specific wavelengths of light using polychromatic polarized light, lasers, light-emitting diodes, fluorescent lamps, dichroic lamps or very bright, full-spectrum light. The light is administered for a prescribed amount of time and, in some cases, at a specific time of day. One common use of the term is associated with the treatment of skin disorders, chiefly psoriasis, acne vulgaris and eczema. Light therapy which strikes the retina of the eyes is used to treat circadian rhythm disorders such as delayed sleep phase disorder and can also be used to treat seasonal affective disorder, with some support for its use also with non-seasonal psychiatric disorders.

Knowledge evaluation exercises:

1. What is linearly polarized light?

a) is the light that, after double refraction, contains only the vector $\vec{E}$;
b) is the light in which the vector $\vec{E}$ presents a single oscillation direction;
c) as an example we can name the extraordinary ray that comes out of the Nicol prism;
d) is the light in which the vector $\vec{E}$ presents more oscillation directions, but has a preferred orientation;
e) as an example we can name the natural light.

2. What are optically active substances?

a) can be dextrorotatory, which rotate the plane of polarized light to the right;
b) those substances that do not rotate the plane of vibration of polarized light it crosses;
c) they can be levorotatory, which rotate the plane of polarized light to the right;
d) organic substances cannot be;
e) Their concentration can be determined by conductometric method.

3. After passing through a crystal of Iceland, light is polarized. Which beam is polarized?

a) only ordinary beam;
b) only nonordinary beam;
c) The reflected beam;
d) scattered beam;
e) both the ordinary and the nonordinary beam.

4. On what does the rotation angle of the vibration plane depend?
a) on the concentration of the optically active solution;
b) on the incidence angle of the light beam on the polarizer;
c) only on the wavelength of the incident light;
d) on the length of the solution layer travelled by the light;
e) only on the nature of the solvent.

5. In the relation for calculating the concentration using the polarimetric method, what does α represent?
a) the refraction angle;
b) the incident angle;
c) the angle formed by electric field vector $\vec{E}$ and the magnetic field vector $\vec{H}$;
d) the rotation angle of the vibration plane;
e) the refraction angle of the Canada balsam.

6. Using the polarimetric method, the angle $\alpha = 2.45^{o}$ is determined for a glucose solution. Given are $[\alpha]_D^{20} = 52.75^{o}/dm$ and the length of the tube $l = 2dm$. The obtained concentration value is:
a) 2.63;
b) 2.18;
c) 2.58;
d) 2.32;
e) 2.44.

7. What can be affirmed about optically active substances?
a) they can be dextrorotatory, which rotate the vibration plane of the polarized light towards the right;
b) are those substances that do not rotate the vibration plane of the polarized light that travels through them;
c) can be levoratorotatory, which rotate the vibration plane of the polarized light towards the right;
d) do not contain molecules with asymmetric structure;
e) cannot be organic substances.

8. In natural light:
a) the electric field vector, $\vec{E}$, oscillates in a single direction;
b) the electric field vector, $\vec{E}$, oscillates in all directions, in a plane that contains the propagation direction of the wave;
c) the electric field vector, $\vec{E}$, has a preffered oscillation direction, which, however, is not unique;
d) the electric field vector, $\vec{E}$, oscillates in all directions, in a plane perpendicular to the propagation direction of the wave;
e) the magnetic field vector, $\vec{H}$, oscillates along a single direction.

10. STUDY OF COLORED SOLUTIONS BY FOTOCOLORIMETRY METHOD

Purposes:

- Study of the phenomenon about light absorption;
- Study of the construction and working principle of photoelectric colorimeter;
- Familiarization with colorimetric method applications in medicine;
- Using photoelectric colorimeter for the determination of the concentration of substances in solution.

Theoretical notions:

Absorption of light. Bouguer -Lambert law

Light, electromagnetic nature and dualistic properties (wavelength and corpuscular), is characterized by multiple sizes as, for example, the flow of light, light intensity, etc. The amount of energy carried by electromagnetic wave through a certain surface per unit of time is called the ***flow of light***.

Light intensity (or light flux density) is called the amount of energy carried by the wave of light in a unit time through a unit surface, perpendicular to the direction of propagation of the wave. The passage of light through a layer of substance, its intensity attenuates. This decrease intensity occurs as a result of the interaction of light with wave substance through which it passes. Wave of light causes the forced oscillations of electrons in atoms and molecules. This consumes a portion of the wave energy, which usually turns to other forms of energy. The phenomenon in which the light intensity attenuation when passing through any substance as a result of the transformation of light energy into other forms of energy, is called the ***absorption*** of light (fig.10.2).

Absorption of light can cause warming substances, ionization, excitation of atoms or molecules, chemical processes, etc. For example, visible and infrared radiation, which is absorbed by the skin, it causes warming the body. Ultraviolet radiation causes photochemical reactions, absorbed in the upper layer of the skin, as a result the skin tans areas, i.e. it forms the pigment color dark Tan. Absorption law of a parallel beam of monochromatic light, in a homogeneous medium was discovered by Bouguer and developed by Lambert.

