9. DETERMINATION OF MINERAL ELEMENTS IN PHYSIOLOGICAL SAMPLES

The organism needs nutrients, water, and vitamins as well as minerals in order to perform its functions in a healthy way. Minerals are found in the human body in very small amounts. The functions of some minerals in the body are as important as vitamins and hormones. Minerals are generally found in nutrients and their deficiencies are not very common.

Studies have shown that 65% of the human body is composed of water and the remaining 35% is composed of organic substances. 27 out of 92 elements found in nature are found in the structure of living things. 11 of these elements are basic elements and others are trace elements that are found in the very low ratio in the body. Although trace elements are found in very low amounts, they have very important tasks for life. All these trace elements that are essential for the living organism are found sufficiently in nature and they are easily taken into the organism with the diet. The basic and trace elements, symbols and percentages found in the human body are given in Table 9.1.

Table 9.1. Elements and their percentages in living organisms

Basic Elements	Symbol	Element (%)	Trace elements	Symbol
Carbon	C	9.50	Iron	Fe
Oxygen	O	25.50	Manganese	Mn
Hydrogen	H	63.0	Zinc	Zn
Nitrogen	N	1.40	Fluorine	F
Calcium	Ca	0.31	Cupper	Cu
Phosphorus	P	0.22	Chrome	Cr
Sulfur	S	0.05	Cobalt	Co
Potassium	K	0.06	Iodine	I
Chlorine	Cl	0.08	Molybdenum	Mo
Sodium	Na	0.03	Tin	Sn
Magnesium	Mg	0.01	Vanadium	V
			Bromine	Br
			Selenium	Se
			Silicon	Si
			Nickel	Ni

As seen in Table 9.1, carbon, hydrogen, oxygen, and nitrogen are the most common elements in living organism body, and they compose 99% of the cells. 50-60% of the cell weight consists of carbon, 8-10% nitrogen, 25-30% oxygen and about 3-4% hydrogen. Instead of these, the other most abundant minerals in the body called macro minerals are calcium, phosphorus, sulfur, potassium, chlorine, sodium and magnesium. Elements that are found in their ionized forms in organism are sodium, potassium, magnesium, calcium, and chlorine.

9.1. The Importance of Salt for the Organism

The sodium ion is generally present in the human organism as chloride salt. These ions, which are taken into the organism in an excess amount within the nutrients, are found in high proportion in body fluids. Sodium chloride should be consumed 3 grams in daily basis. It is found in abundant in sea water than the soil which is due to its relatively high solubility in water. As a result of its low presence in the soil, the amount of sodium in plants is also low. That's why salt is added to the dishes, since sodium intake cannot meet the needs in the body. Sodium, which is found in body fluids protects the body from excess water loss, provides osmotic pressure equilibrium and balance between acid-base levels and, affects the functions of muscles. The amount of sodium varies depending on nutrition. In healthy individuals, the amount of sodium in the body is kept constant within certain limits. The normal level of sodium in the blood is 135-145 mM for patients under 65 years old, and 132-142 mM for those over 65 years of age. As a result of excessive sweating in hot weather, sodium concentration is significantly reduced. The fact that the sodium concentration in the blood falls below normal values is called "hyponatremia". In such cases, nausea, vomiting, exhaustion and muscle cramps may be seen. The increase in the level of sodium in the blood is called "hypernatremia". Excess sodium intake causes edema, fat metabolism deterioration, and hypertension. The urinary sodium level is more sensitive indicator than plasma sodium.

Chlorine is the main anion of the intercellular fluid and the blood plasma. It is chemically present in the form of NaCl and HCl. The stomach acid is in the form of HCl. Gastric acid is produced from NaCl in the blood and it has an important role in the digestion of foods in the stomach. Chlorine is taken into the organism as sodium chloride (NaCl). Therefore, sodium and chlorine metabolism are interrelated. Chloride anion is

used to adjust the acid-base balance and osmotic pressure. Chlorine is found in table salt, meat, milk, and eggs. A large portion of the chlorine taken into the body is excreted in the urine, and a very small portion of the chlorine is taken out of with feces and sweating.

