LABORATORY 4

1. Quantitative determination of hydrogen peroxide

Hydrogen peroxide (as oxidizer) reacts in acidic solution with iodide ions oxidizing them to free iodine, which is titrated with sodium thiosulfate.

$$H_2O_2 + 2\,KI + H_2SO_4 \rightarrow I_2 + 2\,H_2O + K_2SO_4$$

$$I_2 + 2\,Na_2S_2O_3 \rightarrow 2\,NaI + Na_2S_4O_6$$

Hydrogen peroxide is reduced to water. This reaction goes relatively slowly. To accelerate the reaction, proper catalyzer, e.g. ammonium molybdate, can be added. The reactions presented above show that 1 mole of hydrogen peroxide reacts with 2 moles of iodide ions and 1 mole of free iodine is released. Iodine reacts with 2 moles of sodium thiosulfate. Thus, there are 2 moles of thiosulfate per 1 mole of hydrogen peroxide.

Procedure:

Transfer quantitatively the examined sample of H_2O_2 solution to a 100 mL volume measuring flask and fill it with water to the mark. Mix well turning it upside down. Using pipette transfer 10 mL of the solution to the Enlermayer flask, add 10 mL of 1M H_2SO_4, 10 mL of 3% KI and a small crystal of ammonium molybdate. Mix the solution, close the flask with piece of paper and place it in a dark place for 5 min. Titrate the released iodine with 0.1M solution of sodium thiosulfate ($Na_2S_2O_3$) to get light yellow color of the solution. Then add 5 drops of starch and continue titration to get discoloration of the solution. Repeat titration three times and count the average volume used.

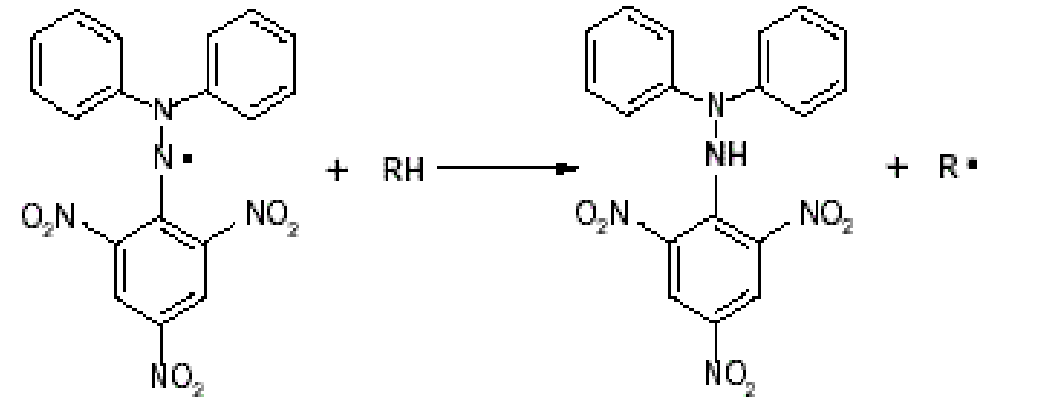
Using the relation presented above (there are 2 moles of thiosulfate per 1 mole of hydrogen peroxide) and knowing that $n_{\text{sodium thiosulfate}} = V_{\text{sodium thiosulfate}} \cdot C_{\text{sodium thiosulphate}}$, calculate the amount of hydrogen peroxide (in grams) in the examined sample (in 100 mL). Molar mass of H_2O_2 = 34 g/mol.

2. Determination of antioxidative abilities of vitamin C and selected infusions

Antioxidants are compounds that, in small concentrations, are able to protect our organism from free radicals action. They are present e.g. in herbs, coffee, tea, cocoa, and other plant products. Plant extracts rich in polyphenolic compounds reveal strong antioxidative properties connected with the presence of several hydroxyl groups. Antioxidative properties of these compounds depend on

the position and the number of hydroxyl groups (higher –OH group number intensifies antioxidative properties).

The aim of the experiment is to familiarize with the method of antioxidative abilities measurement of vitamin C and selected infusions using synthetic radical DPPH (1,1-diphenyl-2-picrylhydrazyl). DPPH is a stable free radical with one unpaired electron on valence shell of one nitrogen atom from nitrogen bridge. Its alcohol solution has dark violet colour. A reduced DPPH can be formed during reaction of this radical with the substance that is able to donate hydrogen. Violet colour of the solution disappears after DPPH reduction. This colour change can be spectrophotometrically checked. A degree of DPPH solution colour change after addition of solution with antioxidant can be a measure of its ability of free radical annihilation.



violet yellow

Procedure:

1. Solutions and infusions of antioxidants:
   a. vitamin C (4 mM solution of ascorbic acid) treated by free radicals (H_2O_2) and the temperature (37 ^oC).
   b. vitamin E and canola oil treated by high temperature without and with the presence of vitamin E. Extraction of vitamin E and oil was carried out before the measurement. Samples of vitamin E and oil were vigorously shaking in isopropanol (with 1 : 3 volume ratio) and the layers were separated (upper alcoholic layer was taken for measurements)
   c. 0.5 % infusions of black, green and white tea.
      1 g of tea was brewed in 100 mL of water at 90 ^oC for 8 min. Then infusion was filtered, cooled to room temperature and diluted 1 : 1 with water.

