OSMOTIC FRAGILITY OF ERYTHROCYTES

Principle:

Cells respond to changes in osmotic pressure. Hypotonic medium induces rupture of the plasma membrane and liberation of the cytoplasmic content. This phenomenon can be easily studied with erythrocytes, which release hemoglobin that is easy to assay, and hemolysis is apparent by a naked eye.
For measurement of hemoglobin released from erythrocytes Drabkin reagent is used. This procedure is based on the oxidation of hemoglobin and its derivatives to methemoglobin in the presence of alkaline potassium ferricyanide. Methemoglobin reacts with potassium cyanide to form coloured cyanmethemoglobin. The colour intensity measured at 400 nm is proportional to the total hemoglobin concentration.
We use human erythrocytes that were washed with physiological solution and thus do not contain plasma.

Chemicals: Drabkin reagent – potassium ferricyanide ($K_3[Fe(CN)_6]$) and potassium cyanide (KCN) in alkaline medium

Procedure:

BE CAREFUL! USE GLOVES AND PROTECTIVE COAT – YOU ARE WORKING WITH A BIOLOGICAL MATERIAL, POTENTIALLY **INFECTIOUS**, AND WITH A **HIGHLY TOXIC** DRABKIN REAGENT!

1. Label 5 eppendorf microtubes 1–5 and pipette 1 ml of the following solutions into them:

- tube 1 – physiological solution (non-diluted)
- tube 2 – physiological solution diluted with water in the ratio 3:1
- tube 3 – physiological solution diluted with water in the ratio 2:1
- tube 4 – physiological solution diluted with water in the ratio 1:1
- tube 5 – physiological solution diluted with water in the ratio 1:5, containing NH_4Cl (NH_4Cl disables remaining membrane pumps)

2. Pipette 50 µl erythrocyte suspension into each microtube, gently mix and let stand for 10 minutes. Then centrifuge 3 minutes at 3 000 × *g* and carefully collect the supernatants for hemoglobin assay.
Note: supernatant is the liquid above the sediment produced during centrifugation.

3. Prepare five glass test tubes 1–5 with 2 ml Drabkin reagent. Add 50 µl of each supernatant from the previous step into the corresponding glass test tube and mix gently.

Measure the absorbances of all the samples at 400 nm against a blank containing the Drabkin reagent only.

Due to the fact that the blood sample is not fresh, we observe partial hemolysis even in test tube 1. The amount of hemoglobin determined in this test tube represents the control value and should be subtracted from the values obtained for all the tubes. The value of hemoglobin

measured in test tube 5 is the maximum obtainable amount and we express it as 100% hemolysis. On this basis, calculate the percentage of hemolysis in test tubes 2–4.

Using a calibration graph (consultable in the laboratory), express your results as the hemoglobin concentration in mg/l.

Evaluation

Test tube	% of hemolysis
2	
3	
4	