**MEDICAL CHEMISTRY AND BIOCHEMISTRY**

**LEARNING OBJECTIVES**

**I. MEDICAL CHEMISTRY (MC)**

**1. GENERAL CHEMISTRY (C1)**

**Lectures (L)**

**L1 Introduction into chemical basis of life. Atoms and elements.**

1. Identify and define the chemical properties and characteristics of atoms, molecules and ions.
2. Describe the concepts of absolute and relative atomic mass. Describe an electron, proton and neutron, isotopes and the amount of the substances.
3. Explain the electronic configuration of atoms and define the concept of orbitals.
4. Present and explain the types of orbitals and the correlation between orbital energy and arrangement of electrons in an atom.
5. List the periodic properties of elements, describe the energy of ionization, electron affinity and the radius of an atom.
6. Explain the importance of these properties in defining the characteristics and chemical reactivity of each chemical element.

**L2 Chemical bonds.**

1. Describe the energy changes in formation of ionic compounds.
2. Recognize the impact of atomic radius and ionic charge on the energy of the crystal lattice.
3. List the differences between ionic and molecular crystals.
4. Recognize and explain the formation of sigma and pi covalent bonds.
5. Explain sp3, sp2, sp-hybridization, clarify the principle of hybridization and the shape of molecules that are formed thereby.
6. Identify the principles of forming single, double and triple covalent bonds with particular reference to the formation of sigma and pi covalent bonds.
7. Explain the concept of polarity of the covalent bond and define electronegativity.
8. Recognize intra- and intermolecular bonds and forces, describe the hydrogen bond in water and in biological systems, with special reference to macromolecules and living organisms.
9. List the characteristics of non-covalent interactions (dipole-dipole, dipole-induced dipole) and their biological role.

**L3 Free particles: the nature of gases.**

1. Relate gas pressure units.
2. Apply the ideal gas law to relate and calculate values for pressure, volume, temperature, and amount of a gas.
3. Describe the relationship between volume and pressure of a gas, at constant temperature, and the number of moles.
4. Describe the relationship between the volume and the number of moles of a gas, at constant temperature and pressure.
5. Describe the relationships of gas variables (p, V, n and T) in the ideal gas law.
6. Apply Dalton’s law of partial pressure to calculate the pressure of gas mixture and calculate the partial pressures of gases in a mixture.
7. Relate molecular weight and velocity of molecules using Graham’s law.
8. Use the kinetic molecular theory to explain ideal gas behavior (p, V, n, T) as well as the average kinetic energy and average velocity.

**L4 Water and aqueous solutions. Colligative properties of solutions.**

1. List the colligative properties of aqueous solutions.
2. Explain the concept of boiling point elevation and freezing point depression.
3. Describe the process of osmosis and specify Van't Hoff equation to calculate the osmotic pressure of a solution.
4. Calculate the molar mass of solutes from measurement data of colligative properties.
5. Recognize the characteristics of different solutions with particular reference to electrolyte solution.
6. Be able to define the degree of dissociation of an electrolytes.
7. Distinguish molar conductivity of strong and weak electrolytes and explain the concept of molar conductivity at infinite dilution.
8. Explain how the size of ion affects molar conductivity of the electrolyte focusing on the hydrogen ion.

**L5 Acids and bases. Buffer solutions.**

1. Identify the term acids and bases by the Brönsted - Lowry theory and conjugate base - conjugate acid relationship.
2. Recognize the importance of dissociation constants and understand the process of calculating the value of dissociation constants.
3. Explain the ionization of water and the derivation of the ionic product of water.
4. Calculate the hydrogen ion concentration and pH of various solutions and interpret its importance.
5. Explain the term "pK" and ways of expressing the acidity of a solution.
6. Explain the term ampholyte and their acid - base behavior and indicate the typical representatives of biological ampholytic molecules with particular reference to water molecule.
7. Clarify the process of salt hydrolysis and the mode of action of salt in aqueous solution in relation to the pH change.
8. Describe the mechanisms involved in regulation of acid-base equilibrium in the body and indicate the pH of body fluids.
9. Recognize the composition and role of buffer solutions, and state the mechanism of action of buffer solutions in biological systems.
10. Explain the Henderson-Hasselbach equation.

**L6 Colloidal-dispersed systems.**

1. Explain the colloidal state of matter.
2. List the properties of colloidal solutions and distinguish lipophilic and lyophobic colloids.
3. Describe the different properties of salt and gel states.
4. List the characteristics of dispersed, associated and macromolecular colloids.
5. Describe the importance of colloidal solution for the living organism, the application of colloidal solutions in medicine, and their properties.
6. Explain the term emulsion and distinguish types of emulsifiers.
7. Recognize the differences of physical and chemical adsorption and give examples of polar and nonpolar adsorbents.
8. Clarify the cause of special properties of colloidal solution. Explain the impact of Donnan equilibrium on the pH in cells, osmotic pressure and the creation of membrane potential.

**L7 Energy in transition: thermochemistry.**

1. Define the difference between the change in enthalpy ΔH and the change in internal energy (ΔU) during a chemical reaction.

2. Explain the difference in standard enthalpies of oxidation of stearic and oleic acid.

3. Explain why nutritional calculations use a lower amount of protein standard molar enthalpy of combustion compared to the amount obtained in the calorimeter bomb.

4. Implement the first law of thermodynamics to the transition of the nutrient enthalpy of combustion in human cells.

**L8 Reactions at equilibrium.**

1. Define equilibrium constant of a chemical reaction.
2. Define dynamic equilibrium.
3. Explain the direction of a chemical reaction when a reaction at equilibrium is subjected to a change of conditions (concentration, pressure and temperature change).
4. Explain partition equilibrium and the principle of gas-liquid chromatography.

**L9 The rate of chemical change.**

1. Define the rate of chemical reaction and its unit.
2. Define the coordinate axes in graphs that give a linear dependence of the rate of first and second order chemical reactions.
3. Compare dependence of reaction half-lives of the first and second order reactions.
4. Define the basic principles of chemical reactions kinetics and the control regulation of the reaction rate.
5. Propose a thermodynamic explanation why catalysts cannot alter the equilibrium of reactions.
6. Define the term transition state and propose a role for the transition state in reducing the activation energy.

**L10 The natural direction of change: the second law.**

1. Define and explain briefly the role of entropy in biochemical reactions.

2. Define and explain the interrelationship between Gibbs free energy (ΔG') and standard free energy (ΔGo).

3. Apply the Gibbs free energy equation to calculate the free energy and equilibrium content of reactants and products in coupled reactions.

4. Explain the synthesis of 2 ATP molecules during glycolysis.

**L11 Chemical energy and electrical energy: electrochemistry.**

1. Define the relation between electrode (half-cell) potential and ion concentration.
2. Explain how measurement of the electromotor force (E) of a cell is used to obtain thermodynamic information, such as the ΔG value of a reaction.
3. Define the relation between equilibrium constant Kc and the standard Eo of a reaction.
4. Define the equation to calculate the Eo of a cell knowing the standard reduction potentials of two half-cells.
5. Indicate significant biological oxidation - reduction systems.
6. Explain the supply of biological systems with energy.

