MINISTRY OF HEALTH OF UKRAINE BOHOMOLETS NATIONAL MEDICAL UNIVERSITY

Department of Medical and General Chemistry

MEDICAL CHEMISTRY

Students workbook

Student _			
Group			

Approved by Methodical Commission of physicochemical disciplines of Bohomole	ts
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The workbook includes laboratory works and questions in all basic topics in medical chemistry: complex compounds; properties of solutions; titrimetric analysis; ion—exchange absorption and partition chromatography; surface phenomena; properties of colloidal solutions; solutions of high-molecular-weight compounds.

The workbook is for students of the medical faculty.

WORK SAFETY

- 1. The students should work in lab coats.
- 2. During work the students should keep silence and order; all operations of laboratory work must be performed carefully in compliance with description.
- 3. Watch closely that reagents don't come in contact with face, hands and clothes.
- 4. Pour alkali and acid into the water but not vice versa while diluting alkalis and concentrated acids. It is forbidden to pour out concentrated acids and alkalis to the sinks.
- 5. Keep the work place clean after finishing of work, wash used chemical utensil, and shut off water valves and gas.
- 6. When determining the odor of the substance direct the air current from the vessel with a light movement of the hand. Carefully breathe in a small amount of the air.
- 7. Address to the instructor in all cases even trivial traumas or burns.
- 8. Food and drink are not allowed in the laboratory.
- 9. After finishing the work in the laboratory, wash your hands carefully.

Student requirements for the completion of the Course

- 1. Respect the subject, as well as those who are working to improve your knowledge.
- 2. Complete all assignments to the best of your ability.
- 3. Take good, concise lecture notes.
- 4. Maintain good attendance of the lectures and practices.
- 5. Ask questions when confused.
- 6. To be able to perform elementary tasks related to medical chemistry.

Your signature at the end of each report is your pledge that this work is your own and you have neither given nor received help from other students.

Medical chemistry course structure

Organization of medical chemistry studies as a course is based on a credit-module system which meets the requirements of Bologna process. The course of «Medical chemistry» consists of two modules:

- «Acid-base equilibrium and complex formation in biological liquids» (7 subjects);
- «Equilibrium in biological systems at a phase interface» (7 subjects).

Current monitoring of student's progress is carried out in the course of every practical class. You will write test and do laboratory work on each class. Conversion of traditional marks for each subject into points:

$$<4> - 17$$
 points
 $<4> - 14$ points
 $<3> - 9$ points
 $<2> - 3$ points.

A grading scale

Stages of the class	Type of control	Points		
Control of initial level knowledge	Test, oral quiz	0–3		
Analysis of theoretical questions, problem solving	Oral quiz, situational problems	0–3		
Laboratory work	Write up a lab report	0–2		
Control of final level knowledge	Test with problems	0–9		

When the study of all subjects of the module is completed the Final test (module control) is carried out. The maximal number of points for module control is 80, minimal – 50. The student is entitled to pass the Final test only if he earned 63 or more points by the time of practical classes. The Final test consists of oral part or practical skills (12 points max), test part (ten 3–points questions, total 30 points) and written part (three 6–points problems and two 10–points problems, total 38 points). Calculate your final mark yourself:

average total points
$$> 113 - \text{«}3\text{»}$$

average total points $\ge 140 - \text{«}4\text{»}$
average total points $\ge 170 - \text{«}5\text{»}$.

We wish you success!

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«THE CHARACTERISTIC REACTIONS OF IONS OF SOME MACRO– AND MICROELEMENTS»

Purpose: to carry out analytical reactions of some cations and anions and memorize the effects of reactions (analytical signals).

Introduction

The aim of qualitative analysis is to establish chemical identity of the sample species, i.e. chemical elements, atom groups, ions or molecules, forming unknown substance or the mixture of substances. The qualitative analysis allows to define authenticity of pharmaceutical preparations and availability of impurity in them. Specific reaction is called a reaction, specific to one ion or molecule and not interfered by other ions in the solution. Specific reactions of all ions were known, however, the number of such reactions is highly limited. Much more numerous are so called selective reactions, which are specific to ions of the similar properties. Selective reactions, specific to a group of ions, are called group selective reactions. Appropriate reagents are used for analytic reactions. So-called specific reagents induce a reaction specific to only one ion; selective reagents are specific for a group of ions.

Reagents and Equipment

Sodium carbonate solution Potassium nitrite solution

Hydrochloric acid solution Silver nitrate solution

Sodium sulfate solution Potassium permanganate solution

Barium chloride solution Hydrogen peroxide solution

Test tubes Test tube rack

Experiment time: about 25 min.

Evaluation

A pipette is a laboratory instrument used in chemistry to transport a measured volume of liquid. Graduated pipettes allow measurement of any volume that would not exceed the volume of pipette's graduated section.

Test 1. The reaction for detection of carbonate ion

Transfer 5–7 drops of sodium carbonate solution to a clean test tube and add 5–7 drops of hydrochloric acid solution.

Observation:
Write the molecular reaction equation (notice that strong hydrochloric acid displaces weak
unstable acetic acid from salt):
Test 2. The reaction for detection of sulfate ion
Transfer 5-7 drops of sodium sulfate solution to a clean test tube and add 2-3 drops o
barium chloride solution.
Observation:
Write the molecular reaction equation (notice that precipitate of barium sulfate is formed):
Test 3. The reaction for detection of nitrite ion
Transfer 5-7 drops of potassium nitrite solution to a clean test tube and add 2-3 drops o
concentrated sulfuric acid solution.
Observation:
Write the molecular reaction equation (notice that nitrous acid decomposes into nitrogen
dioxide NO ₂ , nitric oxide NO, and water):
Test 4. The reaction for detection of silver ion
Transfer 2-3 drops of silver nitrate solution to a clean test tube and add 2-3 drops o
hydrochloric acid solution.
Observation:
Write the molecular reaction equation (notice that precipitate of silver chloride is formed):
Test 5. The reaction for detection of permanganate ion
Transfer 5–7 drops of potassium permanganate solution to a clean test tube, add 5–7 drops
of sulfuric acid and 2–3 drops of hydrogen peroxide solution.
Observation:

Write the molecular reaction equation (notice that this is oxidation-reductio	n reaction and
O ₂ is formed):	

LABORATORY WORK 2 «COMPLEX COMPOUNDS»

Purpose: to prepare the copper complex, iron complex and calcium complex.

Introduction

All organisms, plants and animals, are made up of cells, where chemical reactions take place. Of all the molecules making up the cells we consider a particular group: the complexes or coordination compounds, which are built up from metal cations and ligands. Around 1930 one begun to find out, that small amounts of many metallic elements – today named «trace elements» are vital for the health of plants and animals. Among these elements are aluminum, vanadium, chromium, manganese, cobalt, nickel, copper, tin, selenium, boron, lead and others. In general, the sum of these metals makes up less than 1 per thousand of the weight of the individual but they are essential for the transport of matter within the organism and for the enzymes, the catalysts of metabolism. Many biologically important compounds are coordination compounds (complexes). Chlorophyll, haemoglobin and vitamin B_{12} are coordination compounds of magnesium, iron and cobalt respectively etc. The coordination compounds are finding extensive applications in analytical chemistry and medicinal chemistry.

Reagents and Equipment

Copper (II) sulfate solution Potassium thiocyanate solution

Ammonia solution Calcium chloride solution

Iron (III) chloride solution Ammonia oxalate solution

Test tubes Test tube rack

Ethylenediaminetetraacetic acid disodium salt dihydrate

Experiment time: about 25 min.

Evaluation

Test 1. Cationic complex preparation

Transfer 4–5 drops of copper (II) sulfate solution to a clean test tube and add a few drops of ammonia solution.

or an	inioina solution.
Obse	rvation:
a)	precipitate of copper (II) hydroxosulfate is formed and its color is
b)	precipitate is dissolved in excess ammonia solution and color of formed solution is
Write	e the molecular reaction equations of formation and dissolving of precipitate:
	Test 2. Anionic complex preparation
Trans	sfer 2-3 drops of iron (III) chloride solution to a clean test tube and add 1-2 drops of
potas	sium thiocyanate solution.
Obse	rvation: complex salt K[Fe(NCS) ₄ (H ₂ O) ₂] is formed and its color is
Write	e the molecular reaction equation:
	Test 3. Chelate complex preparation
	sfer 4–5 drops of calcium chloride solution to a clean test tube and add 2–3 drops of onia oxalate solution.
Obse	rvation: precipitate of calcium oxalate is formed and its color is
Write	e the molecular reaction equation:
Add	ethylenediaminetetraacetic acid disodium salt dihydrate Na ₂ H ₂ Y·2H ₂ O to dissolving
of pro	ecipitate of calcium oxalate. Write the molecular reaction equation:

«PREPARATION OF COBALT (II) NITRATE SOLUTION BY DILUTING CONCENTRATED SOLUTION»

Purpose: to demonstrate a method of solution preparation.

Introduction

In laboratory practice, mixtures in a liquid state are usually called solutions. Solutions concentration is their quantitative characteristic. The concentration of a solute in a solution is a measure of how much of that solute is dissolved in the solvent. Ability to estimate the concentration is very important in the study of pharmacology and clinical subjects. One of the methods of solution preparation is dilution of more concentrated solution.

Next equation is used to make calculation:

$$c_1\left(\frac{1}{z}X\right)\cdot V_1(X) = c_2\left(\frac{1}{z}X\right)\cdot V_2(X),$$

where $c_1\left(\frac{1}{z}X\right)$ – molarity of equivalent (normality) of more concentrated solution; $V_1(X)$ – volume of more concentrated solution that will be taken for preparation of diluted solution; $c_2\left(\frac{1}{z}X\right)$ – molarity of equivalent (normality) of diluted solution; $V_2(X)$ – set volume of diluted solution.

Instruction for using of pipette

- 1. Insert the tip of the pipette into the beaker with solution so that it is about 1/4 from the bottom.
- 2. Hold the pipette in your hand, leaving your index finger free to place over the top of the pipette. With your other hand, squeeze the pipette bulb. Press it firmly over the top of the pipette. Release the pressure on the bulb and allow the solution to flow into the pipette until it is above the volume mark.
- 3. Quickly remove the bulb and place your index finger firmly over the top of the pipette. Slowly roll you finger to one side and allow the liquid to drain until the bottom of the meniscus is aligned with the volume mark.

4. Press your index finger firmly on the top of the pipette so no liquid leaks out. Pull the pipette out of the solution and transfer the solution. When the solution stops flowing, touch the pipette once to the side of the receiving beaker to remove any hanging drops.

Reagents and Equipment

Cobalt (II) nitrate solution 100 mL measuring flask

5 mL pipette Beaker

Pipette bulb Distilled water

Experiment time: about 25 min.

Evaluation

1. Calculate volume of cobalt (II) nitrate solution (molarity of equivalent is 0,4 mol/L) that will be taken for preparation of 100 mL of cobalt (II) nitrate solution with molarity of equivalent 0,02 mol/L using the next formula:

$$c_1 \left(\frac{1}{2} \text{Co(NO}_3)_2\right) \cdot V_1 \left(\text{Co(NO}_3)_2\right) = c_2 \left(\frac{1}{2} \text{Co(NO}_3)_2\right) \cdot V_2 \left(\text{Co(NO}_3)_2\right);$$

We remind you that 1 milliliter equals 0,001 liter (to get liters from milliliters you divide the milliliters by 1000) and 1 liter equals 1000 milliliters (to get milliliters from liters you multiply the liters by 1000).

- 2. Prepare glassware:
 - a) rinse the pipette with distilled water, and then with 0,4 mol/L cobalt (II) nitrate solution;
 - b) rinse beaker and measuring flask with distilled water.
- 3. Calculated volume of 0,4 mol/L cobalt (II) nitrate solution transfer to the 100 mL measuring flask using the pipette.
- 4. Adjust the volume to 100 mL with distilled water.
- 5. Close measuring flask and mix the contents.

«HYDROLYSIS OF THE SALTS»

Purpose: to indicate the pH of different aqueous solutions of salts using the acid-base indicators and predict ability of these salts to hydrolysis.