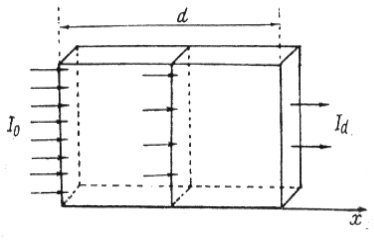


Fig.10.1. Absorbtion of light

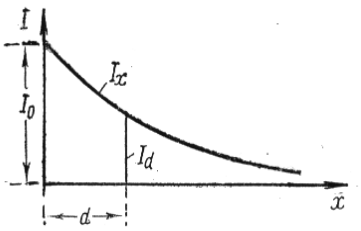


Fig.10.2. Exponential dependence of light intensity

According to this law:

$$I_d = I_o e^{-kd} \quad (1)$$

Where I_0- the incident light intensity, I_d- intensity of light that has passed through a layer of the thickness of the substance d, e – base of natural logarithms ($e = 2{,}72$) and k – coefficient of absorption of natural

substance.

The coefficient k depends on the wavelength of the light and the nature of the substance. The negative power in formula (1) indicates that the intensity of light passing through the substance decrease. Formula (1) is the mathematical expression of Bouguer-Lambert law. This law, in terms of light flows, can be expressed as:

$$\Phi_d = \Phi_0 e^{-kd} \tag{2}$$

where Φ_0- the flow of incident light, Φ_d- the flow of light that has passed through a layer of d thick substance. Because the natural absorption coefficient of the substance depends on the wavelength of the light, the Bouguer-Lambert las, for monochromatic light, is done by:

$$I_d = I_0 e^{-k_\lambda d} \tag{3}$$

where k_λ is the monochromatic absorption coefficient of the substance naturally. The intensity of the light I_a absorbed by the substance, is obtained from:

$$I_a = I_0 - I_d \tag{4}$$

Bouguer-Lambert law establishes that the intensity of the light (or the flow of light) decrease with increasing thickness d of the layer, when passing through it, by an exponential law (see fig.10.1, 10.2). The layers of substances with same thickness, in identical conditions, will always absorb the same part of the light.

In formula (3), if $k_\lambda = \frac{1}{d}$ then

$$I_d = \frac{I_0}{e} = \frac{I_0}{2{,}72}\,. \tag{5}$$

For various substances, the monochromatic absorption coefficient has different values. For example, air's coefficient k_λ value is $0.001m^{-1}$, water - $0{,}4m^{-1}$. Therefore, to decrease the intensity of the incident light about 3 times, we need to use a water layer with a $d = 0.4\ m$ thickness or a layer of air with $d = 10^3\ m$ thickness.

Lambert Beer's law

Researching the monochromatic light absorption in low concentration solutions, Beer has established:

1. absorption of the monochromatic light in colorful solutions takes place according to law Bouguer-Lambert;
2. the monochromatic absorption coefficient of colored solutions is direct proportional with its concentration:

$$k_\lambda = \chi_\lambda C \tag{6}$$

where χ_λ is the monochromatic molar absorption coefficient. This coefficient, at certain temperature and wavelength of light, has a constant value for each substance and therefore, can serve as a characteristic of the substance.

Substituting formula (6) in the Bouguer-Lambert law (3) we will obtain the expression for the Bouguer-Lambert-Beer's law:

$$I_d = I_0 e^{-\chi_\lambda C d} \tag{7}$$

Optical transmission coefficient and extinction of the solution

The ***optical transmission*** (transmission, transparency) of the substance is the ratio of the intensity of the light that passed through the substance I_d and incident light intensity I_0

$$\tau = \frac{I_d}{I_0} \tag{10}$$

In terms of light flows, the optical transmission coefficient can be expressed as:

$$\tau = \frac{\Phi_d}{\Phi_0} \tag{11}$$

The optical transmission coefficient (transparency) of the substance determines which part of the light passes through the substance (the solution) and it can be expressed in %.

The ***extinction*** coefficient (optical density) of the substance determines which part of the light is absorbed by the substance and is done by expression:

$$D = ln\left(\frac{1}{\tau}\right). \tag{12}$$

Applications in medicine

One of such instruments is microcalorimeter photovoltaic MKMF-1, intended to determine the concentration of biochemical components in the painted solutions obtained by the methods of ultramicrosize, microanalysis and macro-analysis. These devices are designed to perform one or more specific types of analyses that are common in medical practice or research. The purpose of these instruments is to reduce the complexity of the analyses. Among the produced equipment - hemoglobinometer, bilirubinometry, saaremetsa. They are usually calibrated directly on the content of this component in units of concentration, and when the measurement is not required recalculation.

Absorption spectra are sources of information about the composition and structure of substances and, therefore, their analysis shows a main method of studying the various properties of the substances, including biological environments. Absorption spectra analysis applies in medicine, for example, to determine the blood oxygen saturation, method called oxyhemometry. This method is based on the variation of the absorption spectrum of blood depending on his oxygen saturation. On the basis of absorption of light were worked out different photometric methods for the study of the solutions, in particular, colorimetry concentration, which is a special case of the photometry and the determination of the concentration of solutions.

