EXPERIMENT NO: 2

DETECTION OF CARBON MONOXIDE AND CYANIDE IN BLOOD

DETECTION OF CARBON MONOXIDE

A) General Information:

a. CARBON MONOXIDE (CO):

Poisoning with carbon monoxide is one of the oldest toxicological events encountered by human beings and acute poisoning and deaths still remain important. CO is a colorless, odorless gas and lighter than air. CO, occurs when carbon compounds (eg solid and liquid fuels) are not fully burnt. Another important source of exposure to CO is the exhaust gases of motor vehicles. In addition, cigarette smoke also contains a significant amount of CO: people exposed to cigarette smoke (both exposed to smoking and passive) are exposed to CO. During the heme catabolism of the human organism, COHb occurs endogenously (0.5% COHb).

CO toxicity is associated with its affinity to hemoglobin. CO's interest in Hb is 200-300 times higher than that of oxygen. CO with Hb creates carboxyhemoglobin (COHb), reduces the capacity of Hb to carry oxygen to tissues. Half of the Hb in the blood was retained by the CO even when the ratio between the CO and oxygen partial pressures in the air was 1/200.

Carboxyhemoglobin (COHb) Saturation Percentage: It refers to the percentage of Hb in the blood that is associated with CO and is an indicator for the severity of poisoning.

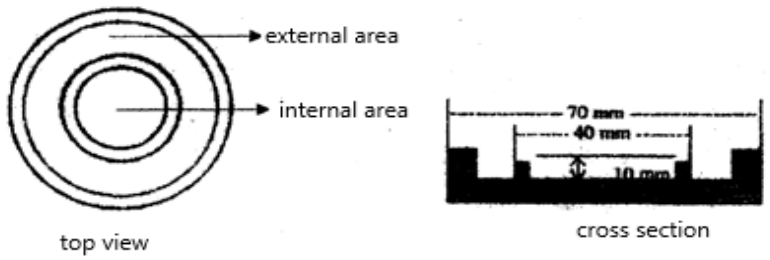
CO's interest in Hb is related to its affinity to ferro (Fe + 2) ions. Hence, Fe + 2 ion-containing Hb and myoglobin (a hemoprotein found in the muscles) are targeted for CO.
In CO intoxications, the oxygen-carrying capacity of the blood to the tissues and, consequently, the tissue oxygen pressure decrease; as well as the effect of CO, it also affects the separation of oxygen from Hb (oxygen's increased interest in Hb and oxygen cannot be released into the tissue).
In general, the severity of intoxication depends on the amount of CO contained in the air, the duration of inhalation and the percentage of COHb saturation.

COHb Saturation percentage	Effect
20%	The symptoms of poisoning begin to appear.
40%	Symptoms of poisoning are exacerbated.
60%	Death

Symptoms of poisoning: feeling of fullness (headache), headache, nausea, vomiting, fatigue, blurred vision, tinnitus, mental confusion, speech difficulty, incoordination of the muscles, pulse acceleration, skin flushing, respiratory acceleration-irregularity. Drowning and loss of consciousness are followed by death. When COHb in blood is above 30%, skin and mucous membranes are colored pink. In acute poisoning, the characteristic pink color at autopsy is noteworthy. If the CO concentration in the air is low and the absorption is slow, cyanosis is seen. Psychomotor neurological reflex disorders, blurred vision, performance changes (in particular, deceleration in time) may occur as a result of low concentrations of CO exposure; this is of particular importance for long-term exposure, traffic accidents where traffic is dense.
Intoxication: It is characteristic that the blood and tissues become more pink than the normal blood color in poisoning with CO. In the event of death, the cherry red color of COHb immediately manifests itself in the whole body skin and mucosa; leather gets bright red color. Analytical toxicological identification is made by determining the amount of COHb in the blood.