Introduction

All biological liquids are water solution with a given rate of pH. Studying of pH of biological liquids enables to determine pathological phenomena taking place in an organism and to prevent diseases. Usually hydrolysis is a chemical process in which a molecule of water is added to a substance. The term salt hydrolysis refers to the reaction of a cation or an anion, or both with water which splits the water molecule into two parts. Salt hydrolysis usually affects the pH of a solution (acidic, basic or neutral).

Background

To predict the acidity or basicity of a salt solution, you need to examine the acidity or basicity of the ions composing salt. Rules for deciding whether a salt solution will be neutral, acidic, of basic:

- 1. *A salt of a strong base and a strong acid*. The salt has no hydrolysable ions and so gives a neutral aqueous solution: pH= 7 (e.g. NaCl).
- 2. A salt of strong base and weak acid. The anion of the salt is the conjugate of weak acid. It hydrolyzes to give a basic solution: pH > 7 (e.g. Na₂CO₃).
- 3. *A salt of strong acid and weak base*. The cation of the salt is the conjugate of the weak base. It hydrolyzes to give an acidic solution: pH < 7 (e.g. NH₄Cl).
- 4. A salt of weak acid and weak base. Both ions hydrolyze (e.g. $(NH_4)_2CO_3$). Whether the solution is acidic or basic depends on the relative acid-base strengths of the two ions. To determine this, you need to compare K_a of the cation (dissociation constant) and K_b of the anion: if $K_a > K_b$ the solution of the salt is acidic; if $K_a < K_b$ the solution of the salt is about neutral.

The pH of salt solutions can be measured by an acid-based indicators shows changes in color at different pH values. The undissociated form of the indicator is a different color than the iogenic form of the indicator. In some indicators, such as phenolphthalein, one of the species is colorless, whereas in other indicators, such as methyl red, both species confer a color.

Reagents and Equipment

Solutions of salts Solution of methyl red indicator

Sulfuric acid solution Solution Solution of phenolphthalein indicator

Sodium hydroxide solution Test tubes

Distilled water Test tube rack

Experiment time: about 60 min.

Evaluation

I. Determination the pH of electrolytes solutions using methyl red indicator.

- 1. Transfer 5 mL of distilled water to a clean test tube and add 3–4 drops of methyl red indicator solution.
- 2. Transfer 5 mL of sulfuric acid solution to a clean test tube and add 3–4 drops of methyl red indicator solution.
- 3. Transfer 5 mL of sodium hydroxide solution to a clean test tube and add 3–4 drops of methyl red indicator solution.
- 4. Transfer 5 mL of distilled water to a clean test tube; add 3–4 drops of methyl red indicator solution and a few crystals of sodium carbonate.
- 5. Transfer 5 mL of distilled water to a clean test tube; add 3–4 drops of methyl red indicator solution and a few crystals of copper sulfate.
- 6. Transfer 5 mL of distilled water to a clean test tube; add 3–4 drops of methyl red indicator solution and a few crystals of sodium chloride.
- 7. Mix the contents of test tubes and notice the color of solutions in each test tube to the Table.

II. Determination the pH of electrolytes solutions using phenolphthalein indicator.

- 1. Transfer 5 mL of distilled water to a clean test tube and add 3–4 drops of phenolphthalein indicator solution.
- 2. Transfer 5 mL of sulfuric acid solution to a clean test tube and add 3–4 drops of phenolphthalein indicator solution.
- 3. Transfer 5 mL of sodium hydroxide solution to a clean test tube and add 3–4 drops of phenolphthalein indicator solution.
- 4. Transfer 5 mL of distilled water to a clean test tube; add 3–4 drops of phenolphthalein indicator solution and a few crystals of sodium carbonate.

- 5. Transfer 5 mL of distilled water to a clean test tube; add 3–4 drops of phenolphthalein indicator solution and a few crystals of copper sulfate.
- 6. Transfer 5 mL of distilled water to a clean test tube; add 3–4 drops of phenolphthalein indicator solution and a few crystals of sodium chloride.
- 7. Mix the contents of test tubes and notice the color of solutions in each test tube to the Table.

Evaluation Table

	Color of	pH of the		
Electrolyte	Methyl red	Phenolphthalein	electrolytes solutions	
H ₂ O				
H ₂ SO ₄				
NaOH				
Na ₂ CO ₃				
CuSO ₄				
NaCl				

III. Molecular and ionic equations of hydrolysis of salts

Write th	e hydrolysis	reactions	for salts	which	undergo	hydrolysis	in the	ionic	and
molecula	forms and r	otice the pF	I of aqueo	ous solut	ion of thes	se salts (acid	ic, basic	or neut	ral):
			·						
			······································		 				
Campara	the pII of so	lta golytion	a madiata	d by one	olygia of l	avidno livaio m	actions	with n	——
•	the pH of sa		s predicte	a by and	arysis or r	iyaroiysis r	eactions	wim p.	П 01
determin	ed experimen	itally.							
			Со	nclusion	1				
Aqueous	solution of	sodium chl	oride is			_; aqueous	solution	of co	pper
sulfate is		; aqu	eous solu	tion of s	odium ca	rbonate is			

«STANDARDIZATION OF THE SODIUM HYDROXIDE SOLUTION»

Purpose: to demonstrate a method of acid-base titration; to determinate of the molarity of equivalent according to the results of titration.

Introduction

Titration is used to determine the concentration of an unknown acid or base. As with other types of titrations, acid/base titrations can be used to determine the concentration of hydronium or hydroxide ions and thus the pH of a solution. Starting with a solution of an unknown concentration of an acid or base, small volumes of a solution of known concentration are added until the neutralization point or equivalence point is reached. At this point, any hydroxide or hydronium ions from the original solution have been neutralized. Titrations are usually performed in the presence of an indicator, a substance that visibly changes when a certain pH is reached. The equivalence point of a titration does not always occur when the pH level reaches 7. If a weak base is titrated with a strong acid, for example, when all the base has been neutralized, the solution will still be slightly acidic. The indicator must therefore be chosen such that its change occurs in the approximate region of the equivalence point.

At the equivalence point:

Number of equivalent weights of titrant = Number of equivalent weights of unknown or

(Normality of the titrant)·(Volume of titrant required to reach end point) = = (Normality of unknown)·(Volume of unknown)

Generally we know the normality of the titrant since it is a standard solution. We also premeasure the volume of the unknown. We then titrate with the standard from a burette into the container with the measured unknown and the chemical indicator until the indicator either turns color or a precipitate indicates that the end point or the equivalence point has been reached. Having the initial and final readings of the titrant burette gives us the volume of the titrant used. The only unknown in the above equation is the Normality of the unknown.

Background

In a titration, the analyte (the substance whose concentration is unknown and sought in the analysis) is reacted with a standard (a substance that reacts with the analyte but whose concentration is known). The analysis uses just enough of the standard to react with all of the analyte, thereby allowing the amount of analyte present to be determined. In this experiment, sulfuric acid (H_2SO_4) is the standard and sodium hydroxide (NaOH) is the analyte.

Consider what happens when a solution of a strong base as NaOH is added to a solution of a strong acid as H_2SO_4 . Since NaOH is strong base, it is completely converted to OH^- and Na^+ ions in solution. Similarly, with the strong acid H_2SO_4 , the solution species are the H^+ and SO_4^{2-} ions. When the solutions are mixed, the H^+ and OH^- ions react with each other to form H_2O molecules. This reaction, referred to as neutralization, is represented by the ionic equation:

$$H_3O^+(aq) + OH^-(aq) \rightarrow 2 H_2O \text{ or } H^+(aq) + OH^-(aq) \rightarrow H_2O$$

Or molecular equation:

$$2NaOH + H_2SO_4 = Na_2SO_4 + 2H_2SO_4$$

As indicator we can use phenolphthalein.

Please, memorize the main concepts of titration:

- Titration: an analytical procedure involving a chemical reaction in which the quantity of at least one reactant is determined volumetrically.
- Standard solution: a solution in which the concentration of a solute is precisely known.
- Usually it is the volume of the standard solution required to react with a given quantity of an analyte that is precisely determined during a titration.
- Titration endpoint: the quantity of reactant in the standard solution added during the titration is stoichiometrically equivalent to the quantity of reactant in the analyte at the titration endpoint.

Reagents and Equipment

Sulfuric acid standard solution Burette

Sodium hydroxide solution 5 mL and 10 mL pipettes

Phenolphthalein indicator solution 250 mL measuring flask

Distilled water Beaker

Experiment time: about 45 min.

Preliminary Activities

- 1. Remember that the burettes are only as clean as they were left by the last user. Clean the burette thoroughly. If the stopcock shows brown stains, consult your teacher about cleaning the stopcock before proceeding.
- 2. Obtain the reagents in the amount you will need.
- 3. Rinse the burette with about 5 mL of the sodium hydroxide stock solution. Be sure to rinse the tip as well. Drain the burette. If the burette drains properly, fill it with the sodium hydroxide solution the lower meniscus must be at zero mark.
- 4. Before proceeding with either the standardization or the titration of the analyte, you should do the following to establish the appearance of the end point of the reaction.
- 5. Place about 25 mL of distilled water in an Erlenmeyer flask and add 3-4 drops of Phenolphthalein indicator solution. Deliver one drop of sodium hydroxide solution and swirl to insure complete mixing. Notice the color of the solution.

Analysis of sodium hydroxide solution

- 1. Measure exactly 10,0 mL of the sulfuric acid standard solution into a 250 mL measuring flask and add approximately 25 mL of distilled water.
- 2. Add 3–4 drops of Phenolphthalein indicator solution.
- 3. Titrate sulfuric acid: use the burette to deliver a stream of titrant to within a couple of mL of your expected endpoint. You will see the indicator change color when the titrant hits the solution in the flask, but the color change disappears upon stirring.
- 4. Approach the endpoint more slowly (add titrant drop by drops) and watch the color of your flask carefully. Use a wash bottle to rinse the sides of the flask and the tip of the burette, to be sure all titrant is mixed in the flask.
- 5. When you have reached the endpoint (for phenolphthalein, the endpoint is the first permanent pale pink that fades in 10 to 20 minutes), read the final volume in the burette.
- 6. Repeat this procedure at least three times.
- 7. Tabulate the data for the sample analyses.
- 8. Use this data to calculate the molarity of equivalent (normality) of sodium hydroxide solution in mol/L.

Evaluation

1. Calculate average volume of NaOH solution used for the titration and notice the result to the Table.

$$\overline{V} = \frac{V_1 + V_2 + V_3}{3} = \underline{\hspace{1cm}} = \underline{\hspace{1cm}} mL$$

2. Convert 10,0 mL of sulfuric acid solution $V(H_2SO_4)$ and \overline{V} in mL of NaOH solution into liters dividing by 1000:

$$1 L = 1000 \text{ mL} = 1.10^{-3} \text{ mL}$$

$$V(H_2SO_4) \text{ mL} \cdot 1 \text{ L} / 1000 \text{ ml} = 10 \cdot 10^{-3} \text{ L}$$

$$\overline{V}$$
 (NaOH) mL · 1L / 1000 ml = \overline{V} (NaOH) ml · 10⁻³ L = ____ · 10⁻³ L

3. Calculate molarity of NaOH solution according to the formula:

$$\overline{V} (NaOH) \cdot c (NaOH) = V \left(\frac{1}{2}H_2SO_4\right) c \left(\frac{1}{2}H_2SO_4\right);$$

$$c(NaOH) = \frac{c\left(\frac{1}{2}H_2SO_4\right) \cdot V(H_2SO_4)}{\overline{V}(NaOH)} = \frac{mol/L$$

Evaluation Table

The molarity of equivalent of sulfuric acid $c(\frac{1}{2}H_2SO_4)$ is _____ mol/L

Volume of the H₂SO₄ solution is _____ L

NaOH titration of sulfuric acid solution					
volume of NaOH	average volume of NaOH	c(NaOH)			
Trial 1 mL					
Trial 2 mL	L	mol/L			
Trial 3 mL					

Conclusion

Normality of sodium hydroxide solution is mol/L.

LABORATORY WORK 6

«BUFFER SOLUTIONS PREPARATION AND THEIR PROPERTIES INVESTIGATION»

Purpose: to prepare the buffer solutions with set pH and measure this pH; to determine the effect of acid, base or water added to buffers on its pH changes.

