COMENIUS UNIVERSITY IN BRATISLAVA
JESSENIUS FACULTY OF MEDICINE

MEDICAL CHEMISTRY EXERCISE BOOK 1

Eva BABUŠÍKOVÁ

Martin 2023

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CONTENTS

PREFACE

"The inhibition of an inhibitor leads to the activation of an inhibitor of an inhibitory pathway. This is the point where most people might be tempted to give up on biochemistry!" *Author: Chris Cooper*

Dear students,

Work in the laboratory is the basis and part of every natural science discipline. Designing an experiment is a unique creative challenge. Although you will not be responsible for the design of our experiments, its execution itself represents a unique opportunity to learn how to think biochemically. In the laboratory, you will verify basic chemical, biochemical, biological, and physical knowledge that you will learn at lectures and seminars. Working in the laboratory requires manual dexterity and theoretical preparation.

“Risk: if you win, you will be happy; if you lose, you will be wise.”
Unknown author

1 BIOCHEMICAL EXPERIMENTS

Biochemistry is science about the chemical foundation and the background of life. The main aim of biochemistry is to understand all chemical processes associated with life at the molecular level. Biochemistry is used in biology, molecular biology, genetics, physiology, immunology, pharmacology, pathology. This scientific discipline is necessary for all sciences dealing with living organisms, medicine as well. Biochemistry is important for understanding health, for its maintenance and is important for understanding and diagnosing diseases and their effective treatment. Biochemical methods and procedures mean to separate, identify, and qualify molecules. Laboratory medical chemistry practicals are useful for students. For biochemical experiments we need:

- Sample: isolated organ, tissue section, cells, blood, plasma, serum, urine, saliva, liquor (cerebrospinal fluid), sputum, exhaled gas, bronchoalveolar lavage, stool,
- Proper methods for the isolation and purification of biomolecules and molecules,
- Proper methods for estimation of structure and amount of biomolecules and molecules,
- Chemicals and biochemical, chemical, physical equipment and machines.

Different chemical compositions of compounds are used for identification and analysis of these compounds. A test method is a method for a test in science such as a chemical test, a biochemical test, and a molecular test. Any test method should be explicit, feasible, effective, and reproducible. The different chemical structure of compounds means different chemical and physical properties of compounds. It is possible to do qualitative (yes / no; present / absent; identification of what is in a sample) and quantitative (a measured value – concentration, amount, activity; find out how much) tests (procedures). There is a variety of methods which can be used for excellent analysis of any compounds. These methods can be divided into subgroups (Figure 1).

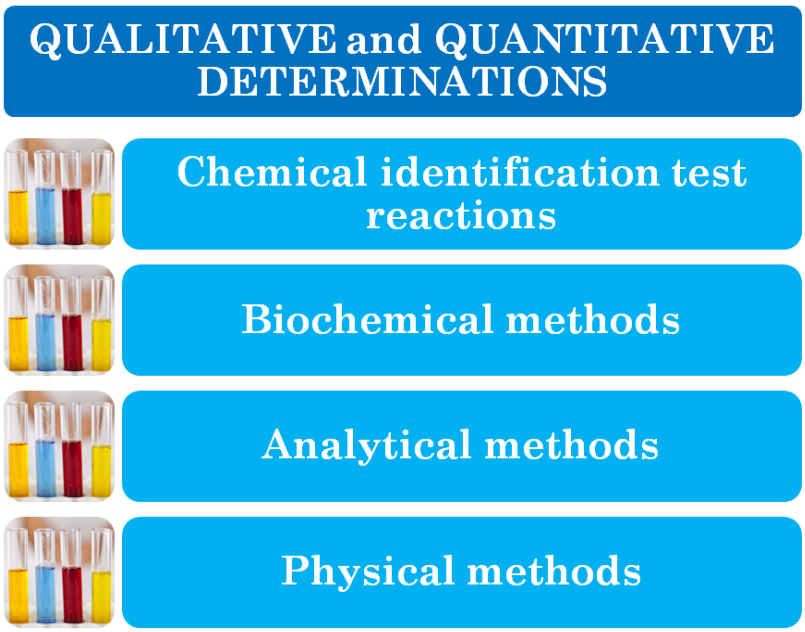

Figure 1: Basic categories for qualitative and quantitative estimation of substances/compounds/biomolecules. The qualitative test is used for the identification of substances/compounds/biomolecules. The quantitative test is for the estimation of the amount/concentration of substances/compounds/biomolecules.

Each qualitative and quantitative test needs control samples (controls): a positive control and a negative control. Positive control absolutely expects to see the result (usually a coloured complex). As a positive control, the analysed substance with a known structure and a known concentration is used. Negative control absolutely expects not to see the result. As a negative control, distilled water is usually used.

1.1 Chemical identification tests

In qualitative analysis, the chemical properties of an unknown substance are determined by systematically reacting the unknown sample with several different reagents. Identification of matter (molecule/compound) is realized on the basis of the specific properties of chemical functional groups present in the structure of an unknown compound. These chemical tests are not specific for direct determination of one concrete type of molecule/compound. It is possible to determinate that a molecule is, for example, a protein, peptide, amino acid, saccharide, phospholipid, or a nucleotide but it is impossible to specify which protein, peptide, amino acids, and so on it is. Usually these tests are very easy to perform, one reaction occurs in one glass test tube and one to three reagents are

necessary. Short incubation from 1 to 10 minutes at room temperature or 100 °C may be necessary. The result of this kind of test is a positive/negative colour change observation or a visible precipitate.

1.2 Biochemical identification methods

It is possible to use a substrate specificity of the enzyme for a biochemical analysis. Enzymatic tests can be used especially for estimation of concentration (amount) of molecules, but it is also possible to use these tests for a molecule identification, qualitative analysis. The majority of molecules can be estimated by these methods.

1.3 Analytical methods

Estimation of concentration (amount), structure (sequence), size, identification, shape/conformation, purity, surface charge, and activity can be done by analytical methods. There are several methods for this purpose. The major principles of analytical methods are based on different physical and chemical properties of constituent elements and functional groups of molecules. Different types of chromatography, spectroscopy, focussing, and mapping are used for detailed and complex analysis of molecules/compounds.

1.4 Biophysical methods

Physical parameters (characteristics) such as density, melting point, boiling point, acidity, basicity, refractive index, optical activity, viscosity, and dipole moment are often used for identification and complex characterisation of molecules/compounds/substances. At present nuclear magnetic resonance (NMR) and mass spectroscopy (MS) are very popular for qualitative as well as quantitate analysis.

Figure 2 represents a schematic diagram for a complex and a detailed analysis of the protein. It is possible to use several methods, but unfortunately, we are limited by the time during the medical chemistry laboratory practicals. The time of an analysis

fluctuates from hours to weeks. Another limiting factor is a current knowledge (students must be able to understand and perform laboratory practical work).

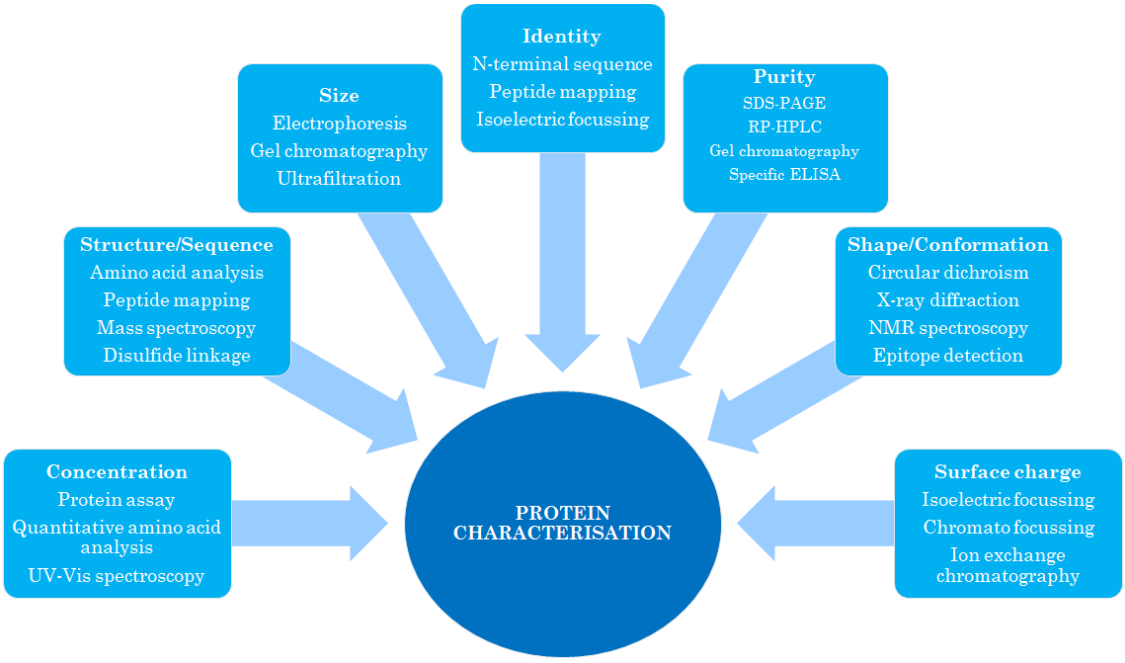

Figure 2: Schematic chart of a complex protein analysis. Any complex protein analysis is a total characterisation of the protein from a chemical point of view and a physical point of view. Protein is a very complex biomolecule. The protein contains more than 100 amino acids that bind together by a peptide bond. Many methods are used for this total characterisation just because it is necessary to know the structure of the protein, namely the primary, secondary, tertiary, concentration, conformation, size, purity.

1.5 BASIC LABORATORY METHODS

Each day in a biochemical laboratory means the use of basic laboratory methods such as weighing, volume measurement, and pipetting. Accurate measurement of quantity and volume of the substances is crucial for the correct result of the experiment.

1.5.1 WEIGHING

Weight is the physical quantity which unit in the SI system is kilogram (kg). Weight measurement – weighing – is an integral part of everyday work in chemical and biochemical laboratories. We distinguish different types of weights in the chemical laboratory depending on the weight of the weighed substance and the requirements for weighing accuracy:

For preparation purposes and weighing of larger weights − it is possible to use technical weights (accurate to 1 – 2 g) which are not very sensitive.

Weighing of smaller weights: we use accurate technical scales (accuracy 10^{-2} – 2.10^{-2} g) and analytical scales (accuracy 10^{-4} – 10^{-6} g).

Chemicals, and compounds are never placed directly on a weighing pan, but on a glass or plastic boat, a clock glass, or a weighing machines are used. The chemicals removed will never return to the storage bottle. After weighing, the scales are cleaned from the weighed chemical with a flannel cloth or brush.

1.5.2 VOLUME MEASUREMENT

Volume is a physical quantity which unit in the SI system (The International System of Units, chapter 3.3) is a cubic meter (m^3; 1 m^3 = 1 000 l). Various measuring vessels are used to measure the volume: volumetric beakers, volumetric flasks, cylinders, and pipettes. Measurable cylinders are used to measure large and approximate volumes (the scale is indicated on the wall of the measuring cylinder), while volumetric flasks are used for the exact measurement (the volume is marked on the wall of the volumetric flask). Always read volume from the bottom of the meniscus (Figure 3). The meniscus is the curved surface of a liquid in a narrow cylindrical container. Pipettes are used for the most accurate measurement of small volumes.

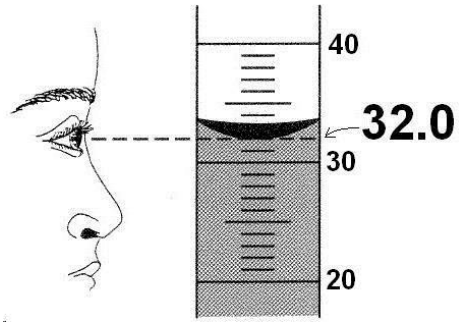

Figure 3: Correct reading of volume. The eye position is in level with the surface of the liquid. Read the liquid level at the bottom of the meniscus. https://calaski.wordpress.com/metric-volume/

1.5.3 PIPETTING

The precise and correct work in the chemical and biochemical laboratories is based on the accurate and correct use of glass, plastic, and automatic pipettes. Either glass pipettes or disposable plastic pipettes should be used. To properly select the pipette for work, you need to know the volume to be pipetted, as well as the range and scale of the pipette. Pipettes are always held vertically. Unused automatic pipettes are held vertically **using a pipette holder**.

Glass and plastic pipettes are most manufactured in a volume range of 1 ml, 5 ml, 10 ml, and 20 ml. The scale, as well as the minimum and maximum volumes that can be pipetted by a pipette, are shown on the pipette walls. Glass and plastic pipettes are used together with an overhead (balloon, pipette boy, pipette man). Never pipette by mouth!

Automatic pipettes are manufactured with a constant or adjustable volume that is placed on the pipettes. Adjustable volume pipettes are most often manufactured in the range of: 0.1 – 2 µl, 0.5 – 10 µl, 2 – 20 µl, 20 – 200 µl, 200 – 1 000 µl and 500 (or 1 000) – 5 000 µl. Pipettes have three positions:

1. Rest position (ready position, upper stop)
2. First stop
3. Second stop.

Tips are used when using automatic pipettes. Tips are usually produced in four different volumes: up to 10 µl, 2 – 200 µl, 200 – 1 000 µl and 500 (1 000) – 5 000 µl. The optimal pipette and the corresponding tip are selected depending on the volume to be pipetted.

The correct pipetting procedure:

1. First, pick a pipette of the appropriate volume range.
2. Set the required volume.
3. Attach the disposable tip. Fit the tip to the end of the shaft. Press down and twist slightly to ensure an airtight seal.
4. Depress the plunger (the pipette knob) to the first stop (Figure 4).
5. Draw up the liquid. Immerse the tip 2 – 3 mm in the liquid. Release the plunger back to the rest position. Wait a second for the liquid to be sucked into the tip. Pipette with a slow, smooth, and continuous action.
6. Ensures that no air is being aspirated and allows you to draw up more than the measured volume. The thumb is carefully pushed out of the button so that bubbles do not get into the tip.
7. Aspirate at 90 degrees, dispense at 30 – 45 degrees.
8. Dispense the liquid. Touch the tip end to the side wall of the receiving vessel. Depress the plunger to the first stop, wait one second, and press the plunger to the second stop to expel all the liquid.
9. Withdraw the pipette, and release the plunger to the rest position.
10. Press the ejector button to discard the tip.
11. Do not hold a pipette when you are not pipetting and store pipettes vertically using a pipette holder.

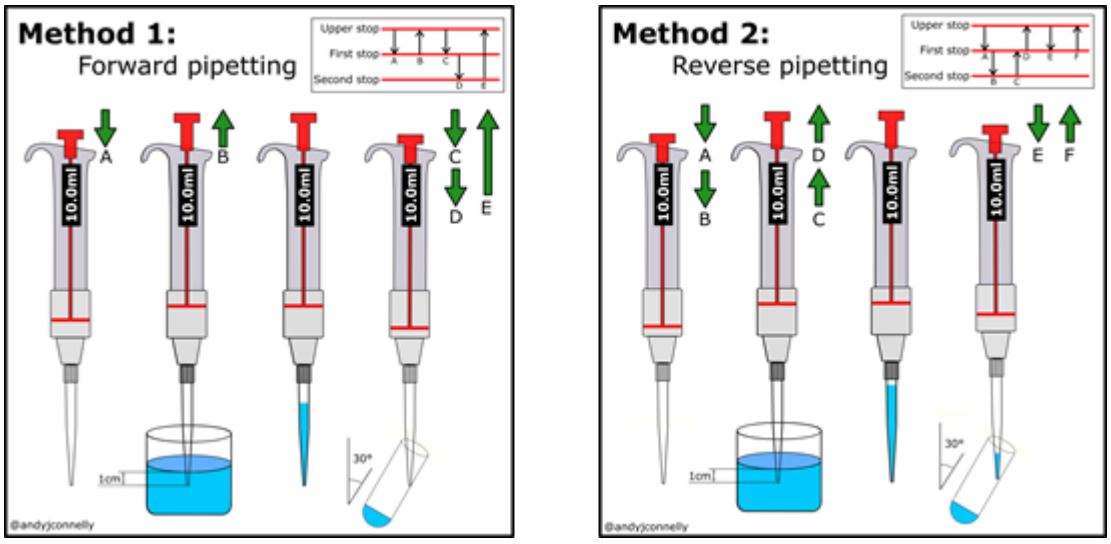

Figure 4: Illustrative diagram of the pipetting procedure. There are two techniques of pipetting, forward and reverse. Forward pipetting is mainly recommended for common aqueous solutions. Reverse pipetting is recommended for solutions with high viscosity heterogeneous solutions (such as blood, serum) or for solutions with a tendency to foam. https://andyjconnelly.wordpress.com/2017/02/12/practical-pipetting-a-guide/

2 LABORATORY SAFETY

The conscious and disciplined approach of students to laboratory exercises is the prerequisite for a successful and correct course of practical training. Very important is also the responsible observance of the laboratory rules and the safety instructions during work in the chemical laboratory. The pandemic situation during 2020 and 2021 is an excellent example that own protection and protection of people in a specific area is crucial for health, for limitation of virus spread, and it is important to have basic knowledge for each specific condition. Therefore, for medical chemistry laboratory practical:

1. Students must be thoroughly theoretically prepared for laboratory practical application.
2. Students work in a pair (unless otherwise stated) at the place designated by the teacher and are required to maintain order.
3. Students use assigned devices for which they are personally responsible.
4. Laboratory practicals are performed according to a specific procedure and according to the instructions of a teacher.

5. On the first practical exercise, students get familiar with the location and method of using fire extinguishers, as well as the safety regulations in the chemical laboratory.
6. Students must wear a laboratory coat, a white coat of natural material.
7. Footwear must completely cover the foot and heel.
8. Long hair should be tied back. Dangling jewellery must be removed.
9. It is forbidden to eat, drink, and smoke in a chemical laboratory!
10. It is forbidden to use a cell phone.
11. Technical problems and injuries that may occur must be reported to a teacher/technician immediately and first aid is provided immediately.
12. Students must not interfere with electrical installation, or water distribution, or handle inappropriately the equipment of a chemical laboratory.
13. Organic solvents, poisons, concentrated acids, concentrated bases, or thick precipitates must not be poured into the sinks. These chemical compounds are poured into special containers.

2.1 WORK WITH CHEMICALS

1. Chemicals should not come into contact with the skin.
2. Solids are scooped up using a plastic spoon when used (for example, during weighing, adding chemicals to a working solution).
3. Chemicals must not be tasted; their fumes should not be inhaled.
4. While working, do not look directly inside the tubes with the reaction mixture and make sure that tubes are not turned with the opening towards the co-workers.
5. Only chemicals that are clearly labeled are used in an experiment.
6. Spilled chemicals must be removed immediately.
7. Be extremely careful when transferring, distilling, or refluxing volatile liquids.
8. Do not return used chemicals to the stock container.
9. With corrosives and corrosive substances, work must be performed with great care and according to the instructions of a teacher.
10. Flammable and irritant substances, as well as concentrated acids and concentrated bases, can only be worked with in a fume hood.
11. To dilute the concentrated acid, pour the acid through the glass rod with a thin stream into the water while stirring and cooling. Never add water to acid.

12. Toxic chemicals are specifically labeled and stored in a lockable container.
13. Never pour water into concentrated acid. Pour the acid slowly into the water. Mixing acid with water is often exothermic.
14. Never pipet by mouth.
15. Before working with poisons, the student is particularly cautioned and instructed about the safety rules before practicing.

2.2 LABORATORY GLASS

Glass is the material most widely used in the chemical laboratory (Figure 5). Although glass material is characterized by high chemical and heat resistance, it is essential to avoid sudden changes in temperature when it is used. The following principles must be followed when working with glass:

1. Glass is a fragile and low-elastic material with which it is necessary to work with care.
2. It is necessary to avoid damaging the surface of glass and glass consumables to reduce the thermal and mechanical resistance.
3. The chemicals are heated only in thin-walled tubes, never in thick-walled tubes.
4. Glass supplies should be washed immediately after use. It is rinsed three times under running water, the remainder of solids can be removed mechanically by a glass washing brush or by a glass rod. The cleaned consumable material is deposited in the collection container and cleaned thoroughly according to the relevant protocol.
5. Figures 5 and 6 represent the basic laboratory glass, tools, and machines, which students will most often use during laboratory practicals.