**Seminar practicals (SP) and practicals (P)**

**SP1 and P1 Basic stoichiometry. Preparation of solutions.**

1. Define the basic rules and work principles in chemical and biochemical laboratory.

2. Define the terms of relative atomic and relative molecular mass.

3. Show different ways to express composition of solutions (different types of portion, concentration, density).

4. Independently solve chemical equations based on given reactants.

5. Independently solve different types of tasks regarding stoichiometry of chemical calculations.

6. Recognize basic laboratory dishes.

7. Independently prepare standard solutions of different concentration, volume and portion in practice.

**SP2 and P2 Optical methods in medical chemistry.**

1. Compare qualitative and quantitative analysis.

2. Describe methods of spectrophotometry and polarimetry.

3. Give examples of optically active compounds.

4. Explain the calibration curve.

5. Prepare the standard solutions of Fe3+ ions.

6. Determine the absorbance of the standard solutions using a blank probe.

7. Construct the calibration curve (absorbance vs. iron mass).

8. Determine the unknown iron mass using the calibration curve.

9. Solve spectrophotometric and polarimetric problem tasks independently.

**SP3 and P3 Gas laws. Ions in solution. Osmotic pressure.**

1. Define ideal and real gases and list variables defining the state of the gas.

2. Explain gas laws.

3. Explain osmosis, osmotic pressure, solution freezing point depressionand boiling point elevation, and compare electrolytes to nonelectrolytes.

4. Determine whether the solution is isotonic, hypertonic or hypotonic.

5. Derive units of measurement and conclude which units are used in which formulas.

6. Differentiate electrolytes and non electrolytes.

7. Predict the number of ions to which an electrolyte ionises.

8. Solve gas laws and problem tasks relating to colligative properties of solutions, on their own.

9. Critically and argumentatively explain the results of the calculations.

**SP4 and P4 Volumetry: neutralisation methods.**

1. Describe the basic principles of quantitative analytical chemical analysis.

2. Define the terms of standard solution, acid and base.

3. Indicate the basic rules for handling chemicals and reagents.

4. Write chemical equations using the neutralisation method.

5. Practice different quantitative methods of chemical analysis.

6. Independently solve chemical equations based on given reactants.

7. Indicate the basic rules for handling chemicals and reagents.

8. Independently apply basic principles of acidimetry and alkalimetry in calculating mass and quantity values for a given acid or base in solution.

**SP5 and P5 Volumetry: oxidation and reduction methods.**

1. Define reactions of oxidation and reduction, and terms of an oxidizing and reducing agent.
2. Dilute a sample and measure the aliquot for analysis.
3. Define standard and indicator for each experimental redox reaction.
4. Calculate the unknown mass of a molecule in a sample based on the analysis of an aliquot and the knowledge of the redox reaction stoichiometry.

**SP6 and P6 Acids and alkalis, pH and buffers.**

1. Define the terms pH, pOH, pK, Kw, acid, base, buffer.

2. Explain the difference between titratable and actual acidity.

3. Calculate the pH of strong and weak acids or bases.

4. Calculate the pH of a buffer solutions using Henderson-Hasselbach equation.

5. Explain the mechanism of buffer action.

6. Prepare independently phosphate buffers of a given pH value.

7. Determine the buffer capacity in relation to strong acid or strong alkali and calculate its value.

8. Test the pH values of given solutions and explain the results by chemical equations.

9. Demonstrate the experiment of suppression of acetic acid dissociation, based on the common ion effect using methyl orange as the indicator.

10. Demonstrate the experiment: dissolution of magnesium hydroxide based on the common ion effect.

# SP7 and P7 Energetics and kinetics of chemical reaction.

1. Describe thermochemical equations.

2. Define and describe entropy and Gibbs free energy using formulas.

3. Define the term of chemical kinetics.

4. List and describe factors that affect the reaction rate.

5. Define reaction order.

6. Calculate unknown thermochemical data using thermochemical equations.

7. Determine direction of a chemical reaction using calculations.

8. Calculate reaction rate constant.

9. Calculate the composition of the reaction mixture vs time from the beginning of the reaction.

10. Calculate the reaction energy using redox reactions.

**P8 Integration of general chemistry**

1. Solve problem tasks independently**2. ORGANIC CHEMISTRY (C2)**

**Lectures (L)**

**L12 Introduction to organic chemistry. Saturated and unsaturated hydrocarbons; physical and chemical properties. Isomers.**

1. Explain the basic chemical properties of the carbon atom in organic compounds.
2. Describe the nomenclature of hydrocarbons (IUPAC).
3. Explain the chemical and physical properties of alkanes (oxidation and halogenation), alkenes and alkynes (electrophilic addition and hydrogenation).
4. Explain the concept of constitutional and structural isomers, and identify different isomers from examples of given compounds.

**L13 Halogenalkanes; nucleophilic substitution, elimination. Optical isomerism; relative and absolute configuration.**

1. Distinguish homolytic and heterolytic cleavage of a molecule and the resulting products.
2. Describe the principle of substitution and elimination reactions of halogenalkanes.
3. Differentiate structural, geometric isomers and conformers.
4. Recognize an asymmetric carbon atom, and differentiate different types of stereoisomers (enantiomers, dia-stereoisomers and meso compounds).
5. Predict the D- and L-configuration of a given compound when compared to the standard.

**L14 Alcohols. Ethers. Aldehydes. Ketones.**

1. Describe the structure of the most important alcohols, as well as their physical and chemical properties.
2. Recognize the structure of ether, and explain the physical properties of ethers.
3. Describe the structure of the most important aldehydes and ketones, and derive their corresponding IUPAC name.
4. Describe the physical and chemical properties of carbonyl compounds, name the most important representatives of aldehydes and ketones.
5. Describe keto-enol tautomerism, and nucleophilic addition to the carbonyl group.
6. Describe the oxidation and reduction reactions of carbonyl compounds, and their role in the body.

**L15 Carboxylic acids and their derivatives.**

1. Describe the structure of lower and higher carboxylic acids, and state their physical and chemical properties.
2. Compare the acidity of various aliphatic and aromatic carboxyl compounds.
3. Present the neutralization and esterification reaction. Show the hydrolysis of carboxylic acid derivatives (ester, amide, anhydride and acyl halide).
4. Describe the role and significance of carboxylic acid derivatives in the body (fats and oils, proteins and ATP).
5. Explain the structural role of phospholipids.
6. Describe the structure and the most important reactions of oxoacids and hydroxycarboxylic acid.

**L16 Cyclic and aromatic hydrocarbons. Sulphur and heterocyclic compounds.**

1. Compare the stability of cyclopropane and cyclohexane, and explain the conformation of cyclohexane.
2. Compare the physical and chemical properties of conjugated and non-conjugated dienes and polyenes.
3. Explain the stability of benzene with respect to the hybrid resonance structure.
4. Show the reaction of electrophilic aromatic substitution.
5. Distinguish between ortho, meta, and para disubstituted benzene derivatives.
6. Explain the neutralization reaction of aliphatic and cyclic amines.
7. Indicate the most important compounds containing sulfhydryl groups and explain the formation of disulfide bonds.
8. Explain the high energy content of a thioester (acetyl-CoA).
9. Recognize the most important heterocyclic compounds (NAD+, FAD, nitrogenous bases, thiophene, furane, pyran).