Introduction

All living things are water-based systems, which mean that they depend heavily on aqueous equilibrium, especially acid-base equilibrium.

Reasons why we should be concerned about pH in biological systems:

- It gives a qualitative measure for many problems in cell biology and related fields.
- Proton dissociable groups are found in macromolecules (such as proteins) as well as the small molecules we have discussed already.
- The cell environment is always buffered at approximately pH 7.
- Experiments such as biological enzymatic assays require a certain pH.

Because all biological processes are dependent on pH, cells and organisms must maintain a specific and constant pH in order to keep their enzymes in the optimum state of protonation. Buffer (solution composed of an acid and its conjugate base) serves to moderate the pH of the solution. Living organize (human body) has such buffer solutions as: phosphoric, carbonic, hemoglobin, protein buffer solutions.

Background

In order to calculate the pH of the buffer solution you need to know the amount of acid and the amount of the conjugate base combined to make the solution. These amounts should be either in moles or in molarities. The K_a of the acid also needs to be known.

We can use the Henderson-Hasselbalch equation to solve this problem:

$$pH = pK_a + log ([salt]/[acid])$$

Assume that a very small percentage of the acid will ionize as the process goes to equilibrium

$$CH_3COOH \implies CH_3COO^- + H^+$$

$$\downarrow \qquad \qquad \downarrow$$
acid conjugate base

Therefore, a very small amount of the conjugate base (acetate ion) can combine with protons (i.e. the reverse reaction) as the process goes to equilibrium

In any case, all these assumptions lead to the conclusion that we can use the starting concentrations of acid (CH_3COOH) and conjugate base (salt – CH_3COONa) as a good estimate of the equilibrium concentrations.

When a strong acid (H₃O⁺) is added to a buffer solution the conjugate base present in the buffer consumes the hydronium ion converting it into water and the weak acid of the conjugate base.

$$CH_3COO^-(aq) + H_3O^+(aq) \implies H_2O + CH_3COOH(aq)$$

This results in a decrease in the amount of conjugate base present and an increase in the amount of the weak acid. The pH of the buffer solution decreases by a very small amount because of this (a lot less than if the buffer system was not present).

When a strong base (OH⁻) is added to a buffer solution, the hydroxide ions are consumed by the weak acid forming water and the weaker conjugate base of the acid. The amount of the weak acid decreases while the amount of the conjugate base increases. This prevents the pH of the solution from significantly rising, which it would if the buffer system was not present.

$$OH^{-}(aq) + CH_3COOH(aq) \implies H_2O + CH_3COO^{-}(aq)$$

We can apply the Henderson-Hasselbalch equation to this problem.

Reagents and Equipment

Acetic acid solutions 50 mL beaker

Sodium acetate solution Glass stick

Hydrochloric solution pH–meter

Sodium hydroxide solution Burette

Distilled water 250 mL measuring flask

Experiment time: about 50 min.

Activities

- 1. Fill the burettes: 1st with the 0,1 mol/L acetic acid solution, 2nd with the 0,1 mol/L sodium acetate solution.
- 2. Transfer the noted in Table volumes of acetic acid solution and sodium acetate solution from burettes to three 50 mL beakers. Mix the contents of beakers.
- 3. Measure by pH-meter pH of the buffer solutions. Notice the pH to the Table. Don't pour out the solution.
- 4. Using the pipette add 1 mL of HCl solution (the molarity is 0,01 mol/L) to the first beaker and 1 mL of NaOH solution of the same concentration to the second beaker. Mix the contents of beakers.

- 5. Transfer the third buffer solution to the 250 mL measuring flask and adjust the volume to 250 mL with distilled water. Mix the contents of measuring flask.
- 6. Measure by pH-meter pH of the prepared solutions. Notice the pH to the Table.

Evaluation

Calculate pH of buffer solutions before (pH_0) and after adding of limited volumes of strong acid (pH_1) , base (pH_2) or water (pH_3) using formulas below and notice them to the Table.

$$=$$
 $+ \lg$ $--- + \lg$ $=$

$$p{H_0}^{(2)} = p{K_d}\left(C{H_3}COOH\right) + lg\frac{c_0(C{H_3}COONa) \cdot V_2\left(C{H_3}COONa\right)}{c_0(C{H_3}COOH) \cdot V_2(C{H_3}COOH)} =$$

$$=$$
 $+ \lg$ $--- + \lg$ $=$

$$pH_0^{(3)} = pK_d(CH_3COOH) + lg\frac{c_0(CH_3COONa) \cdot V_3(CH_3COONa)}{c_0(CH_3COOH) \cdot V_3(CH_3COOH)} =$$

$$pH_1 = pK_d\left(CH_3COOH\right) + lg\frac{c_0\left(CH_3COONa\right) \cdot V_1\left(CH_3COONa\right) - c(HCl) \cdot V(HCl)}{c_0\left(CH_3COOH\right) \cdot V_1\left(CH_3COOH\right) + c(HCl) \cdot V(HCl)} =$$

$$=$$
 + \lg \longrightarrow + \lg $=$

$$pH_2 = pK_d (CH_3COOH) + lg \frac{c_0 (CH_3COONa) \cdot V_2 (CH_3COONa) + c(NaOH) \cdot V(NaOH)}{c_0 (CH_3COOH) \cdot V_2 (CH_3COOH) - c(NaOH) \cdot V(NaOH)} = 0$$

$$=$$
 $+ \lg$ $-- + \lg$ $=$

$$pH_3 = pK_d (CH_3COOH) + lg \frac{c_0 (CH_3COONa) \cdot V_3 (CH_3COONa) \cdot 10}{c_0 (CH_3COOH) \cdot V_3 (CH_3COOH) \cdot 10} =$$

Evaluation Table

$$c_0(\text{CH}_3\text{COOH}) = \underline{\hspace{1cm}} \text{mol/L} \qquad c_0(\text{CH}_3\text{COONa}) = \underline{\hspace{1cm}} \text{mol/L}$$

$$c(\text{HCl}) = \underline{\hspace{1cm}} \text{mol/L} \qquad c(\text{NaOH}) = \underline{\hspace{1cm}} \text{mol/L}$$

	Volume of solutions, L					pН		рН	
\mathcal{N}_{2}		volume of solutions, L					lated)	(meas	sured)
	CH ₃ COONa	CH ₃ COOH	HCl	NaOH	H ₂ O	$pH_0^{(i)}$	pΗ _i	pH ₀ ⁽ⁱ⁾	pHi
1	1.10 ⁻²	1.10 ⁻²	1.10^{-3}	_	_				
2	1,5·10 ⁻²	5.10 ⁻³	_	1.10^{-3}	_				
3	5.10 ⁻³	$1,5\cdot 10^{-2}$	_	_	0,200				

Conclusion

a) compare measured and calculated values pH of acetate buffers before and after adding
the solutions of electrolytes
· · · · · · · · · · · · · · · · · · ·
b) what is effect of addition strong acid and base to the buffer solution and diluting of
ouffer solution?

«DETERMINATION OF BUFFER CAPACITY OF ACETATE BUFFER SOLUTION»

Purpose: to determine the buffer capacity of acid and base for acetate buffer.

Introduction

Buffer systems of liquid mediums of the organism, exactly blood and other biological liquids, are regulators that directly provide fast incomplete compensation of blood pH displacement. Activity of lungs, kidneys, liver and skin more slowly compensate pH shifting, but in a complete way, and buffer systems practically instantly react to pH change. A small change of the pH value causes significant changes in the activity of the organism. Stability of the organism's pH systems is a pledge of its normal vital activity. In many cases monitoring of this value enables to find out different pathologies and to make a right diagnosis.

Background

Buffering capacity is the ability of the buffer solution to resist changes in pH. The buffer capacity is expressed as the amount of strong acid or base that can be added before a significant change in the pH will occur (conventionally, to change pH by one unit). Buffer capacity is measured in mmol/L and calculated by equation:

$$B_{a} = \frac{c\left(\frac{1}{z}acid\right) \cdot V(acide)}{V(buffer \, solution) \cdot \Delta pH}; \qquad B_{b} = \frac{c\left(\frac{1}{z}acid\right) \cdot V(acide)}{V(buffer \, solution) \cdot \Delta pH},$$

where B_a – is buffer capacity of acid; B_b – is buffer capacity of base; ΔpH – the change of pH after adding acid or base.

Reagents and Equipment

Acetic acid solution 25 mL beaker

Sodium acetate solution Glass stick

Hydrochloric acid solution pH-meter

Sodium hydroxide solution Burette

Distilled water Measuring flask

Experiment time: about 40 min.

Activities

- 1. Fill the burettes: 1st with the 0,01 mol/L acetic acid solution; 2nd with the 0,01 mol/L sodium acetate solution.
- 2. Prepare two buffer solutions (10 mL) by mixing of equal volumes of acetate acid and sodium acetate solutions in the 25 mL beakers.
- 3. Measure by pH-meter pH_0 of the buffer solutions. Don't pour out the solution.
- 4. Fill the burettes: 1st with the 0,1 mol/L hydrochloric acid solution; 2nd with the 0,1 mol/L sodium hydroxide solution.
- 5. Add HCl solution to the first beaker till pH changes for one unit (pH₁).
- 6. Add NaOH solution to the second beaker till pH changes for one unit (pH₂).
- 7. Notice the results to the Table.

Evaluation

Calculate the acetate buffer capacities of acid and base using formulas below and notice them to the Table.

$$B_{\text{HCI}} = \frac{c(\text{HCI}) \cdot V(\text{HCI})}{V(\text{buffer solution}) \cdot \Delta pH} = \frac{\text{mol/L}}{\text{mol/L}} = \frac{\text{mol/L}}{\text{mol/L}}$$

$$B_{\text{\tiny NaOH}} = \frac{c(\text{NaOH}) \cdot V(\text{NaOH})}{V(\text{buffer solution}) \cdot \Delta pH} = \frac{\text{mol/L}}{\text{mol/L}} = \frac$$

Evaluation Table

$$c(CH_3COOH) = \underline{\hspace{1cm}} mol/L \qquad c(CH_3COONa) = \underline{\hspace{1cm}} mol/L \\ c(HCl) = \underline{\hspace{1cm}} mol/L \qquad c(NaOH) = \underline{\hspace{1cm}} mol/L$$

№	Volume of solutions, L						pH of buffer solution		B _a , B _b ,
	CH ₃ COONa	CH ₃ COOH	Buffer solution	HC1	NaOH	pH ₀	pH _i	ΔрН	mmol/L
1	5.10 ⁻³	5.10 ⁻³			_				
2	5.10^{-3}	5.10^{-3}		_					

Conclusion

The buffer acetate buffer capacity of acid is ______ and the acetate buffer capacity of base is

«DETERMINATION OF THE HEAT OF NEUTRALIZATION»

Purpose: to determine the heat of neutralization.

Introduction

The heat produced in the reaction between an acid and a base is called the heat of neutralization. Neutralization is a chemical reaction, according to the Arrhenius theory of acids and bases, in which a water solution of acid is mixed with a water solution of base to form a salt and water.

Reagents and Equipment

Sulfuric acid solution Dewar vessels

Hydrochloric acid solution Glass stick

Nitric acid solution Thermometer

Acetic acid solution Cylinder

Sodium hydroxide solution

Experiment time: about 35 min.

Activities

- 1. Using cylinder measure 25 mL of 1 mol/L sodium hydroxide solution and transfer it to the Dewar vessel.
- 2. Measure the temperature (T_1) with the thermometer.
- 3. Using cylinder measure 25 mL of sulfuric acid solution with the molarity of equivalent 1 mol/L and add it to the Dewar vessel. Mix the contents of the Dewar vessel.
- 4. Measure the temperature (T_2) with the thermometer.
- 5. Notice the T_1 and T_2 to the Table.
- 6. Repeat the steps 1–5 for 1 mol/L chloride acid, nitric acid and the acetate acid solutions.

