## LABORATORY 3

## I. ACID-BASE NEUTRALIZATION, TITRATION

Acid-base neutralization is a process in which acid reacts with base to produce water and salt. The driving force of this reaction is formation of a low-energy and stable covalent bond in water, together with the second product, mostly ionized salt. The "neutralization" term does not mean neutral pH , but the state in which the same mole numbers of both acid and base have been mixed. To detect the moment of neutralization, we use an indicator, which for example, can change its color when neutralization is reached. If the reacting partners differ in their dissociation degree, a hydrolyzing salt is formed, and the pH of the "neutralized" solution can be less than 7.0 (for strong acid mixed with weak base) or above 7.0 (for strong base mixed with weak acid). Salts, formed after reactions between alkali or alkaline earth metals bases $\left(\mathrm{NaOH}, \mathrm{KOH}, \mathrm{Ba}(\mathrm{OH})_{2}\right)$ and strong acids $(\mathrm{HCl}, \mathrm{HI})$ do not undergo hydrolysis.

## Acid-base titration

When strong acid and strong base react each other or any of the strong partner reacts with the weak one (acid or base), an essentially irreversible quantitative reaction takes place. The titration is a measurement of the reactant solution concentration. The titration process is stepwise addition from a burette (drop by drop) a standardized solution (solution with known concentration) of base (or acid) to Erlenmayer conical flask containing known volume of acid (or base) solution, in the presence of proper indicator. To calculate the concentration of the examined solution we use the formula:

$$
\begin{aligned}
& C_{1} \times V_{1}=C_{2} \times V_{2} \\
& C_{1}=\frac{C_{2} \times V_{2}}{V_{1}}
\end{aligned}
$$

$\mathrm{C}_{1}$ - unknown concentration of acid (or base) in the Erlenmayer flask
$\mathrm{C}_{2}$ - known concentration of standardized base (or acid) solution in the burette
$\mathrm{V}_{1}$ - volume of the acid (or base) solution in the Erlenmayer flask
$\mathrm{V}_{2}$ - volume of the standardized base (or acid) solution added from the burette to the Erlemnayer flask

The titration process can be ilustrated with titration curve, which is a function of pH change in the titrated solution, as the result of the titrant added from the burette.

The equivalence point is defined as the pH value, in which equal mole numbers of acid and base have been mixed. For titration of 0.1 M strong acid (in the Erlenmayer flask) with 0.1 M strong base (in the buret(te), pH of the mixture rises quickly to pH near 7.0 with sharp slope.

The shape of the titration curve of a weak acid with a strong base is different. When we mix strong base with weak acid, for example NaOH with acetic acid $\left(\mathrm{CH}_{3} \mathrm{COOH}\right)$, a sodium acetate hydrolyzing salt $\left(\mathrm{CH}_{3} \mathrm{COONa}\right)$ is formed and, with acetic acid still present in the Erlenmayer flask, a buffering mixture is formed.

The buffering capacity of the mixture, the greatest when a half of acetic acid is reacted, results in a flat part of the curve, until concentration of acetic acid strongly diminishes. At the equivalence point of the reaction, there is no acetic acid left and resulted hydrolysing salt sodium acetate increases pH of the equivalence point to near 8.0.

## Indicators

Indicators are conjugated acid-base pairs added to a titration mixture in small molar amounts, in order to monitor the pH . The acidic and basic forms of indicators have different colors. The pH range, at which an indicator color begins to change, depends on its $\mathbf{p K}$ ( pH at which molecule of indicator is dissociated in $50 \%$ ). Generally, we must select proper indicator for kind of titration: the indicator should change its color at or near the point of equivalence.

| Indicator | Color of <br> Acidic Form | Color of Basic <br> Form | Range Color <br> Change | pK |
| :---: | :---: | :---: | :---: | :---: |
| Methyl orange | Red | Yellow | $3.1-4.4$ | 3.7 |
| Bromophenol <br> blue | Yellow | Blue | $3.0-4.6$ | 4.0 |
| Methyl red | Red | Yellow | $4.2-6.3$ | 5.1 |
| Bromothymol <br> blue | Yellow | Blue | $6.0-7.6$ | 7.0 |
| Phenolphthalein | Colorless | Pink | $8.3-10.0$ | 9.7 |

## Practical part: Titration procedures

## 1. The colors of some indicators of acidity and alkaline medium

Add reagents to 6 test tubes according the following scheme. Write out your work and formulate a conclusion.