**L17 Amino acids. Carbohydrates.**

1. Describe the general structure of amino acids, zwitter ions and explain the isoelectric point.
2. Consider creating a peptide bond. Explain its properties.
3. Classify amino acids by the nature of the side chain.
4. Explain the reaction of decarboxylation, deamination and transamination of amino acids.
5. Classify carbohydrates according to the number of carbohydrate subunits (mono-, di-, polysaccharides).
6. Classify monosaccharides according to the functional group and the number of carbon atoms.
7. Show glucose and fructose by the Fisher and Haworth projections.
8. Explain the cyclization of glucose and fructose into a six-membered and five-membered ring, respectively, and describe the nature of the resulting linkage (hemiacetal, hemiketal).
9. Distinguish alpha and beta glucose.
10. Consider creating a glycosidic bond. Explain the properties of 1,2-, 1,4-, and 1,6- glycoside bonds.
11. Indicate the most important polysaccharides and differentiate their structural and physical properties (starch, glycogen and cellulose).

**Seminars (SO)**

**SO1 Resonant structures. Isomers. Alkanes, alkenes, alkynes. Alkaloids, phenols, esters, aldehydes. Ketones.**

1. Explain the term of stereoisomerism and stereoisomers.

2. Define geometrical and conformational isomerism.

3. Describe the structure of an alkane, alkene and alkyne and their chemical properties.

4. Describe the structure of an alcohol, phenol and ether, their nomenclature, physical and chemical properties as well as the chemical reactions for their synthesis.

5. Describe the importance of alcohol usage in medicine.

6. Repeat the chemical and physical properties of carbonyl compounds.

7. Name (according to the IUPAC and trivial nomenclature) carbonyl compounds.

8. Solve reactions of nucleophilic addition, nucleophilic substitution and elimination.

9. Define the principle of the aldol condensation reaction.

**SO2 Amines. Sulphur heterocyclic compounds. Amino acids. Carbohydrates. Carboxyl compounds.**

1. Describe the structure, synthesis and chemical reactions of organic compounds with sulphur.

2. Explain the relative configuration, and apply the D- and L- steric category to specific biologically important natural molecules (carbohydrates and amino acids).

3. Draw monosaccharides (glucose, fructose) according to the Haworth projection formula.

4. Compare α and β (1→4), and α and β (1→6) glycosidic bond.

5. Connect amino acids by a peptide bond.

6. Recognize the structure, physical and chemical properties of carboxylic acids.

7. Define the principle of carboxylic acid derivative synthesis.

**Seminar practicals (SP) and practicals (P)**

**SP9 and P9 Qualitative analysis of some organic compounds**

1. Describe the individual functional groups of organic compounds and their general characteristics.
2. Compare the qualitative chemical analysis of organic and inorganic compounds.
3. Compare the results of identifying an alcohol and formaldehyde by the dichromate ion test.
4. Compare the results of determining formaldehyde, glucose and sucrose by Fehling's reagent.
5. Compare the most important precipitation and colored chemical reactions for detecting proteins.
6. Compare the results of determining glycine and proteins using the ninhindrin reaction.
7. Analyse various organic compounds in the sample.

**SP10 and P10 Potentiometric titration of amino acids**

1. List the ionizable groups of the common amino acids and their pKa values.
2. Calculate the pH of an unbuffered aqueous solution of a polyfunctional amino acid and the change in pH that occurs following the addition of a given quantity of strong acid or alkali.
3. Define pI and indicate its relationship to the net charge of a polyfunctional electrolyte.
4. Explain how pH, pKa and pI can be used to predict the mobility of a polyelectrolyte, such as an amino acid, in a direct-current electrical field.
5. Use a pH-meter to measure changes in pH upon the addition of certain amounts of a strong acid or a strong base.
6. Draw the titration curve for an amino acid.
7. Use this curve to estimate the pKa values of the ionizable groups of the amino acid and determine the equivalent volume of acid or base.
8. Calculate the molecular weight of an unknown amino acid and from the table of molecular weight and pKa values conclude which amino acid was in the sample.

**II. MEDICAL BIOCHEMISTRY (MB)**

**Lectures (L) and seminars (SB)**

**BIOCHEMISTRY 1 (B1)**

**1. PROTEIN STRUCTURE AND FUNCTION**

**L18 Amino acids**

1. Describe the contribution of each type of R group of the common amino acids to their chemical properties.
2. Describe the general structure of amino acids, zwitter ions and explain the isoelectric point.
3. Classify amino acids by the nature of the side chain.
4. Define pI and indicate its relationship to the net charge of a polyfunctional electrolyte.
5. Describe the directionality, nomenclature, and primary structure of peptides.

**L19 Structure of proteins**

1. Identify the bond in a peptide that exhibits partial double-bond character and its conformational consequences in a peptide.
2. Explain and illustrate the primary, secondary, tertiary and quaternary structure of proteins.
3. Identify the major recognized types of secondary structure and explain supersecondary motifs.
4. Describe the kind and relative strength of the forces that stabilize each order of protein structure and indicate the present state of knowledge concerning the stepwise process by which protein are thought to attain their native conformation.
5. Identify the physiologic roles of chaperones in protein maturation. Describe multiple chromatographic methods commonly employed for the isolation of proteins from biologic materials.
6. Explain how scientists analyze the sequence or structure of a protein to extract insights into its possible physiologic function.

**L20 Globular proteins**

1. Describe the most important structural similarities and differences between myoglobin and hemoglobin. Sketch binding curves for the oxygenation of myoglobin and hemoglobin and explain why the physiologic function of hemoglobin requires that’s its O2 binding curve be sigmoidal rather than hyperbolic.
2. Identify the covalent linkages and other close associations between the heme and the globin chain in oxymyoglobin and oxyhemoglobin, and describe the structural and conformational changes in hemoglobin that accompany its oxygenation and subsequent deoxygenation.
3. Explain the role of the hindered environment on the ability of hemoglobin to bind carbon monoxide.
4. Explain the role of 2,3-bisphosphoglycerate (BPG) in oxygen binding and delivery.
5. Outline the role of hemoglobin in CO2 and proton transport.

**SB20 Sickle cell anemia**

1. Classify the mutated amino acid of sickle erythrocyte hemoglobin (valine in HbS) in comparison to the normal erythrocyte hemoglobin amino acid (glutamate in HbA): according to the polarity, size and charge.
2. Describe the sequence of physico-chemical processes from the hemoglobin mutation to the occurrence of sickle cell anemia.
3. List and explain variables that increase sickling.
4. Describe different options of sickle cell anemia treatment.
5. Explain possible selective advantage of the heterozygous state.

**L21 Fibrous proteins**

1. Describe the structure of fibril-forming collagens.
2. Specify steps of post-translational modification of procollagen that happen inside and outside of the cell.
3. Integrate the knowledge of post-translational modification of procollagen, lack of vitamin C and scurvy occurrence.
4. Explain the structure of elastin.
5. Explain how α1-antitrypsin deficiency causes emphysema and liver cirrhosis.

**L22 Enzymes**

1. Outline the four principal mechanisms by which enzymes achieve catalysis.
2. Describe how an “induced fit” facilitates substrate recognition and catalysis.
3. Indicate whether ΔG, the overall free energy change in a reaction, is dependent on the reaction mechanism.
4. Indicate whether ΔG is a function of the reaction rate.
5. Outline how substrate concentration affects the rate of an enzyme-catalyzed reaction.
6. Describe the application of the Michaelis-Menten equation to the determination of Km and Vmax.
7. Contrast the effect of increasing substrate concentration on the kinetics of simple competitive and noncompetitive inhibition.
8. Indicate two general ways in which an allosteric effector can modify catalytic activity.