Evaluation

1. Calculate the heat of neutralization for each acid and notice them to the Table:

$$\Delta H_{\text{neutralization}} = \frac{C \cdot [V(\text{base}) + V(\text{acid})] \cdot \Delta T \cdot \rho}{c \left(\frac{1}{z} a c i d\right) \cdot V(\text{acid})},$$

where, C - thermal capacity (4,18 kJ/K); V(acid) - volume of acid solution, L; V(base)	. —
volume of base solution, L; ρ – density of solution (\approx 1 kg/L); $c\left(\frac{1}{z}acid\right)$ – molarity	of
equivalent of acid, mol/L.	

 $\Delta H_{neutralization}$ (CH₃COOH) = _____ = kJ/mol

2. Compare experimental values of the heats of neutralization with reference values:

Evaluation Table

Acid	$c\left(\frac{1}{z}acid\right)$,	Т ₁ , К	Т2, К	ΔΤ, К	ΔH _{neutralization} , kJ/mol		
Ticlu	mol/L	11, 10	12, K	$\Delta 1, K$	Reference value	Experimental value	
H ₂ SO ₄					-53		
HC1					-56		
HNO ₃					-56		
CH ₃ COOH					-47		

5 .	. Write the four neutralization reactions in the ionic and molecular forms:					

«INFLUENCE OF SODIUM THIOSULFATE CONCENTRATION ON THE RATE OF THIOSULFURIC ACID DECOMPOSITION»

Purpose: to calculate the rate of reaction; to determine the decompositions rate depending on its concentration.

Background

Sulfuric acid reacts with sodium thiosulfate with formation of thiosulfuric acid according following steps:

$$Na_2S_2O_3 + H_2SO_4 = H_2S_2O_3 + Na_2SO_4$$
 (fast)

$$H_2S_2O_3 = S + SO_2 + H_2O$$
 (comparatively slow)

According to the chemical kinetics, in case of multi-step reactions, each step will occur at its own distinctive rate. If one step takes place much more slowly than all other steps, it will definitely control the overall reaction rate. The slowest step is called rate determining step. Sulfuric acid concentration remains constant in all experiments, so estimation of conditional reaction rate should be done according to the change in sodium thiosulfate concentration

Reagents and Equipment

Sodium thiosulfate solution Test tubes

Sulfuric acid solution Distilled water

Stop-watch Glass stick

Burettes

Experiment Time: about 50 min.

Preliminary Activities

- 1. Remember that the laboratory glassware is only as clean as they were left by the last user. Clean laboratory glassware thoroughly.
- 2. Obtain the reagents in the amount you will need.

Activities

- 1. Fill three burettes: 1 with 0,1 mol/L sodium thiosulfate solution; 2 with 1 mol/L sulfuric acid solution; 3 with distilled water.
- 2. Pour 1 mL of sodium thiosulfate solution and 4 mL of distilled water from burettes into a clean test tube.

- 3. Pour 3 mL of thiosulfate solution and 2 mL of distilled water from burettes into a clean test tube.
- 4. Pour 5 mL of thiosulfate solution from burette into a clean test tube.
- 5. Mix the contents of test tubes.
- 6. Pour 5 mL of sulfuric acid solution from burette into three test tubes.
- 7. Pour together by pairs the contents of the test tubes and notice to the Table the time when the turbidity arises by means of the stop-watch.

Evaluation

1. Calculate molarity of Na₂S₂O₃ obtained after dilution according to the following formula and notice the results to the Table:

$$c_{i}(Na_{2}S_{2}O_{3}) = c_{i}(H_{2}S_{2}O_{3}) = \frac{c_{o}(Na_{2}S_{2}O_{3}) \cdot V_{i}(Na_{2}S_{2}O_{3})}{V_{i}(mixture)},$$

where: $c_0(Na_2S_2O_3)$ – initial molarity of $Na_2S_2O_3$ solution, mol/L; $V_i(Na_2S_2O_3)$ – volume of $Na_2S_2O_3$ solution; $V_i(mixture)$ – total volume of mixed reagents.

$$V_{i}(mixture) = V_{i}(Na_{2}S_{2}O_{3}) + V_{i}(H_{2}O) + V_{i}(H_{2}SO_{4}), L$$

 V_1 (mixture) =

 V_2 (mixture) =

 V_3 (mixture) =

$$c_{2}(Na_{2}S_{2}O_{3}) = c_{2}(H_{2}S_{2}O_{3}) = \frac{c_{0}(Na_{2}S_{2}O_{3}) \cdot V_{2}(Na_{2}S_{2}O_{3})}{V_{2}(mixture)} = \frac{mol/L}{c_{2}(Ma_{2}S_{2}O_{3})} = \frac{mol/L}{c_{3}(Ma_{2}S_{2}O_{3})} = \frac{mol/L}{c_{3}(Ma_{2}S_{2}O_{3}O_{3})} = \frac{mol/L}{c_{3}(Ma_{2}S_{2}O_{3}O_{3})} = \frac{mol/L}{c_{3}(Ma_{2}S_{2}O_{3}O_{3}O_{3$$

$$c_{3}(Na_{2}S_{2}O_{3}) = c_{3}(H_{2}S_{2}O_{3}) = \frac{c_{o}(Na_{2}S_{2}O_{3}) \cdot V_{3}(Na_{2}S_{2}O_{3})}{V_{3}(mixture)} = \frac{mol/L}{c_{3}(Na_{2}S_{2}O_{3})} = \frac{mol/L}{c_{3}(Na_{2}S_{2}O_{3}O_{3})} = \frac{mol/L}{c_{3}(Na_{2}S_{2}O_{3}O_{3})} = \frac{mol/L}{c_{3}(Na_{2}S_{2}O_{3}O_{3}O_{3$$

Evaluation Table

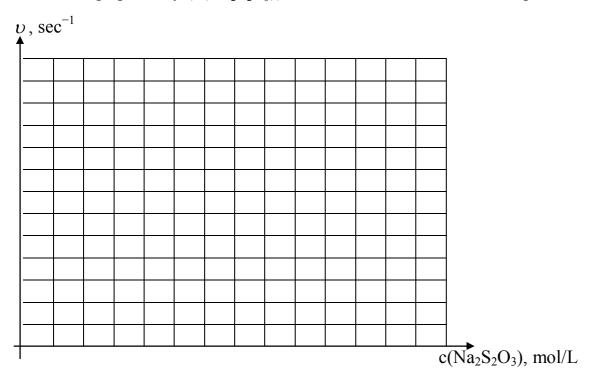
N	V (solution), mL		Time of turbidity arising (τ), sec	Obtained concentration of H ₂ S ₂ O ₃ (c), mol/L	
	$Na_2S_2O_3$ H_2O	H ₂ SO ₄	unsing (t), see		
1	1	4	5		
2	3	2	5		
3	5	0	5		

2. Calculate values of conditional reaction rates (v) according to the following formula:

$$v_1 = \frac{1}{\tau_1} =$$
 $=$ sec^{-1}

$$v_3 = \frac{1}{\tau_3} = --------= \sec^{-1}$$

3. Plot the graph $v = f(c(Na_2S_2O_3))$ and make conclusions about dependence obtained.



Conclusion

The decompositions rate of the $H_2S_2O_3$ is ______versus increasing of its molarity.

LABORATORY WORK 10

«SOLUBILITY PRODUCT CONSTANT. THE CONDITIONS OF PRECIPITATE FORMATION»

Purpose: to determine the conditions of precipitate formations.

Background

In a solubility equilibrium of insoluble salts

$$A_m B_n \implies m A^{n+} + n B^{m-}$$

the equilibrium constant for the equilibrium between an ionic solid and its saturated solution is product of molarity of the dissolved ions at standard temperature:

$$K_{sp}(A_mB_n) = [A^{n+}]^m \cdot [B^{m-}]^n.$$

If the concentration of solute

$$Q(A_mB_n) = [A^{n+}]^m \cdot [B^{m-}]^n$$
,

(Q – ion product quotient: the product of the equilibrium expression when the system is not at equilibrium) is greater than the maximum possible concentration, a precipitate will form.

The precipitate will form if $Q > K_{sp}$ until Q decreases to K_{sp} .

If $Q = K_{sp}$, equilibrium is reached – no precipitate will form.

If $Q < K_{sp}$, any ppt in solution will dissolve until Q increases to K_{sp} .

Reagents and Equipment

Pb(NO₃)₂ solution 1 mL pipette

KCl solution Test tubes

KI solution Distilled water

Experiment time: about 40 min.

Preliminary Activities

- 1. Remember that the test-tubes are only as clean as they were left by the last user. Clean test tubes thoroughly.
- 2. Obtain the reagents in the amount you will need.

Activities

- 1. Transfer 4 drops of the 0,001 mol/L Pb(NO₃)₂ solution to two clean test tubes.
- 2. Add 4 drops of the 0,05 mol/L KCl solution to the first test tube.
- 3. Add 4 drops of the 0,05 mol/L KI solution to the second test tube.
- 4. Notice the observations to the Table.

Evaluation

1. Write tl	he formation re	eactions for sal	ts PbCl ₂ and I	PbI ₂ in the ion	ic and molecul	ar forms:

2. Calculate the molarity of Pb²⁺, Cl⁻, I⁻ in obtained solutions using the next formula:

$$c(x)\cdot V(solution) = c_1(x)\cdot V_1(solution)$$

where c(x) – initial molarity of substance in solution; $c_1(x)$ – resulting molarity of substance in obtained solution; V(solution) – volume of the solution with initial molarity c(x); $V_1(\text{solution})$ – volume of the solution with molarity $c_1(x)$.

$$c_1(x) = \frac{c(x) \cdot V(\text{solution})}{V_1(\text{solution})};$$

 $c(Pb(NO_3)_2 = 0.001 \text{ mol/L}; c(KCl) = 0.05 \text{ mol/L}; c(KI) = 0.05 \text{ mol/L}.$

V(solution) for all substances is 0,4 mL or $4 \cdot 10^{-4}$ L.

$$V_1(solution) = V(solution) + V(solution) = 4.10^{-4} L + 4.10^{-4} L = 8.10^{-4} L.$$

$$c(Pb^{2+}) = \underline{\hspace{1cm}} = mol/L$$

$$c(Cl^-) = \underline{\hspace{1cm}} = \underline{\hspace{1cm}} mol/L$$

$$c(\overline{\Gamma}) = \underline{\hspace{1cm}} = \underline{\hspace{1cm}} = \underline{\hspace{1cm}} mol/L$$

3. Calculate the Q(PbCl₂) and Q(PbI₂) and notice to the Table:

$$Q(PbCl_2) = [Pb^{2+}] \cdot [Cl^{-}]^2 =$$

$$Q(PbI_2) = [Pb^{2+}] \cdot [I^-]^2 =$$

Evaluation Table

Electrolyte	$c_0(x)$, mol/L	$V_0(x)$, mL	Observation	K_{sp} , mol^3/L^3	$Q, mol^3/L^3$
Pb(NO ₃) ₂	0,001	0,4			
KCl	0,05	0,4			
KI	0,05	0,4			
PbCL ₂				$1,7 \cdot 10^{-5}$	
PbI ₂				$8,7 \cdot 10^{-9}$	

4. Compare Q (PbCl₂) and K_{sp} (PbCl₂), Q (PbI₂) and K_{sp} (PbI₂), than make conclusions about formation of precipitate.

	Conclusion	
a) precipitate of PbCl ₂ is	because Q (PbCl ₂)	K_{sp} (PbCl ₂);
b) precipitate of PbI ₂ is	because O (PbI ₂)	Ken (PbI2).

LABORATORY WORK 11

«DETERMINATION OF STANDARD OXIDATION-REDUCTION POTENTIAL»

Purpose: to measure cell potentials depends on chemical activities of species; to determine the standard oxidation-reduction potential.

Introduction

Oxidation-reduction reactions take place in the human organism on different levels including a cell. Electrochemical processes are widely used in scientific research. Methods of electrochemistry such as potentiometry, polarography, as well as methods used for the determination of different ions concentration are widely adopted in medical practice.

Background

$$A_{ox} + B_{red} \implies A_{red} + B_{ox}$$

 A_{ox} is the oxidized form of A (the oxidant); B_{red} is the reduced form of B (the reductant). For such an electron transfer, one may consider two half-cell reactions:

$$A_{ox} + n e^- \implies A_{red} \qquad B_{ox} + n e^- \implies B_{red}$$

For each half reaction:

$$E = E^{\circ} - RT/nF (ln [reduced]/[oxidized])$$

e.g., for the first half reaction:

$$E = E^{\circ} - RT/nF (ln [Ared]/[Aox]),$$

where E = cell potential (voltage), R = gas const., F = Faraday, n = number of e-.