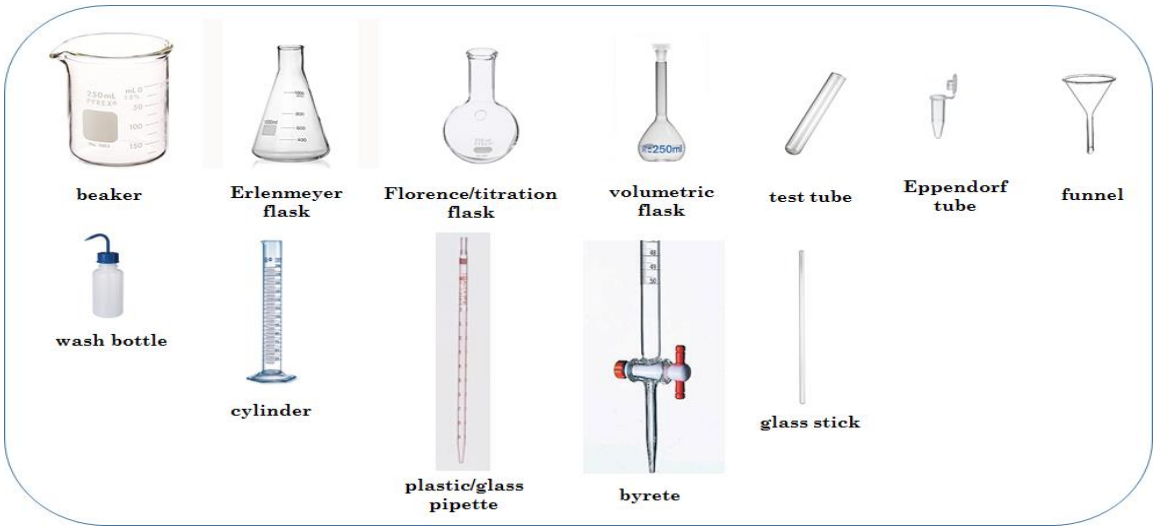

Figure 5: Basic laboratory glass. Laboratory glass is the most commonly used apparatus in biochemical laboratory. Glass is a perfect material for different experiments and it is possible to use it several times. Test tubes can be glass and plastic (centrifugation test tube, test tube for human fluids, Eppendorf tube).

Figure 6: Basic laboratory tools and machines. Many tools are necessary for each experiment. Laboratory practical represents one small experiment. **Water bath** – used for the incubation of a test tube with reagents (a reaction proceeds at specific temperature). Today, we prefer a dry water bath. **Centrifuge** – used for separation of liquid reagents or a sediment (solid molecules) from supernatant (liquid reagent above sediment). **Spectrophotometer** – used to measure absorbance. **Polymerase chain reaction (PCR) cycler** – a routine machine for amplification of a specific segment of deoxyribonucleic acid (DNA). **Automatic pipette boy/pipette boy** – used for pipetting with glass pipettes. **Automatic pipette** – used for pipetting. **Vortex** – used for mixing reagents. **Laboratory beaker stand** – used to hold laboratory glass. **Test tube rack** – used to hold test tubes.

2.3 PREPARATION FOR LABORATORY PRACTICAL ACTIVITY

Theoretical preparation at home for laboratory practical activity is necessary for the successful (accurate and correct) implementation of any experiment, as well as avoidance of possible dangers. Therefore, it is important to know the theoretical part of any practical work, the workflow, and possible clinical correlations. Students need to bring the following tools for each laboratory practical:

1. white laboratory coat from a natural material,
2. writing supplies,
3. calculator,
4. glass handler,
5. notebook,
6. protocol script.

Laboratory report – a laboratory protocol is one of the most important work habits when working in chemical and biochemical laboratories. Experimental protocols are fundamental information structures that support the description of the processes by means of which results are generated in experimental research. A protocol is part of this script. Laboratory practicals will be recognized as completed only after the elaboration of the protocol and the subsequent signing of the protocol by the teaching staff. Only the signed protocol is proof of the practical laboratory performance. Each student performs his own work protocol, although laboratory practicals are usually performed in a pair. The text of laboratory protocol should be clear, lucid, and concise. According to the developed protocol, it should be possible to repeat the whole laboratory exercise exactly.

Laboratory practical includes washing of used laboratory glass.

2.4 LABORATORY PROTOCOL

Each protocol must include the following:

1. Title of laboratory practical
2. Date
3. Name, surname and the number of the study group

4. The principle – contains the most important information about the experiment; this is the basic objective of the laboratory task, a brief theoretical basis of the experiment and experiment methods. It can be a reaction or one or two sentences.
5. Procedure – briefly describe the method (all experimental procedures), either in the form of a contextual text or in the form of concise points. All important details must be provided so that someone could repeat the experiment exactly. It is possible to use also the reference that contains: authors, title of a book (paper), year of edition, pages.
6. Results – present data that are collected from the experiment and summarize the data with:
   a) Measured data obtained during the laboratory work
   b) Observations made during the experiment
   c) Calculations
   d) Tables
   e) Graphs.
7. Discussion – evaluation of the results obtained. The discussion section should explain to the reader the significance of the results. This part of a protocol contains any experimental comments on whether the experiment was performed correctly or not and comments on the problems that occurred during the experiment, indicating possible changes that would make these problems. If the results contained errors, analyse the reasons for the errors. In the case of clinical biochemical experiments, the results obtained are compared with the physiological values. The possible causes of unexpected results (pathological values) are discussed and deduced.
8. Conclusion – a brief summary of the whole laboratory work, complex view to aim, and obtained results of practical exercise.

3 BASIC CHEMICAL LAWS

The law of conservation of mass (principle of mass conservation) – Mikhail Vasilyevich Lomonosov (November 19, 1711, Mishaninskaja, Russia –April 15, 1765, Saint Petersburg, Russia) and Antoine Laurent Lavoisier (August, 26 1743, Paris, France – May 8, 1794, Paris, France)

The mass of all substances (elements, chemicals, compounds) entering the chemical reaction (reactant) is equal to the mass of all chemicals produced by the chemical reaction (s). The total number of atoms of the individual elements in the reactants must be equal to the total number of atoms of the individual elements in the products. The quantity of mass is conserved over time.

The law of define proportion (Proust's law or the law of define composition) – Joseph Louis Proust (September 26, 1754, Angers, France – July 5, 1826, Angers, France)

The weight ratio of the elements making up the compound is always the same and does not depend on its source and the process of preparation of the compound.

The law of combining volumes – Joseph Louis Guy-Lussac (December 6, 1778, Saint-Léonard-de-Noblat, France – May 9, 1850, Paris, France)

The law of combining volumes states that, when gases react together to form other gases and when all volumes are measured at the same temperature and pressure: The ratio between the volumes of the reactant gases and the gaseous products can be expressed as simple whole numbers. There is a linear dependence between the volume and temperature of the gas and the gas pressure is constant.

The law of multiple proportions (basic law of stoichiometry) – John Dalton (September 6, 1766, Eaglesfield, England – July 27, 1844, Manchester, England)

If two elements form more than one compound between them, then the ratios of the masses of the second element which combine with a fixed mass of the first element will be ratios of small whole numbers.

Avogadro's law – Amadeo Avogadro (August 9, 1776, Turin, Italy – July 9, 1856, Turin, Italy)

Equal volumes of all gases, at the same temperature and pressure, have the same number of molecules.

The law of conservation of energy – Julius Robert von Mayer (November 25, 1814, Heilbronn, Germany – March 20, 1878, Heibronn, Germany)

The total energy is unchangeable and is not a function of time. Energy cannot be created or destroyed; instead it can only be transformed from one form to another.

The law of mass action (Law of Guldber – Waage) – Cato Maximilian Gulberg (August 11, 1836, Oslo, Norway – January 14, 1902, Oslo Norway) and Peter Waage (June 29, 1933, Flekkefjord Norway – January 13, 1900, Oslo Norway)

The rate of a chemical reaction is directly proportional to the product of the activities or concentrations of the reactants.

3.1 BASIC CHEMICAL CALCULATIONS

Chemical calculations are a basic and everyday operation in a chemical and biochemical laboratory. It is necessary to prepare each solution from basic compounds which are used for experiments. The result of any reaction is also a compound, and we need to know its concentration or amount. Finally, as a physician, we need to know the amount of a drug, the dilution of a drug per weight of patients. Basic information for everyday calculations in a chemical laboratory is in Figure 7.

The amount of substance has symbol **n** and unit **mol**. Number of particles (N) in 1 mol of compound is given by the Avogadro constant – N_A (6,022 141 79 ± 0,000 031) $\cdot 10^{23}$ mol^{-1}.

The molar mass has symbol **M** and is defined by quotient of the mass (**m**) of a substance (element, compound) and its amount of substance (**n**). The molar mass has unit kg/mol but is usually given by g/mol.

The density (the volumetric mass density) has the symbol **ρ** and is defined by the quotient of the mass (**m**) and the volume (**V**). Density is equal to the mass of a substance divided by the volume of the substance. The density has unit kg/l.

The molar concentration (molarity, amount concentration, substance concentration) has symbol **c** and is given as a quotient the amount of substance diluted substance A (**n**) and the volume of solution (**V**). The molar concentration has unit mol/l.

The mass concentration simplifies the preparation of solution from solid substance and is given by conversion of the molar concentration. The concentration in a solution is the mass of solute per volume of solution.

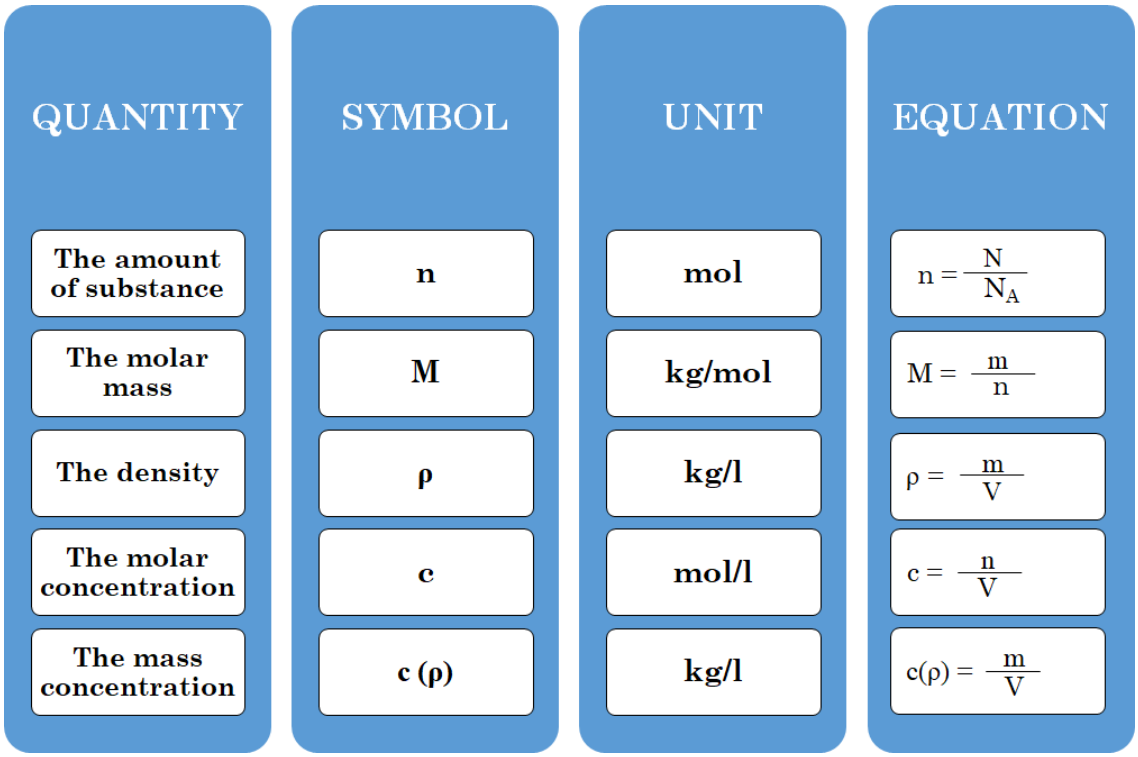

Figure 7: Basic chemical calculations. These calculations are used for solution definition and solution preparation, for determination of biomarker concentration. The density is the mass of sample divided by its volume. The mass concentration is the ratio of minor part i.e. is solute to the major part i.e. solvent.

Sometime, the concentrated (storage) solution must be diluted to yield a less concentrated solution. If a solution is diluted, the volume of the solution increases and its concentration decreases. The total amount of solute is constant (same amount of solute in storage and diluted solution); therefore:

$$c_1 \cdot V_1 = c_2 \cdot V_2$$

If the composition of the solution is expressed by concentration, in the case of isometric mixing of the solutions, the equation applies:

$$c_1 \cdot V_1 + c_2 \cdot V_2 = c_3 \, (V_1 + V_2)$$

The acidity of the aqueous solution is expressed by the pH (hydrogen potential). It is the negative logarithm of the base 10 of the activity of hydrogen ions, but in practice the molar concentration of hydrogen ions is used.

$$pH = - \log c(H^+)$$

Most often used scale of pH in the laboratory is from 0 – 14 (Figure 8). A change in $[H_3O^+]$ causes an inverse change in $[HO^-]$. Therefore, all acidic solutions will contain a low $[HO^-]$, whereas all basic solutions will contain a low $[H_3O^+]$. The acidity or basicity of a solution is often expressed as the pH calculated as the negative logarithm of $[H_3O^+]$. For example, a solution that is 1 M in H_3O^+ has a pH of 0, while a solution that is 0.001 M in HO^- has a pH of 11. The pH can be measured in the laboratory using *acid–base indicators* (pH papers) or pH meters.

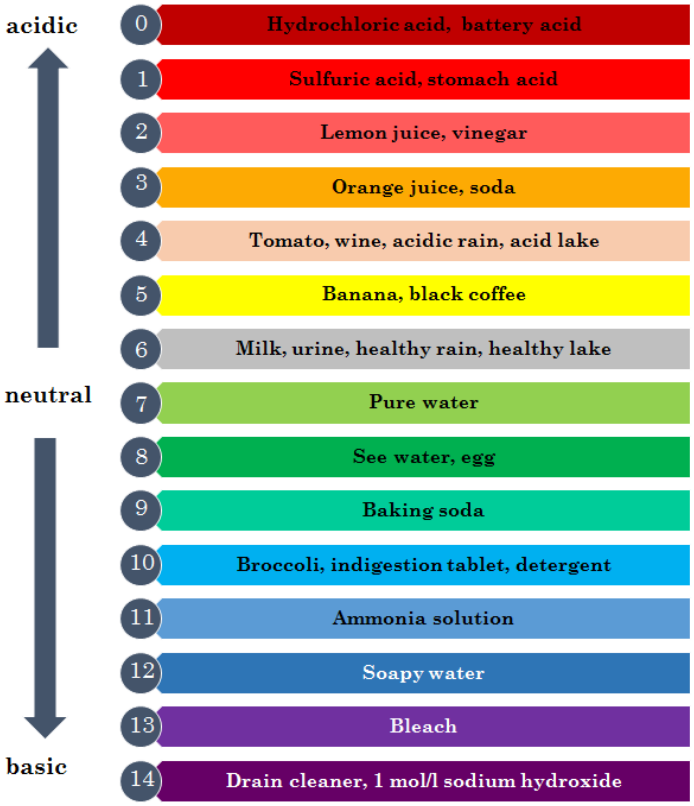

Figure 8: The pH scale. Universal colour pH indicator with practical examples. Some acid-base indicators have a very useful property — they change colour depending on the pH of the solution in which they are. This colour change is not at a fixed pH, but rather, it occurs gradually over a range of pH values. This range is termed the colour change interval. Each pH indicator is defined by a useful pH range.

For pH of a strong acid it is possible to use the molar concentration from which is the deduced concentration of hydrogen ions. It is necessary to take into consideration protic of acid: monoprotic acid: $HCl \rightarrow H^+ + Cl^-$, $c(H^+) = c(HCl)$

diprotic acid: $H_2SO_4 \rightarrow 2\ H^+ + SO_4^{2-}$, $c(H^+) = 2\ c(H_2SO_4)$

Figure 9 shows how to calculate the pH for different types of solutions.

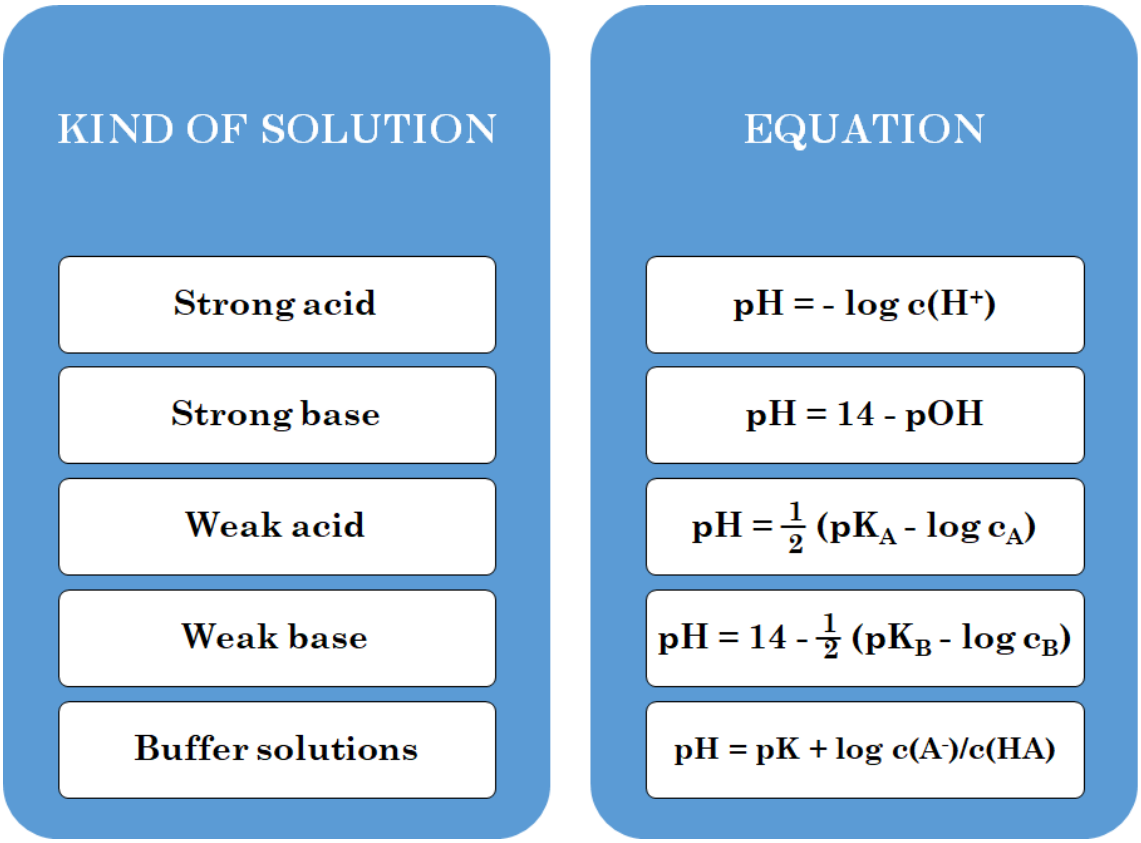

Figure 9: pH equations for different kinds of solutions. c_A – concentration of acid, c_B – concentration of base, $c(A^-)$ – concentration of conjugate base, c(HA) – concentration of acid, K – dissociation constant (A – acid, B – base), pK = -log K

3.2 BASIC ORGANIC COMPOUNDS

Organic compounds are those that have carbon atoms in their structure. Large organic molecules, called macromolecules, can consist of hundreds or thousands of atoms in living systems. Most macromolecules are polymers, molecules that consist of a single unit (monomer) repeated many times. Carbon has four electrons in outer shell and can bond with up to four other atoms (usually H, O, N, or another C). Thus, we will always see four lines connecting a carbon atom to other atoms, each line representing a pair of shared electrons (one electron from carbon and one from another atom). Although carbon is present in all organic compounds, other elements such as hydrogen (H), oxygen (O),

nitrogen (N), sulphur (S) and phosphorus (P) are also common in these molecules. An organic molecule is a molecule that contains carbon atoms (generally bound to other carbon atoms as well as hydrogen atoms). Organic molecules are those that: 1) are formed by the actions of living organisms; and/or 2) have a carbon backbone. Due to the unique ability of carbon to concatenate (form chains with other carbon atoms), millions of organic compounds are known. Study of the properties and synthesis of organic compounds is the discipline known as organic chemistry. Noticeable exceptions are carbon monoxide (CO), carbon dioxide (CO_2), carbonates (e.g. calcium carbonate), carbides (e.g. calcium carbide) and cyanides (e.g. sodium cyanide). Pure carbon compounds such as diamond and graphite are also not organic compounds. The formulas and structural representations of several simple functional organic groups are shown in Figure 10.

