MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION
VOLGOGRAD STATE MEDICAL UNIVERSITY
DEPARTMENT OF PHARMACEUTICAL AND TOXICOLOGICAL CHEMISTRY

LECTURE 3

TOXICOLOGIKAL CHEMISTRY

A group of substances isolated from biological material by mineralization. Ecology of the environment and the prevalence of poisoning with heavy metal compounds and arsenic. Methods for isolation of heavy metal compounds from mineralizate. Compounds of lead, barium, manganese, chromium and silver.

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Characteristics of metal poisons

The human body contains 81 elements of the periodic table of elements

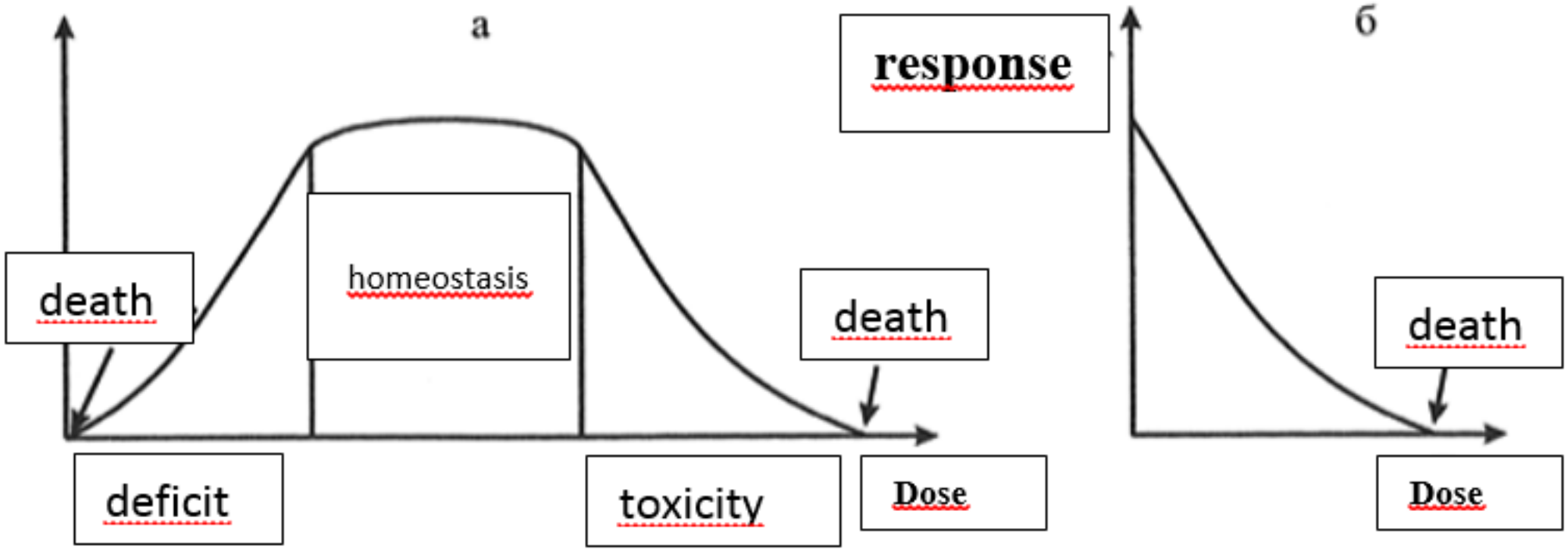
Classification of elements by content in the body:

1. Organogenic elements (C, H, O, N, P, S) $>10^{2}\%$
2. Macroelements (Ca^{2+}, Mg^{2+}, Na^{+}, Cl^{-}) $>10^{-2}\%$
3. Microelements (Cu, Fe, Co, Mo, Mn) 10^{-5}-$10^{-2}\%$
4. Ultramicroelements (Cd, V. Ag) $<10^{-5}\%$

Classification according to the needs of the body:

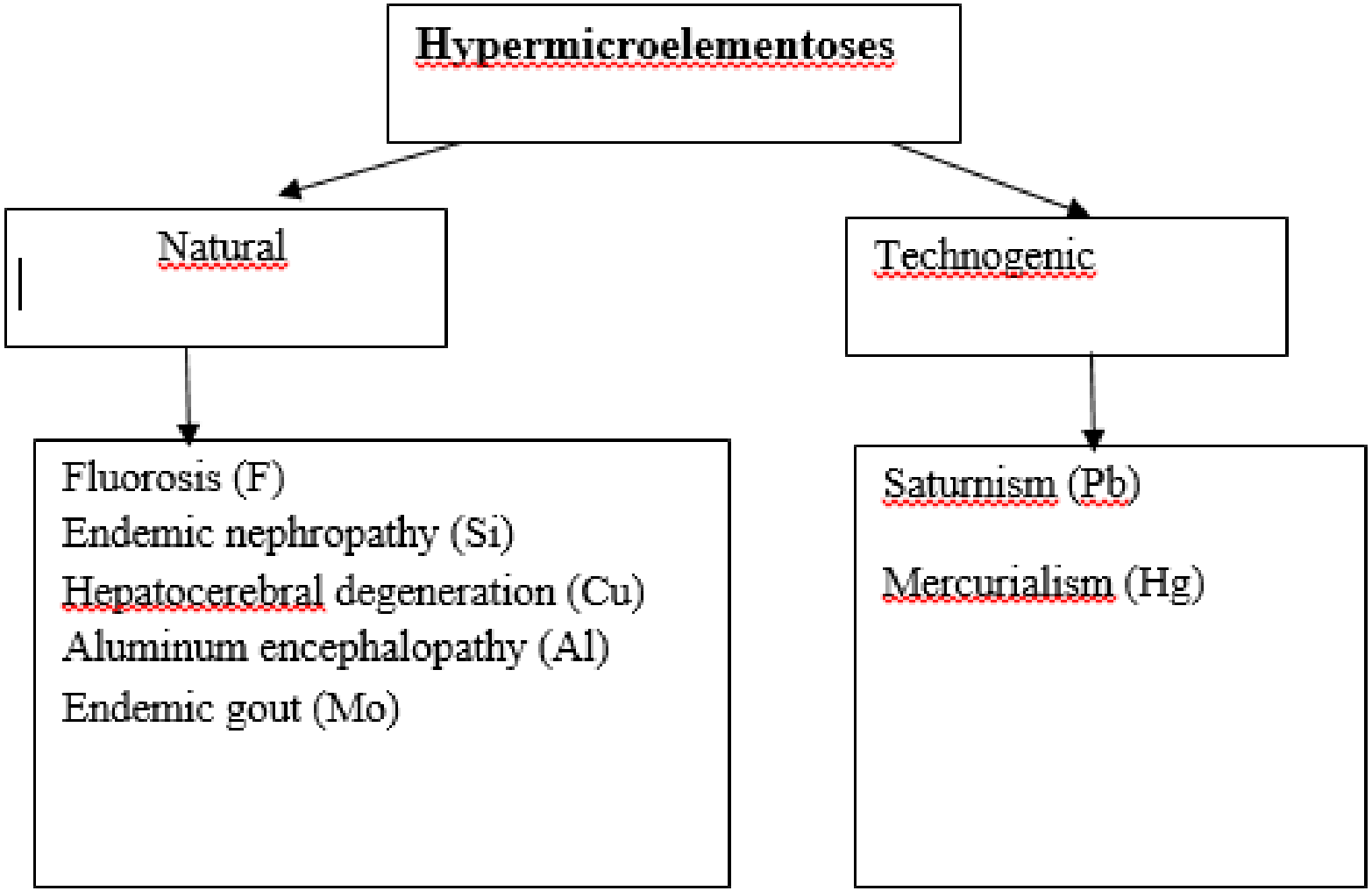
- **Essential elements -** vital elements that are present in the body in constant concentrations (Cu,Zn,Fe,Co,Mo,Mn) a decrease in their concentration leads to the appearance of symptoms of deficiency**.**
- **Conditionaly essential elements -** are present in the body in small concentrations and their biological role is unknown (V,Ni,Cr)
- **Impure substance (admixture) (примесные) -** elements with an unknown biological role, but constantly present in the body.