People with heart diseases, hypertension, nephritis, and also pregnant women can be checked through their urine to determine to meet their desired salt intake. Also, heavy sports and excessive sweating reduce the body's salt content. So, headaches, nausea, diarrhea, leg, and abdominal muscles cramps can be seen. In addition, urinary salt excretion is reduced in acute infectious diseases, edematous chronic nephritis, starvation, severe burns, and diarrhea. About 10-15 grams of salt (NaCl) is excreted in a day with urine.

9.2. The Importance of Calcium

Calcium is the most common element in the human organism (average 1.5 g / kg). Calcium ions (Ca^{2+}) are concentrated in extracellular fluids. 99% of the calcium in the human body is found in bones and teeth as a form of $Ca_3(PO_4)_2$. In the different layers of the teeth, there are phosphate, fluoride, chloride, hydroxide and carbonate compounds exist. A large part of calcium is transported by binding to albumin in the blood. The level of blood calcium in a healthy person is in the level of 9-11 mg / 100 mL (2.25 - 2.75 mM). The values below this limit are called “hypocalcemia” and the values above are called “hypercalcemia”. “Tetanized state” which is an over contraction in muscle can occur as a result of hypocalcemia. If the level of calcium in the blood increases, the stimulation in the nervous system slows down, the muscles become lazy (inactive), loss of appetite and constipation is seen. The daily calcium requirement for an adult is 800 mg. The calcium is absorbed in the small intestine and passed through to the blood and then stored in the bones.

Calcium has a great role in the contraction of the muscles, the function of the heart, during pregnancy and, in the production of milk in the maternity periods. Due to its role in bone development and structure, it is needed to ensure that infants and children should consume adequate amount of calcium. Milk, milk products, green vegetables are rich in calcium. Also, nuts such as almonds, hazelnuts are rich in calcium.

Inadequate intake of calcium which has many physiological functions in the body causes "rachitism" in children and "osteomalacia" in adults.

9.3. The Importance of Inorganic Phosphate

Phosphate plays an important role in the formation of bones and teeth together with calcium. It is found in the form of phosphate ions in the blood, also conjugated from on proteins and lipids. Nucleotides which form nucleic acids have phosphoric acid groups. ATP, which is the energy source of the living organism is also a phosphate compound.

Inorganic phosphate is present in the plasma as HPO_4- and H_2PO_4- ions in the majority. Most of the phosphate ions in the blood are in the form of HPO_4-ions. These ions play an important role in keeping the acid-base balance.

Plasma phosphorus concentration is indicated as the amount of inorganic phosphate. The normal concentration of inorganic phosphate in the plasma is in the range of 2.5-4.5 mg / dL. The phosphorus level of the plasma is closely related to the calcium level. The level of both inorganic substances is in a certain ratio (1/1) with each other in the blood.

9.4. The Importance of Iron

In an organism, iron is found mostly in hemoglobin, myoglobin, cytochrome, peroxidase and catalase enzyme systems. Hemoglobin is a protein of porphyrin derivatives found in the structure of red blood cells (erythrocytes), which gives a red color to the blood and has a role in oxygen transport. Myoglobin is a protein that is found intensely in the red muscles (skeletal muscles) and provides oxygen to the muscles.

The total amount of iron in the body is nearly 4-5 g. 65% of this iron bound on hemoglobin, 4% to myoglobin, 1% to various "hem" compounds and 1% in plasma. Transferrin is a plasma protein that is involved in the transport of iron to the tissues. The remaining 15-30% are also stored in liver parenchymal cells (hepatocytes) as ferritin.

Oxygen is bound to hemoglobin by sharing an electron with the iron atom in its **heme** group. This binding is possible when the iron atom in heme is divalent (Fe^{2+}). Hemoglobin-containing trivalent iron is called 'methemoglobin. In healthy individuals, 3% of hemoglobin can be found in the form of methemoglobin. In order to methemoglobin to be re-bound to oxygen, Fe^{3+} in its structure must be reduced to Fe^{2+}. This process is

carried out by means of methemoglobin reductase and glutathione peroxidase enzymes in the organism