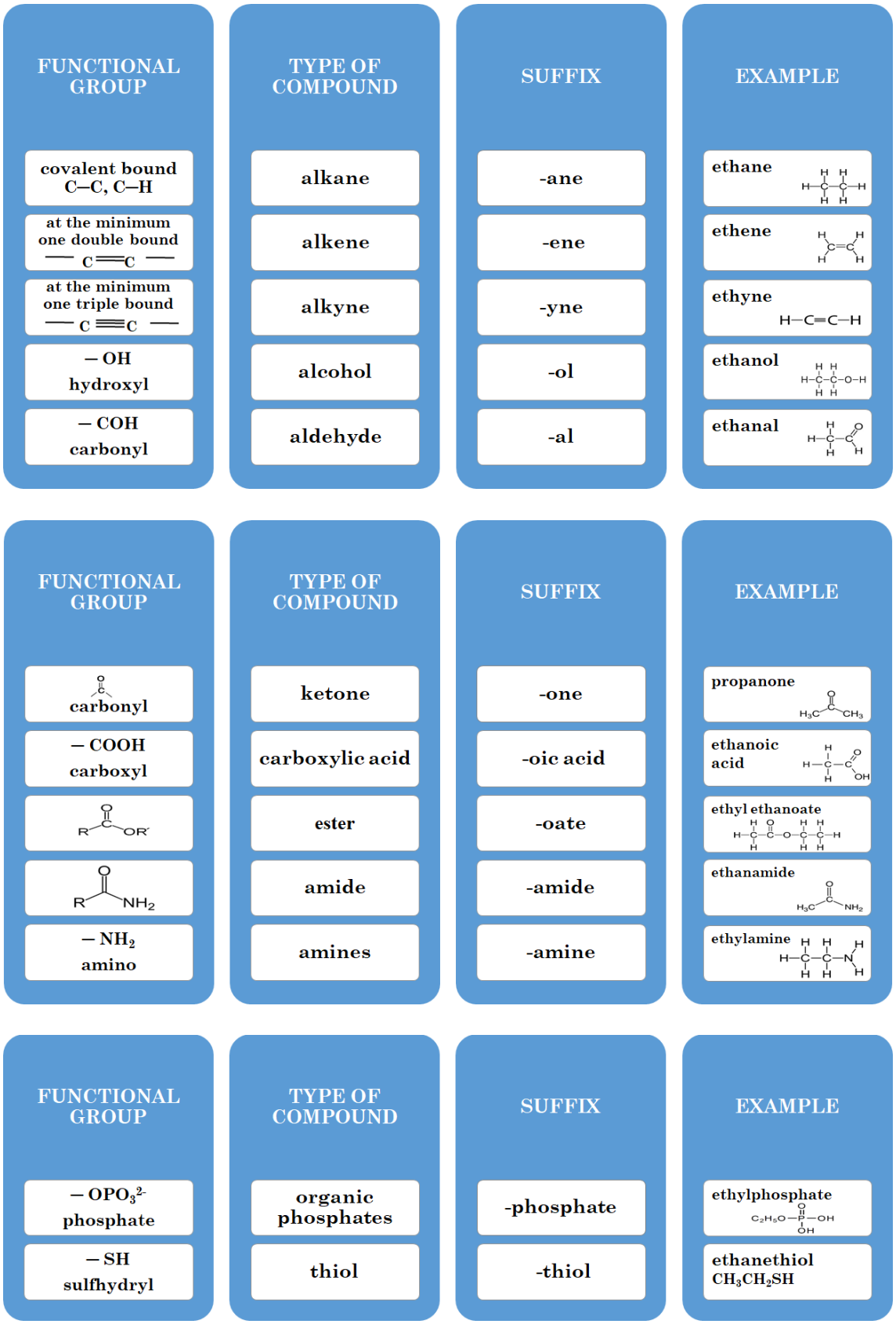

Figure 10: Major types of organic compounds with specific functional groups. Functional groups are groups of atoms in organic molecules that are responsible for the characteristic chemical reactions of those molecules.

3.3 THE INTERNATIONAL SYSTEM OF UNITS

The International System of Units (from the French *Système international d'unités,* SI) is the modern form of the metric system and comprises base units. There are:

- basic units (Figure 11): meter, kilogram, second, ampere, kelvin, candela, mole,
- additional derived units: radian, steradian, hertz, degree Celsius, newton, joule, watt, pascal, coulomb, volt, ohm, siemens, farad, weber, tesla, henry, lumen, lux, becquerel, gray, sievert, katal.

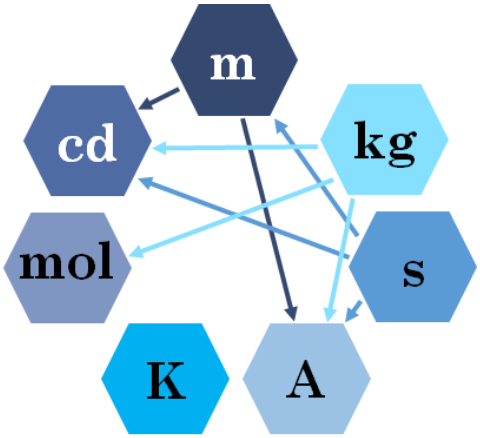

Figure 11: Basic units and reciprocal dependence of their definition. m – meter, kg – kilogram, s – second, A – ampere, K – kelvin, mol – mole, cd – candela.

Basic units represent the building blocks of SI and all the other units can be derived from them. We know 7 basic units (Figure 11, Table 1). Prefixes are added to unit name to produce the multiples and the parts of the main and derived units. They are formed by ten to the third power and are shown in table 2. They are used to clarify the writing of too large or too small values written by the unit.

Table 1: Characteristic of basic units of The International System of Units

UNIT NAME	UNIT SYMBOL	QUANTITY NAME	DEFINITION
meter	m	length	Meter is the distance travelled by light in vacuum in 1/299792458 second.
kilogram	kg	mass	To 19. 05. 2019 Kilogram is the mass of a small cylinder platinum-iridium alloy. Since 20. 05. 2019 The kilogram, symbol kg, is the SI unit of mass. It is defined by taking the fixed numerical value of the Planck constant h to be 6.62607015×10^{-34} when expressed in the unit J·s, which is equal to $kg\cdot m^2\cdot s^{-1}$, where the meter and the second are defined in terms of c and $\Delta\nu_{Cs}$.
second	s	time	Second is the duration of 9 192 631 770 periods of the radiation corresponding to the transition between the two hyperfine levels of the ground state of the caesium (^{133}Cs) at temperature 0 kelvin.
ampere	A	electric current	Ampere is the constant current which, if maintained in two straight parallel conductors of infinite length, of negligible circular cross-section, and placed 1 m apart in vacuum, would produce between these conductors a force equal to 2×10^{-7} newtons per metre of length.
kelvin	K	thermodynamic temperature	Kelvin is 1/273.16 of thermodynamic temperature of the triple point of water.
mole	mol	amount of substance	Mole is amount of substance which contains as many elementary entities as there are atoms in 0.012 kg of clear carbon (^{12}C).
candela	cd	luminous intensity	Candela is the luminous intensity of a source that emits monochromatic radiation of frequency 5.4 x 10^{14} hertz and that has a radiant intensity in that direction of 1/683 watt per steradian.

Table 2: Prefixes of units

PREFIX NAME	SYMBOL	BASE 10	DECIMAL	NAME
yotta	Y	10^{24}	1 000 000 000 000 000 000 000 000	quadrillion
zetta	Z	10^{21}	1 000 000 000 000 000 000 000	trilliard
exa	E	10^{18}	1 000 000 000 000 000 000	trillion
peta	P	10^{15}	1 000 000 000 000 000	billiard
tera	T	10^{12}	1 000 000 000 000	billion
giga	G	10^{9}	1 000 000 000	milliard
mega	M	10^{6}	1 000 000	million
kilo	k	10^{3}	1 000	thousand
hecto	h	10^{2}	100	hundred
deca	da	10^{1}	10	ten
-	-	**10^{0}**	**1**	**one**
deci	d	10^{-1}	0,1	tenth
centi	c	10^{-2}	0,01	hundredth
mili	m	10^{-3}	0,001	thousandth
micro	µ	10^{-6}	0,000 001	millionth
nano	n	10^{-9}	0,000 000 001	milliardth
pico	p	10^{-12}	0,000 000 000 001	billionth
femto	f	10^{-15}	0,000 000 000 000 001	trillionth
atto	a	10^{-18}	0,000 000 000 000 000 001	trillionth
zepto	z	10^{-21}	0,000 000 000 000 000 000 001	trilliardth
yocto	y	10^{-24}	0,000 000 000 000 000 000 000 001	quadrillionth

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

VOLUME MEASUEMENT

Background

Volume measurement is basic and everyday routine in any laboratory. To have the correct volume of any step in an experiment procedure is essential for the right, proper, and reproducible result of any analysis. We will practise preparing different volumes of water to gain skills and confidence for all laboratory practicals, right reading of information on the wall of cylinder, glass, and automatic pipettes and abide by the rules of pipetting. We will pipette various volumes of distilled water: 5.10^{-3} ml, 5.10^{-2} ml, 5.10^{-1} ml, 5 ml and 50 ml.

Principle

Chemicals and Tools

- Distilled water
- Glass pipettes with maximum volume 1, 5 and 10 ml, pipette boy, automatic pipettes, tips, beakers, cylinders, glass test tubes

Procedure

Prepare 4 glass test tubes and one beaker. Add 5.10^{-3} ml, 5.10^{-2} ml, 5.10^{-1} ml, 5 ml of distilled water to test tubes and 50 ml of distilled water to a beaker. Distilled water

pipette with appropriate tools. In the results table, write down which tools can be used to pipet of these volumes.

Results

SAMPLE	VOLUME [µl]	VOLUME [ml]	TOOL AND PIPETTING SCHEME
1			
2			
3			
4			
5			

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

PREPARATION OF 100 ML OF PHYSIOLOGICAL SOLUTION

Background

Water is a universal, necessary, and life keeping solution. Most of the solutions in chemical and biochemical laboratories are prepared with water (distilled, water, or sterilised water). The physiological solution (saline, saline solution) is a solution of sodium chloride with a concentration of 0.154 mol/l (0.9 % weight/volume).

This solution has several applications in medicine:

- Clean wounds, skin abrasions,
- Eye drops,
- Nasal washes,
- Body rehydration (by injection into a vein),
- Dilute drugs (medications).

Principle

Chemicals and tools

- NaCl (Mr = 58.44 g/mol)
- Deionised water
- Beaker, cylinder, volumetric flask, glass stick, automatic balance

Procedure

1. Weigh the calculate mass of NaCl using automatic scales.
2. Add NaCl to the beaker and add 50 ml of deionised water.
3. Dissolve NaCl. Swirl the flask gently if necessary.
4. Add dissolved NaCl to the 100 ml volumetric flask.
5. Rinse the beaker with deionised water and add this water to the volumetric flask.
6. Top the solution to the 100 ml mark with deionised water.
7. Label the physiological solution and store it at 2 – 8 °C.

Caution: Do not simply measure 100 ml of water and add 0.9 g of sodium chloride. This will introduce an error because adding the solid will change the final volume of the solution and throw off the final percentage.

Results

Calculate amount of NaCl needed for 100 ml of physiological solution

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

PREPARATION OF 100 ML OF 0.077 MOL/L SOLUTION OF NaCl

Background

It is possible to use not only solid compounds but also liquid compounds and stock solutions for the preparation of various solutions. Our prepared physiological solution will be used to prepare a NaCl solution with a concentration 0.0077 mol/l.

Principle

Chemicals and tools

- Physiological solution
- Distilled water
- Beaker, cylinder, volumetric flask

Procedure

1. Calculate the necessary amount (volume) of physiological for the preparation of 100 ml 0,077 mol/l NaCl solution.
2. Measure the calculated volume of physiological solution in a cylinder.
3. Add the solution to the 100 ml volumetric flask.
4. Rinse the beaker with distilled water and add this water to the volumetric flask.
5. Top the solution to 100 ml mark with distilled water.
6. Mark the prepared 0.077 mol/l solution and store it at room temperature.

Results

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

PREPARATION OF 100 ML OF 10 % ETHANOL SOLUTION

Background

It is possible to use not only solid compounds but also liquid compounds for the preparation of varied solutions. When the solute is a liquid, it is sometimes convenient to express the solution concentration as a percent of the volume. A very important solution in biochemistry and medicine is ethanol. Ethanol has antiseptic effects – kills bacteria, fungi, and viruses by dissolving their membrane bilayer and denaturing their proteins. All of us used antibacterial gels and disinfectants during the covid19 pandemic months. Much more effective is 70 % ethanol than pure ethanol (96 – 99 %).

Principle

Chemicals and tools

- 96 % ethanol
- Deionised water
- Beaker, cylinder, volumetric flask

Procedure

1. Calculate the necessary amount of 96 % ethanol for the preparation of 100 ml of 10 % ethanol solution.
2. Measure the calculated volume of ethanol in the cylinder.
3. Dissolve ethanol in 50 ml of distilled water.

4. Add dissolved ethanol to the 100 ml volumetric flask.
5. Rinse the beaker with distilled water and add this water to the volumetric flask.
6. Top the solution to 100 ml mark with distilled water.
7. Mark the prepared 10 % ethanol solution and store it at room temperature.

Results

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

THE MEASUREMENT OF pH OF ACIDS AND BASES

Background

Chemicals and solutions are often classified as acidic, basic, or neutral. Measurement and maintenance of pH are very important steps during the preparation of solutions. The acidity of the aqueous solution is expressed by the pH (potential of hydrogen). It is the negative logarithm to the base 10 of the activity of hydrogen ions, but in practice the molar concentration of hydrogen ions is used. Most often used scale of pH in the laboratory if from 0 to 14 (Figure 8). According to the character of the solution, the pH can be calculated using one of the five pH equations (Figure 9). Colorimetric (for example, pH papers), potentiometric, or conductometric methods are used for measurement of pH. The simplest and cheapest measurement of pH is using pH paper (pH strips). The paper is impregnated with chemicals, and the pH papers change colour when they come in contact with the solution (Figure 12). The colour must be compared with a chart to get the number and determine whether the solution is acidic, basic, or neutral.

INDICATOR	pH RANGE	COLOUR RANGE
Crystal violet	0.0 – 2.0	green – blue
Cresol red	0.2 – 1.8	red – yellow
Thymol blue	1.2 – 2.8 / 8.0 – 9.2	red – yellow / yellow – blue
Methyl orange	3.0 – 4.4	red – orange
Bromphenol blue	3.0 – 4.6	yellow – purple
Congo red	3.0 – 5.0	blue-violed– red
Bromcresol green	3.8 – 5.4	yellow – blue-green
Methyl red	4.4 – 6.2	red – yellow
Bromthymol blue	6.0 – 7.6	yellow – blue
Phenol red	6.8 – 8.4	yellow – red
Phenolphtalein	8.2 – 10.0	colorless – fuchsia
Thymophthalein	9.3 – 10.5	colorless – blue
Alizarine yellow R	10.2 – 12.1	yellow – red
Indigo carmine	11.4 – 14.0	blue – yellow

Figure 12: The pH scale and indicators. Basic acid-base indicators and their colour changes.

The simplest and cheapest measurement of pH is by sipping a piece of pH paper in the sample. A pH meter should be used if greater accuracy is required.

Principle

Chemicals and tools

- 0.01 mol/l HCl
- 0.01 mol/l NaOH
- 0.01 mol/l CH_3COOH
- Distilled water
- pH papers
- pH meter
- Beakers, glass test tubes, automatic pipettes, tips

Procedure

1. Add 1 ml of each solution to a new glass test tube (pH paper measurement).
2. Add 5 ml of each solution to a new beaker (pH meter measurement).
3. Measure the pH in each solution with pH paper and pH meter.
4. Calculate the pH of each solution.

Results

SAMPLE	**pH**		
	pH PAPER	**pH METER**	**CALCULATION**
HCl			
NaOH			
CH_3COOH			
H_2O			

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

ESTIMATION OF pH IN COMMON SOLUTIONS

Background

The acidity of aqueous solution is expressed by the pH (potential of hydrogen). It is the negative logarithm to the base of 10 of the activity of hydrogen ions, but in practice the molar concentration of hydrogen ions is used. Most often used scale of pH in the laboratory is from 0 – 14 (Figure 8). According to the character of the solution, the pH can be calculated (Figure 9). Colorimetric (for example pH papers), potentiometric or conductometric methods are used for measurement of pH.

Principle

Chemicals and tools

- Lemon juice
- Orange juice
- Black coffee
- Tap water
- Distilled water
- Saliva
- pH papers
- Glass test tubes, automatic pipettes, tips

Procedure

1. Add 0.5 ml of each solution to new glass test tubes.
2. Measure the pH in each solution with a pH paper.

Results

SAMPLE	KNOWN pH	pH PAPER
LEMON JUICE	~ 2	
ORANGE JUICE	~ 3	
BLACK COFFEE	~ 5	
TAP H_2O	~ 6	
DISTILLED H_2O	~ 7	
SALIVA	~ 7	

Discussion

Conclusion

4 SPECTROPHOTOMETRY

In chemistry, spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is the most common method used in biochemistry. Spectrophotometry is a basic analytical method that relies on the quantitative analysis of molecules/compounds depending on how much light is absorbed by coloured compounds. A brief explanation of the spectrophotometry procedure includes comparing the absorbance of a blank sample that does not contain a coloured compound to a sample that contains a coloured compound (Figure 13). The intensity of such a colour is proportional to the concentration of the compound.

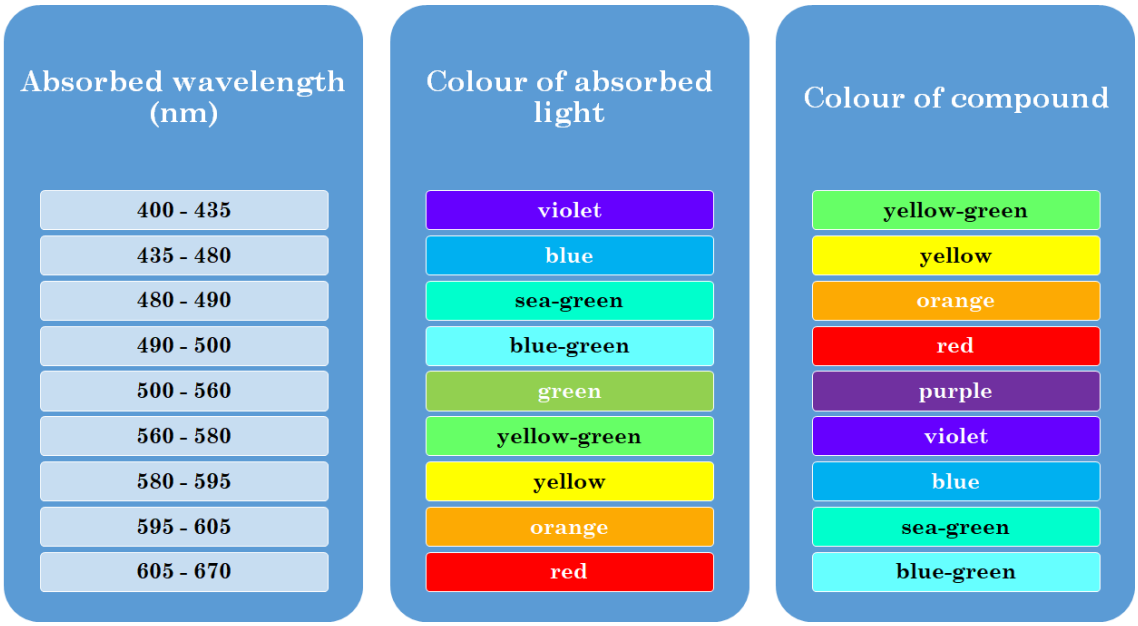

Figure 13: Colours and complementary colours of the visible spectrum. The relationship between the colour of the absorbed radiation and that of the transmitted light. A scheme that correlates colours to their appropriate wavelengths and photon energy.

A spectrophotometer is an instrument that uses light over the ultraviolet range (185 – 400 nm), visible range (400 – 700 nm) or infrared range (700 – 1500 nm) of electromagnetic radiation spectrum (Table 3) and measures the light that passes through a liquid sample.

Table 3: The electromagnetic spectrum. The electromagnetic spectrum is the range of frequencies of electromagnetic radiation and their respective wavelengths and photon energies.

Class	Wavelength
Gamma rays	1 pm
X-rays	10 pm – 1 nm
Ultraviolet	100 – 400 nm
Visible	**400 – 750 nm**
Infrared	750 – 100 000 nm
Microwaves and radio waves	above 1 cm

Spectrophotometric data can also be used in conjunction with the Beer-Lambert law, to determine various relationships between transmittance and concentration, and absorbance and concentration. Transmission and absorption are not linearly related to concentration, but absorbance is linearly related to concentration.

$$\mathbf{I = I_0 \times 10^{-\varepsilon c d}}$$

where

I_0 – the original light intensity,

I – the transmitted light intensity (after the beam of light passes through the cuvette),

c – the concentration in mol/l,

d – the length of path in cm,

ε – the molar extinction coefficient (or molar absorptivity, the characteristic constant of the subjects)

$$\mathbf{A = \log I_0/I = \varepsilon\ c\ d}$$

where

$\log I_0/I$ – the absorbance (A, no unit),

I/I_0 – transmittance (T).

