Methods of Lipid analysis

Done by: Naizabayeva D.
Accepted by: Kenzhebayeva S.S.

Folch method

Principle: lipids are extracted by chloroform-methanol mixture, extract is washed from water soluble impurity, dried and then obtained precipitate is weighted.

*nonpolar solvents (chlorophorm, benzol, diethyl ether): destroy complexes arranged by hydrophobic interactions.

*polar solvents (ethanol, methanol): destroy hydrophilic and electrostatic bonds.

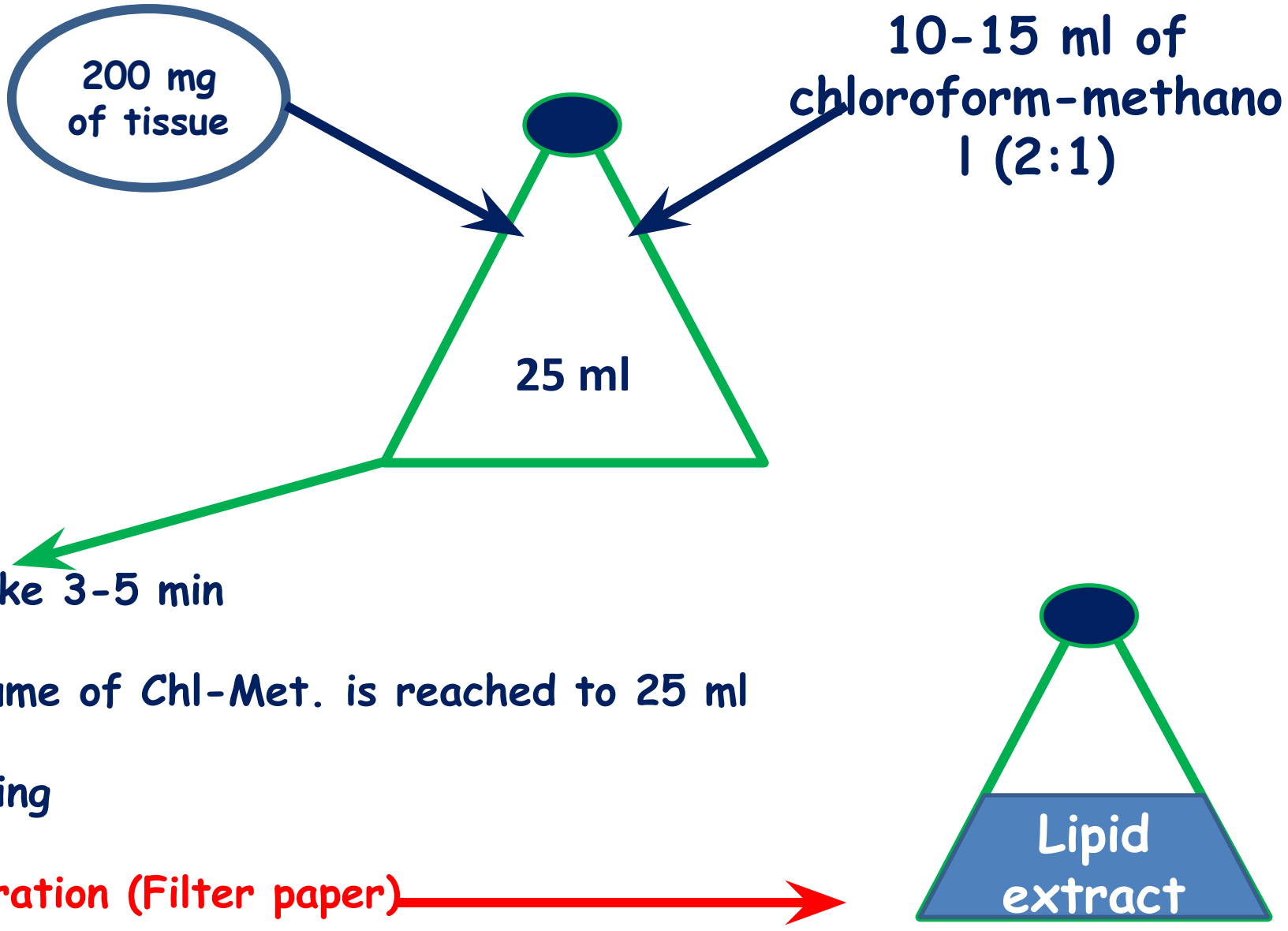
Reagents: Chloroform and methanol (2:1)