This method has a special importance in studying nutrients (substances that are found in very small amounts in the blood and in different tissues of the human body). Using the colorimeter can determine the concentration of nutrients with an accuracy range within the limits *(10^{-4}-10^{-8}) g/ℓ*, used in determination of amount of many substances in blood, urine, saliva, for example: determination of blood glucose, blood urea, serum creatinine, serum proteins, serum choresterol.

In biology, a colorimeter can be used to monitor the growth of a bacterial or yeast culture. As the culture grows, the medium in which it is growing becomes increasingly cloudy andabsorbs more light.

Determination of organic substances and pharmaceutical, for example, only few drugs are colored and most of the drugs are white or colorless. Hence one can add chromogenic reagents to drugs for color

development and whose intensity is directly proportional to concentration.

Equipment and materials:

Photoelectric colorimeter and accessories, distilled water; solutions (4-5) of the substance with known concentrations of colored researched, solutions (1-2) of the same substance with unknown concentrations, gauze.

Description of the facility

Besides being valuable for basic research in chemistry laboratories, colorimeters have many practical applications. For instance, they are used to test for water quality, by screening for chemicals such as chlorine, fluoride, cyanide, dissolved oxygen, iron, molybdenum, zinc and hydrazine. They are also used to determine the concentrations of plant nutrients (such as phosphorus, nitrate and ammonia) in the soil or hemoglobin in the blood.

To determine the concentration of the solutions use colored colorimeters that are of two types: photometers and photo colorimeters. In this work we use photoelectric KF colorimeter 77, which directly measure the optical transmission coefficient and extinction of solutions.

Principle of photoelectric colorimeter can be explained on the basis of the scheme simpler photoelectric colorimeter represented in Fig.10.3.

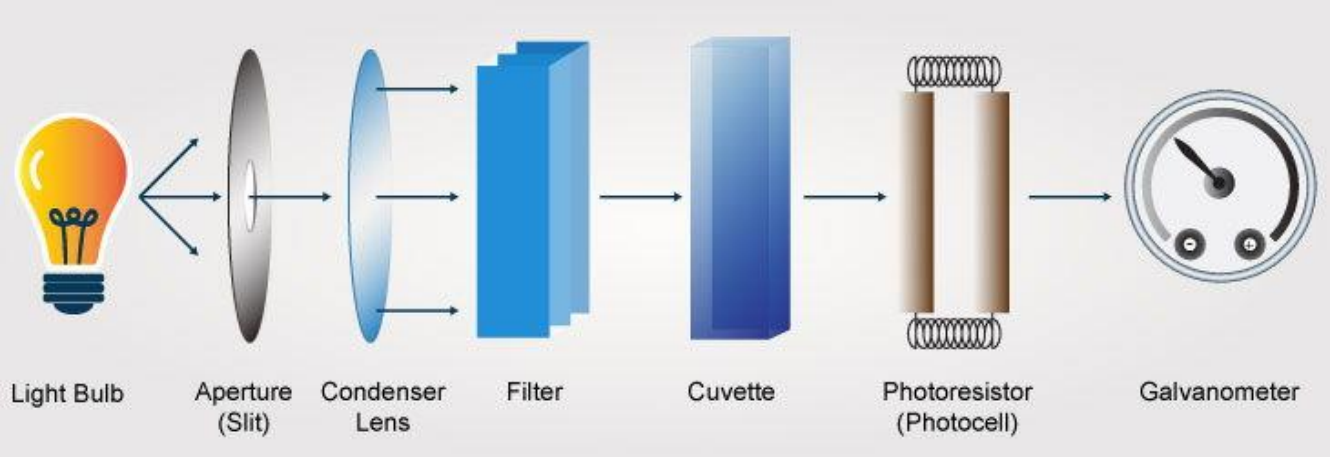


Fig.10.3. Scheme of colorimeter

where S – a source of light, K-glass cuvette, L-lens, CF – photovoltaic cell, F – optical filter, G-galvanometer.

The solution is poured into the tank, investigated k. light at source, passing through the filter F and K with cuvette, falls on the photovoltaic cell CF at the terminals photovoltaic cell is joined galvanometer G, the deviation of the needle which is proportional to the size of the flow of light through the solution.

Working mode:

1. Choose the glass filter and insert it into the filter.
2. Take the test solution (distilled water) a cuvette and read the optical density.

3. Take the standard solution in varying concentration and note down the optical density as C_1, C_2, C_3, C_4, C_5, X_1 and X_2.
4. Make a graph (a graph is plotted taking concentration of standard solution versus the optical density).
5. From the graph, the concentration of the X_1 and X_2 solutions (unknown solutions) can be calculated.
6. Formulate the conclusions.

Table

The number of the solution.	C,%			τ,%	*D*
1.					
2.					
3.					
4.					
5.					
$X_{1.}$					
$X_{2.}$					

Knowledge evaluation exercises:

1.Choose the right relation for the transmission. (Notes: I_0 –incident light intensity, I_d - emerging beam intensity,*d*- the thickness of substance's layer, *k*-the coefficient of absorption).

a) $= \frac{I_d}{I_0}$;

b) $\tau = \frac{I_0}{I_d}$;

c)$\tau = \exp(-kd)$;

d)$\tau = 10td$;

e) $\tau = x_0 \exp(-kd)$;

2.Choose the right statement for Lambert's Law: (Notes: I_0 –incident light intensity, I_d - emerging beam intensity,*d*- the thickness of substance's layer, *k*-the coefficient of absorption).

a)it has the expression:$I = I_0 \exp(kd)$;

b)it is based on observation that the emerging beam intensity increases according to an exponential law when it crosses through a layer of substance;

c) suppose exponential reduction of the light beam's intensity with increasing of the thickness of substance's layer crossed by the light beam;

d) the coefficient of absorption does not depend on the incident light's wavelength;

e) quantitatively describes the absorption of the electromagnetic radiation that crosses through a medium .