Spectrophotometry may be used for:

- Identification of an unknown compound (compound has specific/characteristic absorption spectra),
- Determination of the concentration of the known compound.

To minimize the error, T must control for 20 – 65 % (or A = 0.2 – 0.7). The best results are obtained with $0.1 < A < 1$. When designing the experiment, we must choose an appropriate amount of samples and a proper type of cuvette to make the absorbance among this range. The batch of analysis must include the following solutions:

- Blank – this will help to exclude the absorption from reagents.
- Standard – includes a solution of known concentration of the substance that is to be determined in the test container.
- Test – contains an unknown quantity of the substance (compound).

A blank solution (reference blank, negative control) is used before the first sample is inserted into a spectrophotometer. Other compounds in a solution (or the solvent itself) may absorb the same wavelengths as the compound being analysed. The blank solution is the solvent of our solutions. The blank contains everything found in the sample solution except for the substance being analysed. The spectrophotometer is zeroed with the blank solution (Abs = 0). The reference blank is used for the calibration of a spectrophotometer.

Using of spectrophotometry – absolute measurement

The concentration of unknown compound is possible to calculate if we know the molar extinction coefficient:

$$\varepsilon = A / c\ d$$

Using of spectrophotometry – standard contrast method (relative measurement)

For two solutions of the same substance with different concentrations at the same thickness of the solution layer, the following applies:

$A_1 = \varepsilon\ d\ c_1$ and $A_2 = \varepsilon\ d\ c_2$

$$A_1/A_2 = c_1/c_2$$

If the concentration of the substance in one solution is known (standard, c_s) as well as the absorbance of the standard solution (A_s) and of the test solution (A_t), the concentration of the substance in the solution to be examined is calculated according to the equation:

$$c_t = c_s\ (A_t/A_s)$$

Using of spectrophotometry – calibration curve method

A calibration curve (standard) is used to determine the unknown concentration of a compound in a solution. The instrument is calibrated using several solutions of known concentrations. More standards are required with increasing concentration to accurately determine an unknown concentration of a compound. The absorbance of each known solution is measured and then a calibration curve of concentration versus absorbance is plotted (Figure 14). From the calibration curve, the unknown concentration of the compound is read off on the basis of the measured sample absorbance value.

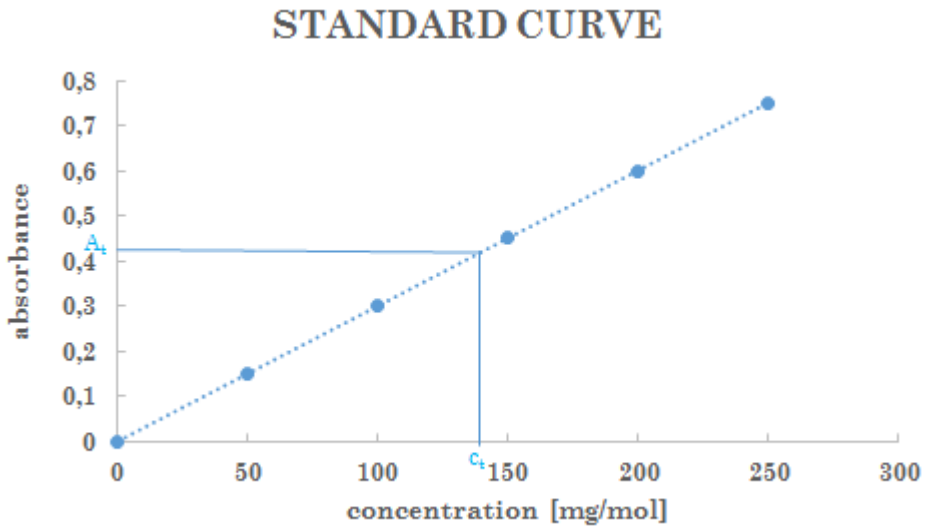

Figure 14: A **standard curve.** A standard curve also known as a calibration curve is a type of graph used as a quantitative research technique. A_t – absorbance of a test sample (unknown solution), c_t –concentration of a test sample determined from the standard curve (unknown solution).

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

DETERMINATION OF CONCENTRATION

DETERMINATION OF UNKNOWN CONCENTRATION OF BOVINE SERUM ALBUMIN – STANDARD CURVE METHOD AND STANDARD CONTRAST METHOD

Background

Biochemical parameters such as concentration can be determined using the calibration curve method or the standard contrast method. A set of standard solutions of bovine serum albumin (BSA) for the standard curve method or one standard solution of BSA for the standard contrast method are necessary. The quantitative analysis of BSA depends on how much light is absorbed by coloured compounds. The absorbance of each solution of known concentration is measured and then a calibration curve of concentration versus absorbance is plotted. The unknow concentration of BSA in the test solution is calculated.

Principle

Chemicals and tools

- Five solutions of BSA: 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml
- Coomasie Blue
- Distilled water
- Glass test tubes, pipettes, tips, cuvettes, spectrophotometer

Procedure

1. Add 100 µl of each standard solution and 700 µl of distilled water to separate glass test tubes.
2. Pipette 800 µl of distilled water (blank) to the new test tube (sample 6).
3. Pipette 100 µl of the sample solution and add 700 µl of distilled water to the new test tube (sample 7).
4. Add 200 µl of Coomassie Blue to each test tube.
5. Vortex and incubate for 5 minutes at room temperature.
6. Calibrate a spectrophotometer using the test tube No. 6.
7. Read the absorbance of each standard and sample at 595 nm.
8. Plot the absorbance of the standards vs. their concentration.
9. Calculate the concentration of the unknown sample using the standard curve and using only the standard sample number 3.

Table 4: Standard curve method

Sample	1.	2.	3.	4.	5.	6.	7.
BSA [µl]	100 from 10 µg/ml	100 from 20 µg/ml	100 from 30 µg/ml	100 from 40 µg/ml	100 from 50 µg/ml	-	-
dH_2O [µl]	700	700	700	700	700	800	700
Sample solution [µl]	-	-	-	-	-	-	100
CB [µl]	200	200	200	200	200	200	200
Vortex and incubate for 5 minutes at room temperature							

BSA - bovine serum albumin, d – distilled, CB - Coomassie Blue

Results

SAMPLE	ABSORBANCE $_{595\ nm}$
1.	
2.	
3.	
4.	
5.	
6.	
7.	

GRAPH

Discussion

Conclusion

5 STRUCTURE AND CHARACTERISTICS OF BIOMOLECULES

There are several compounds in living organisms that are essential for all living systems and they are responsible for diverse roles (functions/actions). The most important molecules in each organism are:

- Proteins,
- Saccharides,
- Lipids,
- Nucleic acids – the common name for all of them is biomolecules.

These molecules are key for all biochemical processes and human life. Most if not all of the diseases have a biochemical basis. Analysis – the determination of the proper structure and activity of biomolecules is essential for diagnosis and right treatment, as well as the development of new strategies for personal diagnosis and personal treatment.

5.1 AMINO ACIDS

Amino acids are organic compounds – functional derivatives of carboxylic acids, containing functional groups amine $–NH_2$ and carboxyl –COOH (Figure 15). Each amino acid also contains a side chain (R), except glycine, the simplest amino acid (one carbon, two hydrogens, one amine group, and one carboxyl group). Amino acids exist as a zwitterion in aqueous solutions under specific conditions. A zwitterion (amphion) is a dipolar ion. It is a molecule with two or more functional groups of which at least one has a positive charge and one has a negative charge, and the net charge of a molecule is zero. The pH at which this happens is the isoelectric point.

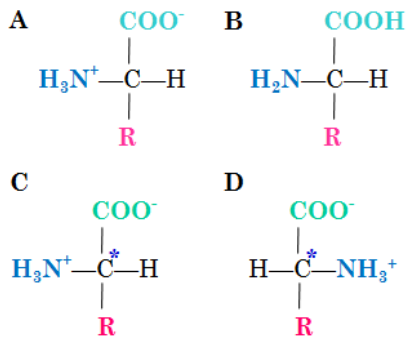

Figure 15: General structural formula of amino acid. The amino acid contains carboxyl group (COOH), amino group (NH_2), hydrogen another functional group (R-group, side chain of amino acid/ distinctive side chain). A – Structural formula of amino acid in biochemical systems and organisms. Amino acids in solution with a specific pH are dissociated. The pH of the human organism is 7.4, therefore the amino acid contains $–COO^-$ and NH_3^+. B – Structural formula of amino acid as an organic compound. C – α,L-amino acid, D – α,D-amino acid. * – common marker of a chiral (asymmetric) carbon.

Human amino acids are α, L – amino acids. Chemical reactions of amino acids follow from their structure. Common reactions for all amino acids are: decarboxylation, deamination, transamination, and condensation. Other reactions depend on the side chains of each amino acid and it is possible to use them for specific evidence. More than 300 amino acids have been described in nature, only 20 are commonly found in mammalian proteins. There are 20 α, L – amino acids (Figure 16) genetically encoded. These amino acids are also called proteinogenic and all of them are part of proteins. Additional 3 amino acids (selenocysteine, pyrrolysine, N-formylmethionine) can be incorporated into proteins by specific translation mechanisms. The nature of the side chain of the amino acid determines the role of the amino acid, which plays in the protein. Therefore, it is useful to classify the amino acid depending on the polarity of the R-group (ionization) in water:

- non-polar side chain,
- polar side chain: uncharged side chain,
  acidic side chain (negative charge),
  basic side chain (positive charge).

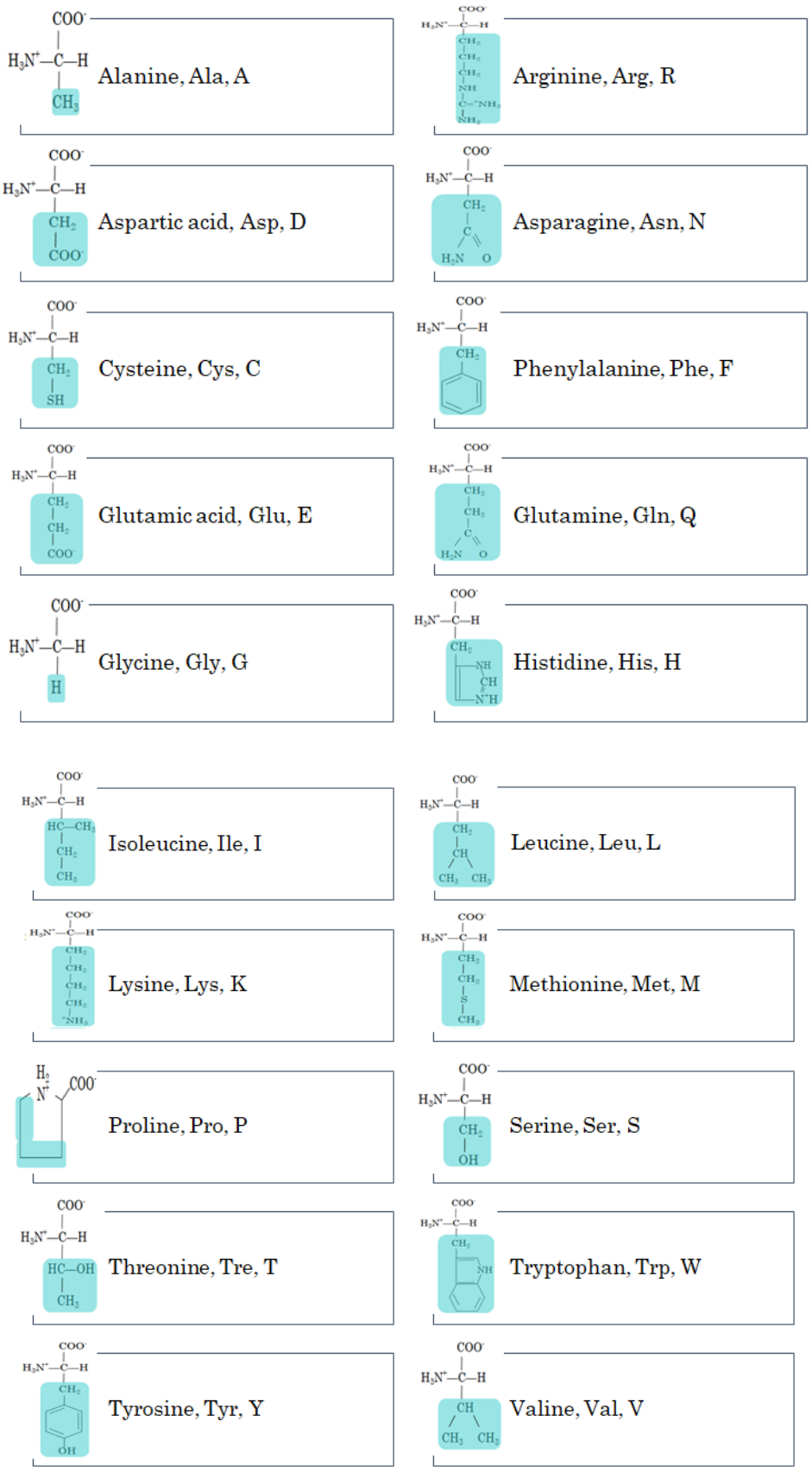

Figure 16: Twenty proteinogenic α,L-amino acids. All proteinogenic amino acids are α and L stereoisomers. Each α,L-amino acid has a 3-letter and 1-letter abbreviation.

Amino acids play several functions in the human body (Figure 17). Alpha-amino acids serve as building blocks of peptides and proteins. Amino acids can act as neurotransmitters or be precursors for biogenic amines, neurotransmitters, mediators, hormones, glucose, ketone bodies, purines, pyrimidines, porphyrins. Non-proteinogenic amino acids act as intermediates during degradation of proteinogenic amino acids. Amino acids and their derivatives can be part of lipids (serine in phospholipids, glycine in bile acids).

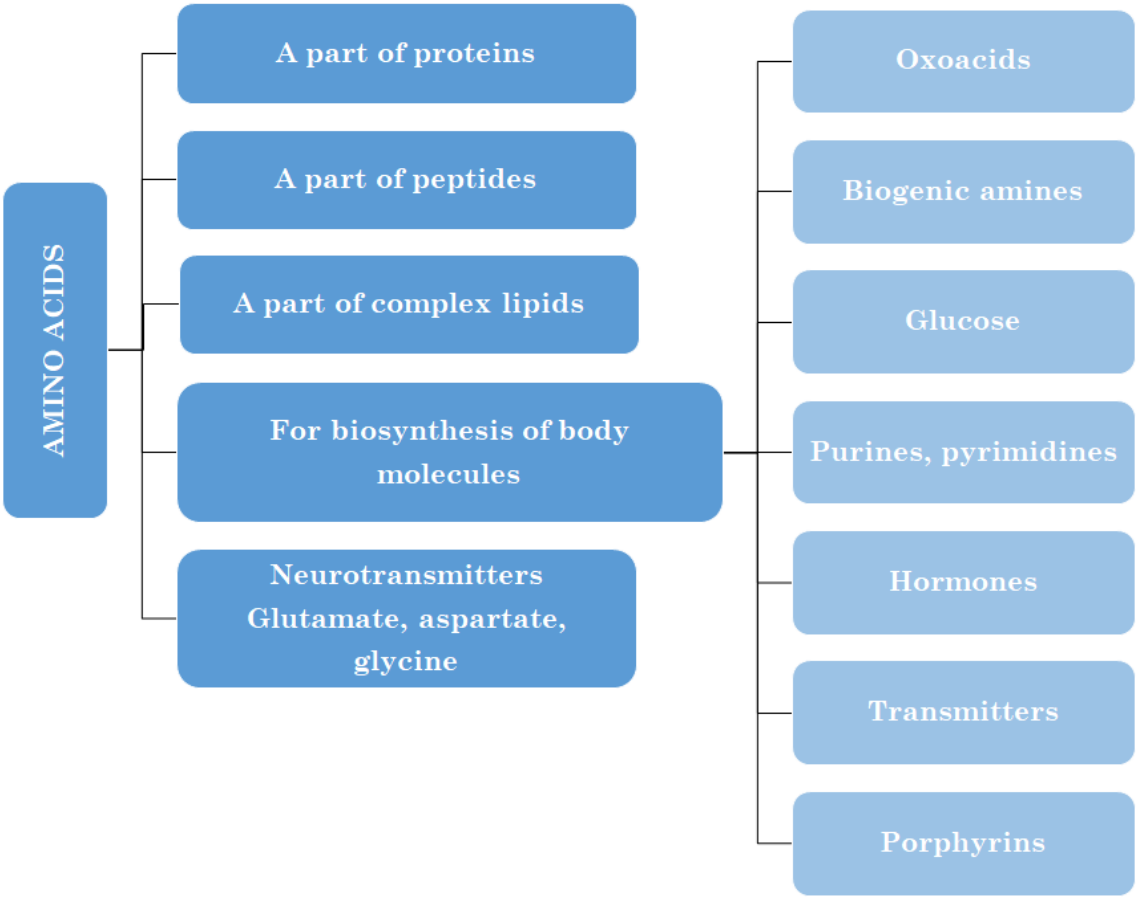

Figure 17: Basic function (activities/roles) of amino acids in the human organism. Amino acids are essential building blocks of peptides and proteins. They can act as precursor for various molecules and act as neurotransmitters.

Amino acids are:

- colourless,
- amphoteric molecules,
- crystalline,

- soluble in water and insoluble in non-polar solvents,
- optically active (rotating the polarized light).

They have high melting points greater than 200 °C.

Amino acids can be analysed by several qualitative tests:

- Ninhydrin test (for α-amino acids),
- Biuret test,
- Xanthoproteic test (for **tyrosine and tryptophan**),
- Lead sulfur test (for amino acids containing sulfur).
- Millon's test (to detect the presence of tyrosine in proteins),
- Sakagucchi test (to detect the presence of arginine in proteins),
- Hopkins-Cole reaction (to detect the presence of tryptophan in proteins).

The ninhydrin test is a colour reaction given by amino acids and peptides on heating with the ninhydrin. This test is used for the detection and quantitation of amino acids and peptides. The Xanthoproteic test is specific to aromatic amino acids. This reaction involves the nitration of benzene circle in alkaline condition. The lead sulfur test is used for the detection of the presence of sulfur. The sulfur of cysteine is converted to inorganic sulphide with concentrated sodium hydroxide (NaOH). Lead acetate is added, and black lead sulphide precipitate is produced. Millon's test is used to detect the presence of phenol (hydroxybenzene) that reacts with Millon's reagent to form red complexes. The Sakagucchi test is used to detect the presence of the guanine group. Arginine reacts with α-naphtol and an oxidizing agent such as bromide water to give a red-colour complex. The Hopkins-Cole reaction is used to detect the presence of an indol group that reacts with glyoxalic acid in the presence of concentrated sulfuric acid (H_2SO_4) to give a purple product.

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF AMINO ACIDS BY NINHYDRIN TEST

Background

Siegfried Ruhemann discovered the reaction of ninhydrin with a primary amino group in 1910. This test is a general test given by all amino acids, and we can use it for qualitative and quantitative analysis of amino acids (esters of amino acids, free amino groups in peptides, and proteins). All amino acids having α amino group, when heated in excess of ninhydrin, give a purple product called Ruhemann's purple (λ_{max} = 570 nm). However, in the case of imino acid, such as proline and hydroxyproline, a different product having a bright yellow colour is formed. Asparagine, which has a free amide group, reacts to give a brown-coloured product. The test is extremely sensitive and it is possible to detect 10^{-6} g of amino acid. The reaction of amino acids with ninhydrin (a powerful oxidizing agent) leads to their decarboxylation, deamination (formation of CO_2 and ammonia) and formation of aldehyde, which has one carbon atom less in its structure. Ninhydrin is reduced to form hydrindantin. Reduced ninhydrin condenses with ammonia and non-reduced ninhydrin molecule, leading to the formation of the purple condensation product (Figure 18).

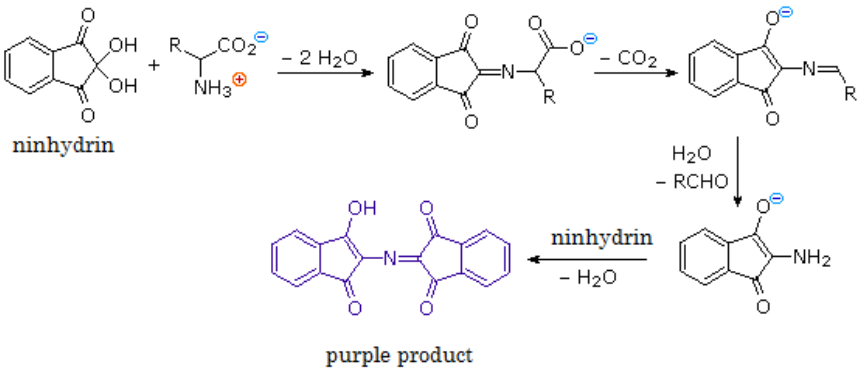

Figure 18: Reactions of the ninhydrin test. Ninhydrin reacts with α,L-amino acid and the result of four follow-up reactions is a specific purple dye condensation product.