Folch method

Procedure:

1. Extraction
2. Purification
3. Drying
4. Weighting of dry lipid extract

Extraction


200 mg
of tissue
10-15 ml of
chloroform-methano
l (2:1)
25 ml
*Shake 3-5 min
*Volume of Chl-Met. is reached to 25 ml
*Mixing
*Filtration (Filter paper)
Lipid
extract

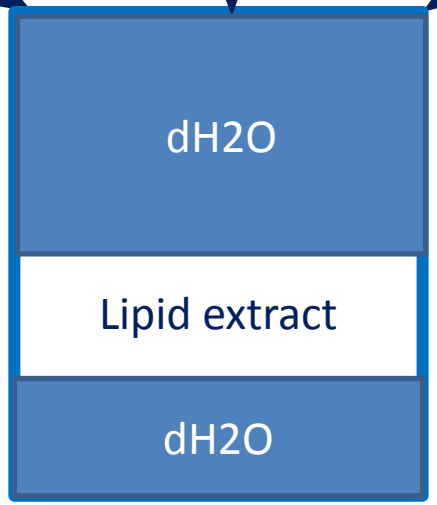
Purification

3) dH2O till the top of the beaker

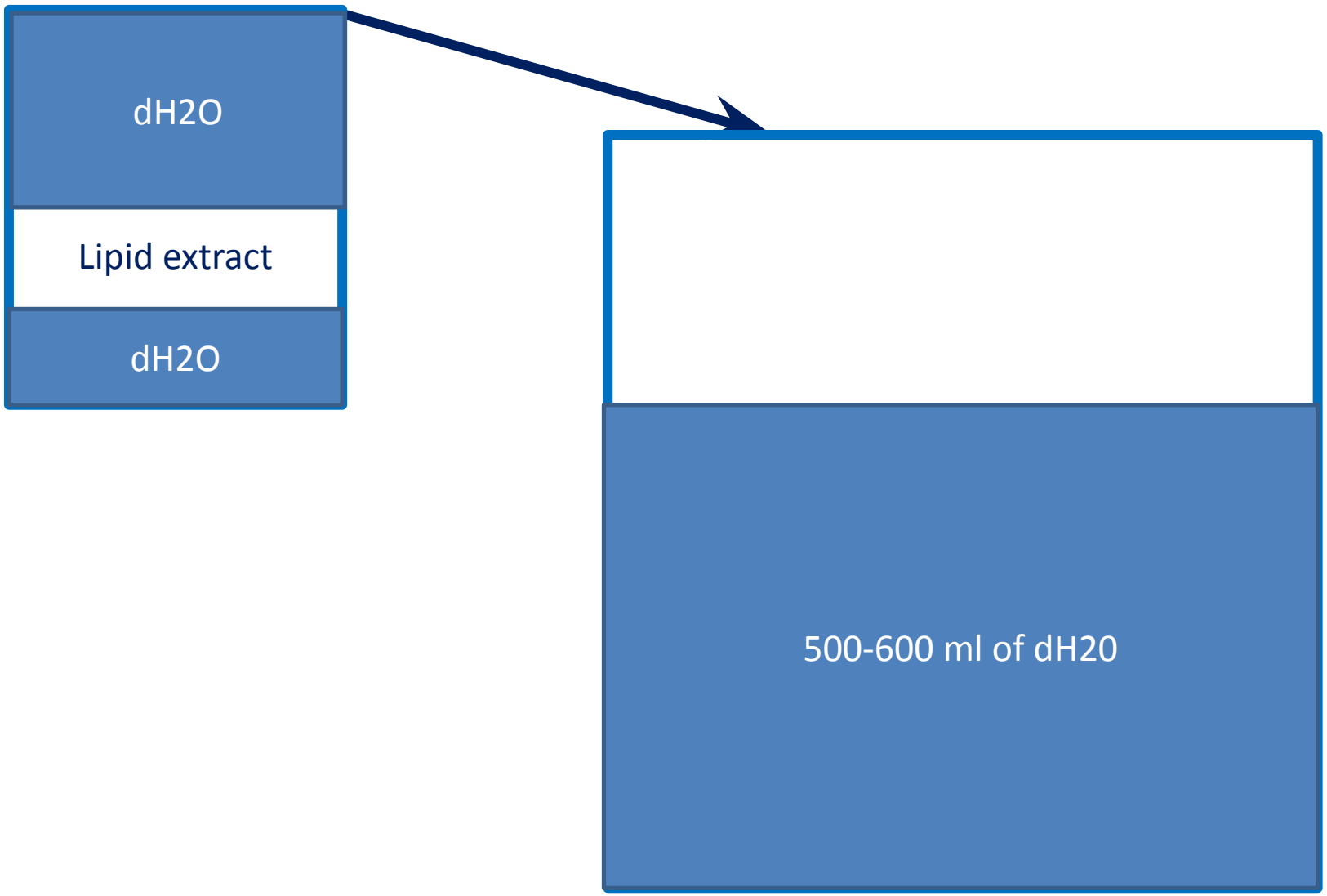
1) 10-20 ml of dH2O

2) 10 ml of lipid extract

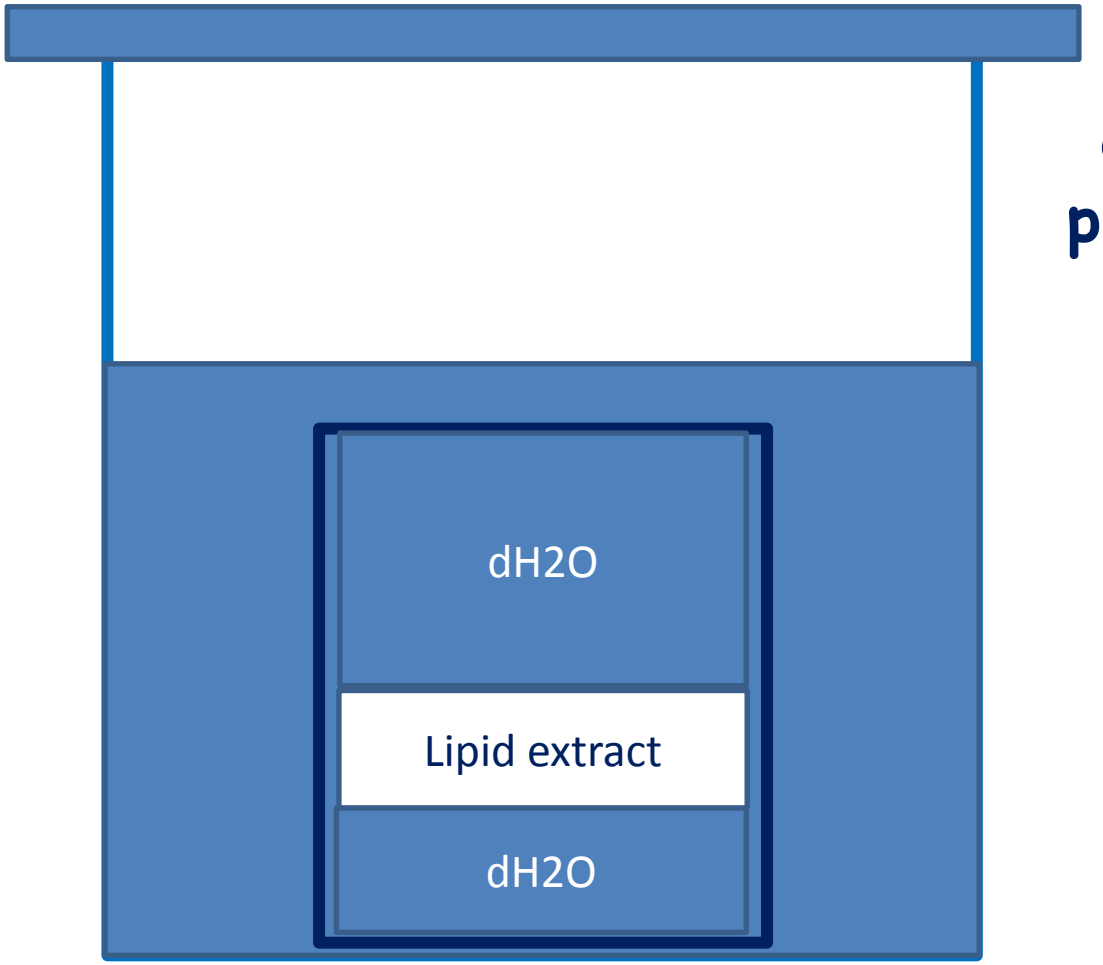
* The tip of pipette should be dived into the water and it should not intensively mix the solution