3.Pick up the right answer for the transmission:

a) it characterizes the decrease of the light beam's intensity when it crosses through a layer of substance;
b)it is a dimentionless physical size;
c) define the ratio between the incident beam intensity (I_0) and the emerging beam intensity(I);
d) it is always subunitar ($\tau < 1$);
e)it is expressed by percentages;

4.The absorption of a solution:
a) is defined by the expression:$A = \lg\left(\frac{1}{\tau}\right)$,(τ-transmission);
b) doesn't have a measurement unit;
c) increases linearly with transmission;
d) according to Lambert-Beer's law, increases in direct proportion with the concentration of the solution;
e)doesn't depend on incident beam intensity.

5.Choose among the right physics meanings, which are contained in Lambert-Beer's Law$I_d = I_0 10^{-\chi_\lambda C d}$:
a) I_d - absorbed light's intensity;
b) I_0- incident beam intensity;
c) C -molar concentration of the solution;
d)d - the thickness of substance's layer crossed by the light;
e) χ_λ - molar absorption coefficient.

6. Which physics measurement described by the Lambert-beer's Law depends on the incident light's wavelength?
a) incident beam intensity;
b) emerging beam intensity;
c) the ratio between $\frac{I}{I_0}$;
d) molar absorption coefficient;
e) the thickness of substance's layer.

7.Choose the right answer:
a) the absorption spectrum of a substance affirms absorption's variation with the incident radiation's wavelength chart;
b) the absorbance,A, of a solution doesn't depend on dissolved substances concentration;
c) the transmission τ defines as: $\tau = \lg\left(\frac{1}{\tau}\right)$;
d) the absorption of a solution that contains several dissolved substances expresses the sum of the solution's absorption for each substance;
e) the transmission is an additive size.

8. The concentration of a solution can be determined by the photometric method if only:
a) the dissolved substance is optically active;
b)the solution meets Malus's Law;
c)the solution absorbs a part of electromagnetic incident radiation;
d) the solvent partly reflects the incident light;

e) the intensity of the reflected light on the surface of cuvette is negligible.

9. In the laboratory medicine the photometry:
a) is used in conjunction with chemical methods which leads to the formation of colored solutions;
b) allows to determine the concentration based on the extent to which the light's wavelength increases with the concentration of the studied compound;
c)it is used only for dosage of the chemical compounds which absorbs in the spectral area of radiation generated by the spectrophotometer;
d)allows to dose all the electrolytes;
e)can be used to measure the intensity of the light emitted by the solution.

10. Pulse Oximetry:
a) is an invasive technique to diagnose the cardiac failure;
b) is based on autofluorescence's measurement of the living tissue;
c) it can be used to determine the heart rate thanks to the periodic variation of the layer's thickness of the investigated tissue;
d) it is a method of determination of the oxygenated hemoglobin concentration and reduced by the absorption's measurement of a piece of tissue at two wavelengths;
e) allows the pulse measurement and the respiratory rate.

11. DISPERSION OF ELECTRICAL IMPEDANCE OF BIOLOGICAL TISSUES

Pursoses:

- Presentation of theoretical notions regarding alternative current parameters and the peculiarities of biological tissues impedance;
- Study of construction and the functional principle of the applied installation;
- Determination of electrical parameters of biological tissue for the different frequencies of the current;
- Familiarization with some applications of the research method in medical practice.

Theoretical notions

Current is the flow of charged particles through a conducting medium, such as a wire. When we talk about electricity, the charged particles we're referring to are almost always electrons. You see, the atoms in a conducting material have lots of free electrons that float around from atom to atom and everywhere in between. The motion of these electrons is random, so there is no flow in any given direction. However, when we apply a voltage to the conductor, all of the free electrons will move in the same direction, creating a current.A curious thing about electric current is that while the electrical energy transfers through the conductor at nearly the speed of light, the electrons themselves move much, much slower. In fact, if you were to walk leisurely alongside a current carrying wire, you would be traveling more than 100 times faster than the electrons.