Principle

Chemicals and tools

- 0.01 mol/l ninhydrin in ethanol
- 0.2 mol/l solution of amino acid 1 (test sample 1)
- 0.2 mol/l solution of amino acid 2 (test sample 2)
- 0.2 mol/l solution of protein (test sample 3)
- Distilled water (control, test sample 4)
- Glass test tubes, automatic pipettes, tips

Procedure

1. Take 1 000 µl of amino acid solution 1 in a dry glass test tube.
2. Similarly, take 1 000 µl of test solutions 2 and 3 in another new dry glass test tubes.
3. Take 1 000 µl of distilled water in a new dry glass test tube as a negative control.
4. Pour 500 µl of ninhydrin solution in each test tubes. Gently mix.
5. Keep the test tubes in a water bath for 5 minutes.
6. Look for the development of a specific colour.

Results

TEST TUBE NAME	OBSERVATION	INTERPRETATION
Test sample 1		
Test sample 2		
Test sample 3		
Test sample 4		

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF AMINO ACIDS BY XANTHOPROTEIC TEST

Background

The xanthoproteic test is used to detect amino acids containing an aromatic ring (tyrosine and tryptophan) in a protein solution that gives yellow colour to nitro derivatives upon heating with concentrated nitric acid (HNO_3). Phenylalanine gives a negative or weakly positive reaction though this amino acid contains an aromatic ring because it is difficult to nitrate under normal conditions. Proteins containing aromatic amino acids in their structure also have a positive xanthoproteic test. The aromatic benzene ring undergoes nitration upon heating to give a yellow-coloured product (nitrated tyrosine or tryptophan). When a base is added to these nitrophenyl groups, they ionize, and derivative salts are produced, the colour changes from yellow to orange (Figure 19).

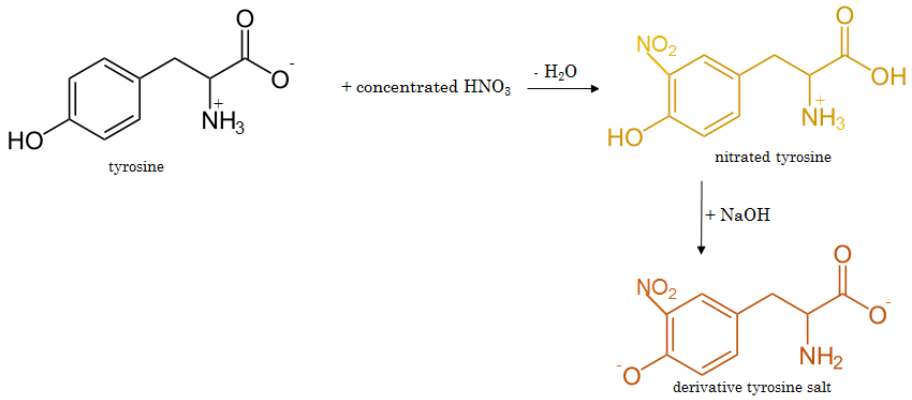

Figure 19: Reactions of the xanthoproteic test. Tyrosine reacts with concentrated nitric acid and results in a specific orange product.

Principle

Chemicals and tools

- 0.05 mol/l phenylalanine (test sample 1),
- 0.05 mol/l tyrosine (test sample 2),
- 0.05 mol/l tryptophan (test sample 3),
- 0.05 mol/l solution of a protein (test sample 4)
- Concentrated nitric acid
- 2.5 mol/l NaOH
- Distilled water (control, test sample 5)
- Glass test tubes, automatic pipettes, tips

Procedure

1. Take 1 000 µl of test solution 1 in a dry glass test tube.
2. Similarly, take 1 000 µl of test solutions 2, 3, 4 and distilled water in another dry glass test tubes.
3. Add 500 µl of concentrated HNO_3 in all test tubes and mix well.
4. Heat the solutions for 2 minutes.
5. Look for the colour development.
6. Cool the solution under tap water.
7. Add 2 000 µl of 2.5 mol/l NaOH to all test tubes.
8. Look for the colour development.

Results

TEST TUBE NAME	OBSERVATION + HNO_3	OBSERVATION + NAOH	INTERPRETATION
Test sample 1			
Test sample 2			
Test sample 3			
Test sample 4			
Test Sample 5			

Discussion

Conclusion

5.2 PROTEINS

Proteins are high-molecular weight compounds that are produced in the process called protein biosynthesis (transcription and translation) by interacting amino acids to form a peptide bond. The building blocks of proteins are 20 amino acids. Proteins are essential parts of living matter, provide many biologically important functions, and participate in every process within the cell (Figure 20). Proteins are active/functional only after folding into unique 3-dimensional structures (primary, secondary, tertiary, and quaternary structure). All proteins have a complex 3-dimensional shape. Protein structure depends on the amino acid sequence in the polypeptide chain – the primary structure. Secondary, tertiary, and quaternary structures characterize the spatial arrangement of proteins. Proteins are not rigid structures. They may change between several related structures while performing their functions. Proteins are due to high molecular weight and electrochemical properties in both solution and colloid state. The different solubility of proteins results from the different ratios of hydrophobic and hydrophilic groups, as well as from their spatial arrangement. Many physical and chemical factors (heat, high energy, mechanical forces, pH, and heavy metal ions) can cause a substantial change in the structure of proteins. A change in the native structure of the protein that does not break the primary structure is called denaturation. Loss of native structure results in loss of properties that condition the biological functions of the protein. Denaturation results in the precipitation of a protein from the solution.

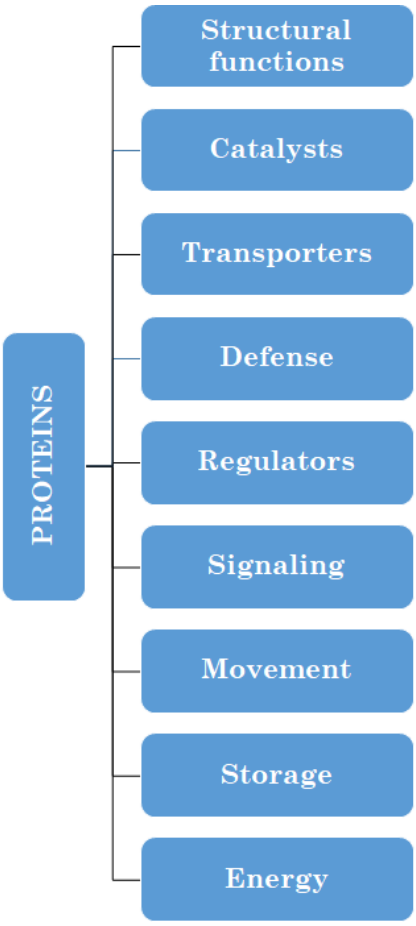

Figure 20: Roles of proteins in the human organism. Structural proteins such as collagen and elastin provide support. Catalyst proteins represent enzymes that are essential for each reaction in a human organism. Regulatory proteins control cell processes. Proteins also play an important part in the immune system (antibodies), in the transport (for example, oxygen transport by haemoglobin), and movement (muscles).

Proteins can be analysed by several quantitative tests:

- Ultraviolet-visible spectroscopy (absorbance at 280 nm),
- Kjeldahl test,
- Dumas method,
- Biuret test,
- The Lowry method,
- The Bradford assay,
- Bicinchoninic acid assay (BCA).

Proteins can be analysed by several qualitative tests:

- Electrophoresis,
- Precipitation methods,

- Colour reactions of proteins,
- Dialysis,
- Proteomics (mass spectrophotometry, X-ray crystallography, NMR spectroscopy).

Precipitation reactions –a protein exists in a colloidal solution due to hydration of the polar group (carboxyl group, amino group, hydroxyl group). They can be precipitated by dehydration or neutralization of polar groups. The formation of a precipitate indicates the presence of proteins. Proteins can be precipitated by salts (such as saturated $(NH_4)_2SO_4$ solution), heavy metal salts (such as lead acetate), alkaloidal reagent (such as picric acid), organic solvents (such as alcohol), heat and acids (such as acetic acid). Proteins give several colour reactions with different reagents as a result of the presence of amino acids or peptide bonds. The Biuret test is used for detecting the presence of peptide bonds (violet-coloured coordination) and is specific to all proteins. This test is positive for proteins and negative for free amino acids; therefore, it is used to differentiate between proteins and amino acids. The xanthoproteic test is positive because of the nitration of the aromatic ring. Millon's test is positive because of the presence of tyrosine in soluble proteins. A reddish-brown precipitate indicates the presence of tyrosine residues that occur in nearly all proteins. The ninhydrin test is a colour reaction given by amino acids and peptides on heating with the ninhydrin. This test is specific to all proteins and amino acids. The Hopkin-Cole test (aldehyde test) is a specific test used for the detection of indole ring and thus, tryptophan in proteins. Sulphuric acid in the presence of mercuric sulfate oxidizes the indole ring of tryptophan, and a violet-purple complex is formed. Nitroprusside's test is specific for proteins containing sulfur (cysteine, cysteine), and it gives a red-purple colour.

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF PROTEIN SOLUBILITY

Background

The solubility of proteins in water depends on the degree of ionization of the molecules. One of the factors that affects the stability of protein solutions is the ionic strength of the environment. A small amount of neutral salts ($MgSO_4$, $MgCl_2$, Na_2SO_4, NaCl, KCl) increases the solubility of proteins in water – salting in. The solubility does not depend on the properties of the neutral salt, but rather on its concentration and on the size of the ionic charges arising from its ionization in solution. Low salt concentration → the solubility increases (salting in). Salt molecules stabilize protein molecule by decreasing the electrostatic energy between the protein molecules, increasing the solubility of proteins. High salt concentration → the solubility decreases, and protein precipitates (salting out).

Principle

Chemicals and tools

- Egg white
- 0.154 mol/l NaCl (test sample 1)
- 0.062 mol/l $MgSO_4$ (test sample 2)
- Distilled water (control, test sample 3)
- Glass test tubes, automatic pipettes, tips

Procedure

1. Take 5 000 µl of test solution 1 in a dry glass test tube.
2. Take 5 000 µl of test solution 2 in another dry glass test tube.
3. Take 5 000 µl of test solution 3 in another dry glass test tube.
4. Take 500 µl of egg white to the all three glass test tubes.
5. Look and explain the solubility of egg white under different conditions.

Results

TEST TUBE NAME	OBSERVATION	INTERPRETATION
Test sample 1		
Test sample 2		
Test sample 3		

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF PROTEINS BY BIURET TEST

Background

The biuret test is a general test for proteins. It gives a colour reaction due to the presence of a peptide linkage in a polypeptide or protein. When proteins are treated with dilute copper sulfate in an alkaline solution, a characteristic purple colour coordination complex of cupric ions is formed with unshared electron pairs of nitrogen in peptide and O_2 of water (Figure 21). The colour and its intensity depend on the size of the molecule, its concentration and minimal two peptide bounds are necessary (small peptides are responsible for red-coloured complexes, proteins for purple-coloured complexes).

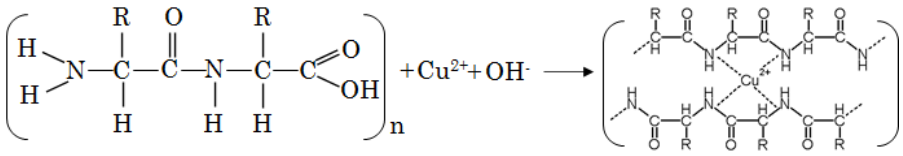

Figure 21: Reaction of biuret reagent with the peptide bond. The biuret test will result in the formation of a violet-purple.

Principle

Chemicals and tools

- 0.062 mol/l $CuSO_4$
- 2.5 mol/l NaOH
- Protein solution (test sample 1)

- Alanine solution (test sample 2)
- Distilled water (test sample 3)
- Glass test tubes, automatic pipettes, tips

Procedure

1. Take 1 000 µl of protein solution in a dry glass test tube.
2. Take 1 000 µl of alanine solution in another dry glass test tube.
3. Take 1 000 µl of distilled water in another dry glass test tube.
4. Add 2 000 µl of NaOH and two drops of $CuSO_4$ to the all three glass test tubes.
5. Look and explain your observation.

Results

TEST TUBE NAME	OBSERVATION	INTERPRETATION
Test sample 1		
Test sample 2		
Test sample 3		

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF PROTEIN DENATURATION BY ACIDS

Background

Protein denaturation (precipitation, coagulation) is a process in which a protein loses its native shape because of the disruption of weak noncovalent chemical bonds and interactions. The solubility of proteins in aqueous buffers depends on the distribution of hydrophilic and hydrophobic amino acid residues on the surface of the protein. Denaturation of proteins results in destruction of secondary, tertiary (and quartery) structures of proteins and loss of protein activities (functions). There are physical and chemical denaturation agents:

- Physical agents: heat, violent shaking (agitation), X-rays, ultraviolet radiation, hydrostatic pressure
- Chemical agents: altered pH, strong acids, strong bases, organic solvents, salts of heavy metals, detergents

Heat and ultraviolet radiation increase molecular motion, which can disrupt the attractive forces, hydrogen bonds, and nonpolar hydrophobic interactions. Violent shaking (agitation) shears of hydrogen bonds in proteins. The change in pH denatures proteins because it changes the charges of the amino acid side chains (disruption of electrostatic attractions and hydrogen bonds). Strong acids and bases disrupt salt bridges. Urea denatures proteins by forming new hydrogen bonds to amino acid side chains that are stronger than the hydrogen bonds between amino acid side chains in the protein. Organic solvents (ethanol and acetone) change the dielectric constant and hydration of the ionized groups. Denaturation of proteins occurs by disrupting hydrophobic interactions. These tests are usually used for protein detection in urine and for deproteinization of serum and plasma.

Principle

Chemicals and tools

- Concentrated HNO_3
- 20 % sulfosalicylic acid
- Protein solution (test sample 1, 2)
- Alanine solution (test sample 3, 4)
- Distilled water (control, test sample 5, 6)
- Glass test tubes, automatic pipettes, tips

Procedure

1. Take 1 000 µl of protein solution two times in two dry glass test tubes.
2. Take 1 000 µl of alanine solution two times in another two dry glass test tubes.
3. Take 1 000 µl of test distilled water two times in another two dry glass test tubes.
4. Add 1 000 µl of HNO_3 to one set of three test solutions (test sample 1, 3, 5).
5. Add 1 000 µl of sulfosalicylic acid to the second set of three test solutions (test sample 2, 4, 6).
6. Look and explain your observation.

Results

TEST TUBE NAME	OBSERVATION + HNO_3	OBSERVATION + sulfosalicylic acid	INTERPRETATION
Test sample 1			
Test sample 2			
Test sample 3			
Test sample 4			
Test sample 5			
Test sample 6			

Discussion

Conclusion

5.3 ENZYMES

Enzymes are biopolymers (proteins and ribozymes) catalysing vitally all biochemical processes. Enzymes as proteins are globular proteins synthesized in living organisms. In addition to proteins, also some ribonucleic acid (RNA) can have an enzymatic activity. Catalytic RNA molecules can be enzymes and they are called ribozymes. The presence and maintenance of a complete set of enzymes is a fundamental prerequisite for metabolism in a living organism (more than 5 000 biochemical reaction types). Enzymes increase the rate of reactions at which reactions approach equilibrium and accelerate reactions by lowering their activation energy. Enzymes are used to accelerate and control the rates of vitally important biochemical reactions in living systems. Enzyme-catalysed reactions are 10^3 – 10^{20} times faster than unanalysed reactions. Enzymes are much larger than their substrates. They contain hundreds to thousands of amino acids. Enzyme contains:

- The catalytic site: 2 – 4 amino acids directly involved in catalysis,
- The binding site: next to the catalytic site,
- The active site: the catalytic site and the binding site,
- The allosteric site: some enzymes contain a specific site for modulators.

Isoenzymes are physically distinct forms of a given enzyme. They differ in their structure, kinetic parameters, distribution in tissues, organs, and subcellular localization. The presence of enzymes allows reactions proceed at the pH and temperature of living organisms. Catalytic activity of enzymes depends on the presence of a precise conformational structure of the polypeptide chains. The catalytic activity, substrate specificity, and stereospecificity are key characteristics of enzymes. Each enzyme catalyses only one or a limited variety of chemical reactions. Enzymes are specific for the substrate (absolute, functional group, and stereospecificity), bond, and type of reaction. There are six groups of enzymes (Figure 22):

- Oxidoreductases,
- Transferases,
- Hydrolases,
- Lyases,
- Isomerases,
- Ligases.

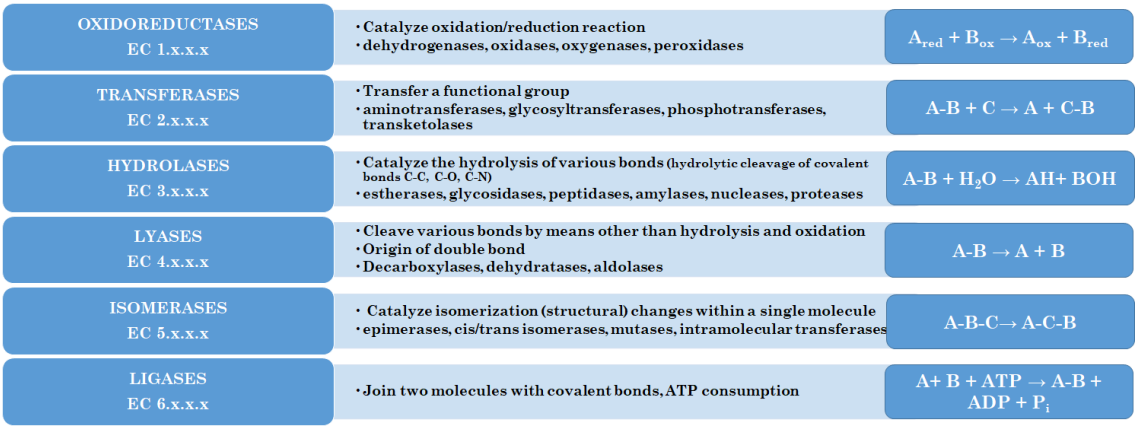

Figure 22: Classification of enzymes. Enzymes are classified according to the reaction they catalyse.

The activity of the enzyme can be regulated by:

- Temperature,
- pH,
- Substrate concentration,
- Product concentration (inhibition by product),
- Covalent modification,
- Allosteric effectors (positive or negative),
- Enzyme concentration (amount).

Enzymes usually need for their catalytic activity a cofactor or a coenzyme. Cofactor is non-protein – inorganic part (ions of metal) of enzyme. Coenzyme is non-protein – organic part of enzyme (Figure 23). The apoenzyme is a protein portion of the enzyme (usually inactive). The holoenzyme is a functional complex (active) of the enzyme and cofactor (coenzyme). Protein and non-protein parts of enzyme are bounded together. The prosthetic group is a coenzyme permanently attached by a covalent bond to the enzyme.

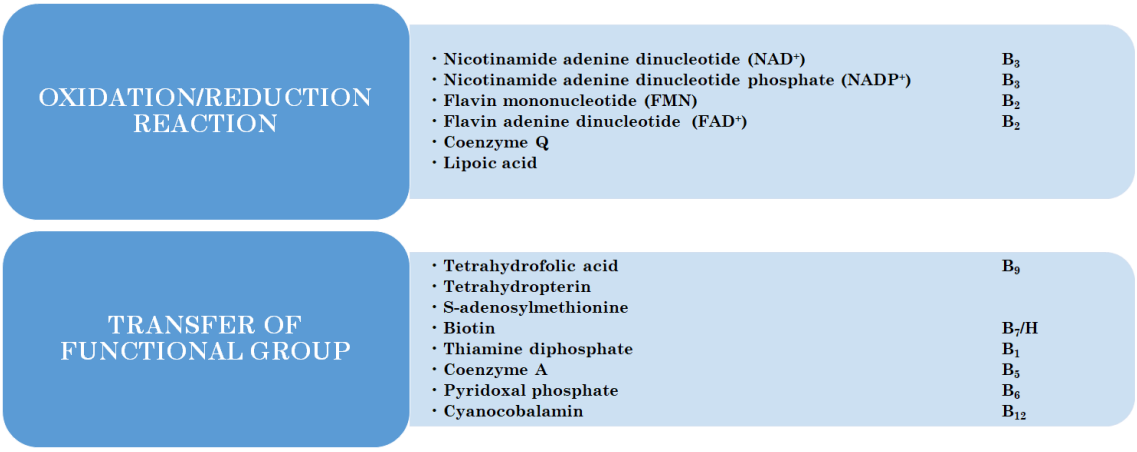

Figure 23: Classification of coenzymes. The coenzyme is essential for the biological activity of the enzyme. Coenzymes act as group-transfer reagents. Hydrogen, electrons, or groups of atoms can be transferred. Coenzymes are synthesized from common metabolites. Vitamin-derived coenzymes are derivatives of vitamins. Vitamins cannot be synthesized by mammals but must be obtained as nutrients.