Dose-response dependencies



а – for the essential elements; б – for the Impure substance (admixture) elements

Hypermicroelementosis is a disease caused by excessive levels of an element in the body.



TOXICOKINETICS of heavy metal compounds **:**

ABSORPTION ROUTES of elements into the body :

- ORAL-GASTRO INTESTINAL TRACT: absorbed in the duodenum and small intestine (diffusion, active transport)
- INHALATION-LUNG – occurs more efficiently because the suction surface area is larger
- DERMAL-SKIN Percutaneous intake occurs more rarely (Hg^{2+})

ELIMINATION:

- Through the skin: exfoliation of epidermis (As); through the sweat gland (Cu,Zn,Cd)
- Through kidneys and intestines: urine and excrement

DETOXIFICATION (natural): metallothioneins (MT(SH)X) are formed in the liver

$$2\ MT(SH)_X + x\ Cd^{2+} \rightarrow MT(S\text{-}Cd\text{-}S)_x\ MT + 2_X H^+$$

they are not excreted from the body, but also do not have a toxic effect

Toxicodynamics of heavy metal compounds :

Mechanism of metal toxicity:

***At the molecular level*:**

- Inhibition of enzymes
- Conformational changes (proteins, nucleic acids) → changes in the rate of biosynthesis, mutations.

At the cellular level:

- Deficiency of important metabolites
- Violation of the structure and permeability of cell membranes.

Organ dysfunction:

- Tumor
- Slowing growth

Binding of "metal poisons“

- binding to amino acids: with -SH (cysteine, methionine); -NH_2 ; -COOH; -OH (serine, threonine) groups
- binding to peptides and proteins - with -NH2; COOH groups
- binding to pteridines (including folic acid), purines, riboflavin, nucleic acids, etc.

Objects of research :

1.living people;

-Blood

- Urine
- vomit
- hair
- nails

2. Corpse (dead people)

-stomach with contents,

- intestines,
- liver,
- kidneys,
- blood
- urine
- bones, etc.

3. non-biological objects -food products,

- liquids, -clothing, etc.

MINERALIZATION

Mineralization (isolation method) – the process of oxidation of a biological object for the release of metal from proteins.

The bonds of metals with most amino acids, peptides, and proteins are strong (covalent). Therefore, more stringent methods are used.

TYPES OF MINERALIZATION:

1. Сухая минерализация (dry mineralization):

1.1. Dry ashing (сжигание)

1.2 Метод сплавления. (fusion method)

2. Wet mineralization (влажная минерализация)

1. Сухая минерализация (dry mineralization): ash formation

1.1 Dry ashing (сухое озоление) - the method is based on heating organic substances to a high temperature with air access

- Small sample of a biological object 1-10г

Method execution:

- Grinding of an object
- Drying in a sand bath
- Charring.
- Wetting with concentrated solution $(NH_4)NO_3$ or HNO_3.
- Drying
- Heating on a flame in a crucible.
- Cooling and, addition : hydrochloric acid - for the presence of manganese, nitric acid for the presence of copper.
- Filtering.
- Evaporation of filtrate to dry
- Dissolution of dry residues in water

1.2 Метод сплавления. (fusion method) The object is heated with molten alkali metal nitrates.

Analis of biological objects for the presents of As, Ag and ect

Method execution:

- Two parts of sodium carbonate and one part of sodium nitrate are added to the object, moistened with water
- Dry
- 5-6g of sodium nitrate is added to the crucible
- Heated until sodium nitrate melts
- Add a mixture of object, nitrate and sodium carbonate
- A new portion is introduced after the combustion of the old one.
- In a porcelain cup, add sodium carbonate, mix,
- It is burned in a crucible
- Cooled, treated with boiling water.

Advantages of dry mineralization methods:

- Directed Research

Disadvantages of dry mineralization methods:

1. Evaporation upon heating (Hg, Tl);
2. Interaction with the crucible material;
3. It is impossible to control the temperature in the crucibles.

2. Wet mineralization mineralizate is formed (transparent or colored liquid)

Oxidizing agents (nitric, sulfuric, perchloric acids), KClO4 – potassium chlorate and perhydrol (30% H2O2).

Destruction to simple compounds.