There are two different types of current in widespread use today. They are direct current, abbreviated DC, and alternating current, abbreviated AC. In a ***direct current*** the electrons flow in one direction, it is represented by the graph from figure 11.1a. Batteries create a direct current because the electrons always flow from the 'negative' side to the 'positive' side.***Alternating current***, abbreviated AC, pushes the electrons back and forth, changing the direction of the flow several times per second, it is represented by the graph from figure 11.1b. In Moldova, the current changes direction at a rate of 50Hz, or 50 times in one second. The generators used in power plants to produce electricity for your home are

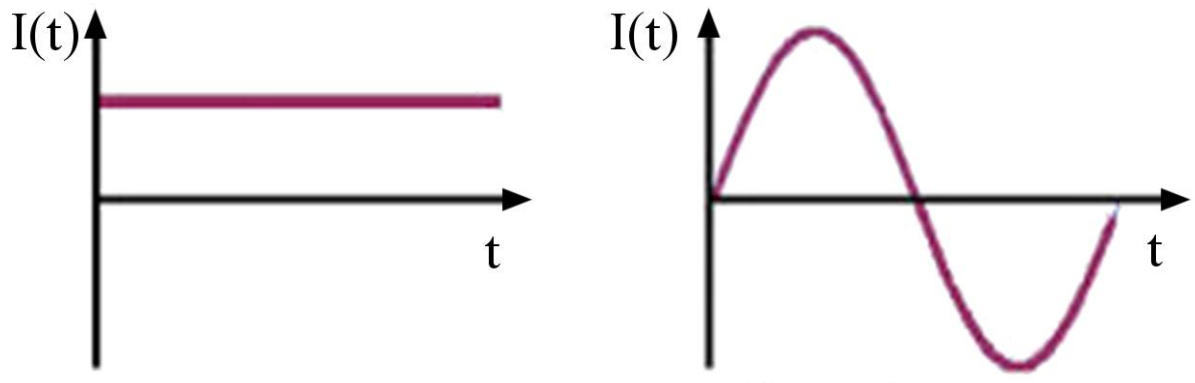


Fig.11.1. Direct (left) and alternating (right) current

designed to produce alternating current. You've probably never noticed the lights in your house actually flicker as the current changes direction because it happens too fast for our eyes to detect.

The AC is produced by the electric field that varies in time by sinusoidal law:

$$E = E_m sin\omega t$$

here *E* is the momentum value of electric field, $E_m$is the maximal value, ω is pulsation. Pulsation depends on the frequency and it is given by the formula:

$$\omega=2\pi\nu.$$

Intensity of the current in a circuit with resistance will vary according to the phase:

$$I = I_m \sin(\omega t + \varphi_0)$$

where φ_0 is phase difference between the intensity and voltage. If the conductor has the solenoid (coil) form, than the self induction phenomenon takes place, which makes the intensity of the alternating current to obtain the same values (zero, maximum, etc.) as voltage, andphase difference being$\varphi_0 = -\frac{\pi}{2}$. For a capacitance (capacitor) the phase difference is$\varphi_0 = \frac{\pi}{2}$. Active resistance does not introduce any phase difference.

For a circuit made from active resistance R, a coil with the inductance L and capacity C, connected in seriesand powered by an AC (fig.11.2), the total resistance or impedance is given by the equation below:

$$Z = \sqrt{(R + R_L)^2 + \left(\omega L - \frac{1}{\omega C}\right)^2}, \qquad (1)$$

where $\omega L = X_L$ is inductive reactance, $\frac{1}{\omega C} = X_C$ is the capacitive reactance and R_L is the resistance of DC of the coil.

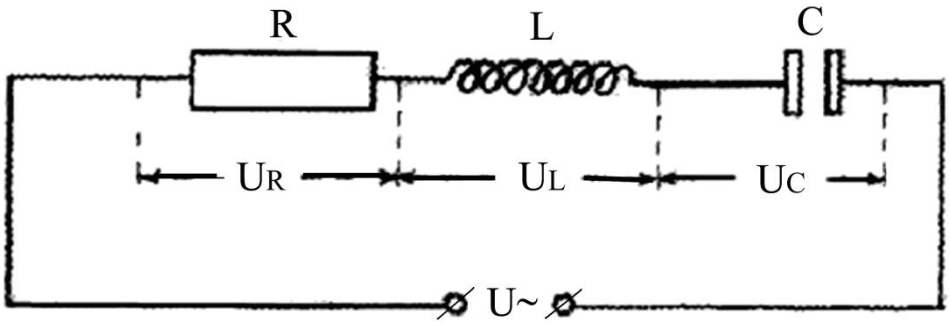


Fig.11.2. Electric circuit

It was found that living tissue inductive reactance missing. Given this fact, living biological tissues impedance is determined from the relation:

$$Z = \sqrt{R^2 + \left(\frac{1}{\omega C}\right)^2}.$$

The presence of capacitive reactance is caused by the cell membranesand depends on their geometric parameters and the environmental.The phenomenon of impedance variation of living tissue depending on the frequency of electric current and it is called ***impedance dispersion***.Graphic this dependence is represented by the ***dispersion curve*** (Fig.11.3). The frequency range in which it appears the dispersion of impedance is called***domain of dispersion***. Often instead of to build dispersion curveis determined the coefficient of polarization (*K*):