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF SUBSTRATE SPECIFICITY OF SACCHARASE AND ALPHA AMYLASE

Background

Hydrolases such as saccharase (alternative names: invertase, sucrase, glucosucrase, beta-h-fructosidase, beta-fructofuranosidase) and α-amylase are enzymes that catalyse hydrolysis of glycosidic bonds in saccharides. Saccharase catalyses the breakdown of the 1,2-glycosidic bond in sucrose (table sugar) into glucose and fructose. Alpha amylase catalyses breakdown of 1,4-glycosidic bond in starch into dextrin and maltose. Both enzymes break the O-glycosidic bond, but due to substrate specificity α-amylase cannot break sucrose and saccharase cannot break starch. Sucrose and starch are non-reducing saccharides, but glucose, fructose dextrines, and maltose are reducing saccharides. This chemical property of saccharides can be used for the detection of reducing saccharides by Fehling's test (13th protocol). The iodine test is used for non-hydrolysed starch (16th protocol).

Principle

Chemicals and tools

- 2 g/l starch
- 0.03 mol/l sucrose
- Saccharase
- α-amylase
- Fehling's reagent – Fehling solution I 70 g $CuSO_4.5H_20$ in 1000 ml distilled H_20

  – Fehling solution II 100 g NaOH, 350 g potassium tartrate in 1000 ml distilled H_20

  Solution I and solution II are mixed in ration 1 : 1 (v/v) before use.
- Lugol's solution (I_2 in KI)
- Glass test tubes, automatic pipettes, tips, water bath

Procedure

1. Four glass test tubes are prepared according Table 5.
2. All samples are incubated for 30 minutes at 37 °C.
3. All samples are examined by the Fehling test and reaction with Lugol's solution.
   i. Prepare 4 ml of Fehling's reagent by mixing Fehling's solution I with Fehling's solution II to one dry plastic test tube. Mix Fehling's reagent well.
   ii. Add 1 000 µl of Fehling's reagent to four dry glass test tubes.
   iii. Add 1 000 µl of test sample 1 to one glass test tube.
   iv. Add 1 000 µl of test sample 2 to the second glass test tube.
   v. Add 1 000 µl of test sample 3 to the third glass test tube.
   vi. Add 1 000 µl of test sample 4 to the fourth glass test tube.
   vii. Mix gently and incubate all four test tubes in a water bath for 3 minutes.
   viii. Look and explain your observation.
   ix. Add 100 µl of Lugol's solution to glass test tubes marked test sample 1, test sample 2, test sample 3 and test sample 4.
   x. Look and explain your observation.

Table 5: Preparation of samples for substrate specificity

SOLUTION	Test sample [ml]			
	1	2	3	4
Starch	2	2	–	–
Sucrose	–	–	2	2
α-amylase	2	–	2	–
Saccharase	–	2	–	2

Results

TEST NAME	TUBE	OBSERVATION + FEHLING'S SOLUTION	OBSERVATION + LUGOL'S SOLUTION	INTERPRETATION
Test sample 1				
Test sample 2				
Test sample 3				
Test sample 4				

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

SEPARATION OF LOW MOLECULAR WEIGHT COMPOUNDS FROM THE SOLUTION OF PROTEINS

Background

Dialysis is the diffusion of ions and small molecules from a colloid solution across a semipermeable membrane to clear water. Osmosis and dialysis are based on the selective permeability of the membranes for molecules. Osmotic phenomena play an important role in biological systems in absorption of fuel and excretion of substances by the kidney and exocrine and endocrine glands function. Dialysis is used to lower the concentration of small molecules in solutions.

Principle

Chemicals and tools

- 0.2 g/mol albumin
- 0.1 mol/l $K_2Cr_2O_7$
- 0.15 mol/l trichloracetic acid
- Distilled water
- Cellophane membrane
- Beaker, glass stick, volumetric cylinder, cuvette, glass test tubes, pipettes, tips, spectrophotometer

Procedure

1. Add 50 ml of distilled water to a beaker.
2. Open the presoaked membrane in distilled water and add 250 μl of albumin solution and 250 μl of $K_2Cr_2O_7$.
3. Close the membrane by pean and dip the membrane into the beaker with distilled water.
4. Each 10 minutes (10, 20, 30 and 40 minutes) mix gently the solution in the beaker and take 2 000 μl of solution to a new dry glass test tube.
5. Measure absorbance at 385 nm.
6. Record diffusion time dependence.
7. After 40 minutes, take 1 000 μl of solution from the beaker to a new dry glass test tube.
8. Add 200 μl of trichloracetic acid.
9. Look, explain your observation, and draw the graph.

Results

GRAPH

Discussion

Conclusion

5.4 SACCHARIDES

Saccharides (sugars, carbohydrates) are biomolecules composed of carbon, hydrogen, and oxygen. Saccharides are aldehydes or ketones of higher polybasic alcohols (polyhydroxylated aldehydes, polyhydroxylated ketones) or their polycondensates with the empirical formula $(CH_2O)_n$. They are divided into four chemical groups based on the number of monosaccharide units:

- monosaccharides,
- disaccharides,
- oligosaccharides,
- polysaccharides (homo/hetero polysaccharides, glycosaminoglycans, glycoconjugates).

Saccharides are essential parts of living matter, provide many biologically important functions, and participate in every process within a cell (Figure 24.). Polysaccharides (glycogen and starch) serve for energy storage, and monosaccharides (glucose) are the most important and fastest utilizable source of energy. Complex lipopolysaccharides, glycoproteins, and proteoglycans are components of membranes and tissues. A saccharide skeleton is a biological precursor of proteins and lipids in biosynthetic pathways. Deoxyribose is part of deoxyribonucleic acids and ribose is part of ribonucleic acids, nucleotides (ATP, cAMP, GTP), coenzymes (nicotinamide adenine dinucleotide – NAD^+, flavin adenine dinucleotide – FAD). Saccharides are important for many biological and physiological processes: cell communication, immune system function, fertilisation, blood coagulation, and organism development. Very important polysaccharides are glycoconjugate. Glycoconjugate is the general classification for saccharides covalently linked to other chemical species, such as proteins, peptides, and lipids.

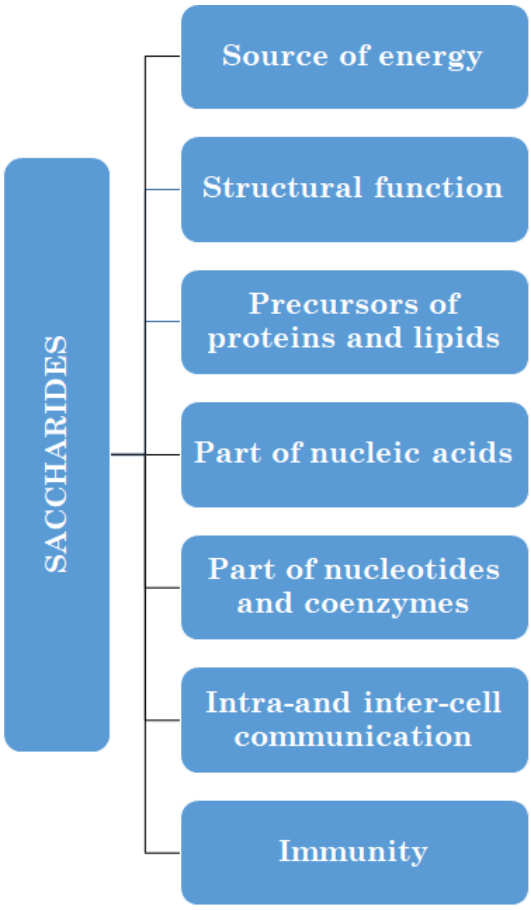

Figure 24: Basic functions of saccharides. Saccharides are the most important and fastest utilizable source of energy. Glucose is the only source of energy for the brain and erythrocytes. Complex lipopolysaccharides, glycoproteins, and proteoglycans are components of membranes and tissues – structural function. The carbon skeleton of saccharides is used as precursor for the biosynthesis of amino acids (proteins) and lipids. Saccharides as a component of complex molecules are essential for other biological and physiological functions such as communication, immune system, fertilization, blood coagulation, organism development, and blood type (blood group).

Qualitative tests for saccharides are as follows:

- Molish's test,
- Benedict's test,
- Fehling's test,
- Barfoed's test,
- Seliwanoff's test,
- Hydrolysis test for sucrose,
- Osazone test,
- Bial's test,
- Iodine test.

Saccharides when treated with concentrated sulfuric acid undergo dehydration to give furfural derivatives. Aldehyde group of an aldose can reduce a number of substances. Ketoses can also reduce substances, but not so easily. Saccharides with free aldehyde or ketone groups can reduce solutions of various metallic ions. The Molish's test is positive for all saccharides. Benedict's test is positive for simple saccharides. The Fehling's test is positive for reducing saccharides. The Barfoed's test is positive for monosaccharides and oligosaccharides. The Seliwanoff's test is a test which distinguishes between ketose and aldose saccharides (ketoses are more rapidly dehydrated than aldoses). The iodine test is used to test the presence of starch, cellulose, and dextrin.

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF SACCHARIDES BY FEHLING'S TEST

Background

The Fehling's test is used to differentiate between reducing and non-reducing saccharides. Fehling's reagent comprises of two solutions, Fehling's solution I and Fehling's solution II. These two solutions are mixed in equal amount before the test. A reducing saccharide reacts with Fehling's reagent in alkaline medium to form an orange to red precipitate. In heating aldoses reduce cupric ion (Cu^{2+}) in Fehling's reagent to cuprous ion (Cu^{+}) with the formation of a red precipitate of cuprous oxide (Cu_2O). The aldehyde group is oxidized to the carboxyl group (Figure 25).

$$CuSO_4.5H_2O + 2NaOH + KNaC_4H_4O_6.4H_2O \rightarrow \text{Fehling's reagent} + Na_2SO_4 + 11H_2O$$

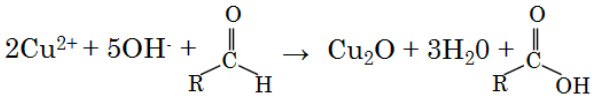

Figure 25: Schematic reaction of aldehyde oxidation with Fehling's reagent. The positive result is detected by reduction of the blue solution of cupric to a red precipitate of cuprous oxide.

Principle

Chemicals and tools

- Fehling's reagent – Fehling solution I 70 g $CuSO_4.5H_2O$ in 1000 ml distilled H_20
  – Fehling solution II 100 g NaOH, 350 g potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$) in 1000 ml of distilled H_2O

  Solution I and solution II are mixed in ration 1 : 1 (v/v) before use.

- 0.1 mol/l glucose solution (test sample 1)
- Distilled water (control, test sample 2)
- Plastic test tubes, glass test tubes, pipettes, tips, water bath

Procedure

1. Prepare 2 000 µl of Fehling's reagent by mixing Fehling's solution I with Fehling's solution II to one dry plastic test tube. Mix the Fehling reagent well.
2. Add 1 000 µl of Fehling's reagent to two dry glass test tubes.
3. Add 1 000 µl of glucose solution to one glass test tube.
4. Add 1 000 µl of distilled water to the second glass test tube.
5. Mix gently and incubate both test tubes for 3 minutes in a water bath.
6. Look and explain your observation.

Results

TEST TUBE NAME	OBSERVATION	INTERPRETATION
Test sample 1		
Test sample 2		

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF SACCHARIDES BY BENEDICT'S TEST

Background

Benedict's test is the test for the presence of reducing saccharides (free aldehyde or ketone group). Benedict's reagent is a bright blue solution and contains copper sulfate pentahydrate ($CuSO_4.5H_2O$), sodium carbonate (Na_2CO_3), and sodium citrate ($Na_3C_6H_5O_7$) in distilled water. When reducing saccharide is heated in the presence of alkaline sodium carbonate, they are converted to reducing species known as enediols. Enediols are powerful reducing agents. They reduce the cupric compounds (Cu^{2+}) present in the Benedict's reagent to cuprous compounds (Cu^{+}) which is responsible for the change in colour of the reaction mixture (Figure 26) and precipitate as insoluble red copper oxide (Cu_2O).

$$CuSO_4.5H_2O + Na_2CO_3 + Na_3C_6H_5O_7 \rightarrow \text{Benedit's reagent}$$

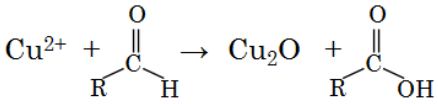

Figure 26: Schematic reaction of Benedict's test. Enendiols are powerful reducing agents that reduce cupric ions to red cuprous oxide – brick-red precipitate.

The colour of the precipitate obtained depends on the quantity of saccharide in the solution; therefore, this test is a semi-quantitative (Table 6, Figure 27). Reaction is nonspecific for reducing saccharides.

Table 6: Approximate concentration of glucose after the Benedict's test.

COLOUR AFTER REACTION	APPROXIMATE GLUCOSE CONCENTRATION [mmol/l]
Blue	0/no reducing sugar
Light green turbidity	till 16
Dark brown turbidity	28.0
Brown turbidity and brick red precipitate	58.0
Red-brown turbidity and brick red precipitate	84.0
Bright red turbidity and brick red precipitate	168.0

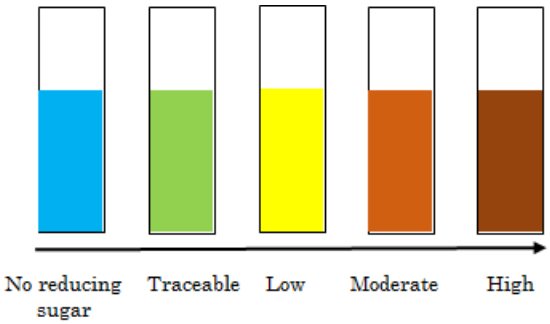

Figure 27: Benedict's test results. The qualitative test produces a colour change from blue to green to yellow to orange to brick-red. The obtained colour correlates with the concentration of reducing saccharide in the solution.

Principle

Chemicals and tools

- Benedict's reagent – solution I 17.3 g $CuSO_4.5H_2O$ in 100 ml distilled H_20
  – solution II 100 g Na_2CO_3, 173 g $Na_3C_6H_5O_7$ in 700 ml distilled H_20

Solution I is added to solution II by continual mixing and filled up to 1000 ml by distilled water.

- Glucose solution (test sample 1)
- Distilled water (test sample 2)
- glass test tubes, automatic pipettes, tips, water bath

Procedure

1. Take 2 500 µl of Benedict's reagent to two dry glass test tubes.
2. Add 200 µl of glucose solution to one glass test tube.
3. Add 200 µl of distilled water to second glass test tube.
4. Mix gently and incubate both test tubes in water bath for 3 minutes.
5. Look and explain your observation.

Results

TEST TUBE NAME	OBSERVATION	INTERPRETATION
Test sample 1		
Test sample 2		

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF SACCHARIDES BY SELIWANOFF'S TEST

Background

The Seliwanoff's test is used to differentiate between aldoses (saccharides containing an aldehyde group) and ketoses (saccharides containing a ketone group). This test is specific to ketohexoses. Ketohexoses are dehydrated and cycled in hot nonoxidizing mineral acids. Ketohexoses treated with hydrochloric acid (HCl) form 5-hydroxymethylfurfural which, upon condensation with resorcinol, gives a cherry red complex (Figure 28). This chemical test distinguishes aldoses from ketoses. Aldoses may react slightly to produce a light pink colour.

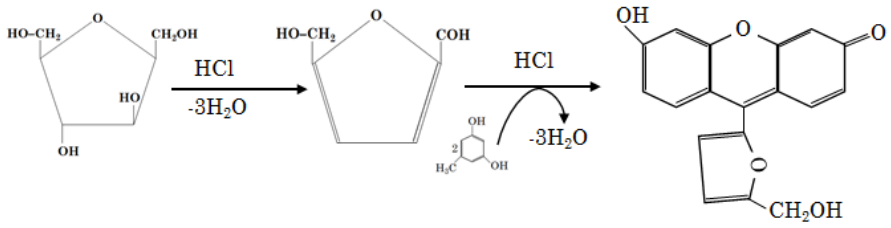

Figure 28: Seliwanoff's test results. Ketoses (fructose) treated with HCl form 5-hydroxymethylfurfural. This molecule reacts with resorcinol and a cherry red product is created.

Principle

Chemicals and tools

- 0.05% resorcinol diluted in 3 mol/l HCl (dissolve 50 mg of resorcinol in 33 ml of concentrated HCl and make it 100 ml with distilled water)
- 0.1 mol/l glucose solution (test sample 1)
- 0.1 mol/l fructose solution (test sample 2)
- 0.1 mol/l sucrose solution (test sample 3)
- 0.1 mol/l ribose solution (test sample 4)
- Distilled water (control, test sample 5)
- Glass test tubes, automatic pipettes, tips, water bath

Procedure

1. Take 2 000 µl of resorcinol solution in five dry glass test tubes.
2. Add 500 µl of glucose solution to the first glass test tube.
3. Add 500 µl of fructose solution to the second glass test tube.
4. Add 500 µl of sucrose solution to the third glass test tube.
5. Add 500 µl of ribose solution to the fourth glass test tube.
6. Add 500 µl of distilled water to the fifth glass test tube.
7. Mix gently and incubate test tubes in water bath for 1 minute.
8. Look and explain your observation.

Results

TEST TUBE NAME	OBSERVATION	INTERPRETATION
Test sample 1		
Test sample 2		
Test sample 3		
Test sample 4		
Test sample 5		

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF SACCHARIDES BY IODINE TEST

Background

The iodine test is a test for polysaccharides. Iodine forms a coordinate complex (deep blue-black colour) between the helically coiled polysaccharide chain and the centrally located iodine within the helix due to absorption. The colour and intensity depend on the length of the polysaccharide. Amylose is a linear chain component of starch and gives a deep blue colour. Amylopectin is a branched chain component of starch and gives a purple colour. Glycogen gives a reddish-brown colour. Dextrins as intermediates during starch hydrolysis give violet, red, and no colour. Starch is often used in analytical methods as an indicator of redox titrations in which triiodide is present.

Principle

Chemicals and tools

- Lugol's solution (potassium iodine with iodine in water)
- 0.1 mol/l starch solution (test sample 1)
- 0.1 mol/l amylose solution (test sample 2)
- 0.1 mol/l amylopectin solution (test sample 3)
- 0.1 mol/l glycogen solution (test sample 4)
- 0.1 mol/l dextrins solution (test sample 5)
- Distilled water (control, test sample 6)
- Glass test tubes, automatic pipettes, tips

Procedure

1. Add 1 000 µl of starch solution to a new dry glass test tube.
2. Repeat step 1. with all other test solutions (amylose, amylopectin, glycogen, dextrins, water).
3. Finally, 6 glass test tubes are prepared.
4. Add 500 µl of Lugol's solution to each test tube (6 test tubes). Mix gently.
5. Look and explain your observation.

Results

TEST TUBE NAME	OBSERVATION	INTERPRETATION
Test sample 1		
Test sample 2		
Test sample 3		
Test sample 4		
Test sample 5		
Test sample 6		

Discussion

Conclusion

5.5 LIPIDS

Lipids are a heterogeneous group of organic compounds that are related more by their physical properties than by their chemical properties. A lipid is an organic biomolecule that is soluble in nonpolar solvents and insoluble in water. There is not an easy characterisation of the chemical structure of lipids. Lipids can be divided into categories:

- Glycerolipids,
- Sphingolipids,
- Saccharolipids,
- Polyketides,
- Sterol lipids,
- Prenol lipids.

There are simple lipids, complex lipids, and derived lipids (Figure 29). Simple lipids are esters of fatty acids with various alcohols:

- Natural fats or oil (alcohol is glycerol),
- Waxes (alcohol is other than glycerol).

Hydrolysis of complex lipids gives a fatty acid with an alcohol and also other groups:

- Phospholipids,
- Glycolipids,
- Lipoproteins.

Derived lipids are cholesterol, steroids, vitamin A, and D.