Advantages of wet mineralization methods

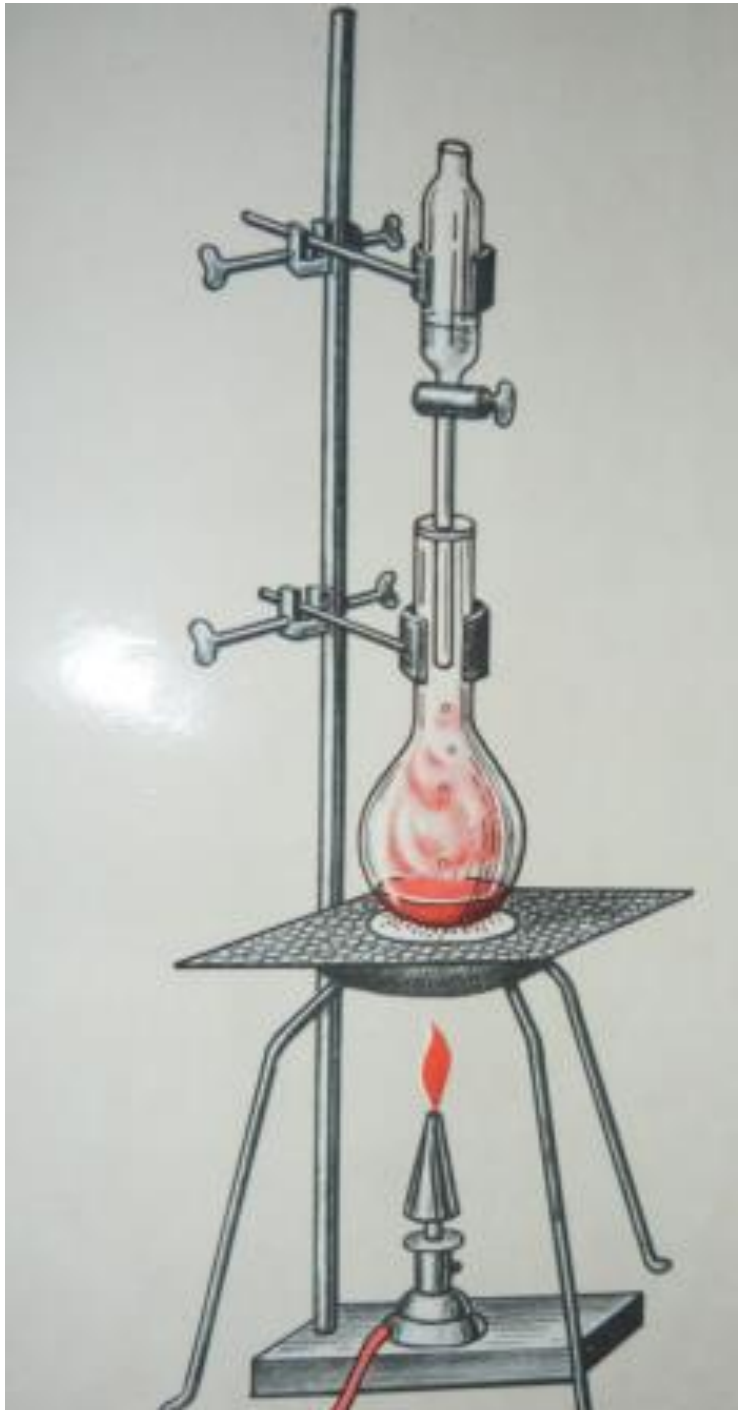
1. Organic substances are rapidly destroyed;
2. High sensitivity;

Disadvantages of wet mineralization methods:

1. Losses Hg
2. Use of concentrated solutions.

Stages of isolation of "metal poisons" from biological objects by the general method:

1. Preparation of the object (100 г)
2. Destruction: oxidizing agents: HNO_3(conc), H_2SO_4 (conc), heating up to 40 minutes
3. Deep oxidation: heating from 4 to 8 hours.
4. Denitration



Denitration- release from nitric, nitrogenous, nitrosylsulfuric acid and nitrogen oxides that interfere with the analysis.

Denitration is carried out using:

- formaldehyde,
- urea,
- sodium sulfite

Denitration with formaldehyde

When formaldehyde reacts with nitric acid, nitrogen is released:

$$4HNO_3 + 5HCHO \rightarrow 2N_2 + 5CO_2 + 7H_2O$$

- • Nitric acid with formaldehyde:

$$4HNO_2 + 2HCHO \rightarrow N_2 + 2NO + 2CO_2 + 4H_2O$$

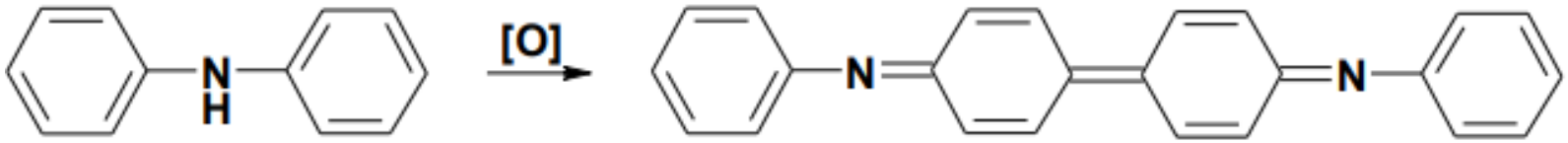
- Nitric oxide (II) is oxidized by air oxygen to nitric oxide (IV), which, when interacting with water, gives nitric and nitric acids which react with formaldehyde.

$$NO + O \rightarrow NO_2$$

$$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3$$

- Nitrosylsuifuric acid decomposes. Nitric acid is formed, reacts with formaldehyde..

Verification of the end of denitration is carried out using diphenylamine in sulfuric acid. The absence of blue staining indicates the complete removal of nitrogen compounds from the mineralize



diphenylamine blue

Ashing accounts for most of the analysis errors.
The best results are achieved with mineralization in a closed system of microwave ovens under the influence of microwave radiation using special sealed Teflon and steel chambers in the presence of an oxidizing reagent - nitric acid, which contain biological samples.

After destruction of biological material using concentrated sulfuric and nitric acid mineralizate is colorless or slightly colored yellow color (due to undestructed organic matter) clear liquid.

In the presence of copper and chromium compounds, the mineralizate may be colored bluish or greenish.

