USP/DSP Jungbauer 28.1.2016 (A)

Literature: • Protein Purification – Rob Scopes (?)
↳ rather practical, no equations; ~~exam~~

! • Biosep. sci & eng. – Harris
↳ a lot of examples from this book

• Biosep. engineering – Mike(?) Ladisch
↳