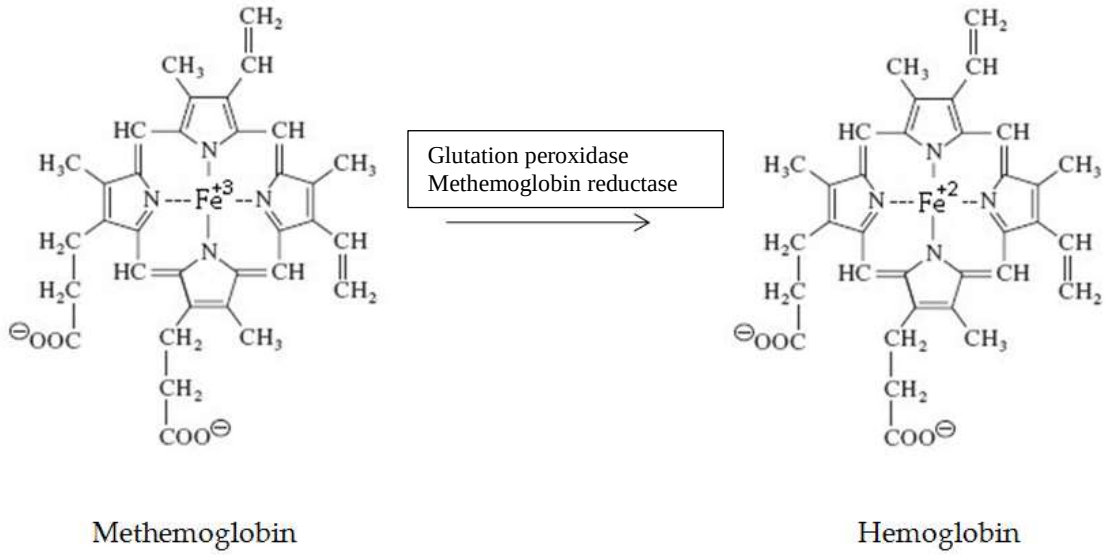

Figure 9.1. Reduction of methemoglobin to hemoglobin

The coordination number of Fe^{2+} in hemoglobin is 6 and the nitrogen of the pyrrole ring is connected to four of these coordination sites, the fifth is nitrogen of the imidazole group of histidine in the globin molecule and the water molecule is bound to the sixth. In the case where water is replaced by O_2, this hemoglobin is called "oxyhemoglobin". Since there are four groups in the hemoglobin molecule, there are four junctions for oxygen.

Hemoglobin that is found in erythrocytes has three main functions:

(1) Transport of O_2 from the lungs to the peripheral tissues.

(2) Transport of CO_2 and protons from the peripheral tissues to the lungs for excretion

(3) Acid hemoglobin / hemoglobinate buffer system (See. Chapter 1).

9.4.1. Importance of oxygen for the organism

Oxygen has a vital role in living organisms to perform their functions. Oxygen is a molecule that should be present for energy production and for the proper functioning of the metabolism in all living organisms and aerobic (oxygen breathing) except some bacteria and anaerobic (oxygen-free breathing) organisms. In the absence of oxygen, the oxygen pressure in the blood drops. In this case, which is called “hypoxia”, anaerobic

metabolism is activated, and energy is produced temporarily. Lactic acid occurs as the final product in anaerobic metabolism. When this condition persists for a long time, lactic acid accumulating gradually causes a decrease in pH of the environment. This is called "metabolic acidosis". The body tries to balance the pH value to a certain degree with various buffer systems. When buffering mechanisms are inadequate, the acidic environment causes disruption of the cellular level and eventually, cell death occurs.

Oxygen binding to hemoglobin: Oxygen binding to hemoglobin is a cooperative event. One O_2 molecule can bind to 4 heme groups of hemoglobin which are in the tetrameric structure. The binding of an O_2 molecule to a heme group increases the interest O_2 binding of the remaining "heme groups". This effect is called "heme-heme interaction" Hemoglobin's affinity for the final bound oxygen is about 300 times greater than that of the first binding. The reversible binding of hemoglobin to oxygen depends on the O_2 partial pressure (pO_2), the pH of the environment, and the amount of 2,3-bisphosphoglycerate present in the environment. The co-operative binding of oxygen changes the partial pressure of oxygen. This allows hemoglobin to deliver more O_2 to the tissues. O_2 concentration and partial pressure of O_2 are high in the lungs. There, O_2 can easily bind to hemoglobin. The resulting oxyhemoglobin (HbO_2) releases most of the oxygen to peripheral tissues for oxidative metabolism where there is low partial pressure.