b. Separation of Volatile Poisons:
It was previously mentioned that distillation method and microdifusion methods were used for the separation of volatile poisons. Let's take a brief look at the method of microdiffusion. In a closed system, the reagent that releases the volatile poison from the biological material to be analyzed is placed in the outer part of the reagent, and the solvent to hold the volatile poison is placed in the inner chamber. The volatile poison, which becomes gaseous at room temperature or at 37°C, diffuses in this closed system and dissolves in the solvent in the inner chamber. This transition event lasts until the balance of vapor pressure in the system is reached. In this way, the volatile poison in the biological material passes to the solvent in the inner chamber. The identification of the volatile compound may be carried out in the microdifusion device by addition of a suitable reagent to the inner chamber, and it is also possible to carry out reactions in the inner chamber by conducting reactions in a tube.

The microdifusion device consists of two chambers of glass or porcelain, similar to the petri dish, and a glass lid that is not sheared out. The external chamber of the microdifusion device is placed with biological material to look for 2-4 g of volatile poison. Add 1mL of the reagent to release the volatile poison. The inner chamber is placed in 2 ml of reagent to absorb this volatile poison and give a color reaction. The device is closed and waited for 2-3 hours.



Conway Microdifusion Apparatus

Detection of Volatile Poisons in Conway Microdifusion Apparatus

Poison	**EXTERNAL AREA**	**INTERNAL AREA**	
	Reagent used to release poison	***Absorbent reagent***	***Color reagent and result***
Alcohol	Saturated Na_2CO_3	Acid + $K_2Cr_2O_7$	yellow → green
Aldehydes	saturated Na_2CO_3	Acid + $K_2Cr_2O_7$	yellow → green
Cyanide	10% H_2SO_4	10% NaOH	Na_2HPO_4 + chloramine T → red
Carbonmonoxide	10% H_2SO_4	$PdCl_2$	Metallic color / Black
Chlorinated hydrocarbons	————	Toluene	%20 NaOH + pyridine → red

B) Principle and Application of the Experiment:

a) Determination of 20% or more COHb saturation in blood:
I. 1 mL of blood which is suspected of poisoning is put in one of the two porcelain capsules, the other is put on normal blood and is slowly heated in the water bath. While normal blood gets charcoal brown-black, the other remains brick red (40% COHb saturation can be detected).
ii. Dilute 1 ml of the sample blood (less than 1 drop with baguette) to 10-15 mL of water in a tube.
these diluted samples are mixed by adding 5 drops of NaOH. Normal blood immediately turns into straw-yellow color, while blood with CO keeps the pink color. This assay is specific for CO, with 20% COHb can be detected. The intensity of the pink color is proportional to the amount of CO; quantitative determination.

b) Determination of COHb by pyrothannic acid:
This test, which is a semi-quantitative specific test, can detect COHb saturation of 20% or more. For this, 0.5 mL of blood is diluted with 4.5 mL of water. Add 5 mL of the pyrothannic acid solution and shake. At the end of the 15-minute standby, the blood containing the CO takes on the pink color, although the normal blood gives a gray-brown precipitate. The intensity of the color depends on the saturation%. The amount of color can be determined by comparing with the standards prepared with blood samples containing a certain amount of CO.

c) CO determination by Conway microdifusion method:
Principle of the Experiment: COHb in blood breaks down with H_2SO_4; released CO reacts with $PdCl_2$ to separate metallic palladium.

External area	ınternal area
1 mL of blood	2 mL of PdCl2
2 mL of 10% H2SO4	

Blood samples:

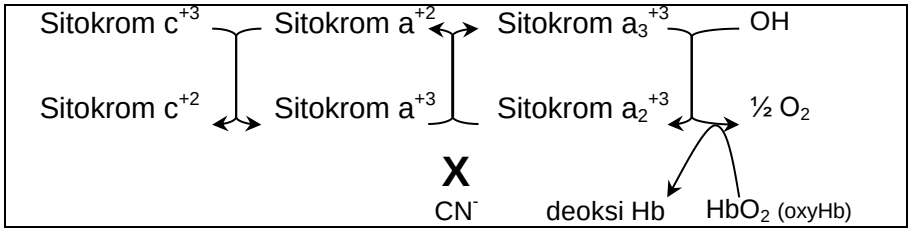
a) Normal blood, b) blood containing 60% CO, c) blood containing 100% CO

To complete the reaction, the apparatus is kept at room temperature for 2 hours or at 37 ° C for 1 hour.

