10-06-25

THYLAKOID PURIFICATION

We start in LAB. In the cold room (4°C) we prepare B_1 with the leaves ~~to~~. We blend it, then centrifuge it at 4°C.

↳ important to not take the stems: they contain fibres we don't want

- We blend some leaves with 100 mL of B1 with the (pulse) setting.
- With a cheese cloth ~~and~~ and a funnel we filter the mixture into bottles.

(Don't blend too much, this causes heat)

- We centrifuge the mixtures at 2000 g in 4°C for 10 minutes

To do this, we first make sure every bottle weighs the same by either adding more buffer or taking out solution.

- we discard the supernatant → + add buffer B_2 and resuspend the pellet (green stuff stuck to the side) with a soft paintbrush to not disturb it too much
- we centrifuge again at 4000 g in 4°C for 10 minutes
- Discard supernatant + add B_3. Resuspend pellet with brush
- We centrifuge at 8000 g in 4°C for 10 minutes
- we resuspend the pellet in B_4.

All of this is done as much in the cold/dark as possible. The blinds in the centrifuge room weren't entirely closed

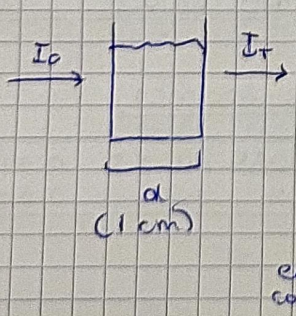
After which we took 1 part of our solution and mixed it with 200 parts acetone. After centrifuging it, the proteins were sunk to the bottom.

We pipetted only the not-solid parts into new vials with these vials we do spectroscopy.

we pipetted around 300 µL into the cuvette → the mixture should be barely green of the spectrometer.

we ran the program and got our graphs on USB to further analyse.

spectroscopy



$T = \frac{I_T}{I_0}$ (transmission)

$\epsilon(\lambda) = \frac{A}{c \cdot d}$

$A = -\log_{10} T$ (absorbance)

$A = \epsilon \cdot c \cdot d$

- ϵ: extinction coefficient
- c: concentration
- d: optical path

We want concentration $C = \frac{A}{\varepsilon(\lambda) \cdot d}$

Data analysis

(1) open excel file

(2) filter from low to high

(3) create extra column (B1 - MIN(B:B)) and drag it down
(second column (first row), whole column)

(4) (fitting program) data → search → solver → solver add-in → ok (tick it)

(5) copy column from spectroscopy, paste it in fitting program under "sample" (values)

(6) click 1, then 2

(7) go to 'chl' → put chl c to 0

(8) data → solver → solve

(9) Atrosso → blue and red should be similar

(10) beta car → 5

(11) solver → solve (good fit in 'fitting')

origin testo:

- chl/car (jasmijn 4.19 0226)
- chla/chlb (jasmijn 2.59)

(12) dilution → 200 → note down chl content (mg/ml)

jasmijn: 1.24

vol tot → 2