**SB22 Enzymes in clinical diagnosis**

1. Define coenzymes, isoenzymes, cofactors and prosthetic groups.
2. List the major coenzymes, cofactors and prosthetic groups in enzyme reactions (vitamin B derivates).
3. Describe the main application of enzyme analysis in clinical laboratory medicine.
4. Describe the application of enzymes and isoenzymes in laboratory diagnosis of myocardial infarction and several disease (creatine kinase, lactate dehydrogenase, alanine and aspartate aminotransferase, gamma glutamyl transferase, acid and alkaline phosphatase, amylase, lipase, ceruloplasmin).

**2. INTERMEDIARY METABOLISM**

**L23 Bioenergetics and oxidative phosphorylation**

1. 1. Compare the mitochondrial content of different tissues and relate this characteristic to the function of a particular tissue.
2. 2. Describe the purpose of the electron transport chain (particularly complexes I, III, and IV) and ATP synthase, their substrates and products and their cellular localization.
3. 3. Discuss how succinate dehydrogenase, mitochondrial glycerol 3-phosphate dehydrogenase and electron-transferring-flavoprotein dehydrogenase transfer electrons to ubiquinone from succinate, cytosolic NADH and fatty acid dehydrogenases, respectively.
4. 4. Explain the biochemical basis for the generation of heat by brown fat and discuss the role of brown fat in infants and the possible role in adults.

**SB23 Regulation of respiratory chain and oxidative phosphorylation**

1. Explain how electron transport and ATP synthase are functionally coupled.
2. Explain how the process of oxidative phosphorylation is influenced by the availability of oxygen and NADH.
3. Explain how the cellular ATP:ADP ratio regulates the rate of ATP production by oxidative phosphorylation.
4. Describe the effects of electron transport chain inhibitors, ATP synthase inhibitors, and uncouplers on oxidative phosphorylation.
5. Describe the biochemical and clinical features associated with ingestion/overdose of electron transport inhibitors (e.g. industrial exposure to cyanide) and uncouplers (e.g. dinitrophenol) of oxidative phosphorylation.

**SB24 Introduction to carbohydrates**

1. Classify and provide structures of carbohydrates: monosaccharides, disaccharides, oligosaccharides.
2. Identify complex carbohydrates.
3. Describe digestion of dietary carbohydrates.
4. Explain abnormal degradation of disaccharides.
5. Explain digestive enzymes deficiencies.

**L25 Glycolysis**

1. Describe the overall purpose of glycolysis, its reactants and products, its cellular localization, and its tissue distribution.
2. Describe the roles of hexokinase/glucokinase, phosphofructokinase-1 (PFK-1), and pyruvate kinase in glycolysis and predict the biochemical and potential clinical consequences in deficiencies of these enzymes.
3. Compare and contrast aerobic and anaerobic glycolysis in terms of the tissues in which they occur, reactants and products, purposes, and the conditions in which they occur.
4. Explain the concept of substrate level phosphorylation and why it is important.
5. Describe the purpose of the reaction catalyzed by lactate dehydrogenase, its reactants and products, cellular and tissue localization, and how it is regulated.
6. Describe the role and fate of the cytosolic NADH produced in glycolysis.

**SB25 Regulation of glycolysis**

1. Compare and contrast the mechanisms for regulating glycolysis including allostery, hormonal regulation and covalent modification.
2. Differentiate the roles of hexokinase and glucokinase in blood glucose regulation.
3. List conditions (inherited genetic defects in various pathways; drugs) that increase the risk of lactic acidosis.
4. Explain the biochemical basis of the hemolytic anemia observed in deficiency of erythrocyte pyruvate kinase.
5. Predict the results of a complete blood count (CBC) and iron studies in a person with pyruvate kinase deficiency that is in hemolytic crisis.

**L26 Tricarboxylic acid cycle**

1. Describe the overall purpose of the pyruvate dehydrogenase (PDH) complex, its reactants and products, its cellular localization, and its tissue distribution.
2. Compare the general structure, regulation, and required cofactors/vitamins of the PDH complex to that of alpha-ketoglutarate dehydrogenase and the branched-chain alpha-keto acid dehydrogenase complex.
3. Describe the overall purpose of the TCA cycle, its cellular localization, and its tissue distribution.
4. Describe the reactants and products of the TCA cycle, as related to the fates of the breakdown products of carbohydrates, fatty acids, and amino acids.
5. Describe the roles of citrate synthase, isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase, succinyl-coA synthetase, succinate dehydrogenase, and malate dehydrogenase in the TCA cycle and predict the biochemical and potential clinical consequences of deficiencies of these enzymes.
6. Explain the effect of the following parameters on the activity of the TCA cycle and the mechanism(s) by which the effect occurs: mitochondrial NADH:NAD+ ratio, ADP:ATP ratio, succinyl-CoA concentration.
7. Describe the central role of the TCA cycle in connecting glycolysis, gluconeogenesis, oxidative phosphorylation, fatty acid metabolism, and amino acid metabolism.
8. Describe the role of TCA cycle intermediates as sources of reactants for biosynthetic pathways.
9. Describe the clinical result of severe thiamin deficiency, and connect the symptoms to the biochemical role of thiamin in the PDH complex and TCA cycle.
10. Explain the rationale for providing thiamin along with glucose to patients with suspected hypoglycemia.

**SB26 Regulation of TCA cycle**

1. Describe the central role of the TCA cycle in connecting glycolysis, gluconeogenesis, oxidative phosphorylation, fatty acid metabolism, and amino acid metabolism.
2. Explain regulation of citrate synthase, iso-citrate dehydrogenase, and α-ketoglutarate dehydrogenase complex.
3. Explain the role of ADP/ATP ratio in regulation of TCA.
4. Explain energy produced by the cycle.
5. Identify anaplerotic reaction of the cycle.

**L27 Gluconeogenesis**

1. Describe the overall purpose of gluconeogenesis, its reactants and products, its cellular localization, and its tissue distribution.
2. Differentiate the enzymes involved in glycolysis vs gluconeogenesis.
3. Explain the contribution of gluconeogenesis to blood glucose regulation.
4. Describe the roles of pyruvate carboxylase, PEPCK, fructose 1,6-bisphosphatase and glucose 6-phosphatase in gluconeogenesis, and predict the biochemical and potential clinical consequences in deficiencies of these enzymes.
5. Evaluate the relative importance of different precursors for gluconeogenesis in feeding, fasting, and exercise.
6. Explain how the activities of glycolysis and gluconeogenesis are regulated in response to fatty acid metabolism and protein metabolism, and in response to insulin and glucagon.

**SB27 Regulation of gluconeogenesis**

1. Explain the role of GLUT transporters in regulation of glucose distribution among various tissues.
2. Compare the activity of glucokinase and hexokinase and explain their role in glucose consumption and storage.
3. Explain the regulation of phosphofructokinase-2 activity and its influence on glycolysis and gluconeogenesis in the liver.
4. Explain the contribution of glycogenesis and glycogenolysis to blood glucose regulation during fed state, fasting state, and exercise.
5. Explain the interrelationship between high blood glucose, β-pancreatic cells and glucokinase.