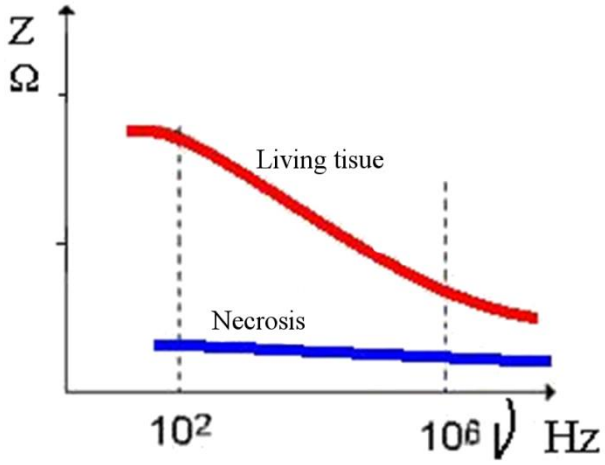


Fig.11.3 Dispersion curve

$$K = \frac{Z_{\omega \min}}{Z_{\omega \max}}$$

where, $Z_{\omega min}$ is the impedance of tissue at the minimum frequency and $Z_{\omega max}$is the impedance of tissue at the maximum frequency. At necrotisation tissue polarization coefficient tends toward one. Note that the above are characteristic of both animal tissues and those of plant.

Electrical properties of tissue

The electrical properties of biological tissues result from the interaction of the applied electromagnetic radiation and the constituents of the tissue at the cellular and molecular level. Three other factors that greatly affect the electrical properties of tissue include: the polarization of water molecules, the polarization of cell membranes which act as barriers to the flow of ions in and out of the cell, and ionic diffusion through the cellular membrane. Human skin tends to have high electrical impedance compared to other biologic tissues due to the properties of the stratum corneum. It has been shown that skin has a statistically higher impedance than the other tissues of the body. These higher impedances are caused by lower water content and the physical structure of the stratum corneum compared to other tissues.

Tissues exhibit the properties ofconductors and dielectrics, they containbothfree and bound (fixed) charges. As a result tissue impedance contains both conductingand dielectric terms. The conductivity term accounts for the movement of freecharges and the relative permittivity term accounts for the movement of boundcharges in the dielectric due to an applied electrical field.The electrical properties of tissue can be described by considering that the current flows in the extracellular fluid atlow frequency and in both the intracellular and extracellular fluids of tissue at highfrequency. The cell membranes will determine how current flows inside the cell. Cellmembranes are composed mainly of proteins and water-insoluble lipids. Therefore, it is expected that impedance will dropwith increasing frequency.

Tissue impedance measurement

The ratio of the potential resulting between two electrodes in contact with tissue to thecurrent injected between two other electrodes will be called transfer impedance. Allmethods for measuring tissue

transfer impedance use electrodes, which inject a knowncurrent into the tissue. Basically, tissue impedance involves the injection of a constant currentinto tissue at different frequencies and measurement of the resultant voltages. Theresultant voltage can be measured by using the same electrodes which were used forcurrent injection (bipolar technique) or a separate pair of electrodes can be used forpotential measurement (tetrapolar technique). Most tissue impedance measurements usethe tetrapolar technique. Theaccuracy of the measurements is dependent on the characteristics of the electronics.

Medical applications

Since the 1930's basic electrical impedance measurementsin biology have been well known.Applications that are already accepted in clinics includethe use of electrical impedance (EI) measurements in functionalstudies of the heart and the cardiovascular system suchas impedance cardiography (ICG), in examination of the digestivesystem and in monitoring of the respiratory system. At present much research effort is being concentratedon cancer detection (mammography) and brain examination.However, in these applications the clinical use of EIT has stillnot proved very successful, despite the fact that many researchgroups are deeply involved in the development of measurementinstrumentation and appropriate software, including newreconstruction algorithms.

- **Impedance spectrometry for tissue characterization**- for the characterisation of tissue spectroscopic studies are performed on the basis of determination of its complex electrical impedance as a function of frequency. The modulus and the phase angle between the excitation current and voltage or the components of resistance and reactance measured over a broad frequency range should be determined. In order to solve the problem of making accurate measurements, special cells for *invitro* and *invivo* measurements are constructed. This method enables tissue to be characterized in different states, such as cancer and ischemia, and practically in clinical conditions allows for the examination of body composition and the evaluation of the extracellular andintracellular water ratio in the region of the body under study.
- **Impedance cardiography (ICG) and pletysmography (IPG)-** Impedance cardiography was introduced for the measurement of cardiac stroke volume, is based on a tetrapolar current method and a simplified model of the thorax. The traditional measurement of the electrical impedance modulus of a body segment, usually for a single frequency in the range 20–100 kHz, is widely used for the detection of respiration signals and for the evaluation of basic haemodynamic parameters, such as stroke volume, cardiac output, intervals of the cardiac cycle and limb blood flow. An application of particularnote is the use of impedance pletysmography and cardiography for the examination of total systemic blood flow (SBF).
- **Electrical impedance tomography (EIT)-** The development of electrical impedance tomography (EIT) depends on advances in the modelling of the organs tested, on the development of reconstruction algorithms and on the construction of instrumentation allowing 2-D and 3-D measurements and analysis. The main advantages of this technology are the relatively low cost of instrumentation and its safety in operation, while the main disadvantages are the limited spatial and temporal resolutions of the method, depending on the limitednumber of electrodes applied and the limited resolution of the reconstruction algorithms.

The methods presented are relatively inexpensive and easy touse in a number of medical applications, which constitute themain arguments for employing EI in clinical practice.