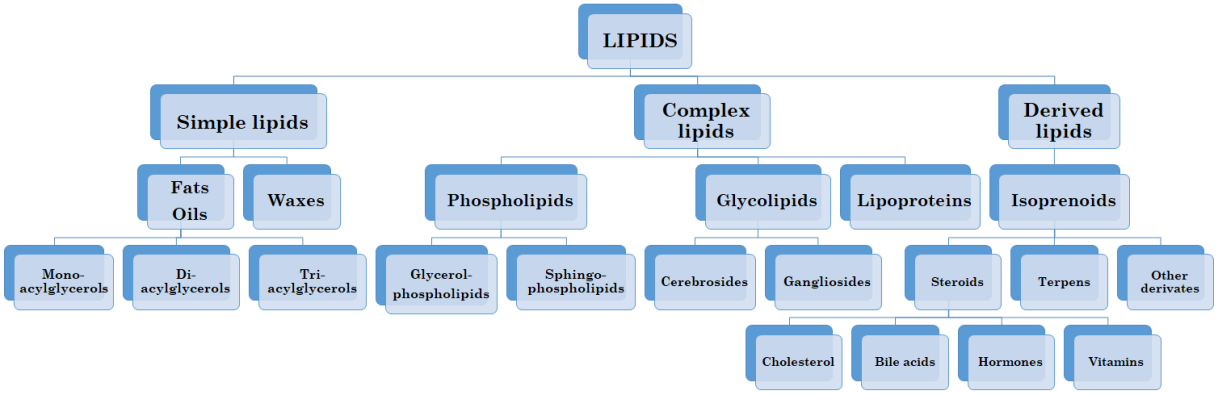

Figure 29: Classification of lipids. There are three types of lipids: 1. Simple lipids, 2. Compound lipids (complex lipids), and 3. Derived lipids. Simple lipids are those compounds that do not have other substances, these are basically ester fatty acids and different alcohols. Natural fats and oils, waxes. Compound lipids are esters of fatty acids and alcohol plus other groups. Phospholipids and sphingomyelin; Sphingolipids; Sulfolipids, Lipoproteins; Lipopolysaccharides. Derived lipids are composed of hydrocarbon rings and a long hydrocarbon side chain. Lipid soluble vitamins; Steroid hormones; Hydrocarbons; Ketone bodies.

Lipids are essential parts of living matter, provide many biologically important functions, and participate in every process within a cell (Figure 30).

The main qualitative tests for lipids are the following:

- Solubility test,
- Spotting test (translucent spot test),
- Acrolein test – used to detect the presence of glycerol or fat,
- Saponification test – the process of making soap that is produced from fats and oils,
- Emulsification test,
- Liebermann-Burchard test – detection of cholesterol and steroids,
- Salkowski's test – test for cholesterol,
- Tests for specific lipid molecules and their parts.

Many tests are preliminary tests that detect the presence of all lipids.

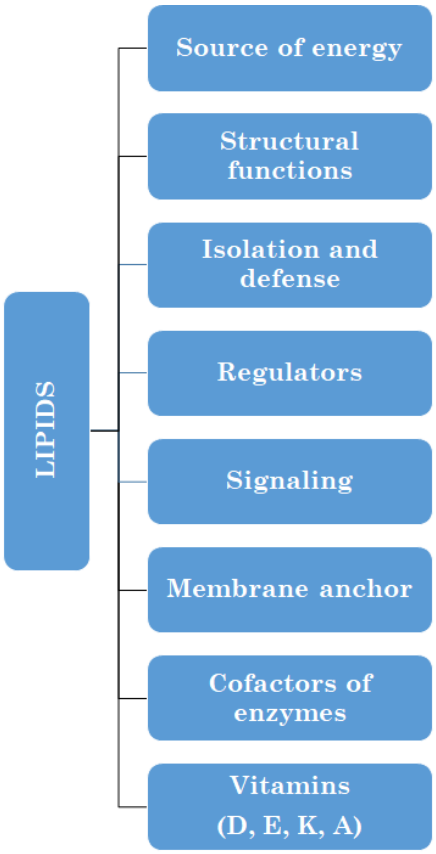

Figure 30: Functions (activities, roles) of lipids. Lipids serve as structural components of biological membranes. They provide energy reserves, predominantly in the form of triacylglycerols. Lipids and lipid derivatives serve as vitamins and hormones. Amphipatic bile acids aid in lipid solubilisation.

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF LIPIDS – SOLUBILITY TEST

Background

Lipids are a heterogeneous group of organic compounds that are related more by their physical properties than by their chemical properties. A lipid is an organic biomolecule that is soluble in nonpolar solvents and insoluble in water. The solubity test is based on the property of solubility of lipids in organic solvents and insolubility in water. The oil will float on water due to a lower specific gravity.

Principle

Chemicals and tools

- ethanol
- chloroform
- sunflower oil (test sample 1)
- olive oil (test sample 2)
- distilled water (control, test sample 3)
- glass test tubes, automatic pipettes, tips

Procedure

1. Take 3 000 µl of chloroform in three dry glass test tubes.
2. Take 3 000 µl of ethanol in three new dry glass test tubes.
3. Take 3 000 µl of water in three dry glass test tubes.
4. Add 5 drops (250 µl) of sunflower oil to a glass test tube with chloroform, with ethanol, and also with water.
5. Add 5 drops (250 µl) of olive oil to a glass test tube with chloroform, with ethanol, and also with water.
6. Add 5 drops (250 µl) of distilled water to the glass test tube with chloroform, with ethanol, and also with water.
7. Shake all test tubes and allow to stand for 1 minute.
8. Look and explain your observation.

Results

TEST TUBE NAME	OBSERVATION	INTERPRETATION
Test sample 1	**Chloroform**	
	Ethanol	
	Water	
Test sample 2	**Chloroform**	
	Ethanol	
	Water	
Test sample 3	**Chloroform**	
	Ethanol	
	Water	

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF LIPIDS – TRANSLUCENT SPOT TEST

Background

If a drop of solution (such as water, alcohol or oil) falls on paper it makes a mark which is bright and translucent when the paper is held up to the light. The mark of some solutions disappears as soon as the liquid evaporates. The lipid will not wet the filter paper u

unlike water. Lipids will form a greasy spot as they are having a greasy texture that will penetrate the filter paper.

Principle

Chemicals and tools

- Sunflower oil (test sample 1)
- Olive oil (test sample 2)
- Ethanol (test sample 3)
- Distilled water (control, test sample 4)
- Filter paper, automatic pipettes, tips

Procedure

1. Take four pieces of filter paper.
2. Add one drop (50 µl) of sunflower oil on one filter paper.

3. Add one drop (50 µl) of olive oil on the second filter paper.
4. Add one drop (50 µl) of ethanol on the third filter paper.
5. Add one drop (50 µl) of water on the last filter paper.
6. Look and explain your observation.

Results

TEST TUBE NAME	OBSERVATION	INTERPRETATION
Test sample 1		
Test sample 2		
Test sample 3		
Test sample 4		

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF LIPIDS – PHOSPHATIDYLSERIN DETERMINATION

Background

Phosphatidylserine is a phospholipid consisting of a glycerol backbone, which is bound to two fatty acids, and serine attached through a phosphodiester linkage to the third carbon of glycerol (Figure 31). All amino acids having α amino group, when heated in excess of ninhydrin, give a purple coloured product called Ruhemann's purple. The reaction of an amino acid with ninhydrin (a powerful oxidizing agent) leads to its decarboxylation, deamination (formation of CO_2 and ammonia) and formation of aldehyde which has one carbon atom less in its structure. Ninhydrin is reduced to hydrindantin. The reduced ninhydrin condenses with ammonia and a nonreduced ninhydrin molecule, which leads to the formation of the purple condensation product.

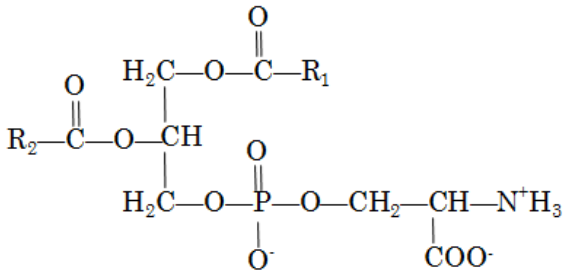

Figure 31: Phosphatidylserine. Phosphatidylserine is a phospholipid that contains: two residues of fatty acids (R_1, R_2), glycerol, phosphate group, and serine.

Principle

Chemicals and tools

- 0.01 mol/l ninhydrin in ethanol
- Mixture ethanol : ether (3 : 1)
- Serum (test sample 1)
- 0.2 mol/l solution of phosphatidylserine (test sample 2)
- Distilled water (control, test sample 3)
- Erlenmeyer flask, glass test tubes, automatic pipettes, tips, water bath, filter paper

Procedure 1 – Preparation of lipid extract

Because lipids are insoluble in water, their extraction from tissues and subsequent fractionation require the use of organic solvents and some techniques that are not commonly used in the purification of water-soluble molecules, such as proteins and carbohydrates.

1. Add 9 ml of mixture ethanol : ether to dry Erlenmeyer flask.
2. Add 1 000 µl of serum.
3. Slowly heat in a water bath until the reaction mixture is boiling.
4. Chill the reaction mixture and percolate (filtrate).

Procedure 2 – Phosphatidylserine determination

1. Take 1 000 µl of lipid extract to a dry glass test tube.
2. Take 1 000 µl of phosphatidylserine to a dry glass test tube.
3. Take 1 000 µl of water to dry glass test tube.
4. Add 500 µl of ninhydrin solution to each test tube.
5. Mix gently and incubate test tubes in a water bath for 2 minutes.
6. Look and explain your observation.

Results

TEST TUBE NAME	OBSERVATION	INTERPRETATION
Test sample 1		
Test sample 2		
Test sample 3		

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF LIPIDS – PHOSPHATIDYLCHOLINE DETERMINATION

Background

Phosphatidylcholine is a phospholipid consists of a glycerol backbone, which is bound to two fatty acids, and choline attached through a phosphodiester linkage to the third carbon of glycerol (Figure 32). Phosphatidylcholine reacts with Dragendorf's reagent and gives a red-orange coloured product. The reaction is not specific (caffeine, alkaloids, and anaesthetics react in a similar way).

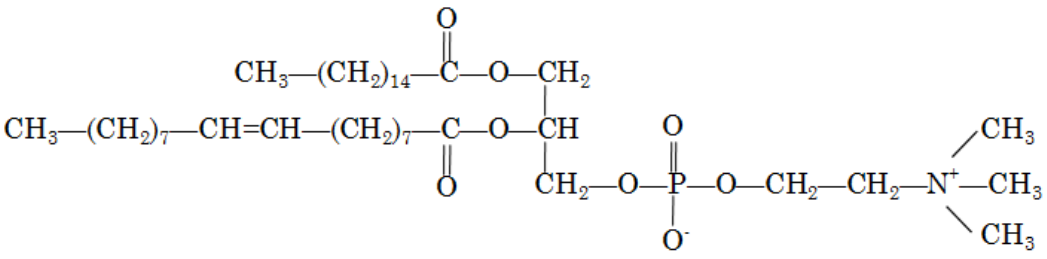

Figure 32: Phosphatidylcholine. Phosphatidylcholine is a phospholipid that contains: two fatty acids, glycerol, phosphate group, and choline.

Principle

Chemicals and tools

- Dragendorf's reagent: Solution A – 1.7 g $Bi(NO_3)_3$ in 100 ml 3.4 mol/l CH_3COOH
  Solution B – 2.4 mol/l KI
  Solutions A and B are mixed with water 4 : 1 : 8 before use.
- Lipid extract (test sample 1)
- 0.2 mol/l solution of phosphatidylcholine (test sample 2)
- Distilled water (control, test sample 3)
- Glass test tubes, automatic pipettes, tips

Procedure

1. Add 500 µl of lipid extract to a dry glass test tube.
2. Take 500 µl of phosphatidylserine to a dry glass test tube.
3. Take 500 µl of water to a dry glass test tube.
4. Add 500 µl of Dragendorf's reagent to each test tube.
5. Look and explain your observation.

Results

TEST TUBE NAME	OBSERVATION	INTERPRETATION
Test sample 1		
Test sample 2		
Test sample 3		

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

EVIDENCE OF LIPIDS – GLYCOLIPIDS DETERMINATION

Background

Glycolipids consist of glycosyl and lipid moieties. A saccharide is attached by a glycosidic covalent bond to lipid. There are several types of glycolipids: glycoglycerolipids, glycosphingolipids – cerebrosides, gangliosides, globosides, lipopolysaccharides, phenolic glycolipids, glycopeptidolipids, nucleoside lipids. Saccharide parts of glycolipids are dehydrated and cyclized to furaldehyde derivatives by the use of mineral acids. These derivatives react with orcinol and produce green coloured complexes.

Principle

Chemicals and tools

- Orcinol solution in 5 mol/l HC1
- Lipid extract (test sample 1)
- 0.2 mol/l solution of glycolipids (test sample 2)
- Distilled water (control, test sample 3)
- Glass test tubes, automatic pipettes, tips, water bath

Procedure

1. Add 500 µl of lipid extract to a dry glass test tube.
2. Take 500 µl of phosphatidylserine to a dry glass test tube.
3. Take 500 µl of water to a dry glass test tube.
4. Add 2 000 µl of orcinol solution to each test tube.
5. Incubate in a water bath for 3 minutes.
6. Look and explain your observation.

Results

TEST TUBE NAME	OBSERVATION	INTERPRETATION
Test sample 1		
Test sample 2		
Test sample 3		

Discussion

Conclusion

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

SEPARATION OF LIPIDS

Background

Lipids of blood serum can be separated by thin-layer chromatography (TLC). It is a physical method of separation in which the components to be separated are distributed between two phases: a stationary phase and a mobile phase that moves in a definitive direction. Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of absorbent material – silica gel or cellulose. This layer of absorbent is called the stationary phase. Because lipids are insoluble in water, their extraction from tissues and subsequent fractionation require the use of organic solvents (the mobile phase) and some techniques that are not commonly used. The method is based on different migration rates of serum blood lipid fractions. Complex mixtures of lipids are separated by differences in their polarity or solubility in nonpolar solvents. As the mobile phase, mixture of petrol ether, ether, and acetic acid (85 : 15 : 2) for separation of neutral lipids is used. The mixture of chloroform, methanol and water (65 : 25 : 4) is used for the separation of polar lipids. The specific retention factor (R_f) of each chemical can be used to aid in the identification of unknown substances. R_f is b/a, b is the distance of the solvent front from the baseline, and a is the distance of the spot center from the baseline. An iodine vapor, phosphomolybdic acid, and carbonizing reagent are then used to identify the separated fractions.

Principle

Chemicals and tools

- 0.055 mol/l phosphomolybdic acid in methanol
- Carbonizing reagent (0.4 mol/l sulfuric acid, 1.5 mol/l ammonium sulfate)
- Petrol ether : ether : acetic acid (85 : 15 : 2)
- Chloroform . methanol : water (65 : 25 : 4)
- Iodine
- Carbonizing reagent (0.4 mol/l sulfuric acid, 1.5 mol/l ammonium sulfate)
- Lipid extract (test sample 1)
- Silica gel thin layer plate, cuvette

Procedure

1. Prepare a silica gel thin layer plate – mark the start line with a pencil.
2. Add 20 µl of lipid extract to the starting line.
3. Add the thin layer plate to the cuvette with a mobile phase and leave it there for 90 minutes.
4. After separation, detection is perpormed with: evaporated iodine, phosphomolybdic acid, and carbonizing reagent.
5. Look and compare your chromatogram with Figure 33.

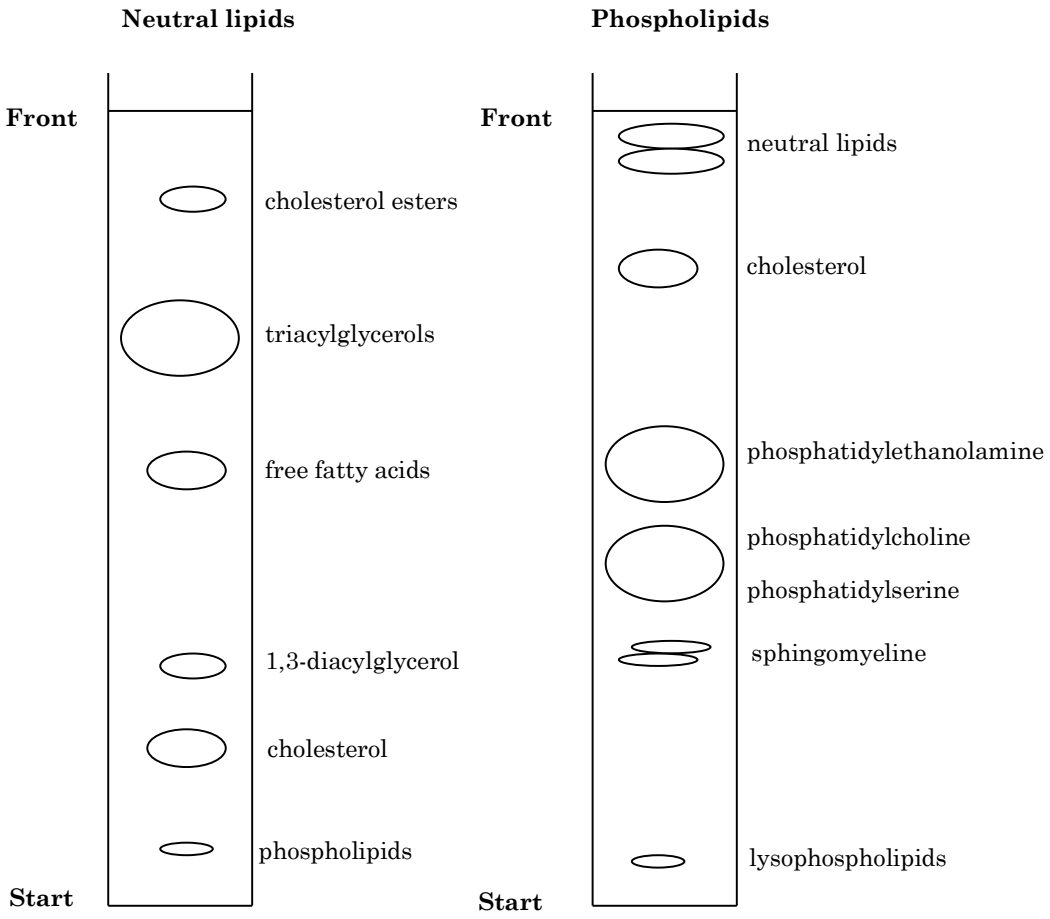

Figure 33: Diagram of the chromatographic separation of neutral lipids and phospholipids of the blood serum. Lipids of the blood serum are separated by the thin-layer chromatography (TLC). Spots are visible after detection of a colourless thin layer plate by detergents. The start is often called the baseline, and the front is also called the solvent front.

Results

Discussion

Conclusion

5.6 BIOLOGICAL MEMBRANES

A biological membrane or biomembrane is an enclosing or separating membrane that acts as a selective permeable barrier within living things. The membrane forms the outer boundary of a living cell or of an internal cell compartment. The outer boundary is the plasma membrane, and the compartments enclosed by internal membranes are called organelles. Biological membranes, in the human body, consist of a bilayer of phospholipid, cholesterol, glycolipids, and embedded integral and peripheral proteins. The lipid bilayer consists of two layers – an outer leaflet and inner leaflet. The components of bilayers are distributed unequally between the two surfaces to create asymmetry between the outer and inner surfaces. Biological membranes are characterised as the fluid mosaic model and are essentially composed of a lipid bilayer. They are amphiphilic – are simultaneously hydrophobic and hydrophilic.

Biological membranes have three primary functions:

- they keep toxic substances out of the cell,
- they contain receptors and channels that allow specific molecules, such as ions, nutrients, wastes, and metabolic products, that mediate cellular and extracellular activities to pass between organelles and between the cell and the outer environment,
- they separate vital but incompatible metabolic processes conducted within organelles.

Membranes are also a medium for rapid biochemical turnover by enzyme systems. Biological membranes transmit extracellular and intracellular signals through receptor molecules. They are responsible for intracellular contact, communication, and recognize immune response. Extrinsic membrane proteins are loosely held to the surface of the membrane and they can be easily separated. Intrinsic membrane proteins are tightly bound to the lipid bilayer and be separated only by the use of detergent or organic solvents.

Transport of substances across the biological membranes

The cross-membrane movement of molecules is selective depending on the nature of molecule. Small molecules can cross the membrane by following ways:

- passive transport – diffusion: small hydrophobic (non-polar) and polar molecules
  – facilitated diffusion: polar and ionic molecules
- active transport – active primary transport
  – active secondary transport.

Passive transport is the transport down the concentration gradient and active transport is against the concentration gradient which requires cellular energy.

Large molecules can cross by:

- endocytosis: phagocytosis and pinocytosis,
- exocytosis,
- transcytosis.