9.5. Experimental Studies

Experiment 9.5.1. Determination of Salt in Urine (Mohr Method)

The principle of the experiment: When urine, which is added potassium chromate as an indicator, is titrated with silver nitrate, chlorine in the urine interact with $AgNO_3$, then it precipitates as AgCI. When there is no chlorine to combine with silver in the environment, excess silver reacts with potassium chromate, and this reaction results in silver chromate in the color of brick-red. The amount of NaCl in urine is calculated as g_{NaCl}/100 mL urine (M_{NaCl} = 58,5 g.mol^{-1}).

$$AgNO_3 + NaCI \longrightarrow AgCI + NaNO_3$$

$$2AgNO_3 + K_2CrO_4 \longrightarrow Ag_2CrO_4 + 2\ KNO_3$$

Experimental Procedure:

Put 2 ml urine in an erlenemayer and add 5 mL of water and 2 drops of 10% potassium chromate and mix them. Titrate the solution with 0.1 N $AgNO_3$. After each drop make sure to mix the mixture until the brick-red color is observed.

At the beginning of the titration, a white precipitate is formed due to the formation of AgCl in the urine, and then the resulting brick-red color indicates the completion of the reaction. Then, the amount of $AgNO_3$ spent from the burette is read. Lastly, the necessary calculations are made through the reactions and the amount of NaCl in the sample is found.

Solutions:

$AgNO_3$ solution (0.1 N): 16.99 g of silver nitrate is dissolved in 100ml distilled water.

Experiment 9.5.2. Calcium Determination in Serum (Clark-Collip Method)

The principle of the experiment:

Calcium, which is in the serum, precipitates as calcium oxalate with the addition of ammonium oxalate. The precipitate is titrated with potassium permanganate in acidic medium. The amount of pink colored formed $MnSO_4$ is proportional to the initial amount of calcium in the serum. Then the amount of calcium (mg Ca / 100 mL serum) in the serum sample is calculated (M_{Ca} = 40 $g.mol^{-1}$).

Not: Note the coefficients and the reduction-oxidation steps (Mn) in the calculations!

$$Ca^{+2} + C_2O_4^{-2} \longrightarrow CaC_2O_4$$

$$CaC_2O_4 + H_2SO_4 \longrightarrow H_2C_2O_4 + CaSO_4$$

$$5\ H_2C_2O_4 + 3\ H_2SO_4 + 2\ KMnO_4 \longrightarrow K_2SO_4 + 2MnSO_4 + 10\ CO_2 + 8\ H_2O$$

Experimental Method: Take **a centrifuge tube** for sample and follow the process shown in the table.

Substances / mL	**Sample**
Distilled water	1
Serum	1.5
%4 ammonium oxalate	0.5

(1) The centrifuge tube is stirred and incubated for 1 hour at 37 ° C in a water bath.

(2) Cool the tube when it is taken from water bath and centrifuge it for 10 minutes.

(3) Dispose the supernatant. Add 2 mL of 2% NH_3 solution precipitate, mix and centrifuge again for 10 minutes.

(4) Add 2 mL of 1 N H_2SO_4 to the precipitate and leave it in the water bath at 60-70^oC until the precipitate is dissolved.

(5) Put the solution in an erlenmayer and titrated while it is still hot with 0.01 N $KMnO_4$ until the pink color, which can remain at least 2 minutes. Read the amount of $KMnO_4$ spent on the burette.

(6) Calculations are made by making necessary calculations and the amount of Ca in the sample.

Solutions:

Stock $KMnO_4$ Solution (0.1 N): 3.16 g $KMnO_4$ is dissolved in distilled water and volume iscompleted to 1 L with distilled water. Solution is filtered after one week later.

Working $KMnO_4$ Solution (0.01 N): Dilute 10 mL stock potassium permanganate solution to 100 ml with boiled distilled water.

H_2SO_4 Solution (1 N): 50 mL 95% concentrated H_2SO_4 is diluted to 1 L with distilled water.