When
$$[A_{red}] = [A_{ox}]$$
, $E = E^{\circ i}$.

 E° is the mid-point potential, or standard oxidation-reduction potential, the potential at which [oxidant] = [reductant] for the half reaction.

Reagents and Equipment

 $K_3[Fe(CN)_6]$ solution Distilled water

 $K_4[Fe(CN)_6]$ solution pH-meter

Burettes Beakers

Experiment time: about 40 min.

Activities

- 1. Switch on the pH-meter. Use reference AgCl-electrode and measuring Pt-electrode.
- 2. Fill two burettes: $1 \text{with } K_3[\text{Fe}(\text{CN})_6] \text{ solution (molarity is 0,01 mol/L); } 2 \text{with } K_4[\text{Fe}(\text{CN})_6] \text{ solution (molarity is 0,01 mol/L).}$
- 3. Prepare three solutions by mixing of solutions $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ varying the volumes of reagents according to the Table in beakers.
- 4. Measure cell potentials (E_{cell}) of prepared solutions (in Volt). Notice the value of E_{cell} to the Table.

Evaluation

1. Calculate the oxidation-reduction potential of systems (in Volt) using and notice to the Table:

$$E_{([Fe(CN)_6]^{3-}/([Fe(CN)_6]^{4-})} = E_{cell} + E_{XC}$$
, where $E_{XC} = 0.222 \text{ V}$.

$$E_{1([Fe(CN)_6]^{3-}/([Fe(CN)_6]^{4-})} =$$

$$E_{2([Fe(CN)_6]^{3-}/([Fe(CN)_6]^{4-})} = =$$

$$E_{3([Fe(CN)_6]^{3-}/([Fe(CN)_6]^{4-})} =$$

2. Calculate the standard oxidation-reduction potential using Peter's equation:

$$E^{0}_{([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})} = E_{([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})} - \frac{0,059}{n} \lg \frac{c([Fe(CN)_{6}]^{3-})}{c([Fe(CN)_{6}]^{4-})},$$

where $c([Fe(CN)_6]^{3-}) = c(K_3[Fe(CN)_6]) \cdot V(K_3[Fe(CN)_6]) / V(solution);$

$$c([Fe(CN)_6]^{4-}) = c(K_4[Fe(CN)_6]) \cdot V(K_4[Fe(CN)_6]) / V(solution);$$

n is the number of electrons of the oxidation-reduction reaction, so n = n

$$c_1(K_3[Fe(CN)_6]^{3-}) =$$

$$c_2(K_3[Fe(CN)_6]^{3-}) =$$

$$c_3(K_3[Fe(CN)_6]^{3-}) =$$

$$c_{1}(K_{3}[Fe(CN)_{6}]^{4-}) = c_{2}(K_{3}[Fe(CN)_{6}]^{4-}) = c_{3}(K_{3}[Fe(CN)_{6}]^{4-}) = E_{1([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})} = E_{1([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})} - \frac{0,059}{n} \lg \frac{c_{1}([Fe(CN)_{6}]^{3-})}{c_{1}([Fe(CN)_{6}]^{4-})} = E_{2([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})} - \frac{0,059}{n} \lg \frac{c_{2}([Fe(CN)_{6}]^{4-})}{c_{2}([Fe(CN)_{6}]^{3-})} = E_{2([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})} - \frac{0,059}{n} \lg \frac{c_{2}([Fe(CN)_{6}]^{3-})}{c_{2}([Fe(CN)_{6}]^{4-})} = E_{3([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})} - \frac{0,059}{n} \lg \frac{c_{3}([Fe(CN)_{6}]^{3-})}{c_{3}([Fe(CN)_{6}]^{3-})} = E_{3([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})} - \frac{0,059}{n} \lg \frac{c_{3}([Fe(CN)_{6}]^{3-})}{c_{3}([Fe(CN)_{6}]^{4-})} = E_{3([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})} - \frac{0,059}{n} \lg \frac{c_{3}([Fe(CN)_{6}]^{3-})}{c_{3}([Fe(CN)_{6}]^{4-})} = E_{3([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})} - \frac{0,059}{n} \lg \frac{c_{3}([Fe(CN)_{6}]^{3-})}{c_{3}([Fe(CN)_{6}]^{4-})} = E_{3([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})} - \frac{0,059}{n} \lg \frac{c_{3}([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})}{c_{3}([Fe(CN)_{6}]^{4-})} = E_{3([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})}$$

Notice the results to the Table.

3. Calculate the average value of E^0 :

$$\overline{E}^{0}_{([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})} = \frac{E^{0}_{1([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})} + E^{0}_{2([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})} + E^{0}_{3([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})}} = \frac{3}{3}$$

Evaluation Table

$$c(K_3[Fe(CN)_6]) = \underline{\qquad} mol/L$$

$$c(K_4[Fe(CN)_6]) = \underline{\qquad} mol/L$$

$\frac{V(K_{3}[Fe(CN)_{6}], mL}{V(K_{4}[Fe(CN)_{6}], mL}$	E _{cell} , V	$E_{i([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-}},V$	$E^{0}_{([Fe(CN)_{6}]^{3-}/([Fe(CN)_{6}]^{4-})}, V$
2/18			
10/10			
18/2			

Conclusion

The value of standard oxidation-reduction potential $\overline{E}^{0}_{([Fe(CN)_6]^{3-}/([Fe(CN)_6]^{4-})}$ is _____

«ADSORPTION OF ACETIC ACID ON ACTIVATED CHARCOAL»

Purpose: to determine adsorption of acetic acid on activated charcoal (carbon).

Introduction

Adsorption is a process that occurs when a gas or liquid solute accumulates on the surface of a solid or a liquid (adsorbent), forming a molecular or atomic layer (the adsorbate). It is different from absorption, in which a substance diffuses into a liquid or solid to form a solution. The term sorption encompasses both processes, while desorption is the reverse process. Adsorption is operative in most natural physical, biological, and chemical systems, and is widely used in industrial applications such as activated charcoal, synthetic resins and water purification.

The processes of adsorption are a part of mechanism of absorption of nutrients and medicinal compounds. Hemosorption method is used for the detoxication of blood. Phenomena of adsorption are the base of chromatography – method of analysis and separation of mixtures of biologically active substances.

In practice, activated carbon is used as an adsorbent for the adsorption of mainly organic compounds along with some larger molecular weight inorganic compounds such as iodine and mercury. Activated carbon, also called activated charcoal or activated coal, is a general term that includes carbon material mostly derived from charcoal. For all three variations of the name, «activated» is sometimes substituted by «active». By any name, it is a material with an exceptionally high surface area. Activated carbon is frequently used in everyday life, in: industry, food production, medicine, pharmacy, military, etc. In pharmacy, activated charcoal is considered to be the most effective single agent available as an emergency decontaminant in the gastrointestinal tract. It is used after a person swallows or absorbs almost any toxic drug or chemical.

Reagents and Equipment

Aqueous solution of acetic acid Distilled water

Aqueous solution of sodium hydroxide 250 mL measuring flasks

Activated charcoal Burettes

Experiment time: about 60 min.

Activities

- 1. Fill the burettes: 1st with 0,1 mol/L NaOH solution; 2nd with acetic acid solution with molarity 0,05 mol/L; 3rd with acetic acid solution with molarity 0,1 mol/L; 4th with acetic acid solution with molarity 0,2 mol/L; 5th with acetic acid solution with molarity 0,4 mol/L.
- 2. Place 1,0 g of activated charcoal in four 250 mL measuring flasks and add 25 mL of acetic acid solutions with different molarity from burettes in each one.
- 3. Adsorption takes 20 min. The contents of beaker flasks should be shaken up periodically.
- 4. In 20 min decant solutions through paper filters. Pour out first 3–5 ml of filtrate.
- 5. Using the pipette transfer 5 mL of filtrate (solution of acetic acid remains after adsorption) from each of measuring flasks to the four clean flasks for titration.
- 6. Add 3–4 drops of Phenolphthalein indicator solution in each measuring flask.
- 7. Titrate filtrates adding 0,1 mol/L NaOH solution from burette till endpoint will reached (for phenolphthalein, the endpoint is the first permanent pale pink that fades in 10 to 20 minutes).
- 8. Read the final volume in the burette and notice them.
- 9. Each filtrate should be titrated 3 times in order to obtain the average value of volume of NaOH solution.

Evaluation

1. Calculate average volume of NaOH solution used for the titration and notice the result to the Table.

$$\overline{V}_1 = \frac{V_1 + V_2 + V_3}{3} = mL$$

$$- V + V + V$$

$$\overline{V}_2 = \frac{V_1 + V_2 + V_3}{3} =$$
 = mL

$$\overline{V}_{3} = \frac{V_{1} + V_{2} + V_{3}}{3} =$$
 = mL

$$\overline{V}_4 = \frac{V_1 + V_2 + V_3}{3} =$$
 mL

2. Calculate equilibrium concentration of CH₃COOH in solution after sorption according to the next formula and notice to the Table:

$$c_{p(i)}(\text{CH}_3\text{COOH}) = \frac{c(\text{NaOH}) \cdot \overline{V}(\text{NaOH})}{V(\text{CH}_3\text{COOH})};$$

$$c_{p(i)}(\text{CH}_3\text{COOH}) = \frac{c(\text{NaOH}) \cdot \overline{V}_1(\text{NaOH})}{V(\text{CH}_3\text{COOH})} = \frac{c(\text{NaOH}) \cdot \overline{V}_2(\text{NaOH})}{V(\text{CH}_3\text{COOH})} = \frac{c(\text{NaOH}) \cdot \overline{V}_2(\text{NaOH})}{V(\text{CH}_3\text{COOH})} = \frac{c(\text{NaOH}) \cdot \overline{V}_3(\text{NaOH})}{V(\text{CH}_3\text{COOH})} = \frac{c(\text{NaOH}) \cdot \overline{V}_3(\text{NaOH})}{V(\text{CH}_3\text{COOH})} = \frac{c(\text{NaOH}) \cdot \overline{V}_4(\text{NaOH})}{V(\text{CH}_3\text{COOH})} = \frac{c(\text{NaOH}) \cdot \overline{V}_4(\text{NaOH})$$

3. Calculate the value of adsorption $\frac{x}{m}$ of acetic acid on charcoal according to the next formula and notice to the Table:

$$\left(\frac{x}{m}\right)_{i} = \frac{\left[c_{o}(CH_{3}COOH) - c_{p(i)}(CH_{3}COOH)\right] \cdot V(CH_{3}COOH)}{m(charcoal)}$$

$$\left(\frac{x}{m}\right)_{1} = \frac{\left[c_{o}\left(CH_{3}COOH\right) - c_{p(1)}\left(CH_{3}COOH\right)\right] \cdot V(CH_{3}COOH)}{m(charcoal)} =$$

= _____=

$$\left(\frac{x}{m}\right)_{2} = \frac{\left[c_{o}(CH_{3}COOH) - c_{p(2)}(CH_{3}COOH)\right] \cdot V(CH_{3}COOH)}{m(charcoal)} =$$

=____=

$$\left(\frac{x}{m}\right)_{3} = \frac{\left[c_{o}(CH_{3}COOH) - c_{p(3)}(CH_{3}COOH)\right] \cdot V(CH_{3}COOH)}{m(charcoal)} =$$

= _____=

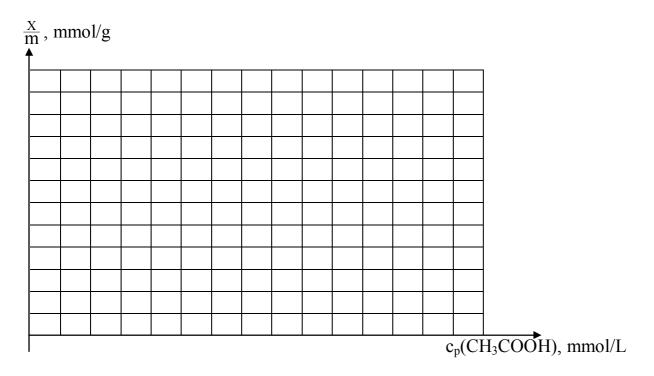
$$\left(\frac{x}{m}\right)_4 = \frac{\left[c_o(CH_3COOH) - c_{p(4)}(CH_3COOH)\right] \cdot V(CH_3COOH)}{m(charcoal)} =$$

= _____=

c (NaOH) =
$$mol/L$$
; V_0 (CH₃COOH) = L ; m (carbon) = g .

