

Lesson 17. Carbon monoxide. Methods of chemical-toxicological analysis.
Spectrophotometric determination of carboxyhemoglobin in blood.

Physico-chemical properties: colorless and odorless gas, which burns with a blue flame. It is a product of incomplete combustion of carbon.



Formed during:

- Incomplete combustion of fuel
- The processing of ferrous and non-ferrous metals,
- Contained in exhaust gases,
- Fires.

The main types of poisoning:

1. Poisoning during a long stay in closed garages where the engine is running
2. Poisoning from fumes (in boiler rooms)
3. Poisoning caused by fires

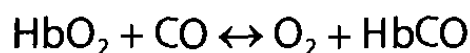
Newborns are more resistant to carbon monoxide

The method of absorption is inhalation

The effect of carbon monoxide on the body – disrupts the oxygen-carrying function of the blood (blood cannot carry oxygen)

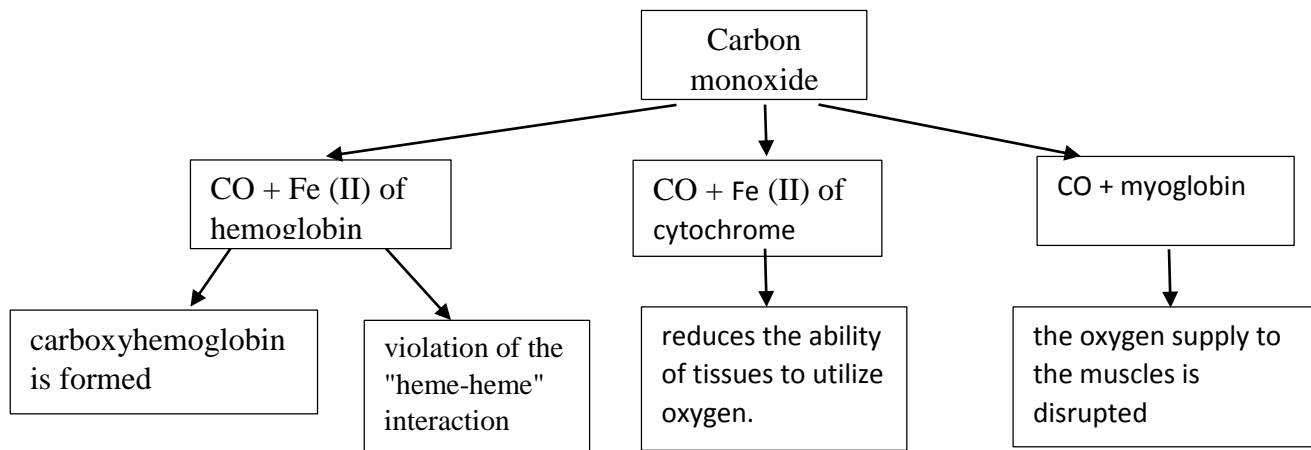
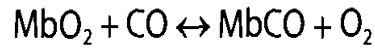
Mechanism of toxic action:

- Carbon monoxide binds to Fe (II) of hemoglobin. as a result, carboxyhemoglobin is formed (greater affinity for hemoglobin than oxygen)



- Carbon monoxide binds to Fe (II) of cytochrome oxidase this reduces the ability of tissues to utilize oxygen.

- CO binds to myoglobin and is fixed by tissues (the oxygen supply to the muscles is disrupted)



Carbon Monoxide Poisoning symptoms



Concentration HbCO %	Symptoms
0-10	-
10-20	Compression of the forehead, headache, redness of the skin
20-30	Mild degree: heaviness in the head, throbbing in the temples, clouding in the eyes, weakness, nausea, frequent vomiting, drowsiness. frequent breathing
30-40	Moderate degree: drowsiness, shortness of breath, loss of consciousness and memory, lethargy, convulsions
40-50	+ rapid breathing and pulse, collapse
50-60	Severe degree: loss of consciousness (days), hallucinations, delirium, convulsions, paralysis, respiratory distress, scarlet skin and mucous membranes.
60-70	+ weakening of breathing and pulse
70-80	Respiratory arrest, death

Factors affecting the course of monoxide poisoning:

- Ethyl alcohol: the more alcohol there is in the blood, the less HbCO is formed
- Prussic acid (HCN) increases the toxic effect of CO
- Nitrogen oxides (II and IV) increases the toxic effect of CO
- Age (newborns are more enduring)

CO is excreted from the body through the respiratory tract in 1 hour by 60-70%, in 4 hours - by 90-96%.

Objects of research: blood, muscles (rarely).

Method of Isolation: CO is determined directly in blood.

Detection

1. Gas chromatography. Sodium carbonate or bicarbonate is added to the blood. CO passes into the gas phase which is injected into a chromatograph. Detection by retention time.

