Task III. Ultrasound and ionising radiation

Required knowledge: Ultrasound; Microscopy; Ionising radiation.

1. Hemolysis of erythrocytes by ultrasound

Main tasks:
Measuring effects of exposure of erythrocytes to ultrasound. To understand what action mechanism is present in the sample during ultrasound application. To understand how to use the Bürker chamber.

Measurement aids and implements:
Ultrasound generator BTL-07, microscope with or without connection to computer, Bürker counting chamber, stand with test tubes, pipettes, flat bottom test tube for sonication, suspension of erythrocytes, saline, cellulose wading, contact medium (paraffin oil).

Procedure:
The experimental sample is in a flat-bottom test tube. It is approximately 3 ml of erythrocyte suspension (horse or sheep blood diluted to 2 %).

1) First of all check in the microscope the sample of erythrocytes in the non-sonicated (control) suspension. It is recommended to use lens with 40× magnification (blue label). Use the Bürker chamber as a glass slide and place the cover glass on top of the sample. Transfer the control sample by the yellow pipette (0.1 ml). The cell suspension is applied to the edge of the cover glass to be sucked into the void by capillary action which completely fills the chamber with the sample.
2) Because of great number of cells, it is required to dilute suspension 10-times more into a clean plastic test tube (i.e. mix 0.1 ml of sample suspension and 0.9 ml saline) Use the large pipette for saline. **It`s required to stirr thoroughly the suspension before each manipulation!**
3) Clean the Bürker chamber by water and tissue and fill the chamber with the new diluted suspension. Put it on the stage of the microscope, find an area with squares of the measuring grid. Count erythrocytes in 20 small squares together (see how to use Bürker chamber). Clean Bürker chamber after counting again.
4) Switch on the ultrasound generator and place the flat-bottom test tube (control sample) on the ultrasound probe. Be sure that there is enough of contact medium (oil).
5) Set the time of sonication – 30 seconds (0.5 min). Set the ultrasound intensity – 0.1 W/cm^2 and press start button. After the first sonication, prepare new diluted sample into a plastic test tube (see point 2). Do the new counting in the Bürker chamber with the sonicated sample in 20 squares again.
6) Repeat the sonication for 30 seconds and every time count erythrocytes in diluted suspension to see the effect of increasing ultrasound exposure. It is recommended to do as many sonications to reduce the number of erythrocytes under 5 (**in all of the 20 small squares together).** Finally, don`t dilute the sonicated suspension and fill the Bürker chamber with undiluted suspension directly from the sonication test tube (flat bottom).
7) Write down (into a table) the number of erythrocytes recounted for 1 ml of **full blood** and corresponding levels of hemolysis (in %) in dependence on the time of sonication. Plot a graph showing the level of hemolysis in dependence on the time of sonification.
8) Use the equation below for the calculation of the number of cells per 1 ml:

$$N = nz/Shx$$

Where N is the number of erythrocytes in **1 mm^3** of undiluted suspension (full blood); n the total number of erythrocytes in x squares (usually 20), z – dilution of suspension (50 in case of the 2% blood, 500 in case of diluted blood), S – area of the small Bürker cell square (0.0025 mm^2) a h – 0,1mm).

2. Measuring ionising radiation absorption

Main tasks:
Determination of half-layer of given material (layer of the material able to reduce the intensity of ionising radiation to the half of the initial value) – lead rubber. To understand what an ionising radiation is and find the way how to protect against it and decrease its dose.

Measurement aids and implements:
Radioactive sample (cesium-137), spectral analyser of gamma radiation with a scintillation detector as a measuring probe, vernier caliper, tweezers, sheets of lead rubber.

Procedure:
1) Turn on the spectral analyser on its front side.
2) Measure the background activity **P** first. For the beginning press button ENT. Wait 60 second (it will stop automatically). Read and write down numbers **only from first column** shown on the display and calculate the average. Reset the display by pressing button ESC after the measurement.
3) By using of tweezers insert the radioactive sample (it is hidden in the lead container) into the chamber below the scintillation detector and measure its activity three times. From the measured values calculate the mean value and subtract the mean background value **P** to get the proper activity of the radioactive sample.
4) Repeat the experiment with reduced activity values by using lead filters. Measure the thickness of the first filter by the vernier caliper and insert it into the upper holder between the radioactive sample and the measuring probe.
5) Use next filter to have more layers and repeat the process described in point 5) also for other added filters. Measure the thickness of each filter. Use as many filters as it is necessary to decrease the number of impulses more than to the half value of activity measured in the sample without filters.
(**Note.** In fact, the activity of the sample cannot be changed by the effect of filters, because the number of disintegrating atoms cannot be influenced in this way. Only the number of photons hitting the detector can change. Further, it is necessary to realise that the detector can register only those particles that the sample emits to the respective spatial angle and are absorbed in the scintillator.)
6) Make a table of measured mean values of sample activity and adequate thicknesses of filters. Draw a graph of dependence of registered photons per unit time on the thickness of absorption layer. Determine the half-layer ($D_{1/2}$) value from the graph and calculate the **linear attenuation coefficient (μ)** of the given material and for the used emitter.

Use this equation:

$$\mu = ln2 / D_{1/2}$$