In the presence of lead, barium, calcium compounds mineralizate, after diluting it with purified water, contains a white precipitate consisting of sulfates of these metals.

To detect “metallic” poisons in extracts from objects use **instrumental and chemical methods** of analysis.

Instrumental methods of analysis

1. **Atomic absorption spectrometry** –the method is based on determining the content of chemicals elements by measuring radiation absorption atomic pairs of the element being determined.

Detection is carried out according to characteristic each element to the resonant transition lines at a certain wave length.

Quantitative determination is carried out by the value light absorption at a certain wavelength for each element. Concentration calculations are carried out according to the calibration schedule or using additive method.

Instrumental methods of analysis

2. Atomic fluorescence analysis

3. Atomic emission analysis

4. X-ray fluorescence analysis

Fractional (chemical) method of analysis “metal poisons”
according to A.N. Krylova

1) the method is developed for the 13 most important “metal poisons”
2) there is an order of allocation and detection of metal ions (scheme of the fractional method and organic reagents (dithizone, ammonium diethyldithiocarbamate, 8-hydroxyquinoline, triphenylmethane derivative))
3) use sensitive and specific reactions, often microcrystal reactions•
4) use at least two reactions: preliminary and confirmatory.
5) use of certain techniques to eliminate interfering effects of foreign elements (dilution, masking, unmasking)

ANALYSIS:

• Diluting the mineralizate with water to 180 ml.

• Separation of white or dirty green sediment filtering.

• Washing the sediment with 10 ml of 1% H_2SO_4 and 10 mlwater.

The filtrate contains Mn2+,Cr3+, Ag+, Cu2+,Zn2+, Cd2+, Bi3+, As3+, Sb3+

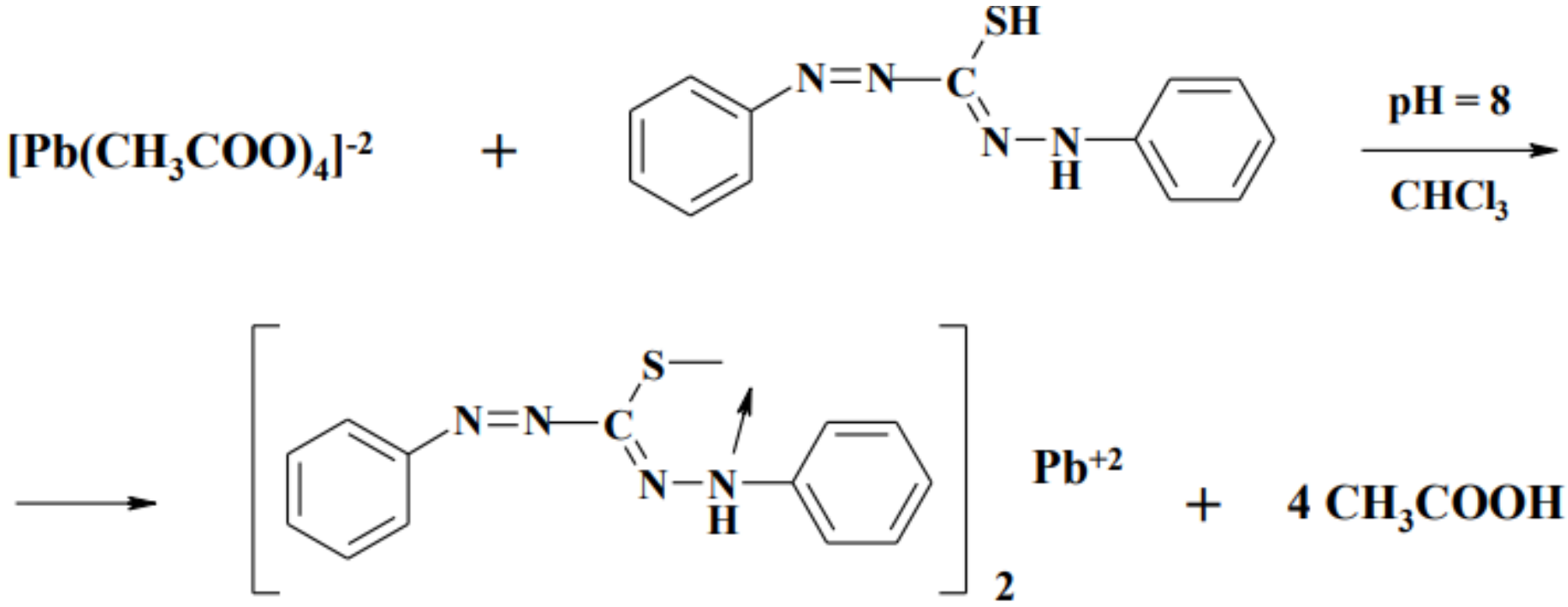
• Separation of $BaSO_4$ and $PbSO_4$ precipitate: rinsing hot solution of ammonium acetate NH_4CH_3COO (5 ml for significant sediment and 1-2 ml for small sediment)

- on a BaSO4 filter
- solution of $Pb(CH_3COO)_2$ in the filtrate

Lead Detection

1. Atomic adsorption spectrometry. Detection is carried out along characteristic lead lines resonant transition at a wavelength of 217 nm.
2. Chemical method. It is carried out using the fractional method, developed by A.N. Krylova.

2.1. Reaction with dithizone (preliminary).



The chloroform layer turns from green to purple-red.