**L28 Glycogen metabolism**

1. Describe the overall purpose of glycogenesis and glycogenolysis, their reactants and products, their cellular localization, and their tissue distribution.

2. Describe the roles of glycogen synthase and the branching enzyme in glycogenesis, and predict the biochemical and potential clinical consequences in deficiencies of these enzymes.

3. Describe the roles of glycogen phosphorylase, debranching enzyme, and glucose 6-phosphatase in glycogen breakdown.

4. Compare and contrast the purpose and regulation of glycogenolysis in hepatocytes vs skeletal muscle.

**SB28 Regulation of glycogen synthesis and degradation**

1. Explain how glycogen synthesis and glycogenolysis are regulated by insulin, glucagon, and catecholamines.
2. Compare the allosteric regulation of glycogen phosphorylase in muscle and in liver and contrast the purpose.
3. Explain how high blood glucose influences glycogen biosynthesis in the liver, and how glycogen biosynthesis is activated.
4. Distinguish the symptoms that arise from glycogen storage diseases that affect the muscle, the liver, and lysosomes, and explain their biochemical basis.

**L29 The pentose phosphate pathway.**

1. For different tissue types, compare and contrast the overall purpose of the pentose phosphate pathway, its reactants and products, and its cellular localization.
2. Describe the role of reduced glutathione in the body, and the contribution of NADPH to its formation.
3. Explain the biochemical basis of the drug-induced hemolytic anemia observed in glucose 6-phosphate dehydrogenase deficiency.
4. Explain the function of transketolase in the non-oxidative branch of the pentose phosphate pathway.

**SB29 Other pathways of hexose metabolism.**

1. Explain how fructose and galactose feed into the glycolytic pathway.
2. Describe the roles of fructokinase and hexokinase in catabolism of fructose and galactose.
3. Identify diseases that arise from defects in the metabolism of fructose and galactose.
4. Describe the biochemical basis for the symptoms seen in aldolase B deficiency (hereditary fructose intolerance).
5. Compare and contrast the symptoms seen in deficiency of galactokinase and galactose-1-phosphate uridyl transferase (GALT) deficiency and explain their biochemical basis.

6. Appraise and present current scientific evidence related to the contribution of fructose to obesity and risk for heart disease

**L30 Glycosaminoglycans, proteoglycans, and glycoproteins**

1. Define the following terms and explain their relationships to one another: glycosaminoglycan, glycoprotein, and proteoglycan.
2. Describe the biochemical basis and common clinical features of the mucopolysaccharidoses.
3. Identify the characteristic properties of proteoglycans and glycosaminoglycans and describe their synthesis.
4. Explain how glycosaminoglycan structure contributes to elasticity of synovial fluid.
5. Compare and contrast the synthesis of O-linked and N-linked glycoproteins.

**3. LIPID METABOLISM**

**SB31 Metabolism of dietary lipids**

1. Describe the process of digestion fatty acid and triglycerides.
2. Describe the process of absorption fatty acid and triglycerides
3. Describe the process of utilization of fatty acid and triglycerides.
4. Explain medical ramification of lipid malabsorption, in particular, with respect to vitamins.
5. Explain the use of dietary lipids by tissues.

**L32 Fatty acid and triacylglycerol metabolism: structure and synthesis of fatty acids**

* 1. Describe the pathway of fatty acid synthesis and in particular the role of acety-CoA carboxylase and fatty acid synthase.
	2. Outline short-term and long-term regulation of fatty acid synthesis.
	3. Explain the concepts of elongation and desaturation of the fatty acid chain.
	4. Describe the synthesis of triglycerides.
	5. Describe the role of dietary omega-3 and omega-6 fatty acids.

**SB32 Regulation of fatty acids synthesis**

1. Explain the interrelationship between blood glucose level and the fate of free fatty acids in adipose tissue.
2. Explain how are fatty acids, from dietary triacylglycerols delivered into adipose tissue and muscles.
3. Explain how is a constant fatty acid concentration maintained in the blood.
4. Explain the metabolic pathways of glucose 6-phosphate in the liver at high blood glucose level.

**L33 Fatty acid and triacylglycerol metabolism: mobilization of stored fats, oxidation of fatty acids, ketone bodies**

* + 1. Compare and contrast the life cycle of various lipoprotein particles with respect to their composition, metabolism and transport.
1. Compare ACAT and LCAT enzymes.
2. Describe the mechanism for activation and transport of fatty acids into mitochondria for catabolism.
3. Outline the sequence of reactions involved in oxidation of fatty acids in mitochondria.
4. Describe the general features of pathways for oxidation of unsaturated, odd-chain and branched-chain fatty acids.
5. Explain the mechanism for the formation of ketone bodies and identify the physiological and pathological roles of those molecules.
6. Describe the mechanism by which hormonal activation of lipolysis in adipose tissue is coordinated with activation of gluconeogenesis in liver during fasting.

**L34 Complex lipid metabolism**

1. Compare and contrast the structure of phosphatylcholine and sphingomyelin.
2. Compare and contrast the roles of phospholipases A2 and C.
3. Describe formation of inositol triphosphate and diacylglycerol and their role in intracellular signaling.
4. Compare and contrast the structure of neutral and acidic glycosphingolipids.
5. Explain biochemical basis and some clinical features in Tay-Sachs and Gaucher disease.
6. Describe biosynthesis of prostaglandins, leukotrienes and a tromboxane.
7. Explain action of cortisol, aspirin and other nonsteroidal anti-inflammatory drugs in eicosanoid biosynthesis.

**L35 Cholesterol metabolism**

1. Distinguish the mechanisms by which cholesterol biosynthesis is regulated by energy availability, hormones, food intake and pharmacological manipulation.
2. Interpret the effect of up-regulating or down-regulating plasma cholesterol levels on intracellular cholesterol synthesis, and the transcriptional regulation of genes that are involved in cholesterol homeostasis.
3. Summarize the process of cholesterol breakdown and elimination from the body.
4. Compare and contrast the structure and function of cholesterol and cholesterol esters.

**SB35 Hypercholesterolemia and synthesis of bile salts**

1. Compare and contrast the structure and function of cholesterol and bile salts.
2. Summarize the process of cholesterol breakdown and elimination from the body.
3. Interpret the effect of up-regulating or down-regulating plasma cholesterol levels on the intracellular synthesis of cholesterol, and the transcriptional regulation of genes that are involved in cholesterol homeostasis.
4. Summarize the different biochemical pathways that could potentially be targeted in the management of hypercholesterolemia.

**SB36 Steroid hormones**

1. Describe pituitary hormone stimulation of steroid hormone synthesis and secretion.
2. Describe first steps of steroid hormone synthesis initiated by ACTH.
3. Compare C17- and C21-hydroxylation in three classes of adrenal gland steroids.
4. Explain 21-α-hydroxylase deficiency and treatment.
5. Describe actions of steroid hormones.
6. Explain action of aromatase.
7. Describe activation of transcription by interaction of steroid hormone-receptor complex with hormone response element.
8. List hormone receptors that are members of a “superfamily” of structurally related gene regulators together with steroid hormone-receptor.