Purification

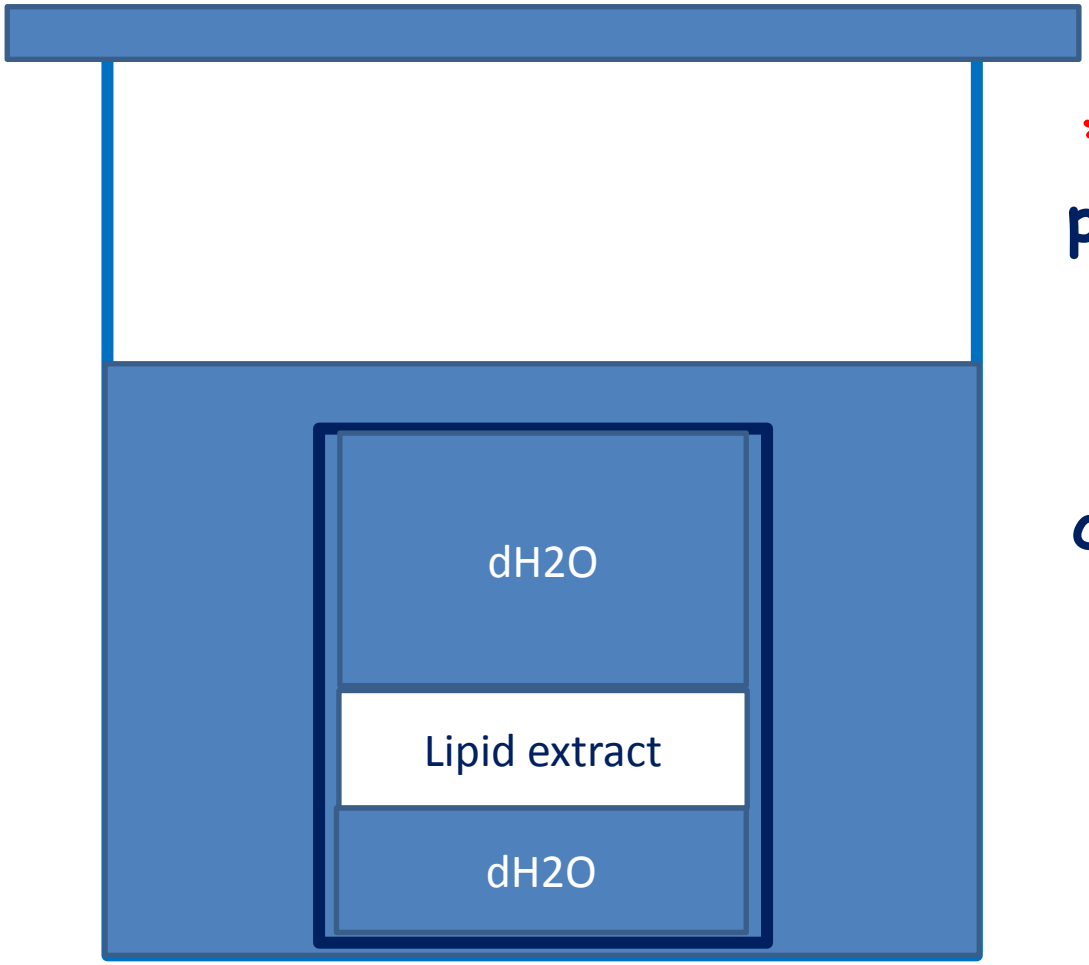


Purification



Covered with glass plate and stayed for a night

Purification

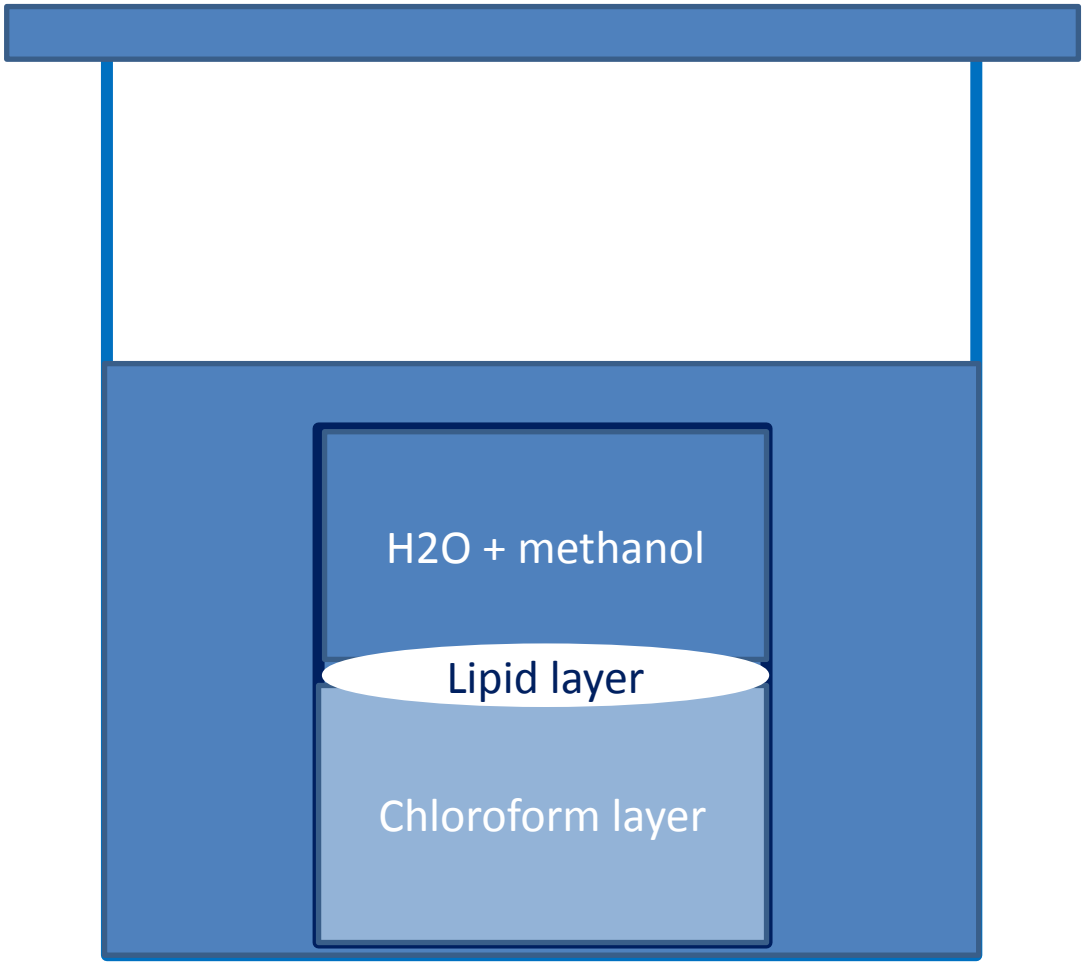


*Covered with glass plate and stayed for a night

*Water soluble components diffused into water

Purification

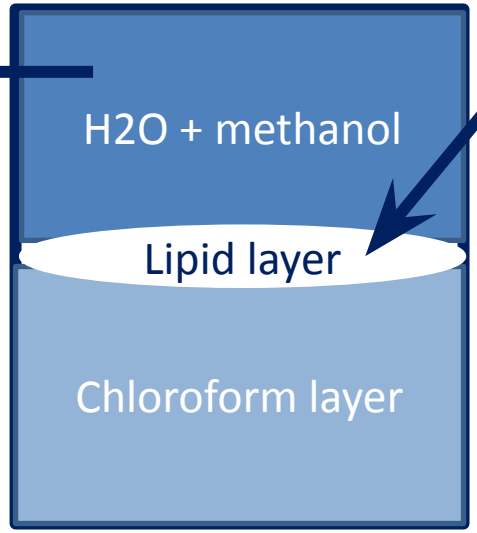
*Next day…



Purification

*Little beaker is taken away from the vessel

1) Methanol-water layer is poured out (2-3 mm is left)



2) 3 ml of methanol is added onto lipid layer to dissolve it

Drying and calculation

*After dissolving of lipid layer, mixture is poured into clean and dry beaker (weighted beforehand)

*Then drying is implemented by vaporization on water bath, and continued in thermostat at 50-60°C

*Dry precipitate is weighted

*Lipid consistency is calculated due to mass of dry precipitate in g per kg of initial studying tissue mass.

Thanks for attention!