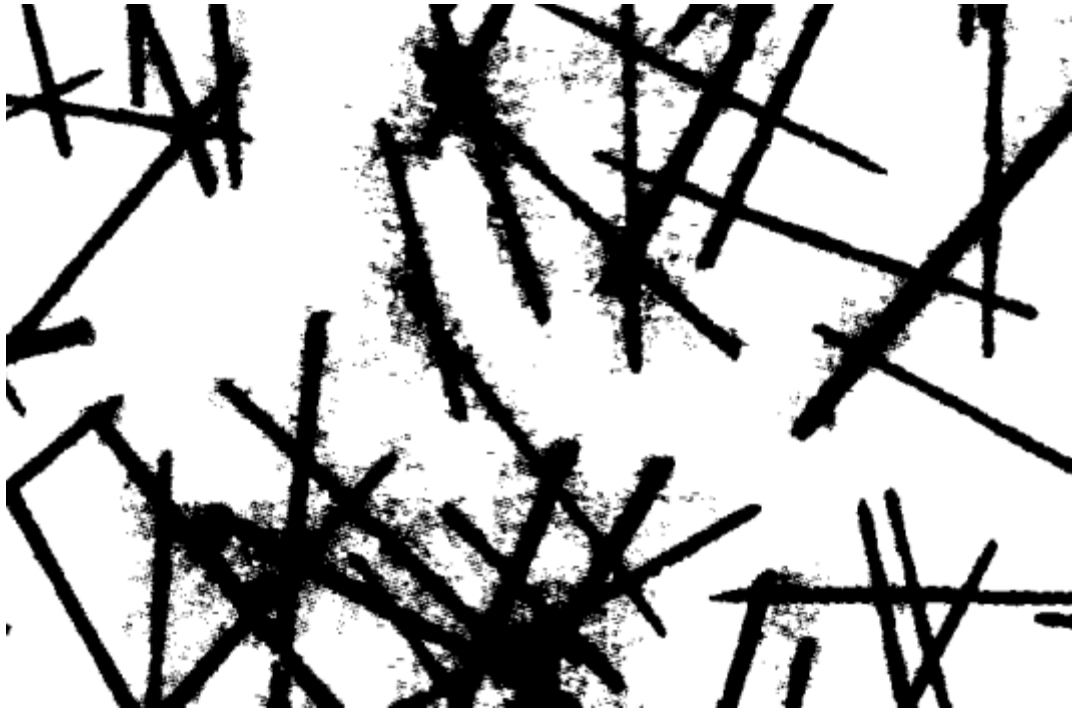
To carry out confirmatory tests for lead a purple-red colored layer of chloroform, containing lead dithizonate - Pb(HDz)2, shake in for 60 s with 0.5-2 ml of 1 M nitric acid solution (independing on the volume and color intensity of the extract). A layer of chloroform restores the green color.

A lead cation is detected in the aqueous phase. Choice reactions (micro- or macrochemical) depends on the volume a queous phase obtained from the destruction of lead dithizonate nitric acid. With a small volume of aqueous phase (0.5 ml) microcrystalscopic reactions are used, with large volume (2 ml or more) - macrochemical.

Microcrystalloscopic reactions to lead

1. Formation of a double salt of cesium iodide and lead. Yellow-green needle-shaped crystals are formed, collected into bundles and spheroids

$$Pb(NO_3)_2 \xrightarrow{KI} PbI_2\downarrow \xrightarrow{KI} K[PbI_3] \xrightarrow{CsCl} Cs[PbI_3]\downarrow$$



Microcrystalloscopic reactions to lead

2. Formation of potassium, lead and copper hexanitrite
Black or brown crystals form in the form of cubes

$$\mathbf{Pb(CH_3COO)_2 + Cu(CH_3COO)_2 + 6\ KNO_2 \rightarrow [K_2CuPb(NO_2)_6] + 4\ CH_3COOK}$$

Macrochemical reactions to lead

1. Reaction with potassium iodide. yellow precipitate soluble in excess reagent.

$$Pb(NO_3)_2 + 2KI \rightarrow PbI_2\downarrow + 2KNO_3 PbI_2 + 2KI \rightarrow K_2[PbI_4]$$

2. Reaction with potassium dichromate. orange precipitate.

$$2Pb(NO_3)_2 + K_2Cr_2O_7 + H_2O \rightarrow 2PbCrO_4\downarrow + 2KNO_3 + 2HNO_3$$

3. Reaction with hydrogen sulfide. black precipitate.

$$Pb(NO_3)_2 + H_2S \rightarrow PbS\downarrow + 2HNO_3$$

4. Reaction with sulfuric acid. white precipitate.

$$Pb(NO_3)_2 + H_2SO4 \rightarrow PbSO4\downarrow + 2HNO_3$$

Grade. Macrochemical reactions can be found in100 g of biological object 0.2 mg of lead.

Quantitation

Lead in mineralizate is determined using:

1. Atomic absorption spectrometry.
2. Extraction-photocolorimetric method for the reaction of lead with dithizone
3. Iodometric determination carried out after dissolving lead sulfate in acetate ammonium

$$2Pb(CH_3COO)_2 + K_2Cr_2O_7 + H_2O \rightarrow 2PbCrO_4 + 2CH_3COOK + 2CH_3COOH$$

$$K_2Cr_2O_7 + 6KI + 7H_2SO_4 \rightarrow 3I_2 + Cr_2(SO_4)_3 + 4K_2SO_4 + 7H_2OI_2 +$$

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

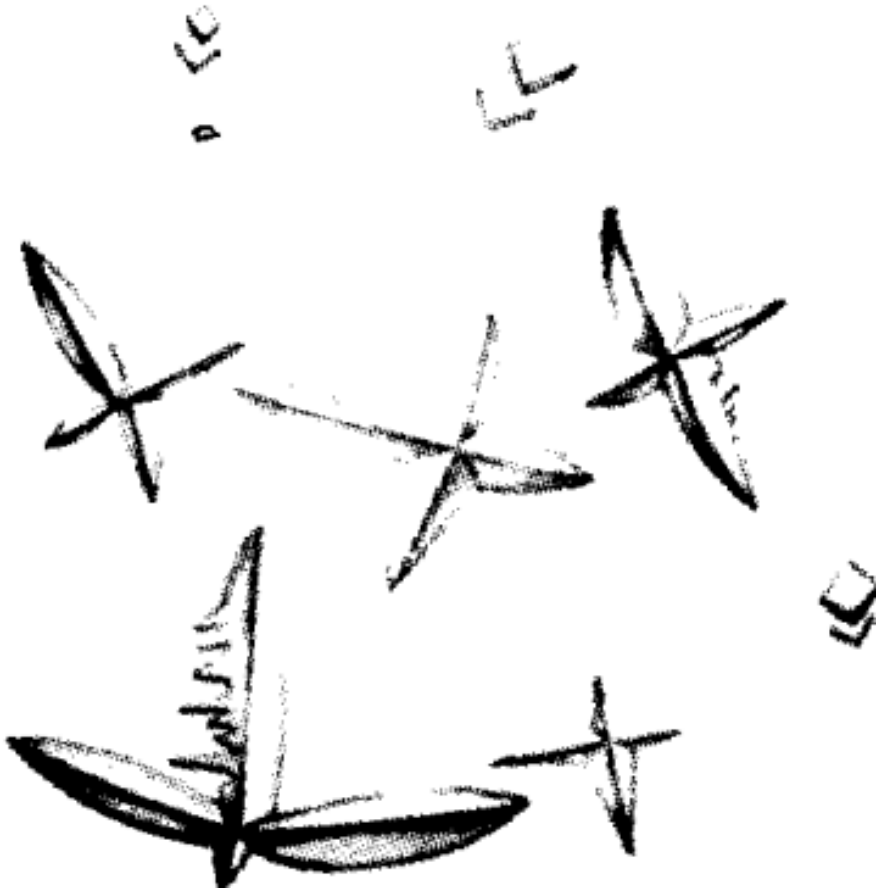
4. Complexometric titration.