No	c ₀ (CH ₃ COOH), mmol/L	\overline{V} (NaOH),	$c_{p(i)}$ (CH ₃ COOH), mmol/L	$\frac{X}{m}$, mmol/g
1	50			
2	100			
3	200			
4	400			

4. Plot the graph of adsorption isotherm, i.e. adsorption against concentration.



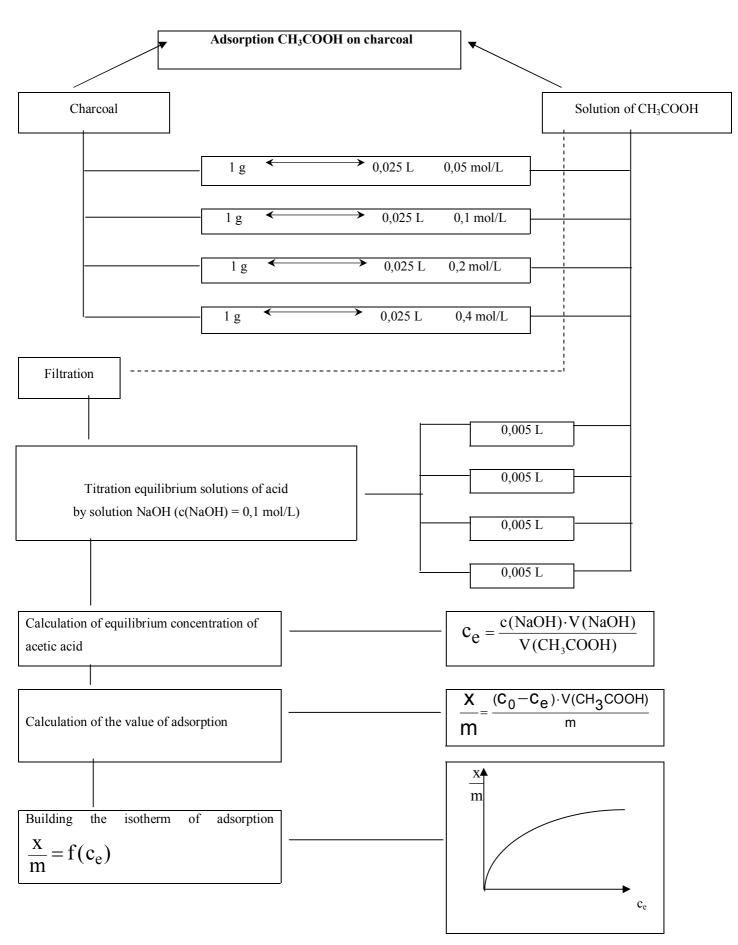
Conclusion

Can you explain how the value of adsorption changes as the equilibrium concentration rises?

Please use the scheme on the next page for performance of this laboratory work.

Algorithm for quantitative calculation of the value

of acetic acid adsorption on activated charcoal (carbon).



LABORATORY WORK 13

«QUANTITATIVE DEFINITION OF CA²⁺ IONS BY ION–EXCHANGE ABSORPTION»

Purpose: to determine quantitative contents of ion Ca²⁺ in solution using ion-exchange absorption.

Background

Ion exchange is of particular importance for human beings since it is one of the intermediatory stages in the chain of complex biochemical and physiological processes. Ionites (ion exchangers) have found a wide application in medicine. In particular they are used to demineralize water, make ionite milk, preserve blood, determine acidity of gastric juices without exploring a probe, etc. In pharmacy ionites (ion exchangers) help to concentrate and purify antibiotics, extract alkaloids from plants, some ionites (ion exchangers) are also used as medicines to bind toxic substances and toxins in gastrointestinal tract.

Reagents and Equipment

Aqueous solution of CaCl₂ Aqueous solution of NaOH

Cationite (cation exchanger) Burette

Beaker flask Column for the ionite (ion exchanger)

Distilled water Phenolphthalein indicator solution

Experiment time: about 60 min.

Activities

- 1. Place the sample of CaCl₂ solution into the column containing a cationite (cation exchanger) in H⁺-form. At first remove some of the distilled water covering the cationite (the water is needed to avoid contact of the cationite with air) in the column.
- 2. Turn the stopcock (tap) in the column to let the liquid drop from the column into the beaker flask. Make sure the cationite (cation exchanger) is always covered by the liquid.
- 3. Rinse the beaker flask containing the analyzed solutions 2–3 times with small amounts of distilled water and pass these portions through column again.
- 4. Rinsing waters have to be collected in the same beaker flask where all eluate was collected.

- 5. Add 15–20 mL of distilled water to the column and collect the eluate again in the same flask.
- 6. On finishing the experiment close the tap and put some more distilled water into the column.
- 7. Add 2–3 drops of phenolphthalein indicator solution to the flask with collected eluate.
- 8. Titrate adding 0,1 mol/L NaOH solution from burette till endpoint will reached (for phenolphthalein, the endpoint is the first permanent pale pink that fades in 10 to 20 minutes). Titrate 3 times to obtain the average value of volume of NaOH solution.
- 9. Notice the results to the Table.

Table

c (NaOH), mol/L	V (NaOH)
	Trial 1 mL
	Trial 2 mL
	Trial 3 mL

Evaluation

1. Calculate average volume of NaOH solution used for the titration:

$$\overline{V} = \frac{V_1 + V_2 + V_3}{3} = mL = L$$

2. Mass of Ca²⁺ (in grams) in the analyzed solution must be calculated according to the formula:

$$m(Ca^{2+}) = c(NaOH) \cdot \overline{V} (NaOH) \cdot M \left(\frac{1}{2}Ca^{2+}\right);$$

$$M\left(\frac{1}{2}Ca^{2+}\right) = M\left(Ca^{2+}\right) \cdot \frac{1}{2}.$$

$$m(Ca^{2+}) =$$

Conclusion

Mass of Ca²⁺ in the analyzed solution is _____

LABORATORY WORK 14

«SEPARATION OF AMINO ACIDS MIXTURE USING PAPER CHROMATOGRAPHY»

Purpose: to perform paper chromatography of a mixture of amino acids; according to the calculated values of distribution coefficients, to determine amino acids in analysis mixture.

Introduction

Chromatography covers physical-chemical methods of separation and analysis of mixtures of gases, vapor, liquids or dissolved substances. They are used to separate the mixtures of materials into the individual species. They work because of the differences in distribution of mixture components between the mobile phase and stationary phase, when the mixture is moved through the stationary phase, the layer of the sorbent. Components of the analyzed mixture are not equally adsorbed on the sorbent; compounds with the higher affinity to the sorbent will be sorbed more strongly and stay for longer on the sorbent, therefore the speed of their moving with the mobile phase is lower. Stationary phase – the sorbent – can be liquid or solid. Mobile phase (the gas or liquid passing the layer of the sorbent) performs the role of solvent and carrier of analysis mixture.

Reagents and Equipment

Chromatography paper Aqueous solution of amino acids

Ninhydrin solution Chromatographic chamber

Pencil Ruler

Experiment time: about 50 min.

Activities

- 1. Mark by a pencil the line (starting line) at 2–3 cm from the top of chromatography paper and then place a drop of the sample of amino acids mixture on this line.
- 2. Let the paper dry for several minutes.
- 3. Place 3–4 mL of the solvent (mixture of phenol with water) into the chromatographic chamber. Then the end of the paper having the drop should be placed in the solvent. Make sure the solvent doesn't touch the spot on the paper. Close up the chromatographic chamber.

- 4. As soon as the solvent rises as high as 16–18 cm up on the paper, take out the paper from the chromatographic chamber and fix the line the solvent reached by pencil (finishing line) and let the paper dry.
- 5. Spray the paper with ninhydrin solution, and then dry it at 50–60 °C.
- 6. When the purple rings develop on the paper, their number tells, how many amino acids were in analysis mixture.
- 7. Measure the way of each amino acid, i.e. the distance from the starting line to the middle of colored ring (X_i) , and the way of solvent, i.e. the distance from starting line to finishing line (X_0) .
- 8. Notice the results to the Table.

Evaluation

1. Calculate the distribution coefficient R_f for each of the amino acids using the formula $R_f = \frac{X_1}{X_2}$ and notice the results to the Table:

$$R_{f(1)} = \frac{X_1}{X_0} = ----=$$

$$R_{f(2)} = \frac{X_2}{X_0} = ----=$$

Evaluation Table

Distance passed by the amino acid X_i , cm	Distance passed by the solvent X_0 , cm	R_{f}

2. Compare calculated R_f with the reference data (Reference Table) and then identify the amino acids in sample.

Reference Table

Amino acid	$ m R_{f}$	Amino acid	R_{f}
Asparagine	0,07	Arginine	0,41
Glutamine	0,16	Tyrozine	0,52
Cysteine	0,19	Alanine	0,55
Glycine	0,03	Leucine	0,79
Methionine	0,30		

LABORATORY WORK 15 «SEPARATION OF DYES MIXTURE USING THIN LAYER CHROMATOGRAPHY»

Purpose: to perform thin layer chromatography of a mixture of dyes.

Introduction

Thin layer chromatography is a method for analyzing mixtures by separating the compounds in the mixture. Thin layer chromatography can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. The mixture of dyes separated through thin layer chromatography on «Silufol» plates. The dyes travel up the chromatography paper at different distances before they cannot remain in solution. The more soluble dyes move further up than the less soluble ones, hence separating from each other.

Reagents and Equipment

«Silufol» plate Chromatographic chamber

Crystal violet dye Pencil

Sudan (III) dye Ruler

Experiment time: about 50 min.

Activities

- 1. Mark the line by a pencil (starting line) at 1–1,5 cm from the top of «Silufol» plate and then place a drop of the sample on this line.
- 2. Place some drop of the crystal violet dye and sudan III dye on starting line.
- 3. Place 3–4 mL of the chloroform into the chromatographic chamber.
- 4. The end of the «Silufol» place in the solvent. Make sure the solvent doesn't touch the spot on the paper. Close up the chromatographic chamber.
- 5. As soon as the solvent rises as higher as possible up on the «Silufol» plate, take out the «Silufol» plate from the chromatographic chamber.
- 6. Fix the line the solvent reached by pencil (finishing line) and let the «Silufol» plate dry.

- 7. Compare the color of colored ring of investigated mixture with the color of colored rings of the crystal violet dye and sudan III dye.
- 8. Make conclusion about the composition of mixture.

Evaluation

Investigated mixture form the colored ring on the «Silufol» plate ant its color is
Sudan III dye form the colored ring on the «Silufol» plate ant its color is
Crystal violet dye form the colored ring on the «Silufol» plate ant its color is
Compare the color and distance from the starting line to the colored ring on the «Silufol» plate passed by the «witnesses» and investigated mixture
Conclusion Investigated mixture consist of

LABORATORY WORK 16 «SEPARATION OF CATIONS Fe³+ AND Cu²+ MIXTURE USING COLUMN ADSORPTION CHROMATOGRAPHY»

Purpose: to perform column adsorption chromatography of a mixture of cations.

Background

Chromatography analysis is used to separate and extract biologically active substrates, such as amino acids, carbohydrates, hormones, enzymes, vitamins and others. Adsorption chromatography was discovered in 1906 by Mikhail Semyonovich Tsvet, a botanist. Adsorption column chromatography is a separation technique that uses a solid stationary phase and a liquid mobile phase. The solid phase is the adsorbent, usually silica gel or alumina. Silica gel is used to separate polar functional groups whereas alumina is used for less polar compounds. In any separation experiment, a mixture (of compounds) is added to the top of the column. The mobile and stationary phases interact with the mixture. The type and the strength of the interaction depend on the nature of the compounds in the

mixture. The differences in structure and polarity cause the compounds to move at different rates through the stationary phase, thus causing a separation.