DETERMINATION OF CYANIDE IN BLOOD:

A) General Information:

Absorbed cyanide (CN-) ions inhibit oxidative enzymes, especially cytochrome oxidase, in all tissues due to their affinity to ferri (Fe + 3) ions. This effect is based on the inhibition of electron transport in the electron transport chain. Electron transfer to molecular oxygen is blocked. Since the oxygen pressure in the periphery is adequate, even higher than normal, oxygen cannot be separated from the oxyhaemoglobin, so cells cannot use oxygen (histotoxic hypoxia).



After a toxic inhalation, symptoms occur within a very short period of time. Symptoms include respiratory failure, headache, fatigue, mental confusion, coma, cyanosis, arterial blood coloration, convulsion due to asphyxia, involuntary defecation and frequent urination. The pulse slows down first, then accelerates. Vomiting occurs before loss of consciousness, and the smell of characteristic cyanide is felt in the vomit (bitter almond smell). Due to high concentrations of oxyhaemoglobin in the blood, flushing of the skin and mucous membranes is seen. Death is due to respiratory arrest and may occur very quickly (within a few minutes); therefore, the intervention must be made urgently.

Therapy;

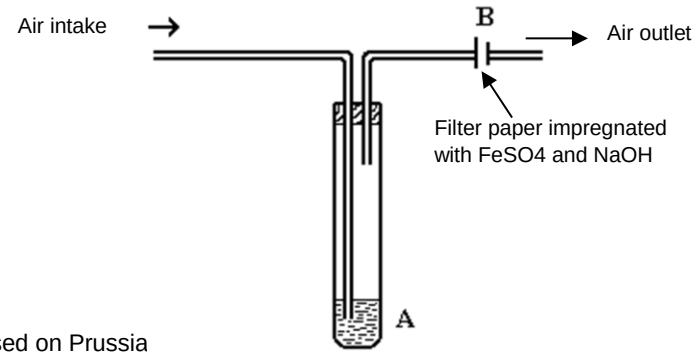
1) Amyl nitrite is quenched (Nitrite provides the oxidation of Hb to MetHb. The aim here is to prevent the inhibition of cytochrome oxidase by binding the free cyanide in the circulation. Until the injection of sodium nitrite, the amyl nitrite is initiated by immediately, letting the effect begin).

2) Sodium nitrite is administered intravenously.

3) Sodium thiosulphate is given (systemic antidote; it converts the cyanide into thiocyanate and thus excretes into the urine)

For the purpose of treatment, other routes can be followed: For example, cobalt edetate is bound with cyanide and excreted in the urine. Another way is to administer the cobalamin compounds. As a result of cobalamin interaction with cyanide, cyanocobalamin, Vitamin B12 occurs.

B) Experiment Preparation: (Gettler and Goldbaum Method):



The experiment is based on Prussia

• A-tube is placed 3 mL of cyanide blood.

• Filter paper impregnated with FeSO4 and NaOH is placed between the B-wings.

• Add 2 mL of 15% TCAA (trichloroacetic acid) solution to the A-tube and seal the tube and allow gas to escape from the environment for approximately 30 minutes.

At the end of the holding time, the impregnated filter paper is removed and immersed in a 30% HCl. In the presence of cyanide, distinct blue color (Prussian blue) occurs. With this assay, 0.005 mg (5µg) cyanide in 10 mL of blood can be identified and quantified.

Impregnation of filter paper: Immerse the Whatman No: 50 filter paper in 10% FeSO4 for 5 minutes and dry. It is then immersed in 20% NaOH. It is removed and dried after 5 minutes.