2. Preparation of 0,5 mM DPPH alcohol solution:

   10.71 mg of DPPH were dissolved in 100 mL of isopropanol. Obtained solution was diluted to get absorbance 0.9 (ʎ = 517 nm; read towards isopropanol).

3. Absorbance measurement:

   Mix 33 µL of examined infusion with 2.5 mL of DPPH solution. Wait 15 min and measure absorbance towards control sample (isopropanol). Absorbance of DPPH solution (A_o) should be also measured.

4. Calculations:

   The ability of the examined infusion to prevent oxidation reaction is calculated from the formula:

$$\textbf{\% of inhibition} = 100 (A_o - A) / A_o$$

   A_o – absorbance of DPPH solution

   A_B – absorbance of examined infusion

5. Results:

Examined solution	A_B	% of inhibition
Vitamin C		
Vitamin C heated at 37 ^{0}C		
Vitamin C + 0.3 % H_2O_2		
Vitamin E		
Canola oil		
Canola oil heated to high temp.		
Canola oil heated to high temp.+ vitamin E		
Black tea		
Green tea		
White tea		

Conclusions:

Compare antioxidative properties of vitamins and antioxidants in studied teas. Evaluate stability of vitamin C and protective role of vitamin E during oxidation of unsaturated fatty acids in oils.

Problems

1. What is solubility of $BaSO_4$ (in mg/100 mL)? $K_{so} = 1 \cdot 10^{-10}$; mol. mass of $BaSO_4$ = 233 g/mole. (Answer: 0.233 mg/100 mL)
2. To 25 mL of 0.2 molar hydrochloric acid solution 75 mL of 0.5 molar hydrochloric acid were added. What is the molar concentration of the solution? (Answer: 0.425 moles/L).
3. Glucose blood concentration is 100 mg% (mg per 100 mL). What is the molar concentration of glucose in blood? (Glucose = 180 g/mole). (Answer: 0.0056 moles/L).
4. In 100 mL of blood there is 350 mg of Na^+ cation. What is the molar concentration of sodium cation in blood? (Na = 23 g/mole) (Answer: 0.15 moles/L).
5. How many mL of 10% (m/v) $CaCl_2$ should be given to a patient in order to increase the level of Ca^{2+} in blood plasma from 2.18 mmol/L to 2.5 mmol/L? The volume of plasma = 5 L, Ht (h(a)ematocrit) = 40% (Ht – a ratio of a volume of red blood cells to the total volume of plasma); ($CaCl_2$ = 111 g/mole). (Answer: 1.06 mL)
6. A patient receives all her nutrition from fluids given through the vena cava. Every 12 hours, 750 mL of a solution that is 4% (m/v) amino acids (protein) and 25% (m/v) glucose (carbohydrate) is given along with 500 mL of a 10% (m/v) lipid (fat). In 1 day, how many grams of amino acids, glucose and lipid are given to the patient? (Answer: 60 g of amino acids, 375 g of glucose and 100 g of lipids)
7. How many mg of Br were introduced into the organism, if a patient received 5 mL of the medicine containing 0.2% (m/v) of KBr ? (KBr = 119 g/mole; Br = 80 g/mole) (Answer: 6.72 mg)
8. The level of vitamin C in the human blood is 1 mg/100mL. what will be the level of this vitamin after injection of 1 mL 2% (m/v) solution of vitamin C (under condition that all the solution will be in the blood. Blood volume = 5L. (Answer: 1.4 mg/100 mL)
9. Many people, with the concentration of ethanol in blood 0.007g/mL reveal alcohol intoxication symptoms. What volume of 40% (v/v) of ethanol with density 0.8 g/mL will evoke these symptoms? We assume that all the consumed alcohol will be in the blood. Blood volume = 5L. (Answer: 109.37 mL)
10. A patient with hypoglycaemia (25 mg of glucose/100 mL of blood) received 5 % (m/v) glucose solution. What volume of this solution should be given to the patient to rise the glucose level to 5.5 mmol/L, under condition that 10% of a given glucose will be metabolized by tissues and 20% will deposit in form of glycogen? Blood volume 4L. (glucose = 180 g/mole). (Answer: 84.56 mL).

11. The level of uric acid in a patient is 0.214 mmoles/L of plasma, solubility of uric acid in human plasma is 7 mg/100mL of plasma at 37°C (mol mass of uric acid is 168 g/mole). Is all uric acid dissolved? (Answer: all uric acid is dissolved)

12. 2 mL of 2% (m/v) medicine solution was mixed with 2 mL of physiological solution of NaCl. 2 mL of diluted solution was injected to a patient. How many mg of medicine were given to the patient? What is percent concentration (m/v) of the medicine in the plasma (volume of plasma - 3L)? (Answer: 20 mg; C_p = 0.00067% (m/v)).

If not mentioned we accept densities of solutions as 1 g/mL.