Barium detection

1. Atomic absorption spectrometry.
2. Chemical method.

1. Recrystallization reaction from concentrated sulfuric acid. (colorless rectangular barium sulfate crystals plates turning into crosses with feathery heterobranched ends)

Grade. Detection limitis 0.05 μg barium perthe test sample.



Dissolution of barium sulfate through reducing reaction.

Part of the sediment is heated on a platinum needle in a reducing parts of the burner flame. The platinum needle is immersed in 2 drops of a 10% solution hydrochloric acid on a glass slide. At the same time, on platinum needle produces barium sulfide, which is partially turns into barium oxide

$BaSO4 + 4CO \rightarrow BaS + 4CO2$

$2BaS + 3O2 \rightarrow 2BaO + 2SO2$

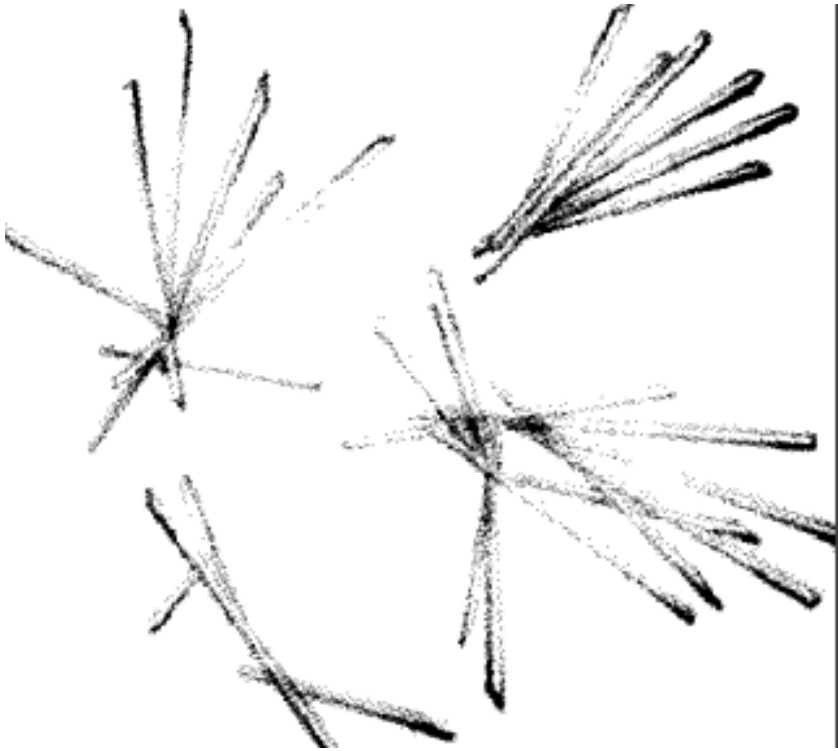
When immersing a platinum needle in a solution of hydrochloric acid barium sulfide and oxide dissolve.

$BaO + 2HCI \rightarrow BaCl2 + H2OBaS + 2HCI \rightarrow BaCl2 + H2S$

Then onto a glass slide containing the solution barium chloride, place a crystal of potassium iodate. The formation of a precipitate of barium iodate is observed

$BaCl2 + 2KIO3 \rightarrow Ba(IO3)2 + 2KCI$

Under a microscope, prismatic crystals collected into spheroids Detection limit - 0.03 μg of barium in the test sample



Quantitation **barium**

1. Atomic absorption spectrometry.

Definition lead by the amount of light absorption at wave length 553.6 nm. Calculation of concentration is carried out according to calibration curve or using the method additives

2. Gravimetric method. Barium after isolation in the mineralizate is in the form of an insoluble sediment barium sulfate. It is reprecipitated from an ammonia solution Trilon B. The precipitate of barium sulfate is filtered off, dried to constant weight and weighed.

3. Complexometric titration.

Detection of manganese in mineralizate.

1. Atomic absorption spectrometry. Detection is carried out by characteristic of manganese resonance transition line at a wavelength of 279.5nm. Evaluation of the method. The detection limit for manganese is 0.1 μg in 1 ml the test sample.
2. Chemical method.

In the mineralizate, manganese is found in the form of lower salts valencies. To detect them, they are converted to permanganic acid byoxidation.

2.1. Reaction with potassium periodate.

$$2MnSO_4 + 5KIO_4 + 3H_2O \rightarrow 2HMnO_4 + 5KIO_3 + 2H_2SO_4$$

2.2.Reaction with ammonium persulfate.

$$2MnSO_4 + 5(NH_4)_2S_2O_8 + 8H_2O \rightarrow 2HMnO_4 + 5(NH_4)_2SO_4 + 7H_2SO_4$$

Grade. Detection limit 1·10-4 mg/ml. The reactions are specific. According to the first reaction. The detection limit for manganese is its content of 0.02 mg per 100 g of object, and according to second reaction - 0.1 mg. The appearance of coloring only in the first reaction may indicate the detection of naturally occurring manganese. Appearance staining by two reactions is evidence of the content in the objectmanganese in quantities higher than the natural norm and requires quantitative definitions.