**BIOCHEMISTRY (B2)**

**4. NITROGEN METABOLISM**

**L37 Amino acids: disposal of nitrogen**

1. Describe protein turnover and indicate the role of ubiquitin in protein degradation.
2. Indicate how the ultimate end products of nitrogen catabolism in mammals differ from those in birds and in fish.
3. Illustrate the central roles of transaminases (aminotranferases), of glutamate dehydrogenase, and of glutaminase in human nitrogen metabolism.
4. Write the equation for an aminotransferase reaction and illustrate the role played by the coenzyme.
5. Represent the reactions that convert NH3, CO2 and the amide nitrogen of aspartate into urea.
6. Indicate the subcellular locations of the enzymes that catalyze urea biosynthesis.
7. Explain why metabolic defects in different enzymes of urea biosynthesis, although distinct at the molecular level, present similar clinical signs and symptoms.

**L38 Amino acid degradation and synthesis**

1. Explain why the absence from the diet of some amino acids is not deleterious to human health.
2. Appreciate the distinction between „essential“ and „nutritionally essential“ amino acids, and identify the amino acids that are nutritionally nonessential.
3. Name the citric acid cycle and the glycolytic intermediates that are precursors of aspartate, asparagine, glutamate, glutamine, glycine and serine.
4. Appreciate the key role of transaminases in amino acid metabolism.
5. Identify the role of tetrahydrobiopterin in tyrosine biosynthesis.
6. Name the principal catabolites of the carbon skeletons of the common amino acids and the major metabolic fates of these catabolites.
7. Outline the metabolic pathways for each of the common amino acids.
8. Draw analogies between the reaction that participate in the catabolism of fatty acids and branched-chain amino acids.

**SB38 Metabolic defects in amino acid metabolism**

1. Identify reactions associated with clinically significant metabolic disorders in the catabolism of amino acids.
2. Provide examples of aminoacidurias.
3. Identify the metabolic defects in maple syrup urine disease and methylmalonic aciduria.
4. Explain the role of phenylalanine hyroxylase in tyrosine metabolism and how its deficiency results in phenylketonuria.
5. Explain how Parkinson’s disease and albinism arise from tyrosine catabolism disorders.

**L39 Conversion of amino acids to specialized products: porphyrin metabolism**

1. Document the role of glycine in the biosynthesis of heme, purines and creatine.
2. Document the role of S-adenosylmethionine as a source of methyl groups in metabolism.
3. Describe the relationship between porphyrins and heme.
4. Define the synthesis of heme.
5. Present how bilirubin is derived from heme and how it is handled in the body.
6. Describe how defective heme synthesis leads to the following porphyrias: AIP (acute intermittent porphyria) and EPP (erythropoietic protoporphyria); and distinguish the signs and symptoms, and treatment options.
7. Describe types of jaundice: neonatal, hemolytic, hepatocellular, and obstructive.
8. Distinguish between direct and indirect measurements of bilirubin and its implications for distinguishing the cause of the jaundice.
9. Explain the significance of neonatal jaundice and describe the common treatment.

**L40 Other nitrogen-containing compounds: catecholamines; thyroid hormones**

1. Describe catecholamine synthesis.
2. Explain pathogenesis and treatment of Parkinson disease.
3. List the precursors and the roles of histamine, serotonin and melanin.
4. Describe creatine synthesis, role and degradation.
5. Describe the synthesis and secretion of thyroid hormones T4, and T3, noting the steps that occur exclusively in the thyroid gland and those in peripheral tissues.
6. Discuss the regulation of iodine uptake by the thyroid gland and the consequences of iodine deficiency.
7. Describe the molecular basis of thyroid hormone action.

**SB40 Signal transduction disorders**

1. Explain biochemical basis of testicular feminization occurrence
2. Explain the disorder of signal transduction during cholera infection.
3. Explain the biochemical basis of night blindness occurrence.
4. Explain signal transduction after α1-adrenergic stimulation and biochemical basis for lithium treatment of mania in patients diagnosed with bipolar disorder.

**L41 Nucleotides. Metabolism of purine and pyrimidine nucleotides.**

1. Name the major purine and pyrimidine bases and identify amino acid and one-carbon metabolites that contribute to the synthesis of these ring structures.

2. Integrate the terminology and define the structural features that distinguish different classes of nucleotide metabolites (such as purine vs. pyrimidine, base vs. nucleoside vs. nucleotide, and ribo- vs. deoxyribo-).

3. Describe the biosynthesis of purine and pyrimidine nucleotides (*de novo* and salvage pathways) with emphasis on the key regulatory steps.

4. Explain the purine salvage pathways and discuss the central role of hypoxanthine phosphoribosyltransferase (HPRT) under physiological and pathophysiological conditions, and in pharmacotherapy (anti-purine chemotherapy).

5. Explain the salvage pathways for uracil and thymine, and their relevance to pharmacotherapy (such as for the treatment of cancer or herpes infections).

**SB41 Regulation of purine and pyrimidine metabolism.**

1. Connect the pentose phosphate pathway to 5’-phosphoribosyl-1-pyrophosphate (PRPP) synthesis and explain the central role of this metabolite in nucleotide metabolism.

2. Identify inborn errors of purine metabolism (such as deficiencies of HPRTase and adenosine deaminase) and compare and contrast their primary clinical presentations.

3. Compare and contrast the effects of 5-flurouracil (5-FU) and methotrexate (MTX) on the synthesis of thymidine.

**5. INTEGRATION OF METABOLISM**

**SB42 Metabolic effects of insulin and glucagon**

1. Explain how glycogen synthesis and glycogenolysis are regulated by insulin, glucagon.
2. Compare the allosteric regulation of glycogen phosphorylase in muscle and in liver and contrast the purpose.
3. Explain how high blood glucose influences glycogen biosynthesis in the liver, and how glycogen biosynthesis is activated.
4. Distinguish the symptoms that arise from glycogen storage diseases that affect the muscle, the liver, and lysosomes, and explain their biochemical basis.
5. Describe the state of hypoglycemia.

**L43 The feed / fast cycle**

* 1. Discuss the action of hormones that regulate fuel metabolism.
	2. Discuss the regulation of metabolism in fed state.
	3. Discuss the regulation of metabolism during fasting.
	4. Integrate metabolism in specific tissues (erythrocytes, liver, muscle, brain).
	5. Discuss the metabolism of muscle at rest and during exercise.
	6. Discuss the regulation of metabolism during stress and trauma.
	7. Identify the metabolic products of ethanol metabolism including acetyl coenzyme A.
	8. Evaluate the metabolic effects and clinical significance of ethanol and its metabolites.

**SB44 Diabetes mellitus**

1. Describe the onset of type I diabetes.
2. Describe the onset of type II diabetes.
3. Explain metabolic changes in type I diabetes: hyperglycemia, ketoacidosis and hypertiacilglyglyerolemia.
4. Explain metabolic changes in type II diabetes: hyperglycemia and dislypidemia.
5. Explain chronic effects and prevention of diabetes.

**SB45 Obesity**

1. Compare and contrast upper and lower body obesity, subcutaneous and visceral fat, and their risks of mortality and morbidity.
2. Describe signals that influence appetite and satiety.
3. Describe changes in blood lipids in obesity.
4. Explain the hypothesis that thermogenin deficit contributes to obesity.
5. Explain the fate of fructose consumed in excess.

