Task III. Ultrasound and ionising radiation

Required knowledge: Ultrasound; Microscopy; Ionising radiation.

1. Hemolysis of erythrocytes by ultrasound

Main tasks:
Check effect of ultrasound on erythrocytes from position of duration of sonication.

Needs for measurement:
Ultrasound generator BTL-07, microscope with connection to computer (or without), Bürker counting cell, stand with test tubes, pipettes, flat bottom test tube for sonication, suspension of Erythrocytes, physiological saline, cellulose wading, contact medium (paraffin oil).

Procedure:
In beaker is prepared suspension of erythrocytes (diluted 2% horse blood).

1) After adequate shuffle pour this suspension into a flat-bottom test tube, suffice approximately 3 mL (half of test tube).
2) First of all count number of erythrocytes this unsonicated (control) suspension. It`s required to dilute suspension 10 times (0.1 mL suspension + 0.9 mL saline).
   It`s required to shuffle suspension before every manipulation!
3) Full Bürker cell and count erythrocytes at least in 20 small squares.
4) Switch on the ultrasound generator and place the flat-bottom test tube with based erythrocytes suspension on the ultrasound probe.
5) Set the time of sonification – 30 seconds (0.5 min). Set the ultrasound intensity – 0.1 W/cm^2
6) Take up 0.1mL from the sonicated suspension after shuffle and dilute by addition of 0.9mL of saline and count total number of the erythrocytes in 20 squares again.
7) The suspension in the flat-bottom test tube sonicate for 30 seconds again. Then proceed by point 6) during sonication. If the sum number of erythrocytes drop under 5 (**total in all of the 20 small squares)** after that don`t dilute the suspension and full Bürker cell with undiluted suspension from base suspension.
8) Accomplish 6 sonifications. If the full hemolysis happen (e.g. after four thirty-seconded sonication), end the experiment.
9) Into a table write down number of erythrocytes recounted for 1mL of **full blood** and level of hemolysis (in %) in depend on the time of sonication. Into graph register level of hemolysis and the time of sonification.
10) For calculation of number of cells in 1 ml use this equation:

$$N = nz/Shx$$

Where N – number of erythrocytes in 1mm^3 undiluted suspension – full blood; n – total number of erythrocytes in x squares (usually 20), z – dilution of suspension (50 in case of 2% blood, 500 in case of diluted blood), S – area of small square of Bürker cell (0,0025mm^2) a h – 0,1mm).

2. Measuring ionising radiation absorption

Main tasks:
Determination of half-layer (layer of the absorption substance as reduce the intension of ionising radiation to the half of the initial value) of given substance – lead rubber.

Needs for measurement:
Radioactive sample (cesium-137), scintillation detector with measuring probe, vernier caliper, tweezers, sheets of lead rubber.

Procedure:
1) Turn on spectral analyser on back side.
2) Set the duration 60 seconds of measuring, press the button TIME repeatedly as necessary on front panel. Press button ENT two times to confirm the time.
3) Measure background activity three times and calculate mean value **P**. For starting the measurement push button START, for resetting the display push button CLEAR. Each measurement take one minute and the green LED control stop to blink at the end of measuring. Read the number of pulses from the display and write it down.
4) By means of tweezers insert the radioactive sample into the bottom place under detector and three times measure its activity (including the background!). From the measured values calculate the mean value and take away the mean background value **P** and thus get the activity proper of the radioactive sample. (Note: For safety reasons in practical there you use preparates with very low activity, without health risk, so they don`t require other special protection)
5) With the vernier caliper measure the thickness of first filter and insert it into the top stand between the radioactive sample and the measuring probe. Measure the activity of the sample three times, when the first filter is used and calculate the mean value and take away the background activity.
6) Use next filter to have more layers and repeat the process described in point 5) also for further filters measure the thickness of filters. Always measure 3 times. Use as many filters as is necessary to decrease the value of impulses more than half of sample of Cs.
(Note. In fact the activity of the sample cannot change by the effect of filters, because the number of disintegrating atoms cannot be influenced. Only the number of particles falling on the detectors changes. Further, it is necessary to realise that the detector can only register those particles that the sample emits to the respective spatial angle and are absorbed in the scintillator.)
7) Make a table of measured, mean values and values acquired after taking away from it the mean background value, compared with thickness of absorption layer. Create a graph of dependence of registered particles per unit of time, on the thickness of absorption layer. Establish from the graph half-layer ($D_{1/2}$) and calculate linear attenuation (μ) coefficient of relaxation of the given emitter.
Use this equation:
$$\mathbf{D_{1/2} = \ln 2/\mu = 0{,}693/\mu}$$