Reagents and Equipment

Aqueous solution of the mixture of cations Fe^{3+} and Cu^{2+} Distilled water Aqueous solution of $K_4[Fe(CN)_6]$ Pipette

Chromatographic column

Experiment time: about 40 min.

Activities

- 1. 10 drops of the solution containing CuSO₄ and FeCl₃ pour carefully along the wall of the column filled with aluminum oxide.
- 2. As soon as Al₂O₃ adsorbs all solution put 3 drops of water into the column.
- 3. Add 10 drops of $K_4[Fe(CN)_6]$ solution (developing agent) into the column.

					Evaluation						
In the	upper	part	of the	column		color	is	observed,	under	it	ions
	 	_ cold	or is obse	erved.							
	lown ch		l reactio	ns betwee	en the compone	ents of t	the 1	mixture and	I the dev	velo	ping
					Conclusion						
In the	upper	part	of the	column	ions		are	adsorbed,	under	it	ions
		are	adsorbed	1.							

LABORATORY WORK 17 «PREPARATION, PURIFICATION AND PROPERTIES OF COLLOIDS SOLUTIONS»

Purpose: to perform methods of preparation of colloids solutions; to determine the charge of colloid particles.

Introduction

<u>Colloidal solutions</u> are very important in the medical field because they can be used to manipulate blood conditions. More specifically, they are often used to regulate colloidal

osmotic pressure, a pressure applied by proteins in the blood to pull water in the vascular system.

Colloids:

- have medium size particles;
- cannot be filtered;
- can be separated by semipermeable membranes;
- scatter light (Tyndall effect in which the path of a beam of light through the colloid is visible due to scatter light).

There are two basic methods of forming a colloid:

- reduction of larger particles to colloidal size, and
- condensation of smaller particles (e.g., molecules) into colloidal particles.

Some substances (e.g., gelatin or glue) are easily dispersed (in the proper solvent) to form a colloid; this spontaneous dispersion is called peptization. A solid (e.g., paint pigment) can be reduced to colloidal particles in a colloid mill, a mechanical device that uses a shearing force to break apart the larger particles. An emulsion is often prepared by homogenization, usually with the addition of an emulsifying agent. The above methods involve breaking down a larger substance into colloidal particles. Condensation of smaller particles to form a colloid usually involves chemical reactions – typically displacement, hydrolysis, or oxidation and reduction.

Reagents and Equipment

Solution of iron chloride (III) Pipettes

Solution of argentum nitrate Beakers

Solution of potassium iodide 250 mL beaker flask

Solution of potassium hexacianoferate (II) Cylinder

Distilled water Test tubes

Experiment time: about 60 min.

Activities

Test 1. Preparation of argentum iodide.

- 1. Using pipette transfer 5 mL of the potassium iodide solution (c(KI) = 0,02 mol/L) to a clean test tube.
- 2. Add some drops of the argentum nitrate solution ($c(AgNO_3) = 0.01 \text{ mol/L}$).

3. Observe and describe the Tyndall effect: for this purpose it is necessary to observe
reflecting a beam of light through the colloid sideways:
Test 2. Preparation of «Berlin blue».
1. Using pipette transfer 10 mL of iron chloride (III) solution (weight percent $\omega = 0.001$)
to a beaker.
2. Add 3 drops of potassium gexacianoferrate (II) solution (weight percent $\omega = 0.001$).
Don't pour out the formed sol!
Observation:
Write reaction:
Write the formula of the colloidal micelle of sol:
3. Using pipette transfer 10 mL of the potassium gexacianoferrate (II) solution to a beaker.
4. Add 3 drops of the iron (III) chloride solution. Don't pour out the formed sol!
Observation:
Write reaction:
Write the formula of the colloidal micelle of sol:
Tost 3 Propagation of the sol of iron (III) hydroxide
Test 3. Preparation of the sol of iron (III) hydroxide. 1. Maggara evently 20.0 mL of the distilled water into a 50 mL backer flesh.
1. Measure exactly 20,0 mL of the distilled water into a 50 mL beaker flask.
2. Add approximately 2 mL of the iron (III) chloride solution (weight percent $\omega = 0.02$) to the beaker flask.
3. Reflux the solution until red-brown coloring is observed.
4. Cool the sol under sheet of water.
Write reaction:

Write the formula of the colloidal micelle of sol (notice, that potential determining ions is FeO^+):

Test 4. Purification of the sol by a dialysis method

- 1. Fill the 50 mL beaker with distilled water on 1/3 of its volume.
- 2. Pour sol of iron hydroxide in dialyzer and place it in a beaker with water.
- 3. In half an hour using pipette transfer 1–2 mL of a solution from a beaker to the test tube.
- 4. Add 5–6 drops of argentum nitrate solution to the test tube.

 Observation:

 Write reaction:

 Conclusion:

Test 5. Determine a sign of a charge of colloid particles by electrophoresis method.

- 1. Fill a U-similar tube on 3/4 of its volume by a colloid solution (prepared in experiment 2).
- 2. Connect electrodes with power unit.
- 3. Let down electrodes in both tube bends.
- 4. Connect a power unit to a current network.
- 5. In 1–2 minutes notice the color of sol in both tube bends:

ne	ar anode
ne	ar cathode
6	Determine a sign of charge colloid particles:

6. Determine a sign of charge colloid particles:

	Conclusions	
How can one prepare the sols?		
How can one purify the sols?		

How to distinguish colloid solution from true one by optical means?

LABORATORY WORK 18 «DETERMINATION OF A COAGULATION THRESHOLD»

Purposes: to determinate coagulation threshold of sol.

Introduction

Colloid particles are too small to be separated by physical means (e.g. filtration).

Colloid particles are coagulated (enlarged) until they can be removed by filtration.

Methods of coagulation:

- heating (colloid particles move and are attracted to each other when they collide);
- adding an electrolyte (neutralize the surface charges on the colloid particles);
- dialysis: using a semipermeable membranes separate ions from colloidal particles.

Protective colloid:

- The addition of large amount of the hydrophile, to a hydrophobic colloid stabilize the system, the hydrophile possibly being adsorbed as a monomolecular layer on the hydrophobic particles. This phenomenon is known as *protection*.
- The added hydrophile is known as *protective colloid*.

Background

Colloidal particles join to larger aggregates. Such an aggregation is called coagulation. During coagulation, hydrosols get clouded; their color is being changed, sedimentation occurs – enlarged particles start to sediment.

According to the Hardy – Schulze rule, coagulation threshold decreases with increasing of the charge of ion causing coagulation. Coagulation of colloid solutions is strongly enhanced by adding of electrolytes. The impact of electrolytes is quantified by coagulation threshold and coagulation capacity of the ions. Coagulation threshold is electrolyte's minimal concentration causing the visible coagulation and expressed in millimoles for one liter of the sol.

Reagents and Equipment

Potassium gexacianoferate (III) solution

Burettes

Potassium dichromate solution

Test tubes

Sol of iron (III) hydroxide

5 mL pipette

Cylinder

Experiment time: about 40 min.

Activities

- 1. Fill burettes: 1st with potassium chromate solution ($c\left(\frac{1}{2}K_2Cr_2O_7\right)=2$ mmol/L); 2nd with potassium hexacyanoferrate (III) solution ($c\left(\frac{1}{3}K_3[Fe(CN)_6]\right)=2$ mmol/L).
- 2. Using the pipette transfer 5 ml of iron (III) hydroxide sol to two test tubes.
- 3. Add potassium chromate dropwise to the first test tube until the iron hydroxide sol starts to coagulate.
- 4. Add potassium hexacyanoferrate (III) dropwise to the second test tube until the iron hydroxide sol starts to coagulate.
- 5. Notice the data to the Table.

Evaluation

1. Calculate the coagulation threshold of electrolytes (in mmol/L) using the formula below and notice the result to the Table.

$$c_{K}(\frac{1}{2}X) = \frac{c(\frac{1}{2}X) \cdot V(X)}{V(sol) + V(X)}.$$

$$c_{K}(\frac{1}{2}K_{2}Cr_{2}O_{7}) = \frac{c(\frac{1}{2}K_{2}Cr_{2}O_{7}) \cdot V(K_{2}Cr_{2}O_{7})}{V(sol) + V(K_{2}Cr_{2}O_{7})} = \frac{c}{V(sol) + V(K_{2}Cr_{2}O_{7})} = \frac{c(\frac{1}{3}K_{3}[Fe(CN)_{6}]) \cdot V(K_{3}[Fe(CN)_{6}])}{V(sol) + V(K_{3}[Fe(CN)_{6}])} = \frac{c}{V(sol) + V(K_{3}[Fe(CN)_{6}])}$$

2. Calculate coagulation power of electrolytes (in a L/mol) using the formula below and notice the result to the Table.

$$V_{K}(X) = \frac{1}{c_{K}(\frac{1}{Z}X)}$$

$$V_{K}(K_{2}Cr_{2}O_{7}) = \frac{1}{c_{K}(\frac{1}{2}K_{2}Cr_{2}O_{7})} = \frac{1}{c_{K}(\frac{1}{2}K_{2}Cr_{2}O_{7})} = \frac{1}{c_{K}(\frac{1}{3}K_{3}[Fe(CN)_{6}])} = \frac{1}{c_{K}(\frac{1}{3}K_{3}[Fe(CN)_{6}]} = \frac{1}{c_{K}(\frac{1}{3}K_{3}[Fe(CN)_{6}]} = \frac{1}{c_{K}(\frac{1}{3}K_{3}[Fe(CN)_{6}]} = \frac{1}{c_{K}(\frac{1}{3}K_{3}[Fe(CN)_{6}]} = \frac{1}{c_{K}(\frac{1}{3}K_{3}[Fe(CN)_{6}]} = \frac{1}{c_{K}(\frac{1}{3}K_{3}[F$$

3. Calculate relative coagulation power of electrolytes:

$$\frac{V_{_{K}}(K_{_{3}}[Fe(CN)_{_{6}}])}{V_{_{K}}(K_{_{2}}Cr_{_{2}}O_{_{7}})} = -----=$$

Evaluation Table

Electrolytes	$c(\frac{1}{Z}X),$ mmol/L	Volume of solution electrolytes V(X), L	Coagulating ion	Coagulation threshold, $c_{\scriptscriptstyle K}\!\!\left(\frac{1}{z}X\right)$ $mmol/L$	Coagulation power, $V_K(X)$, $L/mmol$
K ₂ Cr ₂ O ₇					
$K_3[Fe(CN)_6]$					

 $\label{eq:conclusion} Coagulation power of $K_3[Fe(CN)_6]$ _____ than coagulation power of $K_2Cr_2O_7$.}$

LABORATORY WORK 19 «DETERMINATION OF IRON NUMBER OF A GELATIN»

Purposes: to determine the iron number (protective ability) of a gelatin.

Background

Coagulation phenomenon is essential in many life processes: blood coagulation, milk protein coagulation, digesting of food, etc. Blood groups of people and animals are determined by coagulation reactions. In some cases coagulation is undesirable: using or storing various emulsions, solutions in house holding, industry, pharmacology, etc. In order to increase stability of colloid solutions, surfactants or macromolecular compounds are often used. They protect colloid solutions against coagulation. The protection has a very high biological importance; proteins in living systems protect from precipitation insoluble particles found in water: calcium salts, cholesterol, hinder formation of so-called

«stones» in kidney and liver. Protection efficiency of solutions of macromolecular compounds is expressed as iron number. Iron number is a mass of macromolecular compound (in dry state), required for protection against coagulation of 1 liter of hydrophobic sol of iron hydroxide.

Reagents and Equipment

Gelatin solution Burettes

Sodium chloride solution Cylinders

Sol of the iron (III) hydroxide Test tubes

Distilled water Test tube rack

Experiment time: about 50 min.