**L46 Nutrition**

1. Define calorie, basal metabolic rate, and daily energy expenditure, and describe how these values are measured or calculated.
2. Apply the principles of homeostasis to an understanding of nutrient processing and storage.
3. Describe the general characteristics of a healthy diet.
4. Define essential and list examples of essential, conditionally and non-essential nutrients
5. Define nitrogen balance and explain how it is affected by dietary intake, growth and metabolic stress.
6. Explain the nutritional basis for the major chronic and metabolic conditions and diseases. For example: obesity and cardiovascular disease
7. Describe common biochemical and clinical features of nutritional disorders.

**SB46 Vitamins**

1. Describe the roles of micro- and macronutrients.
2. Describe the structures of vitamins
3. Define a vitamin and describe the metabolism, principal functions, deficiency diseases associated with inadequate intake, and the toxicity of excessive intakes of the vitamins
4. Interpret presenting symptoms associated with a primary deficiency or toxicity of micronutrients, such as osteopenia, nyctalopia (night blindness), microcytic anemia, and megaloblastic anemia.

**6. STORAGE AND EXPRESSION OF GENETIC INFORMATION**

**L47 DNA organization and replication.**

1. Summarize the central dogma of molecular biology, and cite exceptions to the original model.

2. Compare and contrast the structure of DNA and RNA, explaining the difference between the constituent bases, sugars, nucleosides and nucleotides.

3. Describe the double-stranded, helical, and antiparallel chain structure of DNA and how it relates to the processes of DNA replication, transcription, recombination and repair.

4. Compare and contrast the different types of RNA.

5. Summarize the mechanism of DNA replication, and why discontinuous synthesis is required.

6. Explain the process of telomere replication and relate telomere dynamics to aging and disease.

**SB47 DNA repair.**

1. Describe how DNA and DNA processes can be used as therapeutic targets (e.g. anticancer and antibacterial drugs).
2. Compare and contrast polymerase proofreading, direct repair, base excision repair, nucleotide excision repair, mismatch repair, and recombination.
3. Name the different types of mutations that occur in DNA.
4. Summarize why mutations in DNA repair systems can lead to disease, including certain types of cancer (BRCA, HNPCC).

**L48 RNA synthesis, processing and modification.**

1. Describe the universal features of the genetic code and describe its biological relevance.

2. Use the genetic code to predict the amino acid sequence of a protein for a given nucleic acid sequence and demonstrate how nucleotide mutations can lead to alterations in the primary structure of a protein.

3. Summarize the initiation, elongation, and termination of transcription, comparing and contrasting these processes in eukaryotic and prokaryotic cells.

4. Compare and contrast prokaryotic and eukaryotic gene structure.

5. Describe the posttranscriptional processing of eukaryotic mRNA, and explain how diseases may result from alterations in the processing steps and cite examples.

6. Define RNAi and describe its role in regulation of gene expression.

7. Describe the *cis* and *trans* acting elements involved in eukaryotic transcription and summarize their regulation.

**L49 Protein synthesis.**

1. Summarize the three steps of translation: initiation, elongation, and termination. Compare and contrast these processes and their regulation in eukaryotic and prokaryotic cells.

2. Describe the structure and function of chromatin, and summarize the mechanism of remodeling required to make DNA accessible for biological processes.

3. Describe how transcriptional defects may result in diseases and how this knowledge can be used to develop therapeutics.

**SB49 Protein synthesis regulation and inhibition**

1. Describe the major posttranslational modifications and cite examples.

2. Summarize the inhibition effects of various antibiotics on prokaryotic protein synthesis, and potential side-effects of these antibiotics.

**L50 Regulation of gene expression.**

1. Describe the mechanisms of gene regulation, both negative and positive, as exemplified by prokaryotic systems (*lac* operon, *trp* operon, atenuation).

2. Summarize the effect of covalent modification of chromatin on gene transcription (including methylation, histone acetylation and phosphorylation).

3. Summarize the mechanisms of gene expression regulation at the transcriptional level (transcriptional factors, ligand/hormone binding domains, and DNA-binding motifs, such as helix-turn-helix, zinc-finger and the leucine zipper).

4. Describe gene expression regulation at the translational level.

5. Define epigenetics and describe its role in development, imprinting and disease.

**SB50 Gene expression regulation.**

1. Compare and contrast main principles of prokaryotic and eukaryotic gene expression.
2. Define promoters in eukaryotes and prokaryotes.
3. Define enhancer elements and summarize the properties of enhancers.
4. Describe histone covalent modification.
5. Compare the regulation of gene expression by steroid and thyroid hormones.
6. Describe the roles of miRNA and siRNA in gene expression modulation.

**L51 Biotechnology and human diseases.**

1. Describe the principles of nucleic acid isolation (DNA, RNA).
2. Describe the importance and application of restriction enzymes in DNA research.
3. Describe the principles, methods, and applications of blotting (Northern, Southern, Western) and hybridization techniques in biomedical sciences.
4. Describe the principles, methods, and applications of microarray, PCR, and DNA sequencing for clinical and forensic sciences.

5. Describe how recombinant DNA technology is used to clone and express genes

**SPECIAL TOPICS**

**SB52 Blood clotting**

* 1. Recognize the significance of hemostasis and thrombosis in health and disease.
	2. Outline the pathways of coagulation that result in the formation of fibrin.
	3. Identify the vitamin K-dependent coagulation factors.
	4. Provide examples of genetic disorders that lead to bleeding.
	5. Describe the process of fibrinolysis.
	6. Outline the steps leading to platelet aggregation.
	7. Identify the antiplatelet drugs and their mode of inhibition of platelet aggregation.

**Seminar practicals (SP) and practicals (P)**

**SP11 and P11 Serum proteins electrophoresis**

1. Explain the principle and conditions for electrophoresis.
2. List protein fractions that are separated by electrophoresis and their arrangement in elpherogram.
3. Explain factors that affect the speed of individual protein fractions during electrophoresis.
4. Explain the principle of lipoprotein determination using the serum protein elpherogram.
5. Explain the principle of quantitative protein determination in individual protein fractions.
6. Compare densitometric patterns of the γ-globulin fraction in patients with cirrhosis and patients with increased monoclonal antibody level.