2. Chemical reactions

Carbon monoxide forms strong bonds with hemoglobin

Reagents are added to the blood. Blood with carboxyhemoglobin remains bright red, but blood without carboxyhemoglobin changes color

Name of a test	Reagent	Coloring of blood containing HbCO	Coloring of blood without carboxyhemoglobin
Hoppe—Seylor test	with sodium hydroxide	Bright red	Brown
Salkovsky—Katayama test	with ammonium sulfide	Raspberry-red	Gray-green
Horoshkevich—Marx test	with quinine and ammonium sulfide	Light pink	Dirty red
Burker test	with potassium (III) hexacyanoferrate	Red	Yellow
Sidorov test	with potassium (III) hexacyanoferrate and potassium dichromate	Carmine-red	Brown-green
Wetzel test	with potassium (III) hexacyanoferrate and acetic acid	Cherry-red	Gray-brown precipitate
Libman test	with formaldehyde	Red	Brown-black
Rubner test	with lead acetate	Red	Brown
Zaleski test	with copper (II) sulfate	Purple-red	Green

3. Spectroscopic method: hemoglobin absorbs light of a certain wavelength. When light passes through the hemoglobin solution, dark absorption bands appear.

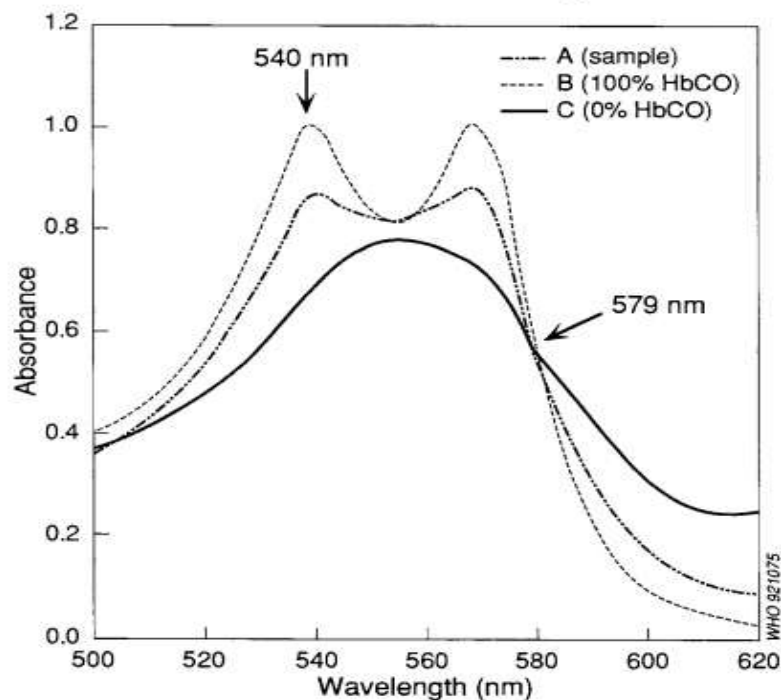
- if there is **no carboxyhemoglobin in the blood**, there are **two absorption bands**. When adding a reducing agent $(\text{NH}_4)_2\text{S}$, - **one wide band**
- if there is **carboxyhemoglobin in the blood**, there are two bands. . When adding a reducing agent. $(\text{NH}_4)_2\text{S}$ the stripes do not disappear, but there is a blackout between the stripes

4. UV-Spectrophotometric determination of carboxyhemoglobin.

In the blood, hemoglobin is in the form of deoxyhemoglobin (Hb) and oxyhemoglobin (HbO_2), and slightly met-hemoglobin. If there is CO in the blood, then the HbCO is formed. All compounds have characteristic absorption spectra in the region of 450-620 nm.

- When interacting with reducing agents, all hemoglobin compounds except COHb are reduced to deoxyhemoglobin.
- Carboxyhemoglobin is not restored, and its absorption spectrum is preserved.

Fig. 11. Spectra obtained using a blood sample from a patient poisoned with carbon monoxide (A), 100% HbCO (B), and 0% HbCO (reduced haemoglobin) (C)



Method of determination of carboxyhemoglobin by WHO (Geneva, 1998)

The blood is divided into 3 parts:

Part A is left unchanged	Part B (standard) is saturated with carbon monoxide up to 100% of the HbCO content	Part C is oxygenated to 100% oxyhemoglobin.
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A reducing agent $\text{Na}_2\text{S}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ is added to each part



Absorption spectra of three solutions are recorded in the region of 500–620 nm.



The optical density of A is measured at 540 and 579 nm



Calculation of the content of carboxyhemoglobina

$$\text{HbCO (\%)} = \frac{A_{540}/A_{579} (\text{раствор A}) - A_{540}/A_{579} (\text{раствор C})}{A_{540}/A_{579} (\text{раствор B}) - A_{540}/A_{579} (\text{раствор C})} \cdot 100.$$

The physiological norm is 1.5–3%

Lethal concentration 60%

Quantitative determination of carbon (II) monoxide is performed by physical-chemical methods:

- **Gas chromatography (GC)** and **gas liquid chromatography (GLC)**, based on the height of the peak in the chromatogram of the compound;
- **UV-spectrophotometry**