Demonstratively experiment

In the class will be demonstrated experiments of electric impedance with live and dead biological tissues. The installation is made up of the electrodes through the milliammeter is connected to a sound generator and parallel to electronic oscilloscopes.

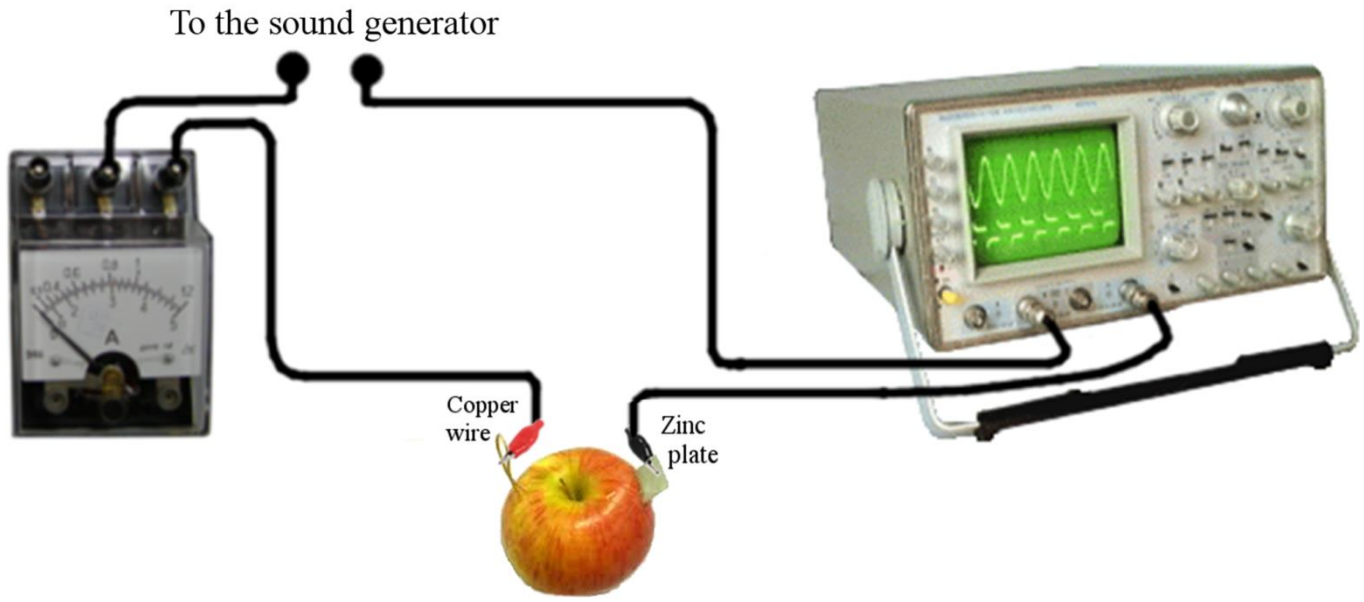


Fig.11.4

ANNEX

Table1. Physical quantities and their SI base units

Physical Quantity	**Unit**	**Symbol**
length	meter	m
mass	kilogram	Kg
time	second	s
electric current	ampere	A
thermodynamic temperature	kelvin	K
amount of substance	mole	mol
luminous intensity	candela	cd

Table 2. Some SI derived units

Physical Quantity	Unit	Symbol
area	square meter	m^2
volume	cubic meter	m^3
frequency	hertz	Hz
mass density (density)	kilogram per cubic meter	kg/m^3
speed, velocity	meter per second	m/s
angular velocity	radian per second	rad/s
acceleration	meter per second squared	m/s^2
angular acceleration	radian per second squared	rad/s^2
force	newton	N
pressure	pascal	Pa
work, energy, quantity of heat	joule	J
power	watt	W
quantity of electricity	coulomb	C
potential difference, electromotive force	volt	V
electric field	volt per meter	V/m
electric resistance	ohm	Ω
capacitance	farad	F
magnetic flux	weber	Wb
inductance	henry	H
magnetic field	tesla	T

Table 3. Decimal multiples and submultiples of SI units

10^3	kilo	k
10^2	hecto	h
10^1	deca	da
10^{-1}	deci	d
10^{-2}	centi	c
10^{-3}	mili	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p

Table 4. Greek alphabet

Capital letters	Description (pronouncing)	Capital letters	Description (pronouncing)	Capital letters	Description (pronouncing)
Αα	alfa	Ii	iota	Ρρ	ro
Ββ	beta	Κκ	capa	Σσs	sigma
Γγ	gama	λ	lamda	Υυ	ipsilon
Δδ	delta	Μμ	miu	Φφ	fi
Εε	epsilon	Νν	niu	Χχ	hi
Ζζ	zeta	Ξξ	xi	Ψψ	psi
Ηη	eta	Οο	omicron	Ωω	omega
Θθ	teta	Ππ	pi		