**SP12 and P12 Urease: inhibitor determination**

1. Define the rate of a chemical reaction and several factors that affect the reaction rate.

2. Explain the transition state theory and co-products in chemical reaction.

3. Define enzyme catalyzed transformation of substrate into product.

4. Explain and show the Michaelis-Menten dependence of enzymatic reaction rate on substrate concentration.

5. Distinguish two main types of reversible inhibitors based on the kinetic parameters.

6. Independently compare cysteine and phenylmercury actetate influence on urease activity in a reaction mixture.

7. Express urease activity in the reaction mixture in form of a number of mmols of urea hydrolysed per minute.

8. Draw conclusions based on the collected results.

**SP13 and P13 Alkaline phosphatase: effect of pH on enzyme activity**

1. Describe the activity of alkaline phosphatase.

2. Predict the influence of pH on the reaction rate.

3. List substrate, product, reaction conditions and reaction flow.

4. Explain the mode of stopping the reaction.

5. Predict the pH at which the reaction rate would be the highest.

6. Define the way of doing the experiment.

7. Test the absorbance using a blank probe.

8. Construct the diagram showing the dependence of the reaction rate on the pH.

9. Indicate the optimal pH for this enzyme-catalyzed reaction.

10. Explain and argue the result.

**SP14 and P14 Alkaline phosphatase: determination of Km and Vmax in the presence**

 **of inhibitors**

1. Describe the application of the Lineweaver-Burk plot to determine Km and Vmax.
2. Compare the effects of the competitive and non-competitive inhibitor on the kinetics of the enzymatically catalyzed reaction.
3. Indicate the reaction catalyzed by alkaline phosphatase and explain its diagnostic significance.
4. Explain the performance of experimental practice with an emphasis on data measurement used to calculate the enzyme activity.
5. Handle automatic pipettes, prepare the reaction mixture including enzyme, substrate, buffer and cofactors.
6. Record the results and apply the equation to calculate the rate of the enzyme-catalyzed reactions.
7. Draw the Burk-Lineweaver plot to show the correlation between reaction rate and concentration of substrate for an uninhibited and inhibited reaction.
8. Determine the reaction parameters: Vmax and Km for an uninhibited and inhibited reaction, and conclude the type of inhibition in the sample (competitive or non-competitive).

**SP15 and P15 Amylase: determination in saliva sample**

1. List amylases.

2. Describe the activity of amylases.

3. List and describe substrate, products, reaction conditions and reaction flow.

4. Point the enzyme thermolability.

5. Explain the determination of enzyme activity.

6. Independently prepare reaction mixtures with heated or non-heated amylase.

7. Explain how to identify the substrate using Lugol solution.

8. Explain how to identify the product of hydrolysis using Fehling reagent.

9. Predict the results of the reaction.

10. Argue the obtained results.

**SP16 and P16 Determination of HbA1c by ion-exchange chromatography**

1. Explain the principles of chromatography.

2. Distinguish basics of chromatography methods used in biochemistry (ion-exchange chromatography, affinity chromatography and gel-filtration).

4. List the advantages of the HPLC chromatographic method.

5. Argue the importance of determining HbA1c in medicine.

6. Describe the diagnostic importance of HbA1c for clinical monitoring of diabetes.

7. Collect HbA1c fractions of haemoglobin from blood using cation-exchange chromatography columns.

8. Determine the absorbance of the HbA1c fraction by spectrophotometric analysis.

9. Determine the absorbance of the total hemoglobin in blood.

10. Determine the percentage of HbA1c in total hemoglobin and compare the data with reference values and interpret the result in terms of diagnosis.

**SV17 and P17 Lipids: separation of skin lipids by thin-layer chromatography**

1. Explain the principle of thin-layer chromatography.
2. List the lipids in the skin.
3. Describe the properties of lipids.
4. Define the Rf term.
5. Independently analyse skin lipids by thin-layer chromatography.
6. Calculate Rf values of skin lipids.
7. Compare Rf values of standards and skin lipids.

**SP18 and P18 Determination of lipoproteins**

1. Describe the structure of lipoprotein particle.

2. Classify classes of lipoproteins.

3. Define HDL and LDL cholesterol.

4. Describe the principle of the method.

5. Explain how to calculate HDL.

6. Prepare reaction mixtures.

7. Test the absorbance of the solutions using a blank probe.

8. Calculate the concentration of HDL in the sample.

9. Compare the result with the reference values.

10. Interpret and argue the result.

**SP19 and P19 Determination of conjugated and unconjugated bilirubin in serum**

1. Describe how bilirubin is derived from heme and how it is transmitted in the body.
2. Explain the chemical structure, metabolism and disorders in the metabolism of bilirubin.
3. Identify types of jaundice and how the findings differ in patients with different types of jaundice.
4. Describe the principle and method of determination of bilirubin concentration in serum.
5. Independently determine the concentration of conjugated and total bilirubin in serum by spectrophotometry and calculation.
6. Describe a calculation of unconjugated bilirubin from values for total and conjugated bilirubin in serum.
7. Compare the collected values to reference values and interpret the results.

**SP20 and P20 Determination of creatinine and the pathological compounds in urine**

1. Describe the synthesis of creatine and the occurrence of creatinine.

2. Link the altered amounts of creatine and creatinine to the health condition of the organism.

3. Describe the quantitative determination of creatinine in the urine sample.

4. List pathological compounds in urine. Link the presence of pathological compounds in urine to health problems.

5. Describe galactose metabolism and galactosemia.

6. Identify pathological compounds in the urine sample. Interpret and critically describe the result.

7. Prepare the reaction mixtures to determine creatinine.

8. Calculate the amount of creatinine in the sample. Compare the result with the reference values.

9. Interpret and critically describe the result.

**SP21 and P21 Determination of iron and iron binding capacity in serum**

1. Describe the metabolism and iron excretion.

2. Explain the biological significance and importance of iron in the organism.

3. Distinguish ferritin and transferrin.

4. Define TIBC and UIBC terms.

5. Describe the reaction principle for the determination of total transferrin concentration (TIBC) and iron concentration in serum.

6. Calculate the iron concentration value and iron-binding capacity value.

7. Compare the collected values to reference values and interpret the results.

**SP22 and P22 Immmunochemical analysis. ELISA**

1. Define the principles of ELISA.

2. Explain direct ELISA method.

3. Explain indirect ELISA method.

4. List the application of ELISA method.

5. Explain how to determine the concentration of an antigen or antibody in the sample.

6. Recognise the proper use ELISA for certain application.

7. Explain the reaction conditions in ELISA.

8. Construct a calibration curve (dependence of OD to standard concentration).

9. Determine the unknown concentration in the sample.

10. Interpret and confront the result.

**SP23 and P23** Determination of vitamin C

1. List the foods rich in vitamin C.

2. Explain the importance of vitamin C intake, particularly regarding medicine.

3. Repeat the principle of titration.

5. Describe the neutralisation reaction of ascorbic acid.

6. Determine the vitamin C concentration in the assigned solution.

7. Compare the obtained values with the original values of the assigned solution.

SP24 and P24 **Hemostasis- clotting time and bleeding time tests**

1. Explain how platelets adhere to the surface of the blood vessels, how they are activated and aggregate in the platelet plug formation.
2. Describe the roles and pathways of clotting factors in blood plasma which activate thrombin and start coagulation.
3. Describe the steps in creating a stable fibrin clot from fibrinogen. Compare the structure of fibrinogen and fibrin by explaining the difference in their blood solubility.
4. Explain the role of vitamin K in blood coagulation process.
5. Explain how different anticoagulants (citrate, oxalate, EDTA and heparin) in drawn blood prevent coagulation.
6. Perform blood coagulation test: prothrombin time (PT), specify the units of measurement and the reference interval. Explain the concept of INR (International Normalized Ratio).
7. Perform blood coagulation test: activated partial thromboplastin time (aPTT), specify the units of measurement and the reference interval.
8. Perform blood coagulation test: fibrinogen, specify the units of measurement and the reference interval.
9. Compare results of blood coagulation laboratory tests.

**P25 Comprehensive final exam (laboratory practicals)**

1. Independently perform the assigned experiments.

2. Independently create time management in laboratory.

3. Calculate assigned data and parameters.

4. Compare and interpret results the assigned experiments.

5. Discuss results of the assigned experiments.