EXPERIMENT NO: 1

ETHYL ALCOHOL DETERMINATION IN BLOOD

A) General Information:

Ethyl Alcohol: Ethyl alcohol can be found in alcoholic beverages in varying amounts depending on the type of drink (eg 4-6% in beer, 8-12% in wine, 40-55% in raki and whiskey).The fatal and acute toxic dose of alcohol varies according to the type of drink taken and the individual factors.Ethyl alcohol, taken orally, is absorbed by a small amount of oral and gastric mucous membranes, while most of it is absorbed from the small intestine.After absorption, it is distributed homogeneously in all body fluids.The metabolism in the organism occurs quickly:First, it is oxidized to acetaldehyde, then to acetic acid; acetic acid is also mainly oxidized to CO2 and water. 90% of the absorbed ethyl alcohol is oxidized; the rest is excreted by urine, breathing air and other body fluids.

Ethyl alcohol is a potent central nervous system depressant. The symptoms of acute alcohol intoxication increase in relation to the concentration of alcohol in the blood. Ethyl alcohol expands coronary and skin vessels; also increases cerebrospinal fluid secretion and pressure.Although it accelerates breathing at low concentrations, it has a depressing effect on heavy poisonings.

The main symptoms of acute alcohol intoxication are:

Nausea, vomiting, dizziness, smell of ethyl alcohol in breathing and vomit, convection of language, sweating, reflex and movement co-ordination disorder, personality change, heat loss (chills)as a result of dilatation of blood vessels in the skin, deceleration pulse, respiratory collapse and coma. The cause of death, respiratory depression.

When the effect of alcohol poisoning passes, acidosis, edema in the brain, swelling of the liver and kidneys are seen.Headache observed after drunkeness is associated with cerebral edema.

Determination and Importance of Ethyl Alcohol in Biological Material:

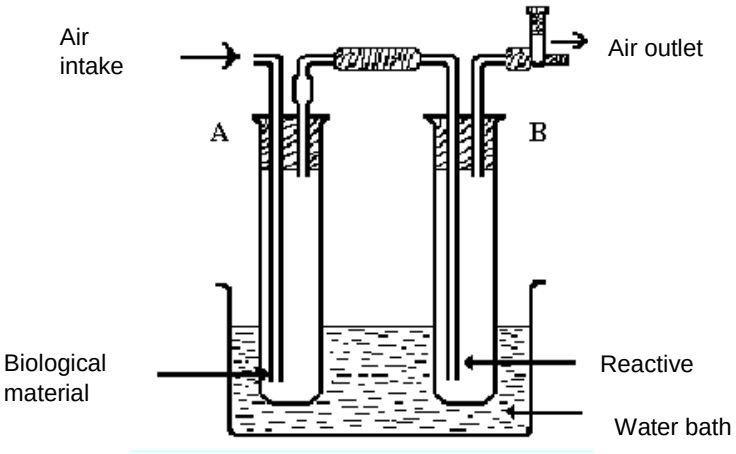
The increasing consumption of ethyl alcohol continues to be a social problem which constitutes a great risk for both health and economy.In particular, it is known that drunk-driving has an important role traffic accidents and accidental deaths.

In many countries it is forbidden to drive while under the influence of alcohol. For this reason, when the blood alcohol level of the driver is above a certain limit, it is considered as a traffic crime.For example; In Norway, 0.05% g, Sweden 0.08% g, Denmark, 0.10% above the detection of alcohol, drunkness is a symptom and in this way is a crime to drive.

In our country, drunk driving are completely banned for commercial vehicle drivers and public service drivers (even trace amounts of alcohol are not allowed).For other drivers, 0.5 Promil was determined as a legal limit, equal to 0.5 gram (0.5 g / L = 0.05 g / 100 mL = 0.05%) alcohol in one liter of blood.This legally accepted value is different in various countries. (PROMOTION: 1/1000 means 1 g alcohol / 1000 mL blood)

Considering its legal implications, the sensitive and precise determination of ethyl alcohol appears to be important in analytical toxicology.

B) Principle and Practice of Experiment:



Apparatus : As shown in the figure, two tubes with a size of 30x210 mm are attached with two-hole mushrooms. After the inlet and outlet pipes are installed, the intermediate connections are made with clean rubber tube. The apparatus is immersed in a boiling water bath and air flow is supplied by the water trompe.

Principle: Alcohol in the blood, urine, cerebrospinal fluid or stomach contents is released by distillation and is retained by the reagent in the second tube (tube B) (Ansties reagent: $K_2Cr_2O_7$ + H_2SO_4) and green color occurs as result of the reaction. (Cr^{+6} in $K_2Cr_2O_7$ is reduced to Cr^{+3}. Cr^{+6} is yellow-orange and Cr^{+3} is green.) The severity of the green color is proportional to the amount of ethyl alcohol. Under test conditions and when working with 4 mL of blood, 0.05 to 0.30 g of ethyl alcohol complies with the Lambert-Beer law. This method is sensitive to 0.01%. g alcohol.

The absorbance of the green solution is read on the spectrophotometer at 605 nm against the blank (blank) assay. The result is read from the calibration chart previously prepared with standard ethyl alcohol.

Experimental Procedure:

a) Add 2 ml of saturated NaOH and 2 mL of saturated $HgCl_2$ to tube A containing the biological material (blood, urine, stomach contents) to be determined to 4 mL of ethyl alcohol. If necessary, the antifoam agent is also added. 9 mL of Ansties reagent is added to tube B.
b) Immediately close the mouth of the tubes and wait 20 minutes in a boiling water bath. In this case, proper airflow is provided with the trompe.
c) After 20 minutes, the tube B removed from the water bath is cooled and carefully poured into a 10 mL glass beaker. The missing volume is completed with water.
d) The test is also carried out under the same conditions as the biological material in which there is no alcohol. (Used as blind in spectrophotometer)
e) The color intensity will be determined by spectrophotometric method. For this purpose, a solution of 10 ml diluted color solution is placed in the spectrophotometer cuvette.After the solution prepared as blank is poured into another cuvette, the absorbance of the colored solution at 605 nm is read against the curve.This result is evaluated from the previously prepared calibration curve and the amount of ethyl alcohol is calculated as % g.

Preparation of the calibration curve:

Firstly, prepare the 2% standard ethyl alcohol solution (by weight). To this end, 2.53 mL of absolute alcohol is added to a 100 mL volumetric flask containing 50 mL of water by means of a pipette or burette. The volume is filled to 100 mL with water in volumetric flask and its mouth is tightly closed.

4 mL blood samples known to be free of alcohol are added from 0 to 0.8 mL of this standard alcohol solution, respectively. Distilled by Modified Bogen method as described above.The optical density of the colors that the standards produce with the Ansties reagent is read against in the spectrophotometer at 695 nm against blank.The calibration curve is prepared by marking these absorbance values and the corresponding alcohol concentration in a graph paper.

The amount of alcohol corresponding to the amount of alcohol (ml) taken in the blood (% g) is shown below (4 mL blood)

Amount of alcohol taken from 2% (mL)	**The amount of alcohol in the blood corresponds to% g**
0	Negative
0.1	%0.05
0.2	%0.10
0.3	%0.15
0.4	%0.20
0.5	%0.25
0.6	%0.30
0.7	%0.35
0.8	%0.40