Table 5. Fundamental physical constants

Speed of light	C=299792458 m/s
Avogadro's number	$N_A=6,022\cdot10^{23}$ mol^{-1}
Molar gas constant	R=8,31 J/(mol·K)
Boltzmann constant	$k=1,38\cdot10^{-23}$ J/K
Charge of electron	$e=1,601892\cdot10^{-19}$ C
Mass of electron	$m_e=9,1\cdot10^{-31}$ kg
Mass of proton	$m_p=1,007276470$ u.a. m.
Mass of neutron	$m_n=1,008665012$ u.a. m.
Permittivity of vacuum	$\varepsilon_0=10^{-9}/36\pi\Phi/m \approx 8,84\cdot10^{-12}\Phi/m$
Magnetic constant	$\mu_0=4\pi\cdot10^{-7}$ H/m ≈ $12,57\cdot10^{-7}$ H/m
Stefan-Boltzmann constant	$\sigma=5,67\cdot10^{-8}$ W/(m$^2\cdot$K^4)
Wien displacement constant	$b=2,9\cdot10^{-3}$ m·K
Planck constant	$h=6,63\cdot10^{-34}$ J·s
The number " π "	π=3,14159
The natural logarithm	e=2,71828
Faraday constant	$9,652\cdot10^7$ K·kg·mol^{-1}

Table 6. The density of water at different temperatures in g/cm^3

	Density	t^0	Density	t^0	Densitatea
0	0,99987	13	0,99940	26	0,99681
1	0,99993	14	0,99927	27	0,99654
2	0,99997	15	0,99913	28	0,99626
3	0,99999	16	0,99897	29	0,99597
4	1,00000	17	0,99880	30	0,99567
5	0,99999	18	0,99862	31	0,99537
6	0,99997	19	0,99843	32	0,99505
7	0,99993	20	0,99823	33	0,99472
8	0,99988	21	0,99802	34	0,99440
9	0,99981	22	0,99780	35	0,99406
10	0,99973	23	0,99757		
11	0,99963	24	0,99732		
12	0,99952	25	0,99707		

ANNEX

Table 7. The density of the alcohol to different temperatures

t, ^{0}C	ρ, kg m^{-3}	t, ^{0}C	ρ, kg m^{-3}
0	806,25	35	776,71
5	802,07	40	772,20
10	797,88	45	767,20
15	793,67	50	762,94
20	789,45	55	785,62
25	785,22	60	754,10
30	780,97		

Table 8. Densities of the aqueous solutions of alcohol (at 20^{0}C)

C, %	ρ, kg m^{-3}	C, %	ρ, kg m^{-3}
5	989,38	55	902,61
10	981,85	60	891,15
15	975,22	65	879,50
20	968,70	70	867,70
25	961,69	75	855,70
30	953,85	80	843,49
35	944,98	85	830,96
40	935,24	90	817,95
45	924,84	95	804,15
50	913,86	100	789,33

Table 9. Absolute viscosity of water between 0-100^{0}C

t ^{0}C	cP	t ^{0}C	cP	t ^{0}C	cP
0	1,7921	21	0,9810	70	0,4061
5	1,5188	22	0,9579	80	0,3565
10	1,3077	23	0,9358	90	0,31365
15	1,1404	24	0,9142	100	0,2838
16	1,1111	25	0,8937		
17	1,0828	30	0,8007		
18	1,0559	40	0,6560		
19	1,0299	50	0,5494		
20	1,0000	60	0,4688		

Table 10. The viscosity of some liquids at a temperature of 20^{0}C

Liquid	The coefficient of viscosity
Acetone	0,33
Alcohol	1,2
petroleum	0,53
glycerine	850
Arterial blood	4,5
Blood Plasma	1,6-2,4
Urine	1,02-1,14

Table 11. The surface tension of water at the temperature of 0-80^0 C

Temperature ^{0}C	Surface tension in dyne/cm.	Temperature ^{0}C	Surface tension in dyne/cm.
0	75,625	45	68,592
5	74,860	50	67,699
10	74,113	55	66,894
15	73,350	60	66,040
20	72,585	65	65,167
25	71,810	70	64,274
30	71,035	75	63,393
35	70,230	80	62,500
40	69,416		

Table 12. Wavelengths

Element	λ, nm	Element	λ, nm	Element	λ, nm
Ba	455,4	H	397,0	Li	460,3
	493,4		410,2		610,4
	553,5		434,0		670,8
	577,8		486,1	Na	589,0
	597,2		656,3		589,6
	614,2	He	388,9	Sr	460,7
	649,6		402,6		638,6
Ca	445,5		447,1		640,8
	487,8		471,3		
	527,0		492,2		
	534,9		501,6		
	559,0		587,6		
	585,7		657,8		
	612,2		706,3		
	616,2	K	404,5		
	643,9		691,1		
Cu	402,3		693,9		
	406,3		766,5		
	427,5		769,9		
	637,8				
	458,7				
	515,3				
	521,8				
	570,0				
	578,2				

Table 13. The radioactive half-lives of nuclei

^{3}H	12262 years	^{32} P	14,3 days
^{14}C	5730 years	^{226}Ra	1622 years
^{55}Co	18,2 hours	^{222}Rn	3,825 days
^{56}Co	80 days	^{90}Sr	28 years
^{60}Co	5,263 years	^{137}Cs	27 years
^{40}K	$1{,}3\cdot10^9$ years	^{130}J	12,3 hours
^{42}K	12,4 hours	^{131}J	8,05 days
^{24}Na	15 days	^{238}U	$4{,}51\cdot10^9$ years

ANNEX

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