Activities

- 1. Fill the burettes: 1st with distilled water, 2nd with gelatin solution (weight percent w = 0.01), 3rd with sodium chloride solution (weight percent w = 0.1).
- 2. Using pipette transfer 2 mL of gelatin solution to one test tube and transfer 1 mL of distilled water to six test tubes.
- 3. Using pipette transfer 1 mL of gelatin solution from the first test tube into the second test tube. Mix the contents of the test tube.
- 4. Using pipette transfer 1 mL of solution from the second test tube to the third test tube, from the third test tube to the fourth, and so on, and finally pour out 1 mL of solution from the last test tube to a sink. Mix the contents of the test tubes.
- 5. Place 10 mL of sol of iron (III) hydroxide in each of test tubes using measure cylinder.
- 6. Add 1 mL of NaCl solution from a burette in each of test tubes. Mix the contents of the test tubes.
- 7. Put test tubes in a test tube rack.
- 8. In 15 min notice to the Table, in which of the test tubes the blush (coagulation) is observed. Put a sign «+» where the coagulation is observed and «-» where there is not.

Evaluation

1. Calculate the mass of gelatin (in mg) in the first test tube (assuming that density of solution 1 g/mL):

$$m_1(x) =$$

and than calculate the mass of gelatin in the remaining test tubes: $m_i = \frac{m_{i-1}(x)}{2}$.

Notice the masses of gelatin to the Table.

Evaluation Table

№ test tube	1	2	3	4	5	6	7
Mass of gelatin, mg							
Coagulation							

2. Calculate «iron number» of gelatin according to the formula:

$$S = \frac{m_1(x) + m_2(x)}{2}$$

where $m_2(x)$ – mass of gelatin in test tube, where coagulation was observed; $m_1(x)$ – mass of gelatin in previous test tube.

S ==	
Con	nclusion
The «iron number» of gelatin is equal to	

LABORATORY WORK 20

«DETERMINATION OF THE ISOELECTRIC POINT OF ALBUMIN OF BLOOD SERUM»

Purpose: to determinate of the isoelectric point of albumin of blood serum.

Background

An important characteristic of proteins is their isoelectric point. It is defined by physicochemical methods based on different physical phenomena. The meaning of isoelectric point (IEP) depends on its structure. If there are more carboxyl groups in the molecule, then its isoelectric point reside within 0<pH<7, if there are more amino groups, then it does within 7<pH<14. The charge of a protein molecule depends on the pH of the medium. A protein is charged negatively in solutions with pH>pH_{IEP}:

$$R - CH + OH - PH > IEP$$

$$COO - R - CH + H2O Anionic form$$

A protein is charged positively in solutions with pH<pH_{IEP}:

$$R - CH + H^{+}$$

$$COO^{-}$$

$$pH < IEP$$

$$R - CH + H_{2}O$$

$$COOH$$

$$COOH$$

$$COOH$$

$$R - CH + H_{2}O$$

$$COOH$$

Reagents and Equipment

Dehydrating substance solution Burettes

Solution of albumin of blood serum Glass stick

Acetic acid solution Test tubes

Sodium acetate solution Test tube rack

Experiment time: about 50 min.

Activities

- 1. Fill the burette with a dehydrating substance solution and the other burette with solution of albumin of blood serum.
- 2. Prepare acetate buffer solutions by mixing of 0,1 mol/L acetic acid solution and 0,1 mol/L sodium acetate solution in the ratio noted in Table.
- 3. Place 1 mL of acetate buffer solution into seven test tubes.
- 4. Place 1 mL of albumin of blood serum solution and 0,5 mL of dehydrating substances solution in each of seven test tubes. The contents of flasks should be shaken up.
- 5. In 5 min notice to the Table in which of the test tubes the blush (coagulation) is observed. Put a sign «+» where the coagulation is observed and «-» where there is not.

Evaluation

1. Calculate value pH for a buffer solution, where the coagulation is observed according to the formula:

$$pH = pK_d(CH_3COOH) + lg \frac{c_0(CH_3COONa) \cdot V(CH_3COONa)}{c_0(CH_3COOH) \cdot V(CH_3COOH)},$$
 where $K_d(CH_3COOH) = 1,75 \cdot 10^{-5} \text{ mol/L};$
$$pK_d(CH_3COOH) = - + lg - + lg - - = + lg = - + lg - - + lg$$

$pH_2 =$	+ lg =	+ lg	=
$pH_3 =$	+ lg=	+ lg	=
$pH_4 =$	+ lg=	+ lg	=
$pH_5 =$	+ lg=	+ lg	=
$pH_6 =$	+ lg=	+ lg	=
$pH_7 =$	+ lg=	+ lg	=

Notice the pH to the Table.

Evaluation Table

№ of test tube	1	2	3	4	5	6	7
V(CH ₃ COONa), mL	8	4	2	1	1	1	1
V(CH ₃ COOH), mL	1	1	1	1	2	4	8
Coagulation							
pH of a solution							
Charge of macromolecule							

2.	IEP	is	equaled	pН	of	a	solution	in	which	the	greatest	coagulation	is	observed

3. Define and notice to the Table a sign of a charge of macromolecules albumin of blood serum (if pH>pH_{IEP} a protein is charged negatively in solutions; if pH<pH_{IEP} a protein is charged positively).

Conclusion

IEP of albumin in blood serum is equaled _____

Important practices for the final test of Module 1 include:

- 1. Identify CO_3^{2-} , SO_4^{2-} , NO_2^{-1} ions in solution. Write the corresponding equations.
- 2. Identify MnO₄⁻, Ag⁺ ions in solution. Write the corresponding equations.
- 3. Evaluate ability of Ag⁺, Al³⁺, Cu²⁺ ions to form complex compounds. Write the corresponding equations.
- 4. Prepare some concentration solution using more concentrated solution.
- 5. Apply a principle of calculation to titrimetric analysis. Estimate concentration and mass of analytes based on titrimetric data.
- 6. Explain the color changes of phenolphthalein solutions after adding to it of some CH₃COOH or NaOH solution.
- 7. Differentiate the using of phenolphthalein and methyl red for acid-base titration.
- 8. Predict the titrants for determination of H₂SO₄, NaOH, CH₃COOH, NH₃, HCl contents in solutions. Write the corresponding molecular and net equations. Estimate concentration and mass of specified analytes based on titrimetric data. Fix end point.
- 9. Predict the pH of salt solutions. Determine the pH of salt solutions by pH-meter.
- 10. Explain the action of following buffers: phosphate, bicarbonate and ammonium.
- 11. Estimate the pH of buffer solutions using Henderson-Hesselbalch equation. Explain the procedure of some pH buffer solutions preparation.
- 12. Determine the buffering capacity. Explain the principle of buffering capacity determination.
- 13. Estimate the osmotic pressure of pharmaceutical product solutions.

Important practices for the final test of Module 2 include:

- 1. Determine the thermal effects of strong and weak acids reactions neutralization.
- 2. Evaluate the opportunity of processes spontaneous
- 3. Explain the mechanism of catalysis action (biological include).
- 4. Estimate the effect of some factors on equilibrium.
- 5. Estimate the precipitate formation based on calculations.
- 6. Explain the principle of potentiometric.
- 7. Explain the using of some electrodes for determination of pH by potentiometric.
- 8. Determine the redox potential by potentiomertric.

- 9. Estimate the oxidizing-reducing ability of systems according to standard redox potentials.
- 10. Predict the direction of oxidation-reduction reactions according to standard redox potentials.
- 11. Estimate the application of adsorbents based on adsorption isotherms.
- 12. Explain the principle of water demineralization by ion exchangers.
- 13. Determine the contents of some ions in solution of pharmaceutical products by ion-exchange.
- 14. Separate the mixture of some species by paper chromatography.
- 15. Explain the principle of paper chromatography.
- 16. Identify amino acids by paper chromatography. Explain the principle of the method.
- 17. Separate the mixture of some cations by column chromatography. Explain the principle of the method.
- 18. Predict ions that form diffuse layer of micelle.
- 19. Prepare sols by method of chemical condensation (reaction of double exchange, hydrolysis).
- 20. Estimate the charge of colloidal particles by method of electrophoresis.
- 21. Purify sols by the method of a dialysis.
- 22. Estimate the effect of electrolytes on the process of sols coagulation.
- 23. Calculate the threshold of coagulation and coagulation ability of electrolytes.
- 24. Determine the «iron number» of gelatin and the IEP of albumin of blood serum.
- 25. Explain the principle of colloidal protection.

Solubility of Ionic Compounds in Water

Key: S = soluble; I = insoluble; D = decomposes in water; U = compound does not exist

Cation	A1 3+	NH ₄	Ba 2+	Cd 2+	Ca 2+	Cr 3+	Co 2+	Cu 2+	Fe 3+	Fe 2+	H 1+	Pb 2+	Mg 2+	Hg 1+	Ni 2+	K 1+	Ag 1+	Na 1+	Sr 2+	Zn 2+
Anion																				
$C_2H_3O_2$	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	I	S	S	S
Br	S	S	S	S	S	S	S	S	S	S	S	S	S	Ι	S	S	I	S	S	S
CO ₃ ²⁻	U	S	I	I	I	U	I	I	U	I	S	I	I	Ι	I	S	I	S	I	I
ClO ₃	S	S	S	S	S	U	S	S	U	U	S	S	S	S	I	S	S	S	S	S
Cl	S	S	S	S	S	S	S	S	S	S	S	S	S	Ι	S	S	I	S	S	S
CrO ₄ ²⁻	U	S	I	I	S	U	I	S	I	I	S	I	I	I	U	S	I	S	I	I
OH ⁻	I	Ι	S	I	I	I	I	I	I	I	H ₂ O	I	I	U	I	S	U	S	I	I
Γ	S	S	S	S	S	S	S	S	S	S	S	I	S	I	S	S	I	S	S	S
NO ₃	S	S	S	S	S	S	S	S	S	S	S	S	S	D	S	S	S	S	S	S
O ²⁻	I	U	S	I	I	I	I	I	I	I	H ₂ O	I	I	Ι	I	D	I	D	I	I
C ₂ O ₄ ²⁻	I	I	I	I	I	S	I	I	S	I	S	I	I	Ι	I	S	I	S	I	I
PO ₄ ³⁻	I	S	I	I	I	I	I	I	I	I	S	I	I	U	I	S	I	S	I	I
SiO ₃ ² -	I	U	S	I	I	U	I	U	U	I	Ι	I	I	U	U	S	U	S	I	I
SO ₄ ²⁻	S	S	I	S	I	S	S	S	S	S	S	I	S	Ι	S	S	I	S	I	S
S ²⁻	D	S	D	I	I	I	I	I	I	I	S	I	D	Ι	I	S	I	S	I	I
SO ₃ ²⁻	U	S	I	I	I	I	Ι	U	U	I	S	I	U	U	I	S	I	S	I	I

Common Logarithm Table

0,m	0,0	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9
n										
1	0,000	041	079	114	146	176	204	230	255	279
2	301	322	342	362	380	398	415	431	447	462
3	477	491	505	519	532	544	556	568	580	591
4	602	613	623	634	644	653	663	672	681	690
5	699	708	716	724	732	740	748	756	763	771
6	778	785	792	799	806	813	820	826	833	839
7	845	851	857	863	869	875	881	887	892	898
8	903	909	914	919	924	929	935	940	945	949
9	954	959	964	969	973	978	982	987	991	996

Example: find the common logarithm for 283500 using Common Logarithm Table.

Find the characteristic of the number: $283500 = 2,835 \cdot 10^5$ (characteristic = 5). Round the number to two significant digits: $2,835 \cdot 10^5 \approx 2,8 \cdot 10^5$. Find the raw with n=2, find the column with m=0,8, the number inside the cell is 0,447 and plus the characteristic of number: $\lg 283500 = \lg (2,835 \cdot 10^5) \approx \lg (2,8 \cdot 10^5) = \lg 2,8 + \lg 10^5 = 0,447 + 5 = 5,447$.

<u>~</u> ⊴	- 16 elium	a 55	0 P 0 8	0 T	83.80 18-8 18-8 6 44 6 44 6 44	on 23 8-8	on 23-18-8	Ctium 6
18 ∨∭A	2 He lium	10 Neon Neon 2-8 20.179	18 Argon 39.948 2-8-8	36 Krypton	25.8 25.8	Xenon 131.29 2-8-18-18-8 86	Radon (222) (223) (224) (224) (225)	118 Ununocti (294)
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