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

DETERMINATION OF COMPOSITION OF BIOLOGICAL MEMBRANES

Background

Thin-layer chromatography is a physical separation method in which the components to be separated are distributed between two phases: a stationary phase and a mobile phase that moves in a definitive direction. Lipids can be fractionated by thin-layer chromatography performed on a sheet of silica gel based on the different absorption of separated lipids to the absorbent. Lipids extracted from tissues are mainly lipids from cell membranes. Cell membranes contain phospholipids, glycolipids, and free cholesterol.

Principle

Chemicals and tools

- 0.13 mol/l sodium citrate
- 0.055 mol/l phosphomolybdic acid in methanol
- Carbonizing reagent (0.4 mol/l sulfuric acid, 1.5 mol/l ammonium sulfate)
- Iodine
- Chloroform : methanol (2 : 1)
- Chloroform : methanol : water (65 : 25 : 4)
- Physiological solution
- Erythrocytes
- Lipid extract from brain

- Lipid extract from liver
- Silica gel thin layer plate, cuvette

Procedure

1. Prepare a silica gel thin layer plate – mark the start line with a pencil.
2. Add 2 x 5 µl of erythrocytes to the starting line.
3. Add 2 x 5 µl of brain lipid extract to the starting line.
4. Add 2 x 5 µl of liver lipid extract to the starting line.
5. Insert the thin layer plate in the cuvette with a mobile phase and leave it there for 120 minutes.
6. After separation make the detection by: evaporated iodine, phosphomolybdic acid, and carbonizing reagent.
7. Look and compare your chromatogram with Figure 34.

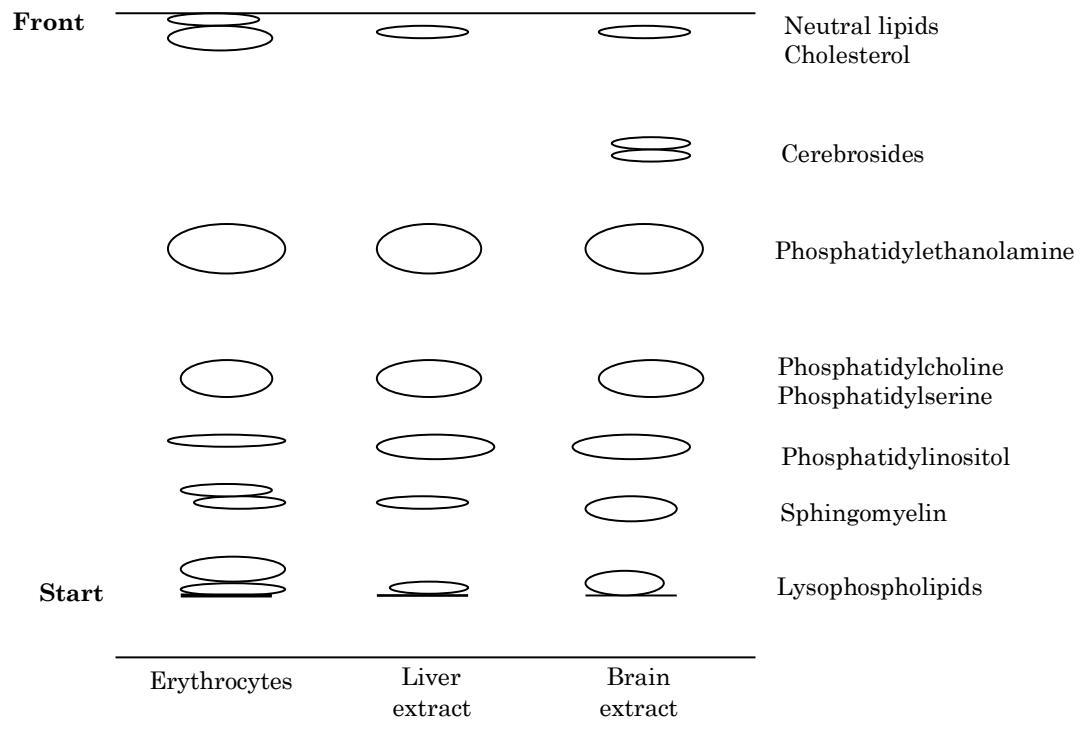

Figure 34: Chromatogram of lipid separation by thin-layer chromatography. Lipids of erythrocytes, liver extract, and brain extract are separated by thin-layer chromatography (TLC). Spots are visible after detection of colourless thin layer plate by detergents. The start is often called the baseline, and the front is also called the solvent front.

Results

Discussion

Conclusion

5.7 NUCLEIC ACIDS

Nucleic acids are bioinformative biomolecules responsible for the storage and expression of genetic information (transfer of information from one generation to the next generation). There are two groups of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nucleic acids are made up of nucleotides, which are monomers of: a nitrogenous base, a 5-carbon saccharide and a phosphate group.

Nitrogenous bases of nucleic acids

A nitrogenous base (nucleobase) of nucleic acids is an aromatic heterocycle. Nitrogenous bases are classified as derivatives of purine or pyrimidine. There are five nitrogenous bases in all living organisms (Figure 35). The purine bases are adenine (A) and guanine (G). The pyrimidine bases are cytosine (C), thymine (T) and uracil (U). Deoxyribonucleic acids contain adenine, guanine, cytosine, and thymine. Ribonucleic acids contain adenine, guanine, cytosine, and uracil.

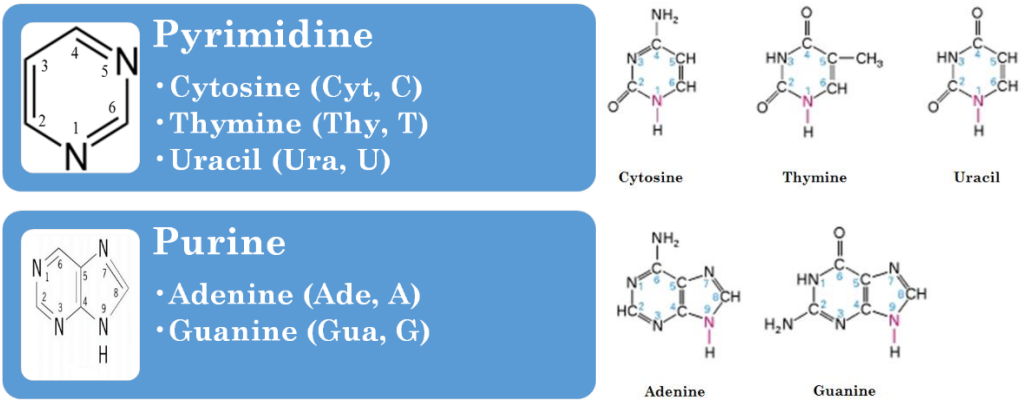

Figure 35: Nitrogenous bases. They are five nitrogenous bases: cytosine, thymine and uracil are pyrimidines; adenine and guanine are purines.

Nucleosides and nucleotides

Nucleosides are glycosylamines consisting of a nitrogenous base and 5-carbon saccharide (either ribose or deoxyribose). Anomeric carbon is linked through a glycosidic

bond to the N9 of a purine or the N1 of a pyrimidine. Figure 36 represents the names of the nucleosides.

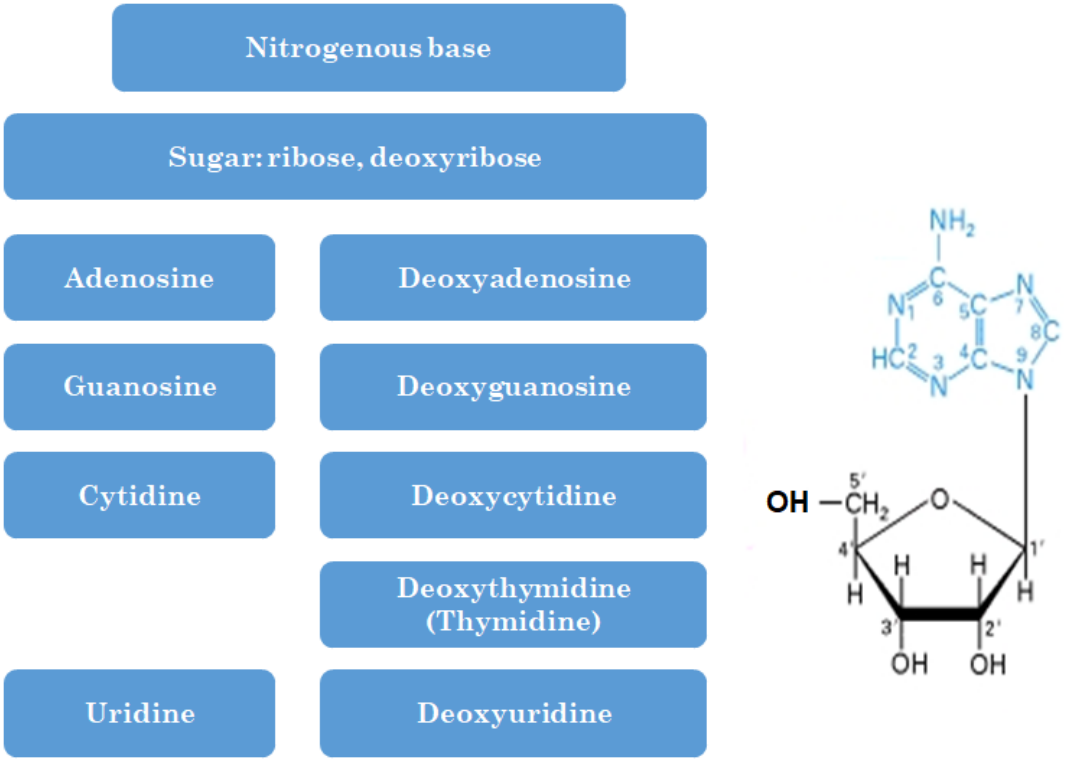

Figure 36: Nucleosides and deoxynucleosides. Nucleosides consist of adenine, guanine, cytosine, uracil, and ribose. Deoxynucleosides consist of adenine, guanine, cytosine, thymine, uracil, and deoxyribose. The structural formula represents adenosine.

Nucleotides are esterified nucleosides. They consist of a nitrogenous base, 5-carbon saccharide, and one or more phosphate groups. They are the basic building blocks of DNA and RNA. Figure 37 represents the types of nucleotides. Nucleotides are not only building blocks of nucleic acids; they play a central role in metabolism (Figure 38).

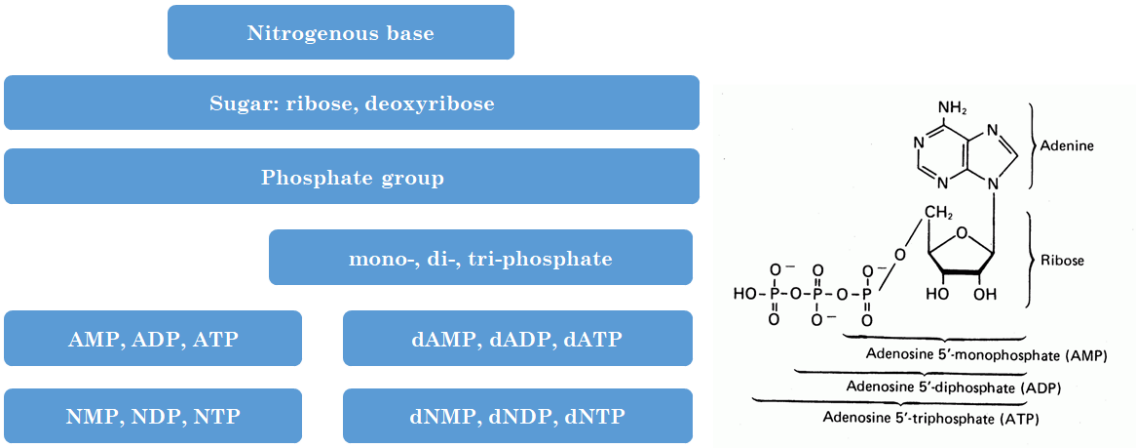

Figure 37: Nucleotides and deoxynucleotides. Nucleotides consist of adenine, guanine, cytosine, uracil, ribose, and one or more phosphate groups. Deoxynucleotides consist of adenine, guanine, cytosine, thymine, deoxyribose, and one or more phosphate groups. Structural formula represents adenosine triphosphate. AMP – adenosine monophosphate; ADP – adenosine diphosphate; ATP – adenosine triphosphate; N – nucleoside; d – deoxy.

The monomer units of nucleic acids	• DNA • RNA, mRNA, tRNA, sRNA, snRNA, vRNA
Nucleotide three phosphates	• Participate in all biochemical processes • A source of energy
Donors of phosphoryl groups	• ATP • GTP
A part of coenzymes	• NAD^+, $NADP^+$, FAD, FMN • Coenzyme A
Signal molecules	• Second messengers: cAMP, cGMP • Regulators: ATP, ADP, ATP/AMP, CTP
Activated intermediates during biosynthesis	• UDP-, GDP-sugars • CDP-glycerol
Synthetic analogs	• In a treatment, research • Chemotherapy, AIDS, transplantation

Figure 38: Functions of nucleotides. Nucleotides play an essential role in the metabolism of all living organisms. Nucleotides are building block of deoxyribonucleic acid and ribonucleic acid. They participate in all biochemical processes. Nucleotides are the main acceptor and donor of the phosphoryl group in metabolism. They are structural components of coenzymes and serve as signal molecules. Some nucleotides are necessary for the activation of intermediates in biosynthesis.

Deoxyribonucleic acid

Deoxyribonucleic acid (DNA) is double-stranded (ds) nucleic acid (with the exception of a few viruses that contain single-stranded DNA (ssDNA). It is a polymer of deoxyribonucleoside monophosphates bind together by covalent 3',5'-phosphodiester bond. Deoxyribonucleoside monophosphate contains: nitrogen base – adenine, guanine (purine),

thymine, cytosine (pyrimidine); deoxyribose and phosphate group (Figure 39). Two chains of DNA are twisted around each other to form a double helix. The two strands of DNA run in opposite directions to each other and are thus antiparallel. The bases of one strand of DNA are paired with the bases of the second strand, so that an adenine is always paired with a thymine and a guanine is always paired with a cytosine. The base pairs are held together by hydrogen bonds: two between A and T and three between G and C. In eukaryotic cells, DNA is associated with various types of proteins (nucleoproteins) present in the nucleus.

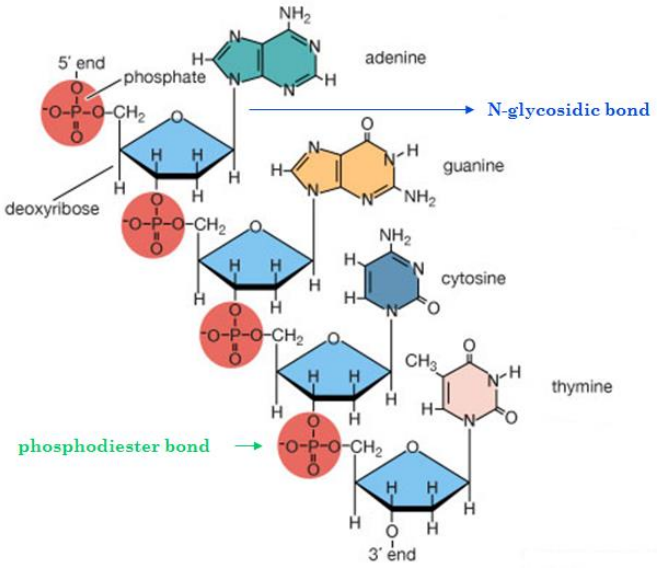

Figure 39: Segment of polynucleotide chain of deoxyribonucleic acid. https://www.britannica.com/science/DNA

Ribonucleic acid

Ribonucleic acid (RNA) is single-stranded (ss) nucleic acid (with the exception of a few viruses that contain double-stranded RNA). However, a single-stranded RNA molecule can form intrastrand double helices by complementary base pairing, as in tRNA. Double-strand RNA (dsRNA) is a signal for gene-specific silencing of expression in a number of organisms.). It is a polymer of ribonucleoside monophosphates that bind together by covalent 3',5'-phosphodiester bond. Ribonucleoside monophosphate contains: nitrogen base – adenine, guanine (purine), uracil (unmethylated form of thymine) and cytosine (pyrimidine); ribose and phosphate group.

There are several types of RNA molecules from which there are the main classes:

- Messenger RNA (mRNA),
- Transfer RNA (tRNA),
- Ribosomal RNA (rRNA).

The other are small RNAs:

- Small nuclear RNA (snRNA),
- Micro RNA (miRNA),
- Small interfering RNA (siRNA).

Messenger RNA

The mRNA carries genetic information from DNA to the ribosome present in the cytosol, where it is used as a template for protein synthesis. Heterogeneous RNA (hnRNA) is a precursor of mRNA. The hnRNA has been transcribed from DNA and it is processed to mature mRNA. The structure of a typical human maturated mRNA contains 5' end: cap, five prime untranslated regions, coding sequence, three prime untranslated regions, and poly-A tail. A 5' cap (also called RNA cap, RNA 7-methylguanosine cap, RNA m^7G cap) is a modified guanine nucleotide that has been added to the front of mRNA shortly after the start of transcription. Its presence is critical for recognition by the ribosome and protection against ribonucleases. Untranslated regions (UTRs) are sections of the mRNA before the start codon and after the stop codon that are not translated. The functions of these regions are probably mRNA stability, mRNA localization, and translational efficiency. Poly-A tail is a long sequence of adenine nucleotides (hundreds) added to the 3' end of hnRNA. This tail promotes export from the nucleus and translation, and protects the mRNA from degradation.

Transfer RNA

Transfer RNA transfers a specific amino acid to a growing polypeptide chain at the ribosomal site of protein synthesis during translation. It has sites for amino acid attachment and an anticodon region for codon recognition, which binds to a specific sequence on the mRNA chain through hydrogen bonding.

Ribosomal RNA

The rRNA is the catalytic component of the ribosomes. Eukaryotic ribosomes contain four different rRNA molecules: 18S rRNA, 5.8S rRNA, 28S rRNA and 5S rRNA. Three rRNA molecules are synthesized in the nucleus and one is synthesized elsewhere. Ribosomal RNA and a protein combine to form a nucleoprotein called a ribosome in the cytoplasm. The ribosome binds to mRNA and performs protein synthesis.

NAME AND SURNAME: _____

GROUP: _____ DATE: _____

ESTIMATION OF COMPONENTS OF RIBONUCLEIC ACID

Background

The isolated ribonucleic acid from yeasts was hydrolysed with sulfuric acid. Ribonucleic acid is hydrolysed to purine bases, ribose, and phosphoric acid. Pyrimidine bases are not hydrolysed in this condition. The hydrolysate of RNA is used for the estimation of: purine bases, ribose, and phosphoric acid by test tube tests. Purine bases (adenine and guanine) react with ammonium solution of $AgNO_3$ to produce a white precipitate of Ag^+. Ribose reacts with orcinol reagent to generate a green-blue coloured complex. The phosphate group (inorganic phosphate) reacts with the ammonium molybdate to yield a yellow precipitate.

Principle

Chemicals and tools

- 0.03 mol/l ammonium solution of $AgNO_3$
- Orcinol reagent (dissolve 10 g of ferric chloride in 1 l of concentrated HCl and add 35 ml of 6 % w/v orcinol in alcohol)

- Ammonium molybdate (dissolve 7.5 g $(NH_4)_6Mo_7O_{24}.4H_2O$ in100 ml of distilled water and then add 100 ml of 32 % HNO_3)
- 0.062 mol/l $CuSO_4$
- 2.5 mol/l NaOH
- RNA hydrolysate (sample 1, 3, 5)
- Distilled water (control, sample 2, 4, 6)
- Glass test tubes, automatic pipettes, tips, water bath

Procedure

Evidence of purine bases

1. Add 1 000 µl of RNA hydrolysate to a new glass test tube. Dropwise 1 000 µl of ammonium solution into the test tube (test sample 1).
2. Add 1 000 µl of distilled water to a new glass test tube. Dropwise 1 000 µl of ammonium solution into the test tube (test sample 2).
3. Look and explain your observation.

Evidence of ribose

1. Add 1 000 µl of RNA hydrolysate to a new glass test tube. Add 1 000 µl of orcinol reagent to the test tube (test sample 3).
2. Add 1 000 µl of distilled water to a new glass test tube. Add 1 000 µl of orcinol reagent to the test tube (test sample 4).
3. Mix both test glass test tubes and incubate for 2 minutes in a boiling water bath.
4. Look and explain your observation.

Evidence of phosphoric acid

1. Add 1 000 µl of RNA hydrolysate to a new glass test tube. Add 300 µl of ammonium molybdate to the test tubes (test sample 5).
2. Add 1 000 µl of distilled water to a new glass test tube. Add 300 µl of ammonium molybdate to the test tubes (test sample 6).
3. Look and explain your observation.

Results

TEST TUBE NAME	OBSERVATION	INTERPRETATION
Test sample 1		
Test sample 2		
Test sample 3		
Test sample 4		
Test sample 5		
Test sample 6		

Discussion

Conclusion

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