| Number of test tubes | 1 | 2 | 3 | 4 | 5 | 6 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| 1 M HCl | 1 mL |  | 1 mL |  | 1 mL |  |
| 1 M NaOH |  | 1 mL |  | 1 mL |  | 1 mL |
| Number of drops |  |  |  |  |  |  |
| Methyl orange | 2 | 2 |  |  |  |  |
| Methyl red |  |  | 2 | 2 |  |  |
| Phenolphthalein |  |  |  |  | 2 | 2 |
| Solution color |  |  |  |  |  |  |

## 2. NaOH standardization with titrated HCl (determination of the concentration of NaOH solution using HCl with known concentration)

Using pipette pour 10 mL of 0.1 M HCl solution into Erlenmeyer flask, add 3 drops of methyl orange. Fill up the burette to zero mark with NaOH solution. Titrate 0.1 M hydrochloric acid solution with NaOH from the burette. The solution will turn its colour to orange (salmon colour) when the equivalence point is reached. Repeat the titration at least two times and notice the volume of NaOH used. Count the arithmetic average of the volumes and use this value for calculation of exact NaOH concentration.

$$
\begin{array}{r}
C_{\mathrm{NaOH}} \times \mathrm{V}_{\mathrm{NaOH}}=\mathrm{C}_{\mathrm{HCl}} \times \mathrm{V}_{\mathrm{HCl}} \\
\mathrm{C}_{\mathrm{HCI}} \times \mathrm{V}_{\mathrm{HCl}} \\
\mathrm{C}_{\mathrm{NaOH}}=\frac{V_{\mathrm{NaOH}}}{}
\end{array}
$$

## 3. Determination of HCl content in student's test sample.

Transfer quantitatively your sample of HCl to a 100 mL volume measuring flask and fill it to the mark with water. Mix well turning it upside down a few times. Using pipette transfer 10 mL of the solution to the Enlermayer flask, add 3 drops of methyl orange, and titrate with NaOH solution (with concentration determined at point 2) until the color changes from red to orange (salmon). Repeat titration two times and count the average volume used. Using the equation, calculate concentration of HCl solution and then amount of HCl in your volumetric flask in grams.

## Example of calculation:

$\mathrm{V}_{\mathrm{HCI}}=10 \mathrm{~mL}, \mathrm{~V}_{\mathrm{NaOH}}=7.2 \mathrm{~mL}, \mathrm{C}_{\mathrm{NaOH}}=0.096 \mathrm{M}$

What is molar concentration of HCl ?

$$
\begin{aligned}
& \mathrm{C}_{\mathrm{NaOH}} \times \mathrm{V}_{\mathrm{NaOH}}=\mathrm{C}_{\mathrm{HCI}} \times \mathrm{V}_{\mathrm{HCl}}
\end{aligned}
$$

How many grams of HCl are there in 100 mL of the solution ?

$$
\begin{aligned}
& C_{\mathrm{m}}=0.069 \mathrm{moles} / \mathrm{L} \\
& \mathrm{~V}=100 \mathrm{~mL}=0.1 \mathrm{~L} \\
& \mathrm{M}_{\text {HCI }}=36.5 \mathrm{~g} / \mathrm{mole} \\
& \mathrm{n}=? \\
& \mathrm{~m}=? \\
& \quad \mathrm{n}=\mathrm{C}_{\mathrm{m}} \times \mathrm{V}=0.069 \mathrm{moles} / \mathrm{L} \times 0.1 \mathrm{~L}=0.0069 \mathrm{moles} \\
& \mathrm{~m}=\mathrm{n} \times \mathrm{M}=0.0069 \mathrm{moles} \times 36.5 \mathrm{~g} / \mathrm{mole}=0.25 \mathrm{~g}
\end{aligned}
$$

## II. 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 polyphenols and flavonoids 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.


## Procedure:

1. Solutions and infusions of antioxidants:
a. 1 mM vitamin C solution.
b. $0.5 \%$ infusions of tea, coffee, cocoa and herbs.

1 g of tea, coffee, cocoa or herb was brewed with 100 mL of water at $90^{\circ} \mathrm{C}$ for 8 min . Then infusion was filtered, cooled to room temperature and diluted $1: 1$ with water.
2. Preparation of $0,5 \mathrm{mM}$ DPPH alcohol solution:
10.71 mg of DPPH were dissolved in 100 mL of ethanol. Obtained solution was diluted to get absorbance 0.9 ( $\Lambda=517 \mathrm{~nm}$; read towards ethanol).
3. Absorbance measurement:

Mix $33 \mu \mathrm{~L}$ of examined infusion with 2.5 mL of DPPH solution. Wait 15 min and measure absorbance towards control sample (ethanol). Absorbance of DPPH solution ( $\mathrm{A}_{\circ}$ ) should be also measured.
4. Calculations:

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

$$
\% \text { of inhibition = } 100\left(A_{\circ}-A\right) / A_{\circ}
$$

$\mathbf{A}_{\mathbf{o}}$ - absorbance of DPPH solution
A - absorbance of examined infusion

## Examples of problems:

1. 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).
2. Glucose blood concentration is $100 \mathrm{mg} \%$ ( mg per 100 mL ). What is the molar concentration of glucose in blood? (Glucose $=180 \mathrm{~g} / \mathrm{mol})$. (Answer: 0.0056 moles/L).
3. What is the molar concentration of hydrochloric acid solution, if for neutralization of 20 mL of the acid, 10 mL of $0.4 \%(\mathrm{~m} / \mathrm{v}) \mathrm{NaOH}$ solution were used ? ( $\mathrm{Na}=23 \mathrm{~g} / \mathrm{mol}$ ). (Answer: $0.05 \mathrm{moles} / \mathrm{L})$.
4. In 100 mL of blood there is 350 mg of $\mathrm{Na}^{+}$cation. What is the molar concentration of sodium cation in blood? (Answer: 0.15 moles/L).
5. How many mL of $10 \%(\mathrm{~m} / \mathrm{v}) \mathrm{CaCl}_{2}$ should be given to a patient in order to increase the level of $\mathrm{Ca}^{2+}$ in blood plasma from $2.18 \mathrm{mmol} / \mathrm{L}$ to $2.5 \mathrm{mmol} / \mathrm{L}$ ? The volume of plasma $=5 \mathrm{~L}, \mathrm{Ht}(\mathrm{h}(\mathrm{a})$ ematocrit) $=40 \%(\mathrm{Ht}-\mathrm{a}$ ratio of a volume of red blood cells to the total volume of plasma); ( $\left.\mathrm{CaCl}_{2}=111 \mathrm{~g} / \mathrm{mol}\right)$. (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 $4 \%(\mathrm{~m} / \mathrm{v})$ amino acids (protein) and $25 \%(\mathrm{~m} / \mathrm{v})$ glucose (carbohydrate) is given along with 500 mL of a $10 \%(\mathrm{~m} / \mathrm{v})$ 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 $0.2 \%(\mathrm{~m} / \mathrm{v})$ of KBr ? $(\mathrm{KBr}=119 \mathrm{~g} / \mathrm{mol} ; \mathrm{Br}=80 \mathrm{~g} / \mathrm{mol})$ (Answer: 6.72 mg )
8. The level of vitamin C in the human blood is $1 \mathrm{mg} / 100 \mathrm{~mL}$. What will be the level of this vitamin after injection of $1 \mathrm{~mL} 2 \%(\mathrm{~m} / \mathrm{v})$ solution of vitamin C (under condition that all the solution will be in the blood. Blood volume $=5 \mathrm{~L}$. (Answer: $1.4 \mathrm{mg} / 100 \mathrm{~mL}$ )
9. Many people, with the concentration of ethanol in blood $0.007 \mathrm{~g} / \mathrm{mL}$ reveal alcohol intoxication symptoms. What volume of $40 \%(\mathrm{v} / \mathrm{v})$ of ethanol with density $0.8 \mathrm{~g} / \mathrm{mL}$ will evoke these symptoms? We assume that all the consumed alcohol will be in the blood. Blood volume $=5 \mathrm{~L}$. (Answer: 109.37 mL )
10. A patient with hypoglycaemia ( 25 mg of glucose $/ 100 \mathrm{~mL}$ of blood) received $5 \%(\mathrm{~m} / \mathrm{v}$ ) glucose solution. What volume of this solution should be given to the patient to rise the glucose level to $5.5 \mathrm{mmol} / \mathrm{L}$, under condition that $10 \%$ of a given glucose will be metabolized by tissues and $20 \%$ will deposit in form of glycogen? Blood volume 4L. (molar mass of glucose = $180 \mathrm{~g} / \mathrm{mole}$ ). (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 $\mathrm{mg} / 100 \mathrm{~mL}$ of plasma at $37^{\circ} \mathrm{C}$ (mol mass of uric acid is $168 \mathrm{~g} / \mathrm{mole}$ ). Is all uric acid dissolved? (Answer: all uric acid is dissolved)
12. 2 mL of $2 \%(\mathrm{~m} / \mathrm{v})$ 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 ( $\mathrm{m} / \mathrm{v}$ ) of the medicine in the plasma (volume of plasma-3L)? (Answer: 20 mg ; $\mathrm{C}_{\mathrm{p}}=0.00067 \%$ ( $\mathrm{m} / \mathrm{v}$ )).

If not mentioned we accept densities of solutions as $1 \mathrm{~g} / \mathrm{mL}$.