Experiment 9.5.3. Determination of Inorganic Phosphate in Serum

The principle of the experiment:

A yellow molybdate complex is formed as a result of the interaction of inorganic phosphate in the serum with sulfomolybdate. A reaction occurs with that complex with ascorbic acid, which acts as a reducing, so it is formed of a dark molybdenum blue where the darkness is proportional to the amount of inorganic phosphate.

Experimental method: Procedures, which is indicated in the following table, are applied respectively for the blank, sample and standards.

For the blank, sample and standards, the procedures indicated in the following tables are applied respectively.

Substances / mL	Blank	Sample	Std 1 (0.01 mg/ml)	Std 2 (0.02 mg/ml)	Std 3 (0.03 mg/ml)	Std 4 (0.04 mg/ml)	Std 5 (0.05 mg/ml)
Standards	-	-	0.5	0.5	0.5	0.5	0.5
Serum	-	0.5	-	-	-	-	-
Distilled water	0.5	-	-	-	-	-	-
Trichloroacetic acid	1.0	1.0	1.0	1.0	1.0	1.0	1.0

The sample tube is centrifuged for 5 minutes. The following procedure indicated below is applied to the crystal-clear solution at the upper part of the solution in the sample tube and to the other tubes respectively.

Substances / mL	Blank	Sample	Std 1 (0.01 mg/ml)	(St 2 (0.02 mg/ml)	Std 3 (0.03 mg/ml)	Std 4 (0.04 mg/ml)	Std 5 (0.05 mg/ml)
Distilled water	3	3	3	3	3	3	3
Sülfomolidbat	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Askorbic asid	1.5	1.5	1.5	1.5	1.5	1.5	1.5

The tubes are stayed in the dark for 15 minutes, then the absorbance values of the sample and standard tubes are measured at 620nm. The measured absorbance values are plotted versus phosphate concentration to draw a standard calibration graph and calculate the phosphate amount in 100 mL blood from the graph.

Solutions:

Trichloroacetic acid (30%): 30 g of trichloroacetic acid is dissolved in distilled water and diluted to 100 mL.

Sulfomolybdate: 5 M H_2SO_4 and 7.5% sodium molybdate solutions of equal volume are mixed.

Ascorbic acid (1%): 1 g of ascorbic acid is dissolved in distilled water and diluted to 100 mL.

Stock standard phosphate solution (1 mg / mL): 0.4393 g of KH_2PO_4 dissolved in water. Add 1 mL of 5M H_2SO_4 and dilute it to 100 mL.

Experiment 9.5.4. Assessment of Oxyhemoglobin-Deoxyhemoglobin Equilibrium

The principle of the experiment:

It is a qualitative study based on the principle of the hemoglobin O_2 binding and releasing.

Experimental Method:

I. Phase: Preparation of oxyhemoglobin solution

3 to 4 ml is taken from the blood sample into a centrifuge tube and it is centifugated for 5 minutes. Then, the supernatant is discarded. Add 5 mL of 0.2 M NaCl solution to remaining precipitate and centrifuge again. Then the supernatant is discarded. The precipitate in the tube is transferred to the separatory funnel with 5 mL of distilled water. Then, add 5 mL of toluene and shake vigorously for 3-4 minutes. At room temperature, the phases are allowed to stand until separated. The liquid phase separated in the bottom is taken into a tube, which is the oxyhemoglobin solution. The top toluene phase is discarded.

II. Phase:

(1) 2 mL Stokes' reagent is put in a tube.

(2) Ammonia solution is added dropwise until a green color is observed.

(3) This solution is added dropwise to the 5 mL oxyhemoglobin solution in a tube until the red color changes to a purple color. The formation of purple color indicates that oxyhemoglobin is converted to deoxyhemoglobin.

(4) Purple-colored deoxyhemoglobin solution is poured into the petri dish and left for 15 minutes. In the meantime, deoxyhemoglobin is re-bound to oxygen than turn to red oxygen oxyhemoglobin.

Solutions:

Stokes solutions: 3 g of tartaric acid is dissolved in 100 mL of water. This solution is prepared by dissolving 2 g of iron II sulfate heptahydrate ($FeSO_4.7H_2O$). The solution should be prepared prior to use.

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