QUANTITATION

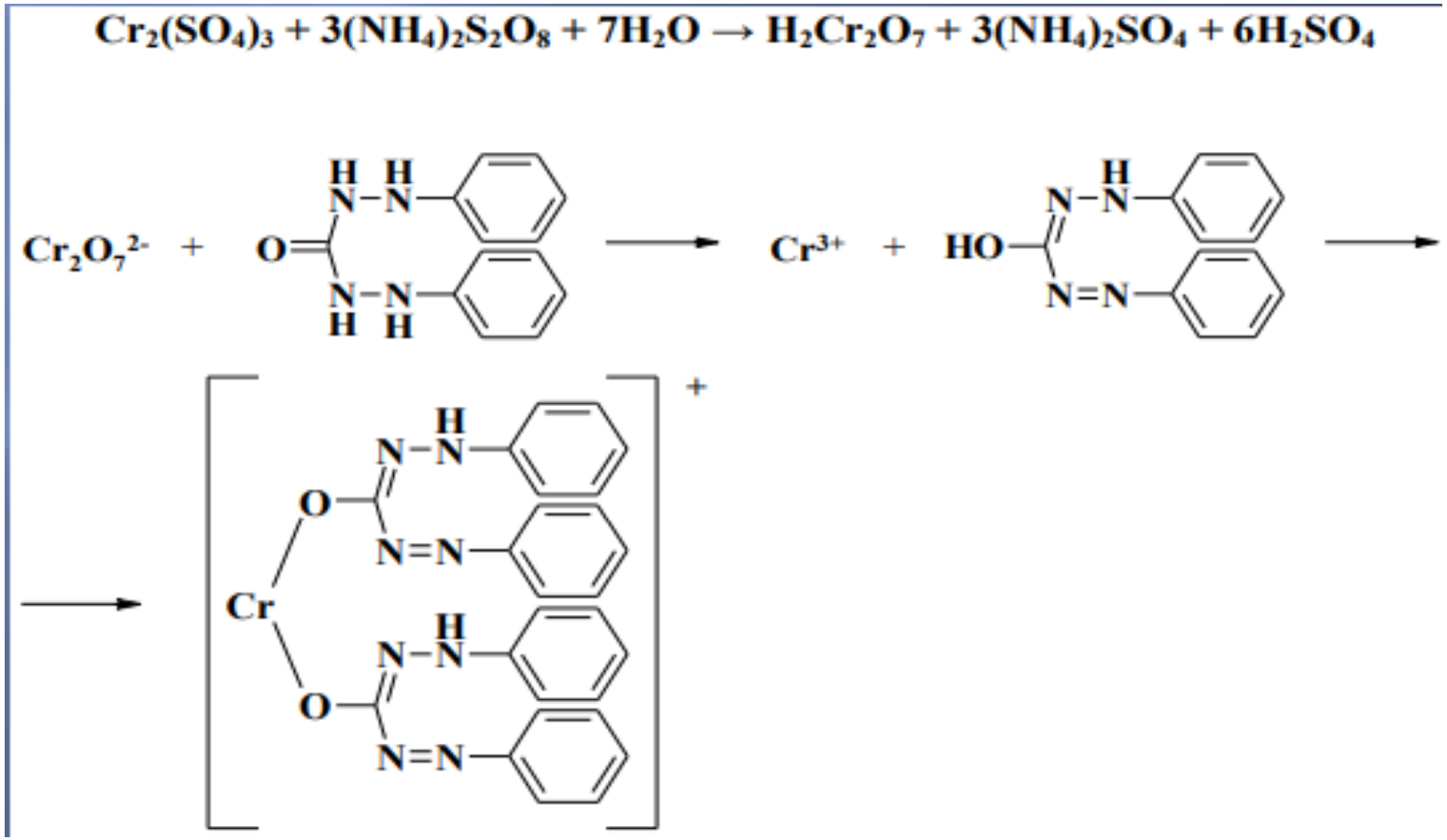
1. Atomic absorption spectrometry. The determination is made by the amount of light absorption at wavelength 279.5 nm. Calculation of manganese concentrationcarried out according to the calibration schedule or method additives

2. The photocolorimetric method is based onoxidation of manganese(II) to manganese(VII). Optical The density of the solution is measured at 525 nm in a cuvette withlayer thickness 10 mm. Calculation of manganese concentration inmineralization is carried out according to the calibration schedule.Manganese can be determined by this method inamount of 0.02-20 mg or more per 100 g of object.

Chromium detection

1. Atomic absorption spectrometry.
2. Chemical method. In the mineralizate, chromium is found inform of chromium sulfate (III) - $Cr_2(SO_4)_3$

2.1. Reaction with diphenylcarbazide (preliminary).formation of a pink or red-violet color



Реакция специфична, предел обнаружения составляет 0,2 мкг хрома в 1 мл минерализата

2.2. Reaction of chromium peroxide formation(confirming).
coloring the ethereal layer blue or blue.

$Cr_2(SO_4)_3 + 3(NH_4)_2S_2O_8 + 7H_2O \rightarrow H_2Cr_2O_7 + 3(NH_4)_2SO_4 + 6H_2SO_4$

$H_2Cr_2O_7 + 4H_2O_2 \rightarrow 2CrO_5 + 5H_2O$

Grade. The reaction is specific and clear for chromium, the limit detection is 2 μg of chromium in 1 ml of mineralizate.

