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ANALYTICAL CHEMISTRY

LABORATORY MANUAL

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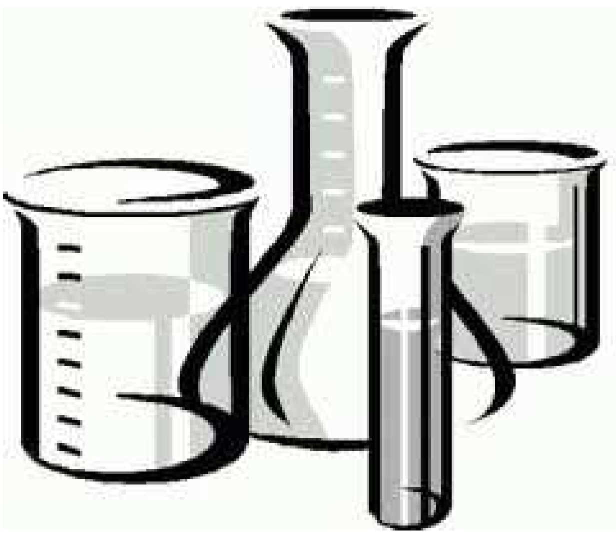


Kaunas, 2012

CONTENT

1. INTRODUCTION

Analytical chemistry – science field evolving and adapting methods, devices and strategy for obtaining information about chemical composition, structure and energy state of substances.

Goals of analytical chemistry: to detect chemical elements, which compose particular substance - **qualitative analysis;** to determine the ratios of different elements in investigative substance **- quantitative analysis.**

Various substances differ from each other by composition, structure, physical and chemical properties. Most of properties can be used to learn about qualities, which distinguish substance from others. These qualities are **analytical signals.** Methods of analysis are based on obtaining analysis signals and measurement of intensity of signals. According to action, which gives analytic signal, methods of analysis are divided into physical and chemical instrumental methods. They are employed in both in qualitative and quantitative analysis.

Chemical methods of analysis – methods based on chemical interaction of atoms, molecules and ions. These methods are employed to detect characterize chemical properties of element or ion. Methods of chemical analysis can be divided according the type of chemical reaction, rate of chemical reaction and advisability (gravimetry, titrimetry, gas analysis, kinetic methods of analysis).

Physical methods are based on different parameters of substance radioactivity, electromagnetic properties, radiation.

Also there are:

- biological methods – based on use of biologically active substances and biological systems.

- biochemical methods – when substances of biological origin are investigated with chemical methods.

Lately combined methods are used more often. Analysis of chemical composition of substance proceeds by following steps: choosing a sample; preparation of sample for analysis; extraction of component to investigate, purification; choosing method and scheme of analysis; disruption or solving of sample, separation and concentration; measurement of physical properties of sample, chemical reagent or product of chemical reaction; calculation of analysis data; estimation of results reliability .

This manual written to help first year veterinary medicine students in analytical chemistry laboratory work. Practical work, described in this book, includes classical, mostly used in practice, essential for VA absolvent methods of chemical and instrumental analysis. Classical methods, for example, titrimetry (volumetric) analysis, are presented, as well as potentiometry, photometry, chromatography. Issue also contains method of qualitative macroanalysis, information on buffer and colloid solutions, Theoretical background is given in each chapter. Main concepts, questions, samples of tasks are also presented. Manual contains safety instructions for chemical laboratory worker and list of chemical reagents with chemical formulas.

2. WORK SAFETY INSTRUCTIONS FOR PERSONS WORKING IN CHEMICAL LABORATORY

2.1. GENERAL PART

2.1.1 Only persons that are introduced to safety rules and first aid methods are allowed work in chemical laboratory. Students' knowledge is tested. Person, introduced to safety rules must sign in safety rules instruction journal.

2.1.2 Student must obey established order in the work place, take care of his or her health and of colleagues' health, perform requirements of this instruction. Students can't use devices, which have defects and must report lecturer about them.

2.1.3 Ill and intoxicated persons are not allowed to work.

2.1.4 All works in chemical laboratory must be performed only if gas and electricity supply systems work correct, and fume hood is functional.

2.1.5 Fire prevention requirements:

2.1.5.1 Avoid actions, which can lead to conditions, favourable to fire.

2.1.5.2 Students must be introduced to main fire elimination measures, coordinate their actions during fire danger.

2.1.5.3 Smoke only in area, specially set on this purpose.

2.1.6 Requirement for electricity safety:

2.1.6.1 Electrical devices can be exploited only according to their instructions, given by manufacturer.

2.1.6.2 Don't use defect sockets, plugs, switches and other defect equipment.

2.1.6.3 Electrical devices must be grounded, if it is required by use rules.

2.1.6.4 Switch off electrical device if current flow outside circuit is noticed.

2.1.6.5 Don't connect to one socket several high power devices, if their requirement of current may exceed permeability of installation cables.

2.1.6.6 Electricity distribution boards must be locked.

2.1.6.7 It is forbidden to fix devices connected to the electrical circuit.

2.1.6.8 Remember, voltage up to 36 volts is not dangerous to human.

2.1.7 Work carefully with laboratory equipment, glassware and devices and start work with them only after learned how to use them. If equipment is broken, report to laboratory worker immediately.

2.1.8 Connection of the devices must be checked by laboratory assistant before use.

2.1.9 If gas, water supply, canalization, electricity system defect is noticed, report to laboratory worker.

2.1.10 If gas flow is noticed, close gas valve and don't switch on any devices, which can induce flame or sparks.

2.1.11 When leaving laboratory, check if all electrical and gas devices are switched off and if no water or gas flow is present. Last leaving laboratory person is directly responsible for this requirement.

2.1.12 Each laboratory must contain: first aid medicaments, sand box for fire extinguish, woolen blanket, resin gloves and shoes, resin carpet for isolation, safety glasses.

2.1.13 If accident took place, help injured person with first aid and call emergency medical service if is needed, use telephone number 112.

2.1.14 Report accident to leader and don't change anything in accident location, unless it causes danger to people. Necessary changes must be noted in act.

2.1.15 Personal care:

2.1.15.1 Work only with clean laboratory robes.

2.1.15.2 Wash hands before and after work with warm water and soap, use disinfection and neutralization measures.

2.1.15.3 Don't keep food at the work place, eat only in special place.

2.2 HANDLING OF REAGENTS AND DEVICES

2.2.1 Flammable Solutions must be hold in thick glass dishes with polished corks. Dishes are hold in metal boxes, covered with asbestos.

2.2.2 It is forbidden to keep in the laboratory more than 3 liter of flammable solutions.

2.2.3 Only one balloon of gas allowed to be kept in laboratory.

2.2.4 When finishing gas using, remaining pressure in the balloon must be at least 0,5kg/cm^3.

2.2.5 Bromine, phosphorus, alkaline metals, concentrated acid supply must be kept in place, safe in case of fire.

2.2.6 On package with chemical reagent must contain label with name of substance and its purity.

2.3. WORK WITH DANGEROUS SUBSTANCES

2.3.1 All experiments with strong smelling, explosive, dangerous to health or volatile substances are performed in fume hood, with protecting glass lowered.

2.3.2 When working with strong smelling, dusty, dangerous to health substances not in the fume hood, respiratory mask and safety glasses must be used.

2.3.3 For new experiment (or laboratory work) or device safety is responsible person, who prepared it.

2.3.4 Flammable substances and heating devices must be handled extremely carefully. Don't heat ether (C_2H5-O-C_2H_5), ethanol (C_2H_5-OH), petrol (Cs-C_9) using opened flame or opened electrical heater. Heat them carefully, on closed electrical cooker or in water bath.

2.3.5 In the flaming volatile, not solving in water substances, flaming active metals fire must be extinguished with sand. They can't be extinguished with water.

2.3.6 Flaming robes and other surfaces extinguish the fire by wrapping in woolen blanket.

2.4. PERFORMING OF CHEMICAL EXPERIMENTS

2.4.1 Use for chemical experiment exact amount of substance, as indicated in laboratory work instruction.

2.4.2. If amount of reagents are not indicated, don't weight or measure volume of them, but amount of reagents can't exceed half of tube or reaction dish volume.

2.4.3. If concentrations of acid or alkaline solution are not indicated, use only diluted reagents.

2.4.4. After use close dishes with reagents with the same corks and put them to their place.

2.4.5. Avoiding reagents' contamination, use clean pipette or paddle.

2.4.6. Not used reagents can't be poured back to the dishes.

2.4.7. When diluting sulfur acid, acid must be poured to water, not on the contrary.

2.4.8. Solutions must be mixed by shaking the tube, not by closing it by finger and inverting.

2.4.9. After use concentrated acids, concentrated alkaline solutions. strong smelling or aggressive reagents are poured not to canalization, but to special dishes. Before throwing them away they must be neutralized (acids - with calcium hydroxide $Ca(OH)_2$ or calcium carbonate $CaCO_3$, alkaline solutions - with acids).

2.4.10. In cases, when small amounts of acids, alkaline solutions, strong smelling or aggressive reagents are poured to sink, big amount of water must be poured at the same time.

2.4.11. Remains of silver (Ag) and other expensive reagents are poured to special dishes.

2.4.12. When heating solutions, direct opened end of tube to side opposite to people.

2.4.13. When using pipette avoid accidentally to pump out solution to mouth

2.4.14. Don't pump out concentrated acids, alkaline solutions by mouth. Use gum pump.

2.4.15. Forbidden to investigate qualities of reagents by tasting. Ali reagents are poisons!

2.4.16. Volatile substances smell carefully directing air toward yourself by wave of hand.

2.4.17. It is forbidden to use laboratory dishes for eating, drinking and keeping food products.

2.4.18. It is forbidden to use dirty dishes to chemical experiments. After work, dishes must be washed immediately.

2.4.19. Alkaline solutions can't be kept in dishes with polished corks.

2.4.20. Gum hose can be pulled on only on glass pipe moistened with water or smeared with vaseline, glycerol $C_3H_5(OH)_3$. Keep glass pipe in hand wrapped in towel.

2.4.21. Gum is cut only with sharp knife moistened with water or smeared with glycerol. When drilling gum cork, smear the drill with vaseline or glycerol.

2.4.22. When corking up dish, keep dish in hand close to opening.

2.4.23. Pouring liquid from bottle keep label on the topside to avoid smearing.

2.4.24. Work place must be kept clean. Poured out reagents and other contaminants must be cleaned immediately.

2.4.25. Keep notes in drawer to avoid contamination with chemical reagents.

2.4.26. In chemical laboratory special laboratory coats should be worn. Put on the laboratory coat before entering the laboratory and put it off after leaving.

2.5 Actions in emergency cases

2.5.1 In all cases of intoxication, injury and fire inform department workers and call emergency services: 112.

2.5.2 If cut with glass remove glass fragments from wound, treat wound with iodine and bandage.

2.5.3 If spilled acid over oneself, wash injured place with big amount of water, neutralize with baking soda ($NaHCO_3$) 1-3 % solution.

2.5.4 If spilled concentrated sulfur acid (H_2SO_4) over oneself, clean injured place with paper or cloth, then wash and neutralize.

2.5.5 If spilled alkaline solution over oneself wash injured place with big amount of water, neutralize with acetic acid (CH_3COOH) solution or saturated boric acid (H_3BO_3) solution.

2.5.6 If reagent accidentally gets to mouth immediately split it away, rinse mouth with water and neutralizing Solutions (baking soda, boric or acetic acid).

2.5.7 Burned spot wash immediately with cold water and bandage.

2.5.8 If intoxicated with chlorine (Cl_2), hydrogen sulfide (Ha_2S) or with other substances via respiratory duct lead patient to fresh air, give him ammonia to smell, adjust cold compress on neck or breast. If is necessary, use artificial respiration and heart massage.

2.5.9 If bromine (Br_2) contacted with skin, wash injured spot with ethanol or petrol, smear with glycerol and bandage.

2.5.10 Ali first aid measurements are in laboratory drugstore.

3. LABORATORY TECHNIQUE, MATERIALS AND FUNDAMENTAL OPERATIONS

Students, who work in a chemistry laboratory, must know the purpose, potential use and all features of the laboratory's equipment, devices and tools. The success of laboratory session is determined by accurately, thoroughly performed operations and actions, named in the description of an experiment, as well as acquirement.

While working in the analytical chemistry laboratory it is necessary to learn how to: a) prepare laboratory glassware, Instruments and filters for work; b) how to filter, to heat and to dry materials; c) to measure liquid volume or weight with technical and analytical balance; d) to assemble chemical equipment; e) how to prepare solutions of required concentration, f) to calculate the required amount of reagents, the yield of reaction products and the relative error, g) to describe accomplished session, h) to draw used devices and chart graphs.

3.1. Cleaning of laboratory glassware

Before use, all glassware should be thoroughly cleaned to prevent errors caused by contaminants. First of all the glassware is washed with tap water, using small amounts of soap or soda and a brush to scrub the glassware. If the glassware is not truly cleaned, the dirt is eliminated while washing with hydrochloric acid or "cleaning mixture": concentrated sulphuric acid (H_2SO_4) is poured in 200 ml of saturated potassium bichromic acid ($K_2Cr_2O_7$) solution mixture reaches 500 ml. The glassware surface is rinsed in such "cleaning mixture" for several minutes, but for ease of use the utensil can be filled with solution or dipped into it and left to stay for longer time. Finally it should be washed with tap water and rinsed with deionized water, tipping and rolling the glassware. If rinsing a pipette, burette or other glassware with a tip, water needs to be discarded through the tip. The clean glassware should be inverted on a paper towel to dry.

3.2. Warming, drying, heating

Spirit lamps, electric and gas burners, water baths, drying and heating ovens, and thermostats are used for warming in the laboratory. Thermal resistant substances are dried in the electric drying ovens (100 - 125 C), and thermal non-resistant substances – in the vacuum drier or desiccator, which is filled with moisture sorbent. The materials heated at 800 - 1000°C temperature lose all volatile impurities, and sometimes they decompose into the thermal resistant compounds. It iš mainly heated in the electric muffle furnace and on the flame of gas burner at times. The heated material is placed in the porcelain, quartz or platinum crucible.

3.3. Filtration

The process usually employed to separate an insoluble solid from a liquid is called filtration. After the filtration process, the liquid that passes through the filter paper (filtrate), and/or the solid that remains on the filter paper (precipitate or residue), can both be used. It is used for filtration:

- **Filters of filter paper** are cut from simple filter paper and first folded in half. Then paper is folded again in squeeze-box form or infundibularly, but not in perfect quarters: the two folded edges should not quite touch; the second edge should be about 3 mm from the first edge. The filter is cut to a diameter that fits snug to the funnel walls, not reaching edges. The liquid with residues is poured into the funnel through the glass stick insomuch that its height would not reach filter's border 10 mm. If only the filtrate is required for further work, it should be filtrated through the folded filter (speed of filtration is faster);
- **Ashless filters** are used to perform quantitative analysis. The density of these filters is indicated on a tag of a cover. We can decide about ashless filter's density from strip color of a cover: red - least density, white - medium density, blue - close;
- **Glass filters** - glass funnels with poured spongy glass plate. The density of filters is marked by four numbers: No. l - least density, No.4 - most density;
- **Vacuum filtration** - Buchner filter with holey porcelain bottom is used and moistened with water and paper filter is placed on it. Paper filter should freely go in a funnel; its edges shouldn't be turned back. Buchner filter that is prepared for filtration is inserted into Bunsen flask, which is connected with water or mechanical pump in order to make vacuum;
- **Warmed filters** are used to filter viscous liquid, saturated and oversaturated solutions.

Collected residues are thoroughly washed several times with small amount of a solvent(water). Another portion of liquid is poured only then, when an initial is out flowed completely. If liquid over the residues is utterly clean, it can be decantated (poured without roiling liquid) before the filtration. It is possible to wash the residues, which remained after the decantation, on several occasions with a solvent (water) again in order to eliminate impurities.

3.4. Measurement

The measurement of liquid volume can be performed using graduated cylinders, volumetric flasks and measuring vessels. Unlike counting, which can be exact, measurements are never exact but are always estimated quantities. Obviously, some instruments make better estimates than others, so more precise liquid volume is measured by calibrated measuring vessels:

- **Pipette** – vessel, used to suck, to drop and to measure liquid. Mohr pipette measures only one, definite and marked on it volume. Graduated pipettes allow measurement of any volume that would not exceed the volume of pipette's graduated section. Such pipettes commonly are graduated with 0.1 ml scale and allow to measure volume in 0.005 ml precision. Semimicropipettes and micropipettes can be graduated with 0.01 and 0.001 ml scale;

• **Automatic micropipette** – instrument, used to suck, to drop and to measure liquid.

• **Burette** - glass tube (generally with 0.1 ml scale), used to drop and to measure liquid volume. Semimicroburettes and microburettes can be graduated with 0.01 or 0.001 ml scale;

• **Measuring flasks** – are used to measure various volumes and to prepare various concentration solutions.

Volume of liquid, which is colorless and moistens surfaces, is measured looking at the bottom of liquid's meniscus in the measuring vessels. Colorful liquid's volume, when we can't see the bottom of meniscus, is measured by deducting according to a top of meniscus. Meniscus should be in a level of a person who measures (Fig. 1).

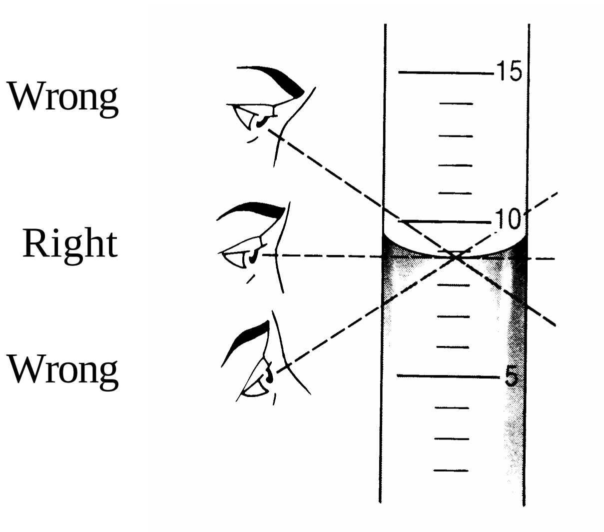


Fig. 1. Measurement of volume by watching meniscus

Liquid density is measured with an aerometer or pycnometer. The density of liquid depends on the temperature. If it is required to estimate more exact density, the liquid needs to be thermostated up to normal temperature or to recalculate the density, which is estimated in any temperature value, into the density of normal condition (273.15 K temperature, 101.325 Pa pressure).

If there is not an aerometer or pycnometer, liquid density can be estimated in a 50 ml or 100 ml measuring flask: it is balanced empty and dry - a g, the same flask with deionized water is weighted – b g, and eventually the same flask filled up to its mark with ascertainable liquid is balanced – c g,

Liquid density is estimated using formula:

$$\rho = \frac{c-a}{b-a} \quad (\text{g/cm}^3) \qquad (1)$$

3.5. Description of the experiment

All procedures of an experimental work in a laboratory should be recorded by legible shrift in a special exercise-book. The description of each new experiment is written in each new page, starting with pointing an experiment number and title. First, a short theoretical introduction is written followed by: a) a purpose of the work; b) calculating formulas; c) equations of reactions; d) "worked out" or calculated results; e) description of observed phenomena, f) drawings of used devices, charts and curves; g) acts of analysis. The work is signed and the date of its performance is noted. Instructor attests performed experiment in the group

Journal and signs in the student's laboratory exercise-book. The size of description is determined by one rule: all work can be repeated and the experiment as well as calculating can be checked, by referring to the exercise-book.

3.6. Laboratory work No. 1

SCALING WITH ANALYTICAL BALANCE

Technical balance is balance with little precision (+10^{-2} g). Analytical balance can weigh up to 200 g of substance in 10^{-4} – 10^{-5} g precision. You must scale carefully, without using sudden movements and strength. It is forbidden to push and to regulate balance (if it's a demand, balance is regulated by laboratory instructor or worker). Before scaling an object on the analytical balance, it must be weighted on technical one. In this case we economize time and extend the work time of analytical balance.

Purpose:

To familiarize with the measurement process and use of the technical and analytical balance. To estimate exact mass of a chosen object. To rate absolute and relative error of technical balance scale.

Procedure:

1) Technical balance is turned on and prepared for weighing – the state of zero is regulated. An object that is chosen for weighing (dry, clean and of room temperature) is scaled and the mass of an object is written into 1st table with precision of l digit after the decimal point.

2) Analytical balance is prepared for work like this: balance is turned on by twisting an arrester against clock arrow, and if there's a need, zero position of the scale is regulated in the way it coincided with a reading line in an illuminated screen. After that the balance is turned off and glass door is opened, the scaling object is **warily (Attention!** It is allowed to put the weighing object and weights, as well as to change milligramic weight-ring's number only then, when the balance is turned off (arrested) placed on the left side of the balance plate. Only chemically inert things can be placed directly on the balance plate. Volatile materials are scaled in the glass weighing bottles that are closed tightly.

The weights are placed on the right side of the balance plate (it is strictly forbidden to touch the weights) using pincers. Their total mass must match the display of the technical balance (for example, if the mass of an object, which was weighted on the technical balance, is 12.9 g, then 10 g and 2 g weights are placed on the analytical balance). The weights, which are heavier than l g and the weighing object itself, must be placed in the centre of the balance plate. The tithes of gramme are estimated before the balance is turned on, whirling the bigger limb (milligram weight-ring's handle) to the fixed position. During the scaling the balance is turned on and while slowly moving an arrester it can be observed what numbers are visible in the scale. Agreeably to on-screen number's mark (+ or -) it is decided, whether it is worth to increase or to decrease the number of weight-rings by the limb. Using the bigger (the tithes of gramme) or the smaller (the hundredth of gramme) limb, it is obtained, that datum-line would stop in the positive part of the scale between 0 and 10.

An obtained value of the weighted object is recorded in a table with 0.0001 precision: integer number is equal to the weight's mass on the plate, first two digits after the comma are seen on the bigger handle, the third and second digits after the comma - in the scale. After balancing, the balance is turned off. The propriety of scaling is checked by the instructor. Then the balance is sorted-out: the weighing object and the weights are removed, glass windows are closed and the limbs are carried back to the previous zero datum.

3) Absolute error **A** of scaling by the technical balance is calculated:

$$\mathbf{A} = \mathbf{M}_{\mathbf{anal.\ bl.}} - \mathbf{M}_{\mathbf{tecn.\ bl.}}\ (\mathrm{g}), \qquad (2)$$

Where: $M_{anal.\ bl}$ – object's, that was weighted on the analytical balance, mass, g;
$M_{tecn.\ bl.}$ – object's, that was weighted on the technical balance, mass, g.

4) Relative error **R** of scaling by the technical balance is calculated:

$$\mathbf{R = ((M_{anal.\ bl.} - M_{tecn.\ bl.}) / M_{anal.\ bl.}) \times 100 \quad (\%)} \qquad (3)$$

5) The result is written in the Table No l:

Table No l. **Scaling with analytical balance**

Scaled object	M techn.bl., g	M anal.bl., g	Absolute error A, g	Relative error R, %
	_ _._	_ _._ _ _ _	_ _._ _ _ _	_ _._

Date: 01-09-2011

Analized by: Alice Jackson __________ (signature)

4. TITRIMETRIC (VOLUMETRIC) ANALYSIS

Titrimetry method is based on the measurement of used for titration chemical reagent's volume, mass or electric streaming duration. Titrimetric methods can be divided into visual and instrumental determination of the titration's end methods; instrumental methods are divided by measured features and used devices into optical, electrochemical, thermometric, radiometric, polarimetric etc.

The quantity of material is estimated by visual volumetric analysis method, measuring the volume of known concentration agent solution, which has reacted with this material. For volumetric analysis quick followed, quantifiable and irreversible reactions need to be chosen. According to the reaction's type, volumetric analysis is divided into these methods:

1) Neutralization;
2) Precipitation;
3) Complexometry;
4) Redoxometry;
5) Hydrolysis (saponification).

Titration – analysis process, when solution is dropped into another known solution's volume till the equivalence point is reached.

Equivalence point - the moment, when reactants react wholly (without residuals).

Law of equivalent proportions states, that **the proportions in which two elements separately combine with a third element are also the proportions in which they combine together** (their quantity is proportional to their equivalents; German scientist V. Richter, 1791 year).

Applying this law, we can see an existing dependence between reacting substances volume and concentration in the equivalence point:

$$n_1 \cdot V_1 = n_2 \cdot V_2 \qquad (4)$$

Where: V_1, V_2 – solutions volumes, cm^3 (ml);
n_1, n_2 – solutions molar concentrations of equivalent (normality; eqv/mol·l).

The (4) equation is called **mathematical expression of equivalent point.**

Solutions molar concentrations of equivalent are represented by the number of solute **equivalents** in one liter of solution and are calculated using formula:

$$n = \frac{m}{E \cdot V} \ \text{(eqv/mol} \cdot \text{l)}, \qquad (5)$$

Where: **m** - solute's mass, g;
E - solute's equivalent;
V - solution's volume, l.

When in l l of solution it is dissolved:

l equivalent of solute, the solution is named - mononormal;
0.1 equivalent of solute- decinormal;
0.01 equivalent of solute - centinormal.

Equivalent is that comparative quantity by weight of an element, which possesses the same chemical value as other elements, as determined by actual experiment and reference to the same standard. Specifically: a) The comparative proportions by which one element replaces another in any particular compound; b) The combining proportion by weight of a substance, or the number expressing this proportion, in any particular compound; as, the equivalents of hydrogen and oxygen in water are respectively l and 8, and in hydric dioxide l and 16. In other words, equivalent of an element or material is its mass, that attaches or exchanges 1,008 amount of hydrogen, 8 amount of oxygen, and attaches or exchanges one mole of electrons.

Solutions' concentration can be represented by **titer (T)** in the volumetric analysis - it is the number of **solute's grammes in the one cm^3 (ml) of the solution (g/cm^3).**

There's a transition between molar concentration of equivalent and titer:

$$T = \frac{n \cdot E}{1000} \quad (g/cm^3), \qquad (6)$$

Where: E - solute's equivalent.

Element's equivalent is calculated using this formula:

$$E = \frac{A}{n}, \qquad (7)$$

where: A - element's atomic mass;
n - element's valence.

E. g., $E_{Na} = \frac{23}{1} = 23$ (g/mol).

Usually **indicators - materials, which show particular state of the chemical system –** are used to highlight the equivalent point. An indicator is chosen for every titration occasion. If the chosen indicator is right, its color is intensely changed in the equivalent point.

Vessels, used in the volumetric analysis:

- exact volume measurement vessels; pipette, automatic micropipette, burette, measuring flasks;
- other vessels: Erlenmeyer (100 - 250 ml conical flasks) flasks, vessels for indicators and solutions keeping, graduated test-tubes, measurement cylinders.

Preparation of vessels for analysis

All vessels that are used in volumetric analysis should be well cleaned and washed with water, scrubbing with a brush, soda or "cleaning mixture", which way of preparation has been mentioned in a paragraph 3.1. Further all vessels are washed with tap or deionized water. Burettes and pipettes are rinsed with a solution, which will be measured by them afterwards.

Compound equivalent is calculated using this formula:

$$E = \frac{M}{n \cdot k}, \qquad (8)$$

Where: M - compound's molar mass;
n - valence of atoms, ions, radicals, which participate(d) in the exchange reaction;
k - the number of these atoms, ions, radicals.

E. g., $E_{Na_2SO_4} = \frac{23 \cdot 2 + 32 + 16 \cdot 4}{1 \cdot 2} = \frac{142}{2} = 71$ (g/mol).

Oxidizer's (reducer's) equivalent is calculated using this formula:

$$E = \frac{M}{e}, \qquad (9)$$

Where: M - compound's molar mass;
e - attached by oxidizer or released by reducer electron number.

E. g., $E_{KMnO_4} = \frac{39 + 55 + 16 \cdot 4}{5} = 31.6$ (g/mol),
($Mn^{7+} + 5\bar{e} = Mn^{2+}$)

4.1. VOLUMETRIC ANALYSIS SOLUTIONS

Investigative solutions' concentration is estimated by titrating them with solutions of known concentration, which can be **standard** (reference solution) and **titrated** (work/process solution).

Standard solutions are prepared by **weight manner** or from the **fixanals** (glass ampoules, filled with exactly scaled quantity of known reagent). Their molar equivalent concentration (normality) is calculated, when weighted quantity of reagent and measurement flask's volume is known. Only proper for particular reaction materials, that have known, constant, unchangeable in the air and solution chemical composition, are used in the preparation of standard solutions.

Titrated (work/process) solutions are prepared from materials, which composition changes depending on instability, volatility, hygroscopicity, ability to react with surrounding CO_2, O_2 or other causes (for ex., HCl, NaOH, $KMnO_4$ etc.). Generated concentration of work solutions is usually approximate, and their exact concentration is estimated by titrating with standard solution.

4.1.1. Laboratory work No. 2

PREPARATION OF STANDARD $NA_2B_4O_7$ SOLUTION

Task: To prepare 250 ml 0.1 n (0.1 normality) standard sodium tetraborate ($Na_2B_4O_7$) solution.

Procedure:

The quantity of sodium tetraborate $Na_2B_4O_7 \cdot 10H_20$ (borax) m (g), which is required to prepare 250 ml 0.1 n solution, is calculated using the formula:

$$m = n \cdot E \cdot V \ (g), \qquad (10)$$

Where: n - molar equivalent concentration (normality) of stock solution;
V - volume of stock solution (l).
E - equivalent of borax, calculated referring to (8) formula:

$$E = \frac{M}{n \cdot k} = \frac{23 \cdot 2 + 10,81 \cdot 4 + 16 \cdot 7 + 10 \cdot (1 \cdot 2 + 16)}{2 \cdot 1} = \frac{381,37}{2} = 190,685 \text{ (g/mol)}$$

(M -mole mass of borax).

Then:

$$m = 0.1 \cdot 190.685 \cdot 0.25 = 4.7671 \text{ (g)}.$$

You need to take clean, dry 100 ml capacity glass and, using the analytical balance, to scale close to calculated (4,7671 g) quantity of borax **a** (g). It is sluiced down with 40 - 70 ml of deionized water and is dissolved, while warming until 50 °C as well mixing with a glass stick. The solution is cooled (glass stick shouldn't be withdrawn), quantifiably transfused into 250 ml measurement flask, then attenuated with deionized water until the mark and well mixed. Prepared solution of borax is poured into the new cleaned glass vessel, which is theretofore rinsed with little volume of prepared borax solution. Sodium tetraborate solution's molar equivalent concentration is calculated using this formula:

$$n_B = \frac{a}{m} \cdot 0.1 \text{ (eqv/mol·l)} \qquad (11)$$

You need to stick etiquette on the vessel with prepared standard solution of borax:

$Na_2B_4O_7$
n = <u>0</u> . _ _ _ _ eqv/mol·l
V-No Alice JACKSON
Matt WALKER
2012-09-19

4.1.2. Laboratory work No. 3

PREPARATION OF TITRATED NaOH SOLUTION

<u>Task:</u> To make 500 ml titrated 0.1 n NaOH solution.

<u>Procedure:</u>

NaOH quantity **m** (g), which is required to prepare 500 ml 0,1 n solution, is calculated referring to (10) formula.

$$m = n \cdot E \cdot V \ \ (g), \qquad (10)$$

Calculated NaOH quantity with an excess of 0.2 g is scaled on the technical balance in 100 ml capacity glass. Then substance is dissolved in 50-60 ml of deionized water, then poured into 500 ml capacity clean glass vessel (it is forbidden to use vessels with cut glass stoppers), also diluted until 500 ml and well mixed. An etiquette, where solution's concentration is not indicated, needs to be stuck on the vessel. Prepared titrated NaOH solution molar equivalent concentrations (normality) will be estimated later.

4.1.3. Laboratory work No. 4

PREPARATION OF TITRATED HCl SOLUTION

<u>Task:</u> to make 500 ml titrated 0.l n HC1 solution.

<u>Procedure:</u>

It is calculated, how many ml of HCl with W_0 concentration is required to take, if you want to prepare 500 ml 0.1 n HCl solution. For this purpose, referring to formula (12), HCI percentage concentration is recalculated into molar equivalent concentration:

$$n_{pr.} = \frac{10 \cdot \rho \cdot W_0}{E} \ \ (equ/mol \cdot l), \qquad (12)$$

Where: W_0 – initial HCl, from which our solution will be prepared, percentage concentration;
ρ – initial HCl dense;
E – HCl equivalent.

Referring to (4) equation, required HCl volume is calculated:

$$V_{pr.} = \frac{n \cdot V}{n_{pr.}} \quad (ml)$$

Where: **V** – volume of stock HCl solution ml (500 ml);
n – normality of stock HCl solution (0,1 n);
$n_{pr.}$ – normality of an acid, our solution is made from, calculated referring to (12) eaquation;
$V_{pr.}$ – volume of an acid, our solution is made from, ml.

Calculated HCl acid **$V_{pr.}$** volume (which can be measured by graduated test-tube) is poured into the clean 0.5 l capacity glass vessel, then diluted until 500 ml and finally mixed. An etiquette, where solution's concentration is not indicated, needs to be stuck on the vessel again.

Prepared titrated HCl solution molar equivalent concentrations (normality) will be estimated later.

4.1.4. Questions and examples of tasks

1. An object of analytical chemistry. Quantitative and qualitative analysis. It's purposes and rnethods of performance.
2. Measurement of material's mass and quantity, measures.
3. Fundamental laws of stoichiometry.
4. Volumetric analysis. Methods of volumetric analysis.
5. Exact volume measurement vessels. Their preparation for analysis.
6. Definition of equivalent. Calculation of element, compound, oxidizer and reducer equivalents.
7. Definition of molar equivalent concentration, its calculation.
8. What solutions are named mono-, deci-, centinormal?
9. Definition of titer and its calculation.
10. Solutions, which are used in volumetric analysis. Define standard and titrated solutions.
11. What is the way of preparation of standard and work solutions? What is fixanals?
12. Calculation of material quantity, required to make a solution.
13. Calculation of solution's molar equivalent concentration (normality).
14. Recalculate solution's percentage concentration into molar equivalent concentration and vice versa.
15. How many g of borax ($Na_2B_4O_7 \cdot 10H_2O$) do you need, if you want to prepare 500 cm^3 0.1 n solution?
16. How many cm^3 of HCl (35 % concentration and $\rho = 1.174$ g/cm^3 density) do you need, if you want to prepare 500 cm^3 of decinormal solution?
17. How many g $Ba(OH)_2$ do you need, if you want to prepare 600 cm^3 0.3 n solution?
18. Calculate NaOH solution's molar equivalent concentration, if 8 g NaOH is in its 500 ml volume.
19. Calculate decinormal H_2SO_4 solution's titer (T).
20. How many ml of 50 % $KMnO_4$ ($\rho = 0.105$ g/cm^3) do you need to take, if you want to prepare l liter 0.005 n solution?

4.2. METHOD OF NEUTRALIZATION

Neutralization method – it is method of volume analysis, based on neutralization reaction of H^+ and OH^- ions. In titration process **concentration of H^+ ions and pH values change.** In dependence of strength of reacting acids and alkaline, pH in equivalent point can be pH=7, pH < 7, pH >7. Substances, which react in neutralization reaction, are mostly colorless, so indicators which color depends on media pH are used.

***pH – hydrogen ion exponent.** It is equal to negative logarithm of hydrogen ions concentration (activity):

$$\mathbf{pH = -lg[H^+] \quad or \quad pH = -lg\, a_{H^+}} \qquad (13)$$

pOH – hydroxyl exponent. It is equal to negative logarithm of hydroxyl ions concentration:

$$\mathbf{pOH = -lg[OH^-]} \qquad (14)$$

transition between these exponents:

$$\mathbf{pOH + pH = 14} \qquad (15)$$

Indicators - weak organic acids or alkali, which dissociated and not dissociated forms differ in color. pH range in which change of indicator color can be visually detected is called indicator color change interval (Table No 2). It can't exceed 2 pH units. The smaller interval of color change, the more sensitive indicator. We can measure concentrations of weak and strong acids and alkali solutions, salt solutions (water hardness) by neutralization method.

Table No 2. **Indicators, their colors and color change ranges**

Row number	Indicator	Color of acidic form	Color change range pH	Color of alkaline form
1	methyl yellow	Red	2.4-4.0	yellow
2	methyl orange	Red	3.2-4.4	yellow
3	methyl red	Red	4.2-6.2	yellow
4	lacmus	Red	5.0-8.0	blue
5	phenolphthalein	Colorless	8.0-9.8	mauve
6	alizarin yellow	yellow	10.1-12.0	violet

pH changes during titration describe titration curves. They are drawn with pH values on y-axis and alkali or acid excess noted on x-axis (Figures No 3 and No 4). For example, when titrate 0.1n HCI solution with 0.1n NaOH solution, amount of HCl decreases subsequently and after reaching equivalent point, amount of alkali starts increase. This change is presented in Table No 3.

Table No 3. **Changes of pH, when 0,1n HCl solution is titrated with 0,1n NaOH solution**

Acid excess				Alkali excess			
Reacted HCl, %	Remained HCl, %	n_{HCl}	pH	NaOH excess, %	n_{NaOH}	pH	pOH
0	100	0.1	1	0	0	7	7
90	10	0.01	2	0.1	0.0001	10	4
99	1	0.001	3	1	0.001	11	3
99.9	0.1	0.0001	4	10	0.01	12	2
100	0	0	7	100	1	13	1

Another description of titration curve – it is dependence of concentration of examined substance on volume of titrant solution, which is used for titration.

Curve is drawn using data from Table No.3. Instant change in curve is called titration jump. In this case biggest pH jump (from pH = 4 to pH = 10) takes place between 0.1 % HCl excess to NaOH excess.

Indicator color change interval between titration jump is chosen. In our case it can be methyl orange or phenolphthalein. When using phenolphthalein error can take place because of atmospheric CO_2, titration error - difference between equivalent point and end of titration. Because that methyl orange is more often chosen when titrate strong acids with strong alkali (or vice versa). In beginning of titration solution of strong acid is red. During titration and decreasing of acid amount solution becomes orange. Titration proceeds until solution turns yellow after one NaOH drop.

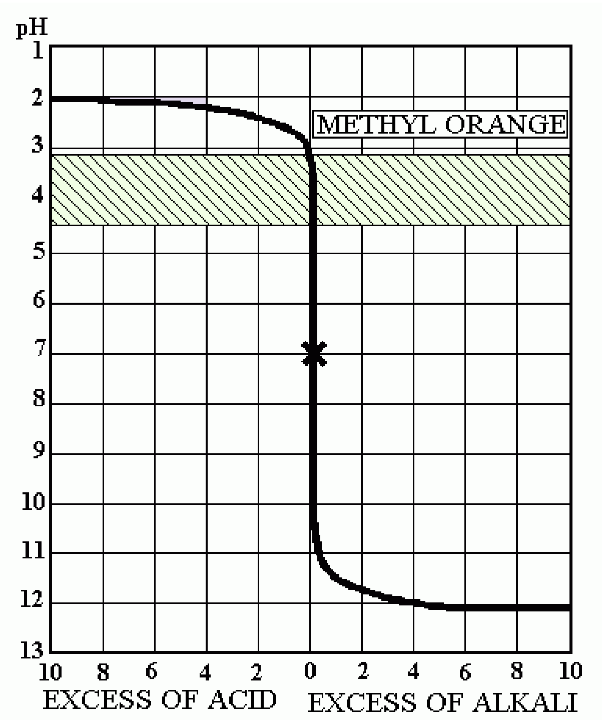


Fig. 2. **Strong acid titration with strong alkali curve.**

When strong alkali is titrated with strong acid, titration changes observed from pH=12 (Fig. No. 2). In the beginning solution is yellow, when amount of alkaline decreases, solution can still remain yellow or change to orange. Titration is recommended to proceed until solution turns red after one drop of HCl (control drop). When calculating HCl volume used for titration, this drop is excluded that is, titration counted from yellow to orange.

4.2.1. Laboratory work No. 5

ESTIMATION OF WORKING HCl SOLUTION CONCENTRATION

Task: Using titration with etalon borax solution estimate working HCl solution concentration. Write a report of analysis.

Procedure:

Prepare titration dishes: burette, pipette, 3 Erlenmeyer flasks (250ml). Wash clean burette with small volume of borax solution. Fill it with etalon borax solution of known concentration (see Laboratory work No. l description). Make sure that air is absent in a burette.

Fix filled burette in vertical position. Wash pipette with l0 ml of investigative solution (in this case HCl). Use pipette to add l0 ml of investigative HCl solution in to three Erlenmeyer flasks. Add 2-3 drops of methyl orange to each flask. Solution becomes red. Note burette data - starting volume for act of analysis (started from, ml). Put Erlenmeyer flask with investigative solution on ceramic tile or white paper to observe changes in color. Titrate with borax solution until yellow color appears. Note burette data for act of analysis (drop to, ml).

During titration following reactions take place:

First borax $Na_2B_4O_7$ salt of weak acid and strong alkali, hydrolyses and then reacts with acid:

$$Na_2B_4O_7 + 7H_2O \rightarrow 2Na^+ + 2OH^- + 4H_3BO_3 \qquad (16)$$

$$2NaOH + 2HC1 \rightarrow 2NaCl + 2H_2O \qquad (17)$$

$$Na_2B_4O_7 + 5H_2O + 2HC1 \rightarrow 4H_3BO_3 + 2NaCl \qquad (18)$$

Then titrate other samples. Difference between results can't exceed +/- 0,2 ml. Calculate volume of borax solution used for titration (used, ml) and average volume (V_B).

Calculate normality of prepared working HCl solution according to mathematical expression of equivalent point. Fill act of analysis as shown in example. Lecturer will check data of titration and calculations. Write estimated molar concentration on label.

Report of analysis No. 1

ESTIMATION OF WORKING HCl SOLUTION CONCENTRATION

For titration:

In burette: $Na_2B_4O_7$, n = _._ _ _ _ (equ/mol·l);

In flasks: l0 ml HCl, n = X (equ/mol·l);

Indicator: methyl orange, 2 drops

Flask number	1	2	3
Titrated until, ml			
Started from, ml			
Used, ml			

Average volume of $Na_2B_4O_7$ **V_B = _. _ _ ml**

$$n_{HCl} = \frac{n_B \cdot V_B}{V_{HCl}} = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \text{(equ/mol} \cdot \text{l)}$$

DETERMINED: The concentration of working solution n_{HCl} = _. _ _ _ _ equ/mol·l

Date

Analysis performed by:

(signature)

4.2.2. Laboratory work No. 6

ESTIMATION OF WORKING NaOH SOLUTION CONCENTRATION

Task: Estimate molar concentration of prepared NaOH solution by titration with HCl of known concentration. Write a report of analysis.

Procedure:

Fill burette with HC1 solution of estimated normality. Add 10 ml of NaOH solution to three Erlenmeyer flasks using pipette, add 2-3 drops of indicator - methyl orange. Solution becomes yellow. Titrate with HC1 until orange color. Note data of titration in table of act of analysis. Calculate normality of NaOH solution accordingly to titration data, write the report No 2. Put estimated molar concentration of solution on the label.

Report of analysis No. 2

ESTIMATION OF NaOH SOLUTION CONCENTRATION

For titration:

In burette: HC1, n = _._ _ _ _ (equ/mol·l);

In flasks: l0 ml NaOH, n = X (equ/mol·l);

Indicator: methyl orange, 2 drops

Flask number	1	2	3
Titrated until, ml			
Started from, ml			
Used, ml			

Average volume of HCl **V_{HCl} = _. _ _ ml**

$$n_{NaOH} = \frac{n_{HCl} \cdot V_{HCl}}{V_{NaOHl}} = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \text{(equ/mol} \cdot \text{l)}$$

DETERMINED: The concentration of working solution n_{NaOH} = _. _ _ _ _ equ/mol·l

Date

Analysis performed by:

(signature)

4.2.3. Laboratory work No. 7

Test No. 1

ESTIMATION OF AMOUNT OF WEAK ACID

When titrate weak acid with strong alkali product of reaction is salt of this acid and strong alkali:

$$CH_3COOH + NaOH \rightarrow CH_3COONa + H_2O \qquad (19)$$

Salt hydrolyses:

$$CH_3COO^- + Na^+ + H_2O \rightarrow CH_3COOH + Na^+ + OH^- \qquad (20)$$

From (20) equation we can see that **$[OH^-] > [H^+]$.**

Because of salt hydrolysis pH>7 in equivalent point (in this case pH= 8.87). We can view pH changes on titration curve.

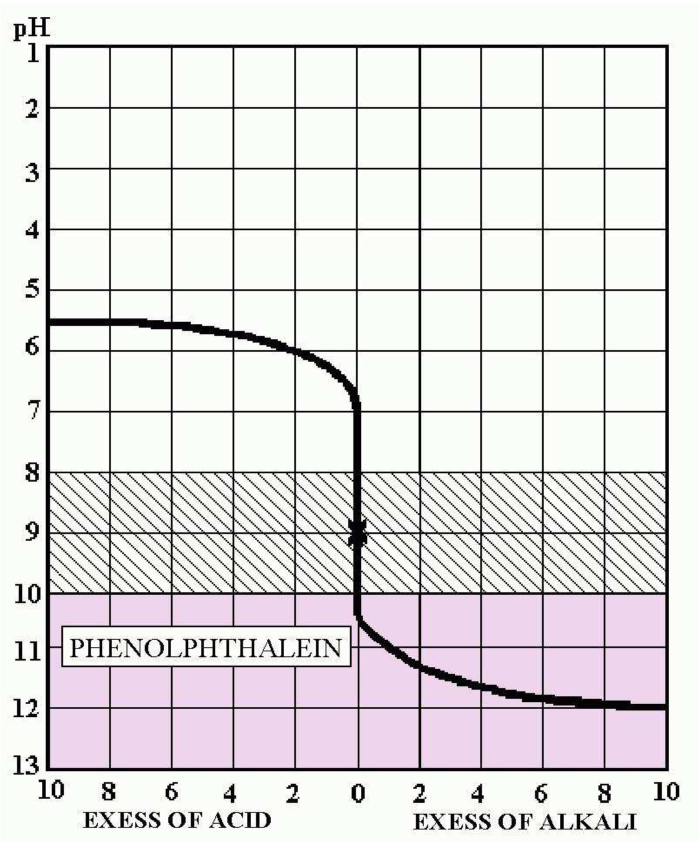


Fig. 3. **Weak acid titration with strong alkali curve**

Color change pH range of phenolphthalein fits to equivalent point, but methyl orange stretches outside titration curve range, so indicator phenolphthalein is suitable to this titration. Remember, when use phenolphthalein solution must be mixed gently, not stirred, because of CO_2 present in air, titration error can take place:

$$CO_2 + H_2O \rightarrow H_2CO_3 \qquad (21)$$

You can heat solution because solubility of CO_2 decreases in higher temperature. Finish titration, when mauve (purplish) color persists for 30 seconds.

When titrate weak alkali with strong acid resulting salt hydrolyses, so at equivalent point pH<7. Choose indicator considering this.

Task: Titrate solution of weak acid. Using titration results calculate molar equivalent concentration of acid (n), titer (T) and amount of weak acid in solution in grams (m), relative error of analysis. Write a report of analysis.

Procedure:

Take clean distilled water washed measure flask Write students first and last name, date and student's journal number on the flask label. Take control sample - unknown amount of weak acid solution. Dilute solution to l00 ml with distilled water, cork it and mix thoroughly. Fill burette with NaOH solution of known concentration. Add l0 ml of solution to three flasks of Erlenmeyer. Add equal amount of indicator phenolphthalein to each flask. Solution must be colorless. Titrate until mauve (purplish) color appears and persists for 30 seconds. Calculate n_r using titration data. Lecturer gives n_{fac}. Calculate relative error R on this data. If R doesn't exceed 5%, calculate titer T and amount of weak acid m (g) Calculate equivalent of organic acid by dividing molecular weight of weak acid by a number of carboxyl groups. Write the report of analysis No 3.

Report of analysis No. 3

ESTIMATION OF CONCENTRATION OF WEAK ACID

Received: solution of unknown volume and concentration of weak acid.

Diluted to: 100 ml

For titration:

In burette: NaOH, n = _._ _ _ _ (equ/mol·l);

In flasks: l0ml __________ acid, n n = X (equ/mol·l);

Indicator: phenolphthalein, 3 drops

Flask number	1	2	3
Titrated until, ml			
Started from, ml			
Used, ml			

Average volume of NaOH **V_{NaOH} = _. _ _ ml**

$$n_{w.a.} = \frac{n_{NaOH} \cdot V_{NaOH}}{V_{w.a.}} = \_\_\_\_\_\_\_\_\_\_\_\_\_\_ = \_.\_\_\_\_\_ \text{ (equ/mol} \cdot \text{l)}$$

$n_{fac.}$ = _._ _ _ _ (equ/mol · l) (lecturer gives)

$$R = \frac{|n_{w.a.} - n_{fac.}|}{n_{fac.}} \cdot 100 = \_.\ \_\ \_\ \%$$ (Relative error can't exceed 5%)

$$E_{w.a.} = \frac{M_{w.a.}}{\text{amount}_{(-COOH)}}$$

$$T = \frac{n \cdot E}{1000} = \_\_\_\_\_\_\_\_\_\_\_\_\_\_ = \_.\ \_\_\_\_ \text{ (g/ml)}$$

$$m_{w.a.} = T \cdot V = \_\_\_\_\_\_\_\_\_\_\_\_\_\_ = \_.\ \_\_ \text{ (g)}$$

DETERMINED: Weight of weak acid ________ m = _. _ _ g

Date

Analysis performed by:

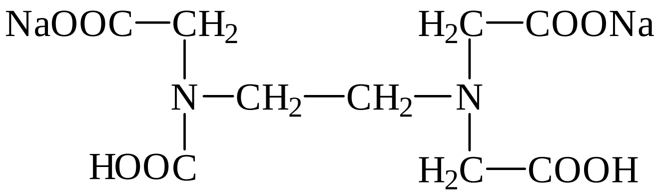
(signature)

4.2.4. Questions and tasks on neutralization method

1. What method is named neutralization method and why?
2. What is equivalent point and how is it expressed mathematically?
3. Indicators, selection of indicators for neutralization method. Color change range of indicators.
4. Character of titration curves, when strong acid iš titrated with strong alkali (or vice versa).
5. Character of titration curves, when weak acid iš titrated with strong alkali and weak alkali is titrated with strong acid. Why in these cases pH isn't equal?
6. Calculation of molar concentration of solution according data of titration.
7. Calculation of solution titer (T) and amount of substance (m).
8. Absolute and relative error. Calculation of errors.
9. Dishes used in volumetric analysis and their preparation for work.
10. Why when use phenolphthalein for titration, solution can't be intensively mixed?
11. What investigations employ methods of volumetric analysis? What physiological liquids can be analyzed by these methods?
12. How much HC1 solution (in ml) with T= 0,0008 g/ml is required to titrate 50ml, 0,15n NaOH?
13. During titration of l0ml NaOH 8ml of 0,1 n HCl solution were used. Calculate NaOH titer (T).
14. What is molar equivalent concentration of NaOH solution, if 9 ml of 0,1 n HC1 solution were used to titrate l0ml of this NaOH solution?
15. During titration of l0ml $Ba(OH)_2$ 50ml of 0,4n HNO_3 solution were used. What amount of $Ba(OH)_2$ (in grams) is present in l00ml of $Ba(OH)_2$ solution?
16. 4,7452g of $Na_2B_4O_7 \times 10H_2O$ was dissolved in distilled water and diluted until 250ml. During titration of l0 ml HCl solution, 12,5ml of borax solution were used. What is molar equivalent concentration of analyzed HC1 solution?

4.3. METHOD OF COMPLEXOMETRY

This method of volume analysis is based on reactions of complex compound formation. Complexometric method has found a wide application since the discovery of organic derivatives, in most cases those of a-amino acids, so called complexones. With numerous ions of metals they form stable complex compounds, called complexonates. One of the complexonates most often used for analysis is disodium ethylendiamintetraacetate (EDTA), also called Trilon B, Complexone III, Titriplex, Versene:

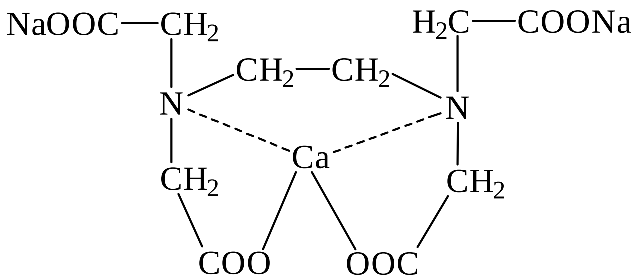


(22)

This is colorless, water-soluble, easy purified substance, abbreviated as $Na_2H_2Tr \cdot 2H_2O$. With ions of different divalent and trivalent metals it forms stable complexonates:

$$Ca^{2+} + Na_2H_2Tr \cdot 2H_2O \longleftrightarrow Na_2[CaTr] + 2H^+ \quad (23)$$

whose structure can be presented like this:



(24)

In case metal ions are colorless, complexonates obtained will be also colorless. If metal ions have a color, complexonates obtained will also be colored. In order to push the equilibrium of complexonate formation to the right, substances binding H+ ions, such as ammonia buffer ($NH_4OH + NH_4Cl$), may be added to the solution.

During the analysis of solutions of calcium and magnesium salts, colorless complexonates are formed; therefore for the visualization of the equivalence point metalochromic indicators (HIn) are used. With metal ions they form intensively colored unstable complexes:

$$Me^{2+} + In^{-} \rightarrow [MeIn]^{+} \quad (25)$$

When the analysis solution is titrated with Trilon B, in the beginning it reacts with free metal ions, which are in excess in the solution, if compared to the indicator:

$$Me^{2+} + H_2Tr^{2-} \rightarrow [MeTr]^{2-} + 2H^{+} \quad (26)$$

and then with the complex $[MeIn]^+$:

$$[MeIn]^{+} + H_2Tr^{2-} \rightarrow [MeTr]^{2-} + In^{-} + 2H^{+} \quad (27)$$

The equivalence point is reached, when the whole complex $[MeIn]^+$ is transformed to the complexonate and titrated solution changes its color, i.e. gets a color of the free indicator. For example, a solution of the indicator Eriochrome Black T has a bright blue color; while with Ca^{2+} and Mg^{2+} ions it forms the red complexonate. During the titration with Trilon B, at the equivalence point, the solution becomes blue. Ethanol solution of this indicator is not stable and has to be used in 10 days after preparation, therefore usually its dry mixture with KCl is prepared (1 : 200).

4.3.1. DETERMINATION OF WATER HARDNESS

Water hardness is caused by dissolved calcium and magnesium salts. It is expressed by millimoles of Ca^{2+} and Mg^{2+} ions in a liter (mmol/l). The water is soft, if there is less than 4 mmol/l of Ca^{2+} and Mg^{2+} ions, it is moderately hard in case of 4-8 mmol/l, hard in case of 8-12 mmol/l, and very hard in case of more than 12 mmol/l. Water hardness can be either temporary (carbonate hardness, TH) or permanent (non-carbonate hardness, PH). Temporary hardness is caused by calcium and magnesium hydrocarbonates $Ca(HCO_3)_2$ and $Mg(HCO_3)_2$ dissolved in water, while permanent hardness is maintained by sulfates, chlorides, silicates, phosphates, and nitrates of calcium and magnesium. Temporary and permanent water hardness form total water hardness (GH).

Elimination of calcium and magnesium salts from water is called water softening. Water can be softened by physical and chemical methods, as well as by distillation. TH can be eliminated by water boiling:

$$Ca(HCO_3)_2 \rightarrow CaCO_3 \downarrow + CO_2 \uparrow + H_2O \quad (28)$$

$$Mg(HCO_3)_2 \rightarrow MgCO_3 \downarrow + CO_2 \uparrow + H_2O \quad (29)$$

Chemical methods comprise softening by slaked lime, soda, sodium phosphate Na_3PO_4, sodium tetraborate $Na_2B_4O_7$, or other materials, which form insoluble carbonates, phosphates, borates of calcium or hydroxide with magnesium salts:

$$Ca(HCO_3)_2 + Ca(OH)_2 \rightarrow CaCO_3 \downarrow + 2H_2O \quad (30)$$

$$CaSO_4 + Na_2CO_3 \rightarrow CaCO_3 \downarrow + Na_2SO_4 \quad (31)$$

$$MgSO_4 + Ca(OH)_2 \rightarrow Mg(OH)_2 \downarrow + CaSO_4 \quad (32)$$

$$3CaCl_2 + 2Na_3PO_4 \rightarrow Ca_3(PO_4)_2 \downarrow + 6NaCl \quad (33)$$

$$CaCl_2 + Na_2B_4O_7 \rightarrow CaB_4O_7 \downarrow + 2NaCl \quad (34)$$

On the industrial scale, water is often softened by means of ion exchange using natural or synthetic macromolecular compounds, called ion exchangers (ionites). Ionites exchanging their cations with those from the water are called cationites. Water is softened by sodium and hydrogen cationites. As sodium cationites it is possible to use natural and synthetic alumosilicates, which are called permutites ($Na_2[Al_2Si_2O_8] \cdot nH_2O$; the general formula Na_2R). They adsorb Ca^{2+}, Mg^{2+} ions from water, releasing Na^+:

$$Na_2R + Ca(HCO_3)_2 \rightarrow CaR + 2NaHCO_3 \quad (35)$$

$$Na_2R + MgSO_4 \rightarrow MgR + Na_2SO_4 \quad (36)$$

Hydrogen cationites (simplified formula HR) are synthetic resins, whose hydrogen can be exchanged by ions of any metals:

$$2RH + Ca(HCO_3)_2 \rightarrow CaR_2 + 2H_2CO_3 \quad (37)$$

$$2RH + MgSO_4 \rightarrow MgR_2 + H_2SO_4 \quad (38)$$

Acids, which are formed, go to the solution. When such water is run through HO^- anionites (simplified formula ROH), HO^- ions of the anionites neutralize hydrogen ions of the acids. Therefore, running water through the system of cationites and anionites allows to get chemically pure water:

cationite process: $$2RH + CaCl_2 \rightarrow R_2Ca + 2HCl \quad (39)$$

anionite process: $$ROH + HCl \rightarrow RCl + H_2O \quad (40)$$

It is possible to regenerate ionites: sodium cationites by 5-10% NaCl solution; hydrogen cationites by H_2SO_4 dilute solution, anionites by alkali solutions:

$$CaR + 2NaCl \rightarrow Na_2R + CaCl_2 \quad (41)$$

$$2NaR + H_2SO_4 \rightarrow 2HR + Na_2SO_4 \quad (42)$$

$$R_2SO_4 + 2NaOH \rightarrow 2ROH + Na_2SO_4 \quad (43)$$

4.3.1.1. Laboratory work No. 8

DETERMINATION OF WATER TEMPORARY HARDNESS

An amount of calcium and magnesium hydrocarbonates in water can be determined titrating by hydrochloric acid:

$$Ca(HCO_3)_2 + 2HCl \rightarrow CaCl_2 + CO_2\uparrow + 2H_2O \quad (44)$$

$$Mg(HCO_3)_2 + 2HCl \rightarrow MgCl_2 + CO_2\uparrow + 2H_2O \quad (45)$$

Task: To titrate by pretitrated HCl solution the unknown water and, for comparison, distilled water. According to data of the titration, to estimate water temporary hardness (TH), in mmol/l. To prepare the report of the analysis.

Procedure:

Prepare the vessels for titration: a burette, a pipette, a volumetric flask of 100 ml, and 3 Erlenmeyer flasks. Place 100 ml of the unknown water measured by pipette or volumetric flask to 2 Erlenmeyer flasks. Add 2-3 drops of methyl orange to each flask. Titrate by HC1 solution of known normality. Estimate average amount **a** (ml) of HCl used. For comparing place 100 ml of distilled water to an Erlenmeyer flask, add 2-3 drops of methyl orange and titrate by the same HCl solution. The amount of HCl used for this titration is **b** (ml). The volume of HC1, which has reacted Ca^{2+} and Mg^{2+} ions, is the following:

$$V_r = a - b \quad \text{(ml)} \quad (46)$$

The amount of HCl used for the reaction is equal to the amount of Ca^{2+} and Mg^{2+} ions, which were present in the solution, i.e. water temporary hardness:

$$TH = \frac{V_r \cdot n_r \cdot 10}{2} = V_r \cdot n_r \cdot 5 \quad \text{(mmol/l)} \quad (47)$$

Report of analysis No. 4

ESTIMATION OF WATER TEMPORARY HARDNESS

Received: about 250 ml of water for analysis (the unknown)

For titration:

In burette: HC1 n = 0.1008 equ/mol·l;

In flasks No. l, 2: 100 ml of the unknown in each;

In flask No. 3: 100 ml of distilled water;

Indicator: methyl orange, 2 drops

Flask number	1	2	3
Titrated until, ml			
Started from, ml			
Used, ml			

Average volume (flasks 1, 2) **V = _. _ _ ml**

Volume of HC1 reacted **V_r = _._ _ ml**

Water temporary hardness:

$$TH = \frac{V_r \cdot n_r \cdot 10}{2} = V_r \cdot n_r \cdot 5$$

$$TH = V_r \cdot n_r \cdot 5 \cdot = \text{\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_} \ (\text{mmol/l})$$

DETERMINED: Temporary hardness of analyzed water TH = _._ _ mmol/1

Date

Analysis performed by:

(signature)

4.3.1.2. Laboratory work No. 9

DETERMINATION OF WATER TOTAL HARDNESS

Task: To titrate by Trilon B the unknown water in alkaline medium obtained using ammonia buffer. According to data of the titration, to estimate water total hardness (GH). To calculate water permanent hardness (PH). To prepare the report of the analysis.

Procedure: Prepare the vessels for titration. Transfer 100 ml aliquots of the unknown water to 3 Erlenmeyer flasks. Add 5 ml of ammonia buffer ($NH_4OH + NH_4C1$) and a bit (size of a safety match's head) of the solid indicator Eriochrome Black T to each flask. Thoroughly mixed content of the flask gets red color. Titrate by Trilon B solution of known concentration. Titration is completed, when one drop of Trilon B solution changes the color of the solution from purple red to green blue, and the addition of one more drop for the control does not change the color any more. According to the data of the titration, calculate water total hardness (GH):

$$GH = \frac{n_{tr.} \cdot V_{tr.} \cdot 1000}{2 \cdot V_{H_2O}}, \qquad (mmol/l) \qquad (48)$$

The permanent water hardness (PH) is obtained when the temporary hardness (TH) is subtracted from the total one (GH):

$$PH = GH - TH, \qquad (mmol/1) \qquad (49)$$

Prepare the report of the analysis.

Report of analysis No. 5

ESTIMATION OF WATER TOTAL HARDNESS

Received: about 350 ml of water for analysis (the unknown)

For titration:

In burette: Trilon B $n_{tr.}$ = 0.05 equ/mol·l;

In flasks No. l, 2,3: 100 ml of the unknown and 5 ml of ammonia buffer in each;

Indicator: Eriochrome Back T.

Flask number	1	2	3
Titrated until, ml			
Started from, ml			
Used, ml			

Average volume **$V_{tr.}$ = _. _ _ ml**

Water total hardness: $$GH = \frac{n_{tr.} \cdot V_{tr.} \cdot 1000}{2 \cdot V_{H_2O}}$$

$GH = V_{tr.} \cdot n_{tr.} \cdot 5 \cdot =$ ______________________ (mmol/l)

$PH = GH - TH =$ ______________________ (mmol/l)

DETERMINED: Total hardness of analyzed water: **GH = _. _ _ (mmol/1);**

Permanent hardness: **PH= _. _ _ (mmol/1);**

Date

Analysis performed by:

(signature)

4.3.2. Questions on complexometry

1. What kind of volumetric analysis is called complexometry?
2. Describe complex compounds.
3. Coordination bond. Its formation according to donor-acceptor mechanism.
4. The structure of complex compounds according to A. Verner.
5. Examples of complexones. Trilon B.
6. Explain the equation of Trilon B reaction with divalent ions of metals.
7. Indicators for complexometry. What causes the changes of their color?
8. Water hardness, types of it and the expression.
9. Which salts maintain temporary and permanent water hardness? Write down the formulas of these salts.
10. Which volumetric methods are used for determination of temporary and total water hardness?
11. How is the temporary water hardness determined and calculated?
12. How are the total water hardness and the permanent one determined and calculated?
13. Methods of water softening. Write down the equation for the reaction, when the temporary water hardness is removed by water boiling.
14. Chemical methods of water softening, equations of the reactions.
15. Water softening by sodium and hydrogen cationites and anionites.
16. Regenerating of ionites used for water softening.

4.4. METHODS OF REDOX TITRATION

These are volumetric methods, based on oxidation/reduction reactions. According to the oxidizing agent used for titration, those methods may be divided into:

Permanganatometry: As a work solution, standardized $KMnO_4$ solution is used. In storage, its concentration changes under the influence of environment. Therefore $KMnO_4$ solution is prepared of approximately 0,05 n, and before the work the concentration is standardized against the standard solution of oxalic acid:

$$2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 = 2MnSO_4 + 10CO_2 + K_2CO_3 + 8H_2O \qquad (50)$$

In the beginning of titration, the solution is heated to 70°C to increase the rate of the reaction (oxalic acid formed decomposes at elevated temperature). Further on the reaction proceeds smoothly, because Mn^{2+} ions produced act as a catalyst. This phenomenon is called autocatalysis. In acid medium, the purple MnO_4^- ion is reduced to colorless Mn^{2+} ion:

$$MnO_4^- + 8H^+ + 5\bar{e} \rightarrow Mn^{2+} + 4H_2O \qquad (51)$$

In this case the equivalence point is detected by the purple color, appearing after the addition of the first excess drop of $KMnO_4$. In this way, the minimal excess of the colored oxidant plays the role of indicator in permanganatometry. This method is usually used for determination of a reducing agent; however, it can be applied to determine an oxidant as well. For this purpose, to an aliquot of an oxidant the strictly determined amount of a reductant is transferred in the certain excess. This excess of the reductant is then titrated by work solution of $KMnO_4$. Such an indirect titration is called **retitration.**

By the permanganatometry, it is possible to determine the amounts of the following substances: nitrites, thiocyanates, hydrogen peroxide, ferrous salts, aldehydes, carboxylic and hydroxy acids and their salts.

Iodometry: As standard solution I_2 solution is used, and the following reaction takes place during the titration:

$$I_2 + 2\bar{e} = 2I^- \qquad (52)$$

Iodometry allows to determine an amount of both reductant and oxidant. For determination of reductant, titration is carried out using iodine solution, which is prepared weighting iodine crystals, purified by sublimation. In this case the reaction (48) proceeds to the right. For determination of an oxidant, the solution to be analyzed is reacted with I ions in excess; in this stage the part of I^- ions, which is equivalent to the amount of the analyzed oxidant, is oxidized to free iodine (I_2), i.e. the reaction (48) proceeds to the left. I_2 produced iš then titrated by the solution of thiosulfate:

$$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI \qquad (53)$$

Both products of the reaction have no color, therefore for the detection of the equivalence point 0,05-0,1% starch solution is used. Titration is carried out until the single drop of $Na_2S_2O_3$ makes the solution colorless. Iodometry finds wide application for determination of oxidizing agents - chromates, hypochlorites, free halides, and reducing agents - sulfldes, sulfltes, arsenic ions.

Bromatometry: During titration the following reaction takes place:

$$Br_2 + 2\bar{e} = 2Br^- \qquad (54)$$

Chromatometry: During titration in acid medium, the following reaction proceeds:

$$Cr_2O_7^{2-} + 14H^+ + 6\bar{e} \rightarrow 2Cr^{3+} + 7H_2O \qquad (55)$$

Cerimetry.
Vanadatometry.

4.4.1. Laboratory work No. 10

DETERMINATION OF THE CONCENTRATION OF $KMnO_4$ WORK SOLUTION

Task: To determine molar concentration of equivalents of $KMnO_4$ work solution, standardizing it against standard oxalic acid $H_2C_2O_4$ solution.

Procedure: Prepare vessels for titration.

Fill burette with $KMnO_4$ working solution. Pipet 10 ml aliquots of standard oxalic acid ($H_2C_2O_4$) solution to the each of three Erlenmeyer flasks. Transfer 5 ml aliquots of 2 n H_2SO_4 solution to the each flask. Heat the content of the flask up to 70°C and titrate until the single drop of potassium permanganate is enough for the solution to get purple. If the amount of acid is not enough, brown precipitate of MnO_2 can be formed. Calculate the normality of $KMnO_4$ solution. Prepare the report of the analysis.

4.4.2. Laboratory work No. 11

Test No. 2

DETERMINATION OF THE AMOUNT OF IRON (II)

In acid medium, ions of divalent iron (Fe^{2+}) are easy oxidized by $KMnO_4$ to Fe^{3+}:

$$2KMnO_4 + 10FeSO_4 + 8H_2SO_4 \rightarrow 2MnS0_4 + 5Fe_2(SO_4)_3 + K_2SO_4 + 8H_2O \qquad (56)$$

Task: To titrate the unknown solution of divalent iron compound with $KMnO_4$ work solution of known concentration. According the titration data, to calculate n (equ/mol·l), T (g/ml) and m (g) of the unknown, as well as the error R (%).

Procedure: Prepare vessels for titration.

Submit 100 ml volumetric flask to obtain the unknown solution of Fe^{2+} salt. Dilute the solution to 100 ml and mix thoroughly. Pipet 10 ml aliquots of the prepared solution with the analyte to the each of three Erlenmeyer flasks and add 5 ml aliquots of 2n H_2SO_4 solution to the each flask. Titrate with $KMnO_4$ solution of restandardized concentration in the burette. According to the data of the titration, estimate **normality (n), titer (T), the amount (m) of divalent iron and the error of the work (R).** Make the report of the analysis (see the form of the report). Write down the redox reactions taking place during the titration.

4.4.3. Questions and exercises on redox titration

1. What is redoximetry based on?
2. Definition of oxidation – reduction phenomenon. Main oxidants and reductants.
3. Oxidation degree, reactions of oxidation and reduction. Formation of equations for these reactions.
4. Methods of redoximetry. Their classification.

5. Permanganatometry. Titrated and standard solutions and indicators for permanganatometry.
6. Reduction of $KMnO_4$ in acid, neutral and alkaline medium.
7. What is the difference to determine oxidant and reductant in permanganatometry? How to determine an amount of divalent iron?
8. What materials can be analyzed by permanganatometry?
9. Iodometry. What is called retitration?
10. Which amount (in grams) of $KMnO_4$ is dissolved in 470 ml of 0.05 n solution used for permanganometry?
11. 16 g of $FeSO_4$ is dissolved in one liter. Which amount of this solution can be oxidized by 25 ml of 0.ln $KMnO_4$ solution?
12. What is a molar concentration of $KMnO_4$ equivalents, if 95 ml of its solution were titrated by 50 ml of the solution, where 1.8 g of Mohr salt $(NH4)_2Fe(SO_4)_2 \cdot 6H_2O$ was dissolved?
13. How many ml of 0.045n oxalic acid is needed for titration of 50 ml of $KMnO_4$ solution, whose titer T = 0.004 g/ml?
14. How many ml of 0.01n iodine solution are needed for titration of 150 ml of the solution, where 0.24 g of Na_2S2O_3 has been dissolved?
15. What amount (in grams) of iodine had been dissolved in 50 ml of the solution, which was titrated with 60 ml of 0.01n $Na_2S_2O_3$ solution?
16. Balance the following redox equations:

$KMnO_4 + HC1 \rightarrow MnCl_2 + C1_2 + KCl + H_2O$

$KMnO_4 + FeSO_4 + H_2SO_4 \rightarrow MnSO_4 + Fe_2(SO_4)_3 + K_2SO_4 + H_2O$

$KMnO_4 + Na_2SO_3 + H_2O \rightarrow MnO_2 + Na_2SO_4 + KOH$

$Cr_2(SO_4)_3 + Br_2 + KOH \rightarrow KBr + K_2CrO_4 + K_2SO_4 + H_2O$

$Fe + HNO_3 \rightarrow Fe(NO_3)_3 + N_2O + H_2O$

$KMnO_4 + CO + KOH \rightarrow K_2MnO_4 + K_2CO_3 + H_2O$

$Na_2SO_3 + KMnO_4 + H_2O \rightarrow Na_2SO_4 + MnO_2 + KOH$

$MnSO_4 + KOH + KClO_3 \rightarrow K_2MnO_4 + KC1 + K_2SO_4 + H_2O$

$K_2Cr_2O_7 + FeCl_2 + H_2SO_4 \rightarrow Cr_2(SO_4)_3 + K_2SO_4 + FeCl_3 + Fe_2(SO_4)_3 + H_2O$

$NaBr + MnO_2 + H_2SO_4 \rightarrow MnSO_4 + Na_2SO_4 + Br_2 + H_2O$

5. QUALITATIVE ANALYSIS

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. Qualitative composition of materials can be determined by physical, physical-chemical and chemical methods of analysis. The methods of chemical analysis are based on the observation of changes occurring in chemical reactions and rest on the statement of the theory of electrolyte dissociation about the **additivity** of ion's properties, i. e. the same properties of the same ion in spite of the mixture and the properties of a solution being the sum of the properties of the ions presented.

Unknown material is converted to the compound with well-known characteristic properties. Such a conversion is called **analytic reaction**, while the substance inducing such a change is the **reagent**.

Analytic reactions are irreversible ion exchange reactions of various types in electrolyte solutions. Clearly expressed external effect of those reactions (analytic signal) evidences the presence of the ion in the solution. For example, when the unknown solution is affected by the reagent and the precipitate is formed or dissolved, or the color of the unknown solution changes or the gas is produced, here we have the analytic signal. Analyzer has to be well acquainted with the external effect of analytic reactions, analytic signal.

Reactions in electrolyte solutions proceed between ions. Ions in electrolyte solutions react irreversibly, i.e. **ion exchange reaction is irreversible, when difficulty soluble compounds, gases, weak electrolytes (acids, bases, water), complex compounds are formed**. In the equations of ion reactions, difficulty soluble compounds, gases, weak electrolytes are indicated as molecules, while strong electrolytes as ions. For example, white precipitate of difficulty soluble barium sulfate is formed, when sodium sulfate reacts with barium chloride.

Molecular equation of the reaction:

$$BaCl_2 + Na_2SO_4 \rightarrow BaSO_4\downarrow + NaCl \qquad (57)$$

Ionic equation of the reaction:

$$Ba^{2+} + 2Cl^- + 2Na^+ + SO_4^{2-} \rightarrow BaSO_4\downarrow + 2Na^+ + 2C1^- \qquad (58)$$

Short ionic equation of the reaction:

$$Ba^{2+} + SO_4^{2-} \rightarrow BaSO_4\downarrow \qquad (59)$$

Solubility of compounds determines formation of precipitate. Since there are no totally insoluble substances, dynamic equilibrium gets established between the precipitate and solution. At constant temperature, multiplication of the concentrations of difficulty soluble electrolyte ions in saturated solution is constant and called **solubility product,** defined as **L**.

During the above reaction $BaSO_4$ is formed. Its solubility product is the following:

$$L = \left[Ba^{2+}\right] \times \left[SO^{2-}\right] = 1{,}1 \times 10^{-10} \qquad (60)$$

An important characterization of an analytic reaction is its **sensitivity,** i. e. possibility to detect minimal amounts of test material. **Sensitivity of an analytic reaction is expressed quantitatively as the minimal amount of explored substance (ion) in mg (milligrams), minimal concentration of explored substance (ion) in solution C_{lim} or the limit of dilution (e.g. 1 : 100 000), which is still detectable by expected external effect of the analytic reaction.**

Subject to the amount of the material required for the qualitative analysis, it can be classified as macro-, micro and semimicro-analysis. Qualitative analysis can be carried out in solution or in dry state (reactions of „pearls”).

Another one important characteristic of analytic reaction is its **specificity.**

Specific reaction is called a reaction, specific to one ion or molecule and not interfered by other ions in the solution. For example, NH_4^+ ion analytic reaction with NaOH is specific one. When the reaction mixture is heated, only salts of ammonium produce ammonia:

$$NH_4Cl + NaOH \rightarrow NaCl + NH_3\uparrow + H_2O \qquad (61)$$

$$NH_4^+ + Cl^- + Na^+ + OH^- \rightarrow Na^+ + Cl^- + NH_3\uparrow + H_2O \qquad (62)$$

$$NH_4^+ + OH^- \rightarrow NH_3\uparrow + H_2O \qquad (63)$$

An external effect of this reaction is the odor of produced ammonia gas.

The work of an analyzer would be very easy, if 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. The fewer ions in the group, the higher selectivity of the reagent.

Selective reagents, which sedimentate from solution ions of the certain group, are called group reagents. According to the group reagents, ions are divided to **analytic groups.** For example, 6 n HCl solution is the group reagent of the first analytic group of cations, Ag^+, Hg^{2+}, Pb^{2+} , which form with it insoluble white chlorides.

There are several ways to classify ions to analytic groups. Organic reagents of high sensitivity can be also applied for qualitative analysis of inorganic compounds. For instance, oxyquinoline (C_9H_6NOH), alizarin [$Cl_4O_2(OH)_2$], benzidine ([$Cl_2H_8(NH)_2$], diphenylamine [$(C_6H_5)_2NH$] and other organic reagents can be used to detect microelements (Cu^{2+}; Zn^{2+}; Co^{2+}; Ni^{2+}; Cd^{2+}) in soil, plants and products of farming.

Analytic reactions are used to determine not a chemical substance, but its cations and anions, because analytic reactions are ion exchange reactions in electrolyte solutions. Therefore, data of the analysis allow to consider the chemical formula of the compound. For example, if Fe^{2+} and SO_4^{2-} ions were found in the unknown solution, the conclusion may be drawn that the analyte is $FeSO_4$ salt. The most widely spread cations (there are about 25 of them) and about the same of anions can form around six hundred neutral salts. For their analysis it is enough to know fifty analytic reactions. Much more complicated is the determination of the formula of an organic compound, where next to qualitative analysis the quantitative one has to be carried out.

Determination of one ion in solution is to be done in a certain order, since some ions interfere the detecting of other ions, i.e. they hinder characteristic effects or induce secondary phenomena, impeding the analysis. During the analysis, the certain sequence of the reactions has to be kept, which is called **analysis procedure or systemic analysis; every ion is to be analyzed after detection or separation of ions, which hinder the analysis**. For the systemic analysis, one should know not only analytic reactions of certain ions, but also the methods of separation or removal of ions or ion groups. For this reason cations and anions are divided to analytic groups according to their group reagent. Cations may be divided to five analytic groups due to different solubility of their chlorides, sulfates and carbonates (there are also another ways of their grouping). All cations interfering with anion detection, can be precipitated by Na_2CO_3 as carbonates. Grouping of anions is more varied and less important than that of cations. For instance, the following ions are attributed to the first analytic group of anions: SO_3^{2-}; SO_4^{2-}; CO_3^{2-}; SiO_3^{2-}; PO_4^{3-}; CrO_4^{2-}; AsO_4^{3-}. Group reagent $BaCl_2$ with those anions forms precipitate of insoluble salts, which are soluble in acids, except $BaSO_4$. So, the complicated task of analysis is simplified into few more elementary tasks due to division of ions to analytic groups with the help of group reagents. Nowadays ions extensively are precipitated by chromatographic methods of ion exchange, where macromolecular compounds with acid or basic properties work as sorbents, cationites and anionites.

Quick qualitative detection of some ions can be accomplished by the reaction of flame coloration. Subject to qualitative composition of analysis solution, flame gets the certain colors. The color of the flame is an indicator of the presence of the particular ion in the solution.

5.1. Laboratory work No. 12

ANALYTIC ION REACTIONS

Task 1: To carry out analytic reactions of cations and anions presented in the table; to put down and memorize the effects of reactions (analytic signals). To write down equations of analytic reactions in molecular, ionic and short ionic form.

Task 2: To perform reactions of flame coloration by Ca^{2+}, Ba^{2+}, K^{+}, Na^{+} cations.

Procedure 1: Carry out the reactions l to 15 (Table No. 4) transferring l ml of solutions l and 2 into clean test tubes (measuring not required).

Put the observed effect of the reactions to Table No 4. For example, the first reaction for detection of Ag^{+} ion is to be carried out in the following way: Transfer l ml of $AgNO_3$ (solution No. 1) without measuring to a clean test tube (the height of the solution in test tube to be about l cm). Transfer about l ml 6n HC1 solution (solution No. 2) to the same test tube. Observe the effect of the reaction, when white precipitate is formed; write it down to the Table No. 4.

Table No. 4. **ANALYTIC ION REACTIONS AND THEIR EFFECTS**

Serial No	**Ion to detect**	**Solution No. 1** (with the ion to detect)	**Solution No. 2** (the reagent)	**Reaction effect** (analytic signal)
1.	Ag^{+}	$AgNO_3$	HCl	**white precipitate**
2.	Hg_2^{2+}	$Hg_2(NO_3)_2$	HCl	
3.	Hg_2^{2+}	$Hg_2(NO_3)_2$	KJ	
4.	Pb^{2+}	$Pb(NO_3)_2$	HCl	
5.	Pb^{2+}	$Pb(NO_3)_2$	KJ	
6.	Hg^{2+}	$Hg(NO_3)_2$	KJ (drops)	
7.	Cu^{2+}	$CuSO_4$	NH_3	
8.	Fe^{2+}	$FeSO_4$	$K_3[Fe(CN)_6]$	
9.	Fe^{3+}	$FeCl_3$	NH_4CNS	
10.	NH_4^{+}	NH_4Cl	NaOH	
11.	CO_3^{2-}	K_2CO_3	HCl	
12.	CO_3^{2-}	K_2CO_3	$BaCl_2$	
13.	SO_4^{2-}	Na_2SO_4	$BaCl_2$	
14.	Cl^{-}	NaCl	$AgNO_3$ (drops)	
15.	NO_3^{-}	$NaNO_3$	$FeSO_4$, H_2SO_4	

At the end of the report write the equation of the reaction in molecular, ionic and short ionic form:

$$HCl + AgNO_3 \rightarrow AgCl\downarrow + HNO_3 \quad (64)$$

$$H^+ + Cl^- + Ag^+ + NO_3^- \rightarrow AgCl\downarrow + H^+ + NO_3^- \quad (65)$$

$$Ag^+ + Cl^- \rightarrow AgCl\downarrow \quad (66)$$

Then carry out the analytic reaction of the next ion, observe, memorize, put down to the table the effect of the reaction (analytic signal), write down the equations of the reaction and so on.

Analytic reaction for detection of Cu^{2+} ion (Table No. 4, reaction 7) is to be carried out in the following way: mix l ml of $CuSO_4$ solution with l ml of NH_4OH solution. At first, bluish white precipitate of copper hydroxide is formed (l stage of the reaction):

$$CuSO_4 + 2NH_4OH \rightarrow Cu(OH)_2\downarrow + (NH_4)_2\ SO_4 \quad (67)$$

$$Cu^{2+} + SO_4^{2-} + 2NH_4^+ + 2OH^- \rightarrow Cu(OH)_2\downarrow + 2NH_4^+ + SO_4^{2-} \quad (68)$$

$$Cu^{2+} + 2\ OH^- = Cu(OH)_2\downarrow \quad (69)$$

This precipitate of $Cu(OH)_2$ is dissolved in excess ammonia, and tetraamonia ion is formed (2 stage of the reaction). The solution becomes blue; the color intensity depends on the concentration of Cu^{2+}:

$$Cu(OH)_2 + 4NH_3 \rightarrow [Cu(NH_3)_4](OH)_2 \quad (70)$$

$$Cu^{2+} + 2OH^- + 4NH_3 \rightarrow [Cu(NH_3)_4]^{2+} + 2OH^- \quad (71)$$

$$Cu^{2+} + 4NH_3 \rightarrow [Cu(NH_3)_4]^{2+} \quad (72)$$

Analytic reaction for detection of NO_3^- ion (Table No. 4, reaction 15) should be carried out in the following way: transfer l ml of $NaNO_3$ solution to a clean test tube, add a little amount (with a head of safety-matches) of $FeSO_4$ crystals, which are solved in the solution on the tube shaking. Carefully pour by a wall of the inclined tube in slight flow about l ml of concentrated H_2SO_4. Between unmixed layers of the solutions of different density, the greenish brown „ring of nitrate” is formed. The reaction proceeds in two stages. In the first stage, ferrous sulfate reduces NO_3^- to NO:

$$6FeSO_4 + 3H_2SO_4 + 2HNO_3 \rightarrow 3Fe_2(SO_4)_3 + 2H_2O + 2NO \quad (73)$$

$$6Fe^{2+} + 8H^+ + 2NO_3^- \rightarrow 6Fe^{3+} + 2NO + 4\ H_2O \quad (74)$$

In the second stage, NO and ferrous sulfate form greenish brown, unstable complex compound, ferrous nitrosyl sulfate:

$$NO + FeSO_4 \rightarrow [Fe(NO)]SO_4 \quad (75)$$

$$NO + Fe^{2+} \rightarrow [Fe(NO)]^{2+} \quad (76)$$

I^- and Br^- ions interfere with this reaction, forming „rings” of another color.

2. For flame coloration reactions, use platinum wire after washing it by concentrated hydrochloric acid and heating on gas burner flame. Dip the heated platinum wire into analysis solution and then into the colorless flame of gas burner. After examination of flame color of ions, presented in the table No. 5, put down the data obtained to the table:

Table No. 5. **REACTIONS OF FLAME COLORATION BY CATIONS**

Serial No.	Cation	Color of the flame
1.	K^+	
2.	Ca^{2+}	
3.	Ba^{2+}	
4.	Na^+	

5.2. Laboratory work No. 13

Test No. 3

ANALYSIS OF A SALT

Task: To carry out the qualitative analysis of the unknown salt; i.e. to determine the cation and the anion of the salt. To prepare the report of the salt analysis. To write down equations of analytic reactions.

Procedure: Dissolve the received unknown salt in test tube in distilled water. If the salt does not dissolve, heat it. Prepare the salt solution for analysis (SA) in such an amount, which would be enough for the whole analysis. Analytical ion reactions for the salt analysis are given in the Table No 4 of the experiment No 10; just only instead of solution l the SA has to be taken. The analysis sequence is presented in the form of salt analysis report. The analysis may be divided into two parts:

5.2.1. Cation determination

At first, analytic reaction of NH_4^+ ion is carried out. Pour about l ml of SA and l ml of NaOH to a clean test-tube. The expected analytic signal is the odor of ammonia (mixture of the reaction may be heated). In case the odor of ammonia gas is felt, the conclusion may be drawn about the presence of NH_4^+ ion in SA. If the odor of ammonia is not felt, it means the absence of NH/ ion in SA. Write down the effect of performed analytic reaction and conclusion about the presence or absence of the ion into the column of conclusions of analysis report. Then perform the analytic reaction of CO_2^{2-}. Pour l ml of SA and l ml of HCl into a clean test tube. The expected effect of the reaction (analytic signal) is discharge of CO_2^{2-} gas. The effect of performed analytic reaction allows to conclude about the presence or absence of CO_2^{2-} in SA. Write down the effect of the analytic reaction and the conclusion to the analysis report once more. If under the treatment with HCl white precipitate was formed, it shows the presence of the first analytic group cations Ag^+, $Hg2^{2+}$, Pb^{2+} in the solution. To determine, which of those cations is presented in SA, perform the specific reaction of Ag^+, Hg_2^{2+}, Pb^{2+} ions (see Table No. 4). After that carry out analytic reactions for detection of Fe^{2+}, Fe^{3+}, Cu^{2+} ions (see Table No. 4). If there is no expected effect of analytic reaction or some another effect, make a conclusion about the absence of the expected ion in SA. If none of above reactions shows any cation in SA, the possibility is that Ca^{2+}, Ba^{2+}, K^+ or Na^+ is present in the salt solution. It should be determined by the flame coloration reactions (see the Laboratory work No. 8).

5.2.2. Anion determination

Some cations (Ag^{+}, Hg_2^{2+}, Hg^{2+}, Pb^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, Ca^{2+}, Ba^{2+}) hinder anion analysis; therefore, they should be removed from the analysis solution. $NH4^{+}$, K^{1} or Na^{+} do not interfere with anion analysis, thus they may be left in the solution. In order to remove interfering cations, boil the rest of SA left after cation analysis for ten minutes with Na_2CO_3. Carbonate precipitate, formed during the boiling, is filtrated and thrown away. Portion the filtrate in two parts. Neutralize one part of the filtrate by HNO_3 for detecting of Cl^{-}, SO_4^{2-}ions (Table No. 4). Neutralize another part of the filtrate by HC1 for detecting NO_3^{2-} ion (Table No. 4, reaction 15).

Prepare the analysis report. Give the analytic reactions of ąualitative determination for the cation and the anion detected in molecular, ionic and short ionic equations,

5.3. Questions on qualitative analysis

1. The aims and methods of qualitative analysis.
2. How is analytic reaction defined? Requirements for analytic reactions.
3. Irreversible reactions in electrolyte solutions. Equations of those reactions.
4. Definition of solubility product.
5. Definitions of reagent and specific, selective, group reagent.
6. Analytic reactions of cations and anions (qualitative reactions of ion determination).
7. Analytic signal as an external effect of analytic reaction.
8. Reactions of flame coloration.
9. Write down analytic reactions of ions from Table No 4 (Laboratory work No 10) in molecular, ionic and short ionic form. Give the analytic signals of those reactions (reaction effects).
10. What is the sequence of the analysis and why is it essential for qualitative analysis?
11. How to eliminate cations interfering with the analysis? Which chemicals are used to neutralize the solution after removal of interfering cations?

Report of analysis No. 6

SALT ANALYSIS

In the test tube No. __ approx ___ g of ______________ salt (describe the obtained salt: colour, crystal shape, etc.) was received.

Serial No.	Operation performed	Reaction effect (analytic signal)	Conclusion
1	The salt solved in distilled water	Dissolved	SA, salt solution for analysis, obtained
2	SA + NaOH on heating	No odor of NH_3 gas	No NH_4^+
3	SA + HCl		
4	SA + KI		
5	SA + NH_4OH		
6	SA + $K_3[Fe(CN)_6]$		
7	SA + NH_4CNS		
8	Reaction of flame coloration		
9	SA + $AgNO_3$		
10	SA + $BaCl_2$		
11	SA + $FeSO_4$ + conc. H_2SO_4		

The conclusion: The formula of the salt obtained is_____________.

Analytic reaction of qualitative determination of _________ ion:

Date

Analysis performed by:

(signature)

6. POTENTIOMETRY

Potentiometric analysis is based on measurement of electromotive force of reversible **galvanic cells.** In potentiometry usually one uses the galvanic cell of two electrodes, immersed into the same solution or into two solutions of different content, which are connected by liquid contact.

Electrode with a potential depending on the concentration (activity or effective concentration) of the certain ions in the solution is called **indicator electrode.** For pH measurements, the glass electrode is usually used as indicator electrode. In order to measure the concentration of other ions presented in the solution (e.g. NO_3^-, Na^+, Cl^-, etc.) membrane electrodes are applied. The state-of-art membrane electrodes of ferments make possible to measure directly the concentration of some organic substances in solutions.

To measure the potential of indicator electrode, the second electrode is immersed into the solution, and its potential is not dependent on the concentration of ions in the solution. Such an electrode is called **reference electrode.** As reference electrode the calomel one is often used. In modern pH-meters for measurements it is often enough of indicator electrode alone.

Electrode potential is measured by a device called **potentiometer** (often simply **pH-meter, ionmeter).** Those instruments are calibrated in units of pH (of pI in ionmeters) or millivolts.

The main advantage of potentiometric methods compared to other techniques is that they are simple and quick. The time for the potential of indicator electrodes to get steady is short. Therefore this method can be used for investigations of reaction kinetics, for automatic control of technological processes. Potentiometric measurements can be carried out in viscous, turbid, colored solutions, without filtration or distillation. Glass-calomel electrodes are used to measure pH in solutions with strong oxidants, reductants, proteins, etc. There are the glass electrodes designed for the special measurements. They allow to measure pH in a drop of a liquid, in human perspiration of skin, in tooth cavity. When pH of stomach juice is measured, small glass electrodes are swallowed (in this case the calomel electrode stays in the mouth).

Potentiometry is sample saving method of measurements, therefore the analyzed solution can be used for the further investigations.

Accuracy of the measurements is about 0,01 unit of pH.

6.1. Laboratory work No. 14

ANALYSIS OF BUFFER SOLUTIONS

Buffer solutions are such solutions, where the concentration of hydrogen ions stays constant on dilution or adding the certain amount of strong acids or alkalis to them. The quality of the solutions to keep the constant concentration of hydrogen ions (constant pH) is called **buffer activity.**

Buffer solutions are made of:

- weak acid and its salt of strong base (buffer systems of acetate, hydrocarbonate, proteins, phosphate, hemoglobin);
- weak base and its salt of a strong acid (buffer systems of ammonia).

A number of biological liquids of living systems are buffer solutions. For instance, pH of human blood is 7,36 and does not change due to hydrocarbonate, hemoglobin, protein and some other buffer systems in the blood. Constant pH of bio liquids and tissues is necessary for normal run of metabolism reactions, other biochemical processes.

Task: To prepare acetate buffer solution, calculate and measure pH of it. To determine the buffer capacity of the prepared solution by alkali and by acid as well. To find out the influence of dilution on pH of buffer solution.

6.1.1. Preparation of buffer solution

Procedure: Prepare 30-40 ml of acetate buffer solution. For this purpose transfer the indicated by supervisor amount of 0,1 n acetic acid and 0,1 n sodium acetate from burettes to 100 ml water-glass.

pH of the buffer solution prepared (pH_c) is calculated using the following formula:

$$pH_c = -\lg C_{H^+} = -\lg\left(K_a \cdot \frac{V_a}{V_s}\right) \qquad (77)$$

Where: K_a – dissociation constant of acetic acid; $K_a = 1{,}8 \cdot 10^{-5}$;
V_a – volume of 0,1n acetic acid solution, ml;
V_s – volume of 0,1n sodium acetate solution, ml.

Then use a universal indicator slip to determine (approximately by the scale of colors) pH of prepared buffer solution. Put down the data to Table No. 8.

Table No. 6. **DETERMINATION OF pH OF BUFFER SOLUTION**

Components of buffer solution, ml		**pH of the prepared buffer solution**			
0,1 n solution of acetic acid	0,1 n solution of sodium acetate	calculated **pH_c**	according to universal indicator	measured by pH-meter **pH_0**	after dilution **pH_d**

Write the measured value of buffer solution pH into the Table No. 6.

6.1.2. Determination of buffering capacity

Capability of different buffer solutions to resist to changes of pH on adding acid or alkali is not the same. It depends on the total concentration of species forming the buffer system, as well as the ratio of ingredient concentrations. Therefore each buffer solution is characterized by **buffering capacity,** a number of equivalents of strong acid or alkali, which changes pH of l liter of the buffer solution by one unit.

Procedure: Pipet 10 ml aliquots of the buffer solution prepared in the first part of the experiment to each of two flasks. Add 3 drops of the indicator (methyl orange) to the first flask and titrate it by 0,ln HC1 solution until rose orange ($pH_1 = 3{,}4$). Calculate the buffer capacity B by acid (see the example of calculations).

Add 3 drops of the indicator (methyl red) to the second flask and titrate it by 0,1n NaOH solution until yellowish color ($pH_1 = 6{,}3$). Calculate the buffer capacity by alkali.

For the calculations use the following formula:

$$B = \frac{gE_{NaOH(HCl)}}{pH_1 - pH_0} \qquad (78)$$

Where: B – buffer capacity by acid or alkali, in equ;
$gE_{NaOH(HCl)}$ – the number of equivalents of the alkali or acid used to titrate l liter of the buffer solution, in equ;
pH_0 – the buffer solution pH measured by pH-meter;

pH_1 – pH at the end of titration. Put down the results of calculations to the Table No. 7.

Table No. 7. **DETERMINATION OF BUFFERING CAPACITY**

Usage of 0,1n HCl solution, ml	Usage of HC1 **gE_{HCl}**, gE	Buffering capacity by HCl **B_{ac}**, gE	Usage of 0,1n NaOH solution, ml	Usage of HC1 **gE_{NaOH}**, gE	Buffering capacity by NaOH **B_{alk}**, gE

6.1.3. Influence of dilution on pH of buffer solution

Procedure: Measure by volumetric cylinder the volume of the buffer solution left after the previous experiment and dilute it by the same volume of distilled water. After mixing of the solution, measure by pH-meter pH of the diluted buffer solution (pH_d). Put the data obtained to the Table No. 6. Make a conclusion on the influence of dilution on pH of the buffer solution.

6.1.4. Examples of calculations

1. Calculate pH of buffer solution, prepared of 30 ml of 0,1n acetic acid solution and 10 ml of 0,ln sodium acetate solution:

$$pH = -\lg\left(1.8\cdot10^{-5}\cdot\frac{30}{10}\right) = -\lg\left(5.4\cdot10^{-5}\right) = -\lg 5.4 - \lg 10^{-5} = -0.73 + 5\lg 10 = -0.73 + 5 = 4.27$$

2. Calculate the buffering capacity of buffer solution with pH_0 = 5,5 10 ml of which was titrated by 2,0 ml of 0,1n NaOH solution (the indicator was methyl red).

First of all we calculate how many gramequivalents of NaOH would be used to titrate l liter of the buffer solution:

$$gE_{NaOH} = \frac{V_{NaOH}\cdot n_{NaOH}\cdot 1000}{10\cdot 1000} = \frac{2\cdot 0.1}{10} = 0.02 \quad \text{(equ)}$$

Because on titration pH has changed from 5,5 (pH_0) to 6,3 (pH_1), so the buffering capacity by alkali (B_{alk}) is equal:

$$B_{alk} = \frac{gE_{NaOH}}{pH_1 - pH_0} = \frac{0.02}{6.3 - 5.5} = \frac{0.02}{0.8} = 0.025 \quad \text{(equ)}$$

Thus, pH of the buffer solution would change by l unit on adding 0,025 equ NaOH to l liter of this solution.

In the same way the buffering capacity by acid (B_{ac}) can be calculated.

6.2. Questions on potentiometry

1. pH and the methods of its determination.
2. Describe the main point of the methods of potentiometry.
3. Classification and working principle of electrodes.
4. Buffer systems, their composition and the mechanism of action.
5. pH of buffer solutions. Calculations and measurement.
6. Buffering capacity and how to measure it.
7. Biological importance of buffering systems.

7. CHROMATOGRAPHY

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.

The methods of chromatography are classified as follows:

1. According to the physical nature of a mobile phase:
   - liquid chromatography (when a mobile phase is liquid);
   - gas chromatography (when a mobile phase is a gas).
2. According to the mechanism of interaction between the material and the sorbent:
   - sorption chromatography (divided in turn into molecular chromatography where the interaction is based on intermolecular forces of Van-der-Vaals, and chemosorption chromatography, where the sorption is caused by various chemical reactions: ion exchange, precipitation, complexation, redox, etc.);
   - gel-chromatography (separation of mixture components due to diffusion of molecules of dissolved materials into the pores of the sorbent).
3. According to the way of chromatographing:
   - frontal chromatography;
   - elution chromatography;
   - displacement chromatography, etc.
4. According to the applied techniques:
   - planar chromatography (thin-layer chromatography, chromatography in paper);
   - column chromatography;
   - capillary chromatography, etc.

In nowadays for the analysis of proteins and other materials **filtration through gels (gel-chromatography)** is widely used. In this case a stationary phase is a liquid in pores (small cavities) of a solid sorbent. The size of pores determines the volume of molecules able to get into them, thus the length of the way for the material to pass through the column depends on its molecular weight. It allows to use this method for determination of molecular weight of materials with the same chemical structure.

7.1. Laboratory work No. 15

SEPARATION OF METAL ION MIXTURE BY CHROMATOGRAPHY IN PAPER

Chromatography in paper is based on the different solubility of components of analysis mixture in not-mixing liquids: mixtures of water and organic solvents. In this case water may be practically called the stationary phase, because it moves through the paper capillaries much slower than organic solvents and is adsorbed in microdrops on cellulose fibers of the paper. If the component is easier soluble in organic solvents, it is gradually accumulated in the organic phase and moved more rapidly in the paper.

The ratio between the length of the component way and that of the solvent is called **distribution coefficient (R_f)** and in normal conditions is a constant.

Task: To separate cation mixture by means of chromatography in paper. According to the given distribution coefficients (R_f) of cations, to determine, which cations are in the mixture.

Procedure: Transfer 10-15 ml of the solvent (mixture of HCl and acetone; volume composition is 8% of concentrated HC1, 87% of acetone and 5% of water). Cut the strip of chromatography paper of 20 cm length, 2 cm width. Mark with a pencil the start line on the strip (at about 2 cm from the edge). In the center of this line transfer by a capillary a drop of analysis mixture of cations in the way that the spot would not be larger than 2-3 cm in diameter. Circle the spot by a pencil and dry in heat desiccator (or above the sand bath). Repeat this operation 2-3 times.

Fix the prepared paper strip with the analysis solution in a cylinder with the solvent so that its end would be immersed into the solvent no more than 0,5 cm. The spot is to be above the solvent level, the strip may not touch the wall of the cylinder. Cover the cylinder with the lip and wait for the solvent to rise above the start line for 8-10 cm. It takes about 1-1,5 hours in the room temperature. After the chromatography, remove the paper strip from the cylinder and dry carefully. Measure the distance L from the start line to the front of the solvent.

In order to detect the certain cations, fill a capillary by the appropriate reagent and touch by it the area of chromatogram, in which the cation is supposed to be. Occurrence of the characteristic color confirms the presence of the cation in the mixture.

According to R_f values, calculate the rising height l of the certain cations.

Table No. 8. **VALUES OF CATION DISTRIBUTION COEFFICIENTS R_f AND REAGENTS TO DETECT THE CATIONS**

Cation	R_f	Reagents
Cd^{2+}	0,10	Sodium sulfide (Na_2S)
Ni^{2+}	0,13	Dimethylglyoxime, ammonia (gas)
Al^{3+}	0,15	Alizarin, ammonia (gas)
Co^{2+}	0,54	Potassium rhodanide (KSCN), saturated solution
Pb^{2+}	0,70	Potassium iodide (KI), 10% solution
Cu^{2+}	0,77	Potassium hexacyane ferrate (II), 10% solution
Zn^{2+}	0,94	Dithizone, 1% solution in chloroform

7.2. Laboratory work No. 16

PAPER CHROMATOGRAPHY OF α-AMINO ACIDS

Task: To perform paper chromatography of a mixture of α-amino acids. According to the calculated values of distribution coefficients, to determine α-amino acids in analysis mixture.

Procedure:

1. Prepare the special rounded chromatographic (or filter) paper. The paper diameter is to be l cm larger than that of Petri dishes to be used.
2. Mark by a pencil a point in the center of the paper and transfer by syringe, capillary, or pipette a drop of analysis mixture of α-amino acids, and dry carefully on heating.
3. In the same place drop the second droplet of the analysis solution and dry it carefully again.
4. To one part of a Petri dish transfer the carrier (mixture of butanol – glacial acetic acid – water; volume ratio is 4:1:5) to cover bottom of the dish and make sure it touches the bended strip of chromatography paper.
5. After placing on the dish the chromatography paper with amino acids transferred (make sure the bended strip of paper is immersed into the carrier), cover with the second part of Petri dish of the same size and wait for chromatography proceed (for about l hour) in the environment saturated by carrier vapor.

6. When the carrier runs for 6-7 cm diameter, open the dish, remove chromatography paper and mark with a pencil the front line of the carrier, and dry for 5-10 min in heat desiccator at 70-80 °C.

7. After drying spray the paper with 0,2% ninhydrin solution in acetone and dry it once more for 5-10min at 80°C.

8. When the purple rings develop on the paper, their number tells, how many α- amino acids were in analysis mixture.

9. For qualitative determination of ct-amino acids of the analysis solution, calculate their distribution coefficient R_f. In order to do this, measure the way of every ct-amino acid made on the paper (a_1, a_2, and so on), i.e. the distance from the mixture application point to middle of colored ring. Calculate also the way of the carrier (b), i.e. the distance from the mixture application point to the front line of the carrier (in cm or mm).

10. Calculate R_f for each α-amino acid:

$$R_f = \frac{a}{b} \qquad (79)$$

11. From the Table No. 11 determine which α-amino acids were present in the analysis solution.

Table No. 9. **DISTRIBUTION COEFFICIENTS (R_f) OF SOME α-AMINO ACIDS WITH BUTANOL AS A CARRIER**

α-amino acid	**Distribution coefflcient R_f**
Cystine	0.13
Lysine	0.16
Arginine	0.18
Glycine	0.34
Alanine	0.39
Tyrosine	0.52
Valine	0.56
Phenylalanine	0.66
Leucine	0.72

7.3. Questions on chromatography

1. Describe the method of chromatography.
2. Classification of chromatography methods.
3. Separation of mixtures by means of chromatography in paper.
4. Distribution coefficient, its calculation.
5. Gel-chromatography. Why is this method applicable for determination of molecular weight of macromolecular compounds?
6. What distance will cover the substance on paper during paper chromatography, if its distribution coefficient R_f = 0.28 and the solvent has made 12.5 cm way?

8. SPECTROSCOPY

Spectroscopy (methods of spectral analysis) is a group of methods of physical analysis. In this analysis the intensity of light emission by atoms or molecules of the analyte or absorption of electromagnetic radiation by the material is measured.

Spectroscopy can be used for both qualitative and quantitative analysis: The frequency (wavelength) of the emitted or absorbed light depends on the composition and structure of the material, whereas the value of analytic signal is proportional to the amount of the material.

Methods of spectral analysis are of high sensitivity; it is possible to detect even small quantities of analysis substance.

Spectral analysis methods are classified according to the way the changes of energy of atoms and molecules cause the occurrence of analytical signal.

In case the material is dissociated to atoms, the analysis methods are attributed to the group of **atomic analysis** (spectral analysis of atomic emission, spectral analysis of atomic absorption, atomic fluorescence analysis, etc.). Such methods are widely used for evaluation of contamination of biological objects by heavy metals, other substances.

In the methods of **molecular spectral analysis** the substance does not get disintegrated. Analytical signal occurs on the impact of electromagnetic radiation upon the material. This causes the changes of molecule energy. Those are the methods of this group: spectral analysis of molecular absorption (in optical range of spectrum), luminescence analysis, etc.

Electromagnetic radiation is of the dual nature; it behaves both as waves and as particles (corpuscles) in the same time. It can be characterized by different parameters, the main of which for the Spectroscopy is a **wavelength X:** the distance between two nearest points of the same phase of a wave. It is expressed in parts of meter: cm, μm (10^{-6} m), nm (10^{-9} m) or angstroms (1Å = 10^{-10} m).

When the beam of light with intensity I_0 falls on the cell (cuvette) with the sample solution, the part of the beam (I_a) will be absorbed and the part (I) will be transmitted through the solution.

This can be expressed by the following equation:

$$I_0 =- I_a + I \qquad (80)$$

The value of I_a depends on the number of material particles absorbing the light in the solution, through which the light has passed, i.e. concentration and thickness of the layer of the solution.

With measured I one can calculate I_a, because they are related by quantitative dependence, described in the **law of Buger-Lambert-Beer:**

An amount of monochromatic (of the certain wavelength) radiation absorbed is exponentially proportional to the concentration of the light absorbing substance in the solution, which has transmitted the light, and the path length of the solution.

$$I = I_0 \cdot 10^{-a'Cb} \qquad (81)$$

Where: a′ – coefficient of the proportion;

C – molar concentration of the solution, in mol/1;

b – thickness of the solution layer (cuvette), in cm.

Logarithm of (84) equation gives the following:

$$\lg \frac{I_0}{I} = a' \cdot C \cdot b \qquad (82)$$

The logarithm of the ratio of incoming light intensity I_0 and transmitted light intensity I is called **optical density of the solution.** Its symbol is A (D or A in literature):

$$A = a'Cb \qquad (83)$$

For absolutely transparent solutions A = O, absolutely nontransparent one has $A \to \infty$.

Proportionality coefficient, which depends on the wavelength and is characteristic to the molecule of light absorbing substance, is called a **molar absorptivity** ε the dimension is l/(mol·cm)/. The numeric value of this coefficient shows the capability of the substance to absorb the light.

In case the substance concentration in the solution is 1 mol/l and the path length is 1 cm, the optical density of the solution at the certain wavelength (A_λ) is equal to the molar absorptivity (ε_λ).

The value of the optical density for estimation of the concentration may be used only in case the Buger-Lambert-Beer law is applicable for the solution. The conditions for this law to be kept are the following:

The value of the optical density for estimation of the concentration may be used only in case the Buger-Lambert-Beer law is applicable for the solution. The conditions for this law to be kept are the following:

1. Wavelengths o f the light transmitted through the solution and the light absorbed by analyte are the same.
2. No chemical changes occur in the light absorbing material.
3. Concentration of the analyte in the solution is low enough and on diluting or concentrating no changes of its chemical properties occur.
4. There are no fluorescenting substances or light scattering particles (suspensions) in the solution.

Subject to the method the analytic signal is registered, the **methods of spectral analysis of molecular absorption** are classified as photometric (visual colorimetry, photocolorimetry) and spectrophotometry.

In quantitative analysis photometric methods are used in case, when the solute absorbs the electromagnetic radiation in the optical range of spectrum (ultraviolet, visible and infrared area of radiation). For biological investigations especially suitable are photometric methods of analysis, based on measurement of visible light absorption by the solution.

Visible light is an electromagnetic radiation of wavelength from 400 to 760 nm.

Visual methods of colorimetry (methods of Standard series, path length changing) are applicable only for the analysis of colored solutions of the substances. In this case color intensity of analyte solution is visually (by eye) compared to the color intensity of standard solution of the same material. Concentration of the analyte solution in the method of path length changing is estimated according the **Beer** law:

If the color intensity of two solutions of the same material is the same, the ratio of the substance concentration in the solutions is in converse proportionality with the ratio of the path lengths.

When color intensities of standard and analyzed solutions are equal, the following statement can be made:

$$A_{std} = A_{anal} \qquad (84)$$

From equations (85) and (86) we derive:

$$C_{anal}\ b_{anal} = C_{std} \cdot b_{std} \qquad (85)$$

According to the Beer law:

$$C_{anal} = \frac{C_{std} \cdot b_{std}}{b_{anal}} \qquad (86)$$

Accuracy of visual methods of analysis depends on sensitivity of the observer's eye and the wavelength of transmitted light. The eye is most sensitive (distinguishes and compares) blue and red solutions.

Visual analysis methods can be carried out in field conditions as well.

Accuracy of the analysis increases, when the intensity of the light transmitted through the solution is measured instrumentally, by photo colorimeters and spectrometers. They can measure radiation intensity not only in visible, but also in ultraviolet and infrared areas of spectrum. Therefore, the analysis can be carried out not only for colored, but also colorless solutions, absorbing the radiation in those ranges.

Methods of photometric analysis due to comparatively simple and inexpensive instrumentation, quickness of the analysis and accuracy are prevailing in investigations of biological objects.

8.1. Laboratory work No. 17

DETERMINATION OF Fe^{3+} AMOUNT BY MEANS OF CALIBRATION DIAGRAM

In photocolorimetry and spectrophotometry the following main methods of quantitative analysis are applied: calibration diagram method, the method of comparing, the method of addition, etc. The most suitable for the common analysis is the method **of calibration diagram.**

For this method one should:

1. Prepare 4-8 Standard (of known concentration) solutions of analysis material;
2. Measure optical densities of Standard solutions;
3. Plot the calibration diagram in coordinates „Concentration - optical density”;
4. Measure optical densities of analysis solutions with the cuvettes of the same path length and at the same wavelength;
5. Find the concentration of the analyte from the calibration diagram.

According to the Buger-Lambert-Beer law, all points in the diagram should be in a line, crossing the O point of coordinates. In case the diagram is non-linear, for quantitative analysis it can be used only in the concentration interval with least deviation from the line.

The concentration range of the calibration diagram should involve all concentrations of analysis solutions.

Calibration diagram may be used only for the measurements with the same instrument.

Task: To prepare colored Standard solutions for the calibration diagram from Fe^{3+} ion solution of the known concentration, with KCNS or NH_4CNS. To measure optical density of these solutions and plot the calibration diagram. To prepare colored analysis solution of Fe^{3+} ions and measure its optical density. From the calibration diagram, to estimate Fe^{3+} ion concentration in the solution.

Procedure:

1. Transfer to test tubes from burettes the aliquots of Fe^{3+} ion solution of known concentration (c = 0,01 mg Fe^{3+}/1ml), distilled water and KCNS or NH_4CNS solutions as given in Table No. 13.
2. Measure optical density of these solutions by photoelectrocolorimeter or spectrophotometer. Use a blue filter for photoelectrocolorimeter. Measure at 550 nm wavelength with the spectrophotometer.
3. Put down the values of optical density A to the Table No. 9.
4. Use the graph paper to plot the calibration diagram. In abscise axis put the concentration of Fe^{3+} ion solution (mg/ml), in ordinate axis put the optical density A.
5. Dilute the analysis solution of Fe^{3+} ions in 100 ml volumetric flask by distilled water to the mark and mix thoroughly the stopped flask.
6. Pipet 10 ml aliquot of the prepared analysis solution of Fe^{3+} ions to the test tube and add from burettes l ml of KCNS or NH_4CNS solution. Mix the solution thoroughly.
7. Measure instrumentally the optical density A_{sol} of the analysis solution.
8. Find from the calibration diagram the concentration of Fe^{3+} ions in the analysis

solution and put it to the Table No. 9.

Table No. 10. **CALIBRATION DIAGRAM FOR QUANTITATIVE DETERMINATION OF Fe^{3+}**

Test tube No	Fe^{3+} ion solution, ml	H_2O, ml	KCNS or NH_4CNS solution, ml	Fe^{3+} ion concentration, mg/ml	Optical density A of the solution
1	8	2	1	0.008	
2	7	3	1	0.007	
3	6	4	1	0.006	
4	5	5	1	0.005	
5	4	6	1	0.004	
6	3	7	1	0.003	
7	2	8	1	0.002	
Concentration of Fe^{3+} ions in the analysis solution:				**C_{sol} =**	**A_{sol} =**

8.2. Questions on spectroscopy

1. Description of methods of spectral analysis and their classification.
2. What processes occur, when electromagnetic radiation passes through a solution?
3. The law of Buger-Lambert-Beer.
4. Optical density. Molar absorptivity. Their determination.
5. Visual methods of colorimetry. Beer law.
6. Methods of spectral analysis for colored and colorless solutions.
7. Absorption spectrum, plotting of it.
8. Calibration diagram, how to make it. Determination of solution concentration by means of calibration diagram.

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APPENDIX

MAIN MATERIALS OF CHEMICAL LABORATORY

Title	Formula	Type*
Acetone	CH_3COCH_3	FL
Acetic acid	CH_3COOH	
Ammonia	NH_3	FA
Ammonium hydroxide	NH_4OH	
Ammonium sulfate	$(NH_4)_2SO_4$	
Carbon dioxide	CO_2	
Carbon monoxide	CO	TG
Carbonic acid	H_2CO_3	
Nitric acid	HNO_3	AM
Barium alkali (hydroxide)	$Ba(OH)_2$	AM
Petrol	$C_6H_{14}...C_{10}H_{22}$	FL
Borax	$Na_2B_4O_7 \cdot 10H_2O$	
Boric acid	H_3BO_3	FA
Butane	C_4H_{10}	FG
Chlorine	Cl_2	TG
Hydrochloric acid	HCl	AM
Ethane	C_2H_6	FG
Ether	$C_2H_5OC_2H_5$	FL
Ethyl alcohol	C_2H_5OH	
Ortho-phosphoric acid	H_3PO_4	AM
Phosgene	$COCl_2$	TG
Glycerol	$C_3H_5(OH)_3$	FA
Iodine	I_2	FA
Calcium hydroxide (slacked lime)	$Ca(OH)_2$	AM
Calcium carbonate (chalk, limestone)	$CaCO_3$	
Calcium oxide (burnt lime)	CaO	AM
Potassium dichromate	$K_2Cr_2O_7$	
Potassium carbonate (potash)	K_2CO_3	
Potassium permanganate	$KMnO_4$	FA
Potassium alkali	KOH	AM
Crystal soda	$Na_2CO_3 \cdot 10H_2O$	
Methane	CH_4	FG
Sodium chloride (white salt)	$NaCl$	
Sodium alkali	$NaOH$	AM
Sodium hydrocarbonate (backing soda)	$NaHCO_3$	FA
Propane	C_3H_8	FG
Sulfuric acid	H_2SO_4	AM
Hydrosulfide	H_2S	TG
Soda	Na_2CO_3	AM

*Abbreviations:

AM - aggressive materials
FA - materials for the first mcdical aid
FG - flararaable gas
TG - toxic gas
FL - flammable Iiquids

DENSITY AND CONCENTRATION OF HYDROCHLORIC ACID SOLUTION

HCl MM 36,47

Density, g/cm	$C_{\%}$	Density, g/cm^3	$C_{\%}$	Density, g/cm	$C_{\%}$	Density, g/cm	$C_{\%}$
1.000	0.36	1.050	10.52	1.100	20.39	1.150	30.14
1.005	1.36	1.055	11.52	1.105	21.36	1.155	31.14
1.010	2.36	1.060	12.51	1.110	22.33	1.160	32.14
1.015	3.37	1.065	13.50	1.115	23.29	1.165	33.16
1.020	4.39	1.070	14.50	1.120	24.25	1.170	34.18
1.025	5.41	1.075	15.49	1.130	25.22	1.175	35.20
1.030	6.43	1.080	16.47	1.135	26.20	1.180	36.23
1.035	7.46	1.085	17.45	1.140	27.18	1.185	37.27
1.040	8.49	1.090	18.43	1.145	28.18	1.190	38.32
1.045	9.51	1.095	19.41	1.150	29.17	1.195	39.37
						1.198	40.00

DENSITY AND CONCENTRATION OF SODIUM ALKALI SOLUTION

NaOH MM 40,01

Density, g/cm	$C_{\%}$	Density, g/cm^3	$C_{\%}$	Density, g/cm^3	$C_{\%}$	Density, g/cm	$C_{\%}$
1.000	0.16	1.140	12.83	1.280	25.26	1.420	38.99
1.010	1.05	1.150	13.73	1.290	26.48	1.430	40.00
1.020	1.94	1.160	14.64	1.300	27.41	1.440	41.03
1.030	2.84	1.170	15.54	1.310	28.33	1.450	42.07
1.040	3.75	1.180	16.44	1.320	29.26	1.460	43.12
1.050	4.66	1.190	17.35	1.330	30.20	1.470	44.17
1.060	5.56	1.200	18.26	1.340	31.14	1.480	45.22
1.070	6.47	1.210	19.16	1.350	32.10	1.490	46.27
1.080	7.38	1.220	20.07	1.360	33.06	1.500	47.33
1.090	8.28	1.230	20.98	1.370	34.03	1.510	48.38
1.100	9.19	1.240	21.90	1.380	35.01	1.520	49.44
1.110	10.10	1.250	22.82	1.390	36.00	1.530	50.50
1.120	11.01	1.260	23.73	1.400	36.99		
1.130	11.92	1.270	24.65	1.410	37.99		

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ANALYTICAL CHEMISTRY LABORATORY MANUAL

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