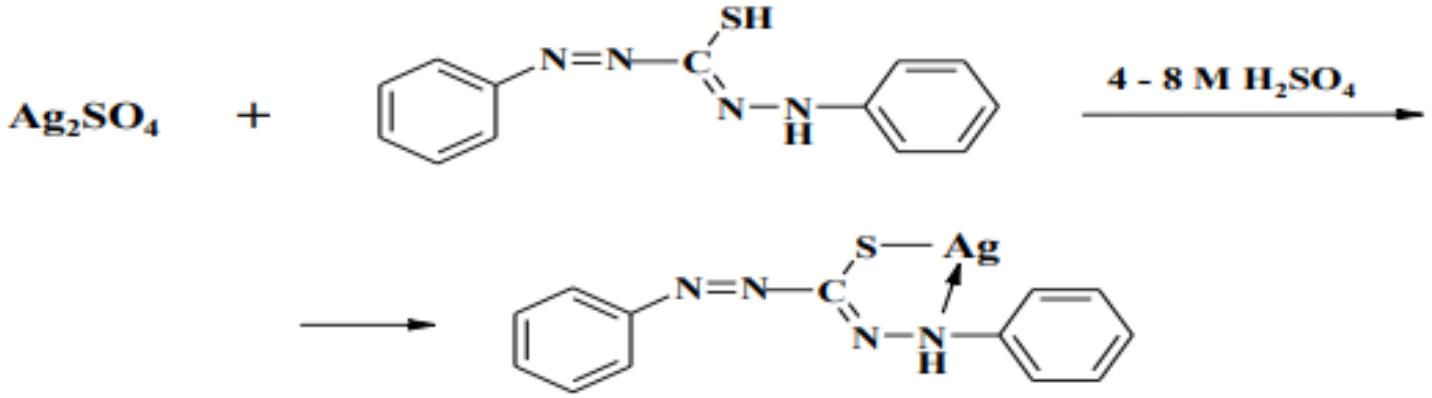
Quantitation

1. Atomic absorption spectrometry.

2.The photocolorimetric method is based on obtaining colored compound with diphenylcarbazide. Optical the density is determined at 546 nm in a cuvette with a layer thickness of 20 mm. To calculate the amount of chromium in the mineralizate, use calibration chart. This method determines chromium in an amount of 0.1-20 mg per 100 g of object.

Detection of silver in mineralizate

1. Atomic absorption spectrometry. Detectionis carried out along the resonance line characteristic of silvertransition at a wavelength of 328.1 nm. Silver Detection Limit0.1 μg in 1 ml of test sample.
2. Chemical method.Reaction with dithizone (preliminary).



Mercury with dithizone forms dithizonate, which is orange-yellow in color. For differences dithizonates the colored chloroform layer is separated and shake with HCl solution. Silver dithizonate breaks down and is released silver chloride, and the golden color turns green. Mercury dithizonateis not destroyed, and the golden color of the chloroform layer does not disappear. The detection limit is 0.04 μg of silver in 1 ml of mineralizate. The reaction has forensic chemical significance if the result is negative.

Isolation of silver from mineralizate.
add 0.5 g sodium chloride, heat and a white precipitate forms silver chloride is filtered off. The sediment is examined for silver compounds by test reactions, and the filtrate by all other cations. The precipitate is dissolved in a certain volume of 25% ammonia solution.

$AgNO_3 + NaCl\ (crystalline) \rightarrow AgCl \downarrow + NaNO_3$

$AgCl + 2NH_4OH \rightarrow Ag(NH_3)_2Cl + 2H_2O$

The solution is analyzed by reactions.

1. Reaction with potassium dichromate. red or red-brown sediment(crystals in the form of rectangular and rhombic plates of orange-red color.) or coloring.
2. $2AgNO_3 + K_2Cr_2O_7 \rightarrow Ag_2Cr_2O_7 + 2KNO_3$

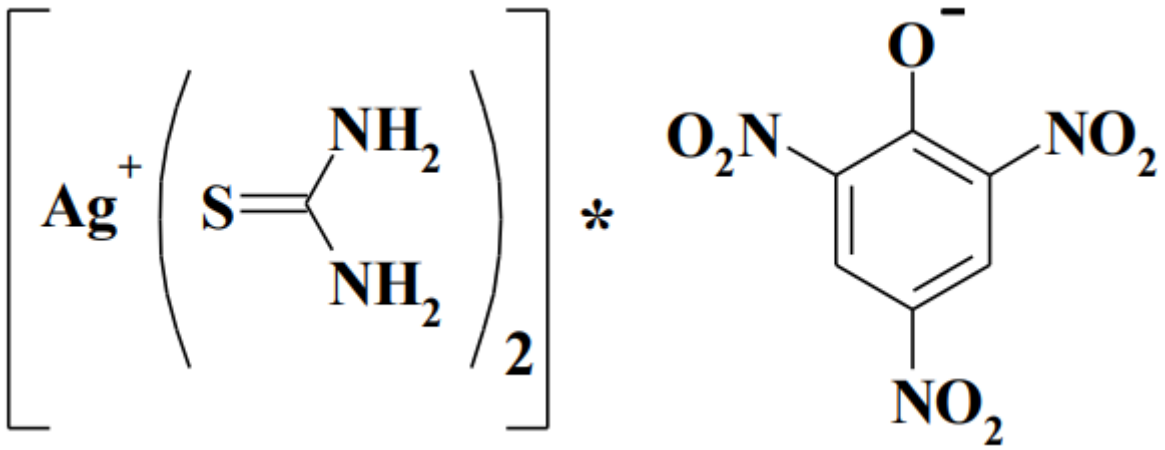
Detection limit - 0.15 μg silver per test sample

2. Obtaining crystals of ammonia chloride complex silver

The resulting ammonia solution is left for glass slide. After removing excess ammonia under microscope observe the formation of characteristic small colorless crystals and intergrowths of tetrahedrons and triangles

The detection limit is 0.05 mg of silver in the test sample.

3. Reaction with thiourea and potassium picrate. Mix 2-3 drops of the resulting ammonia solution with saturated solutions of thiourea and picric acid. In the presence of silver ions, crystals are formed in the form yellow needles and rosettes.



4. Reaction with gold chloride and rubidium chloride. 1-2drops of ammonia solution of the precipitate are evaporated to dryness. O nthe remainder is applied with a drop of a solution containing rubidium chloride sand gold. After 5-10 minutes, the formation of garnet-red prismatic crystals and intergrowths from them is observed.

2AgCl · 3AuCl3 · 6RbCl

Grade. The detection limit is 0.1 μg of silver in the sample.

Quantitation

1. Atomic absorption spectrometry.
2. Titrimetric method. Into the separatory funnelplace 50 ml of mineralizate, add 1 ml of 0.01% solutiondithizone and 3 ml of chloroform. The mixture is constantly shaken andtitrate with 0.01 M ammonium thiocyanate solution until changegolden yellow to green color. Definition of silvertitrimetric method is possible when its content is 100g organ in an amount of 2-20 mg or more.
3. Extraction-photocolorimetric method. IN The method is based on the interaction reaction of silver ions with dithizone. Silver dithizonate is extracted with tetrachloridecarbon. Optical density is determined at wavelength426 nm in a cuvette with a layer thickness of 10 mm. Calculations are carried out according tocalibration chart. The method allows you to determine in 100 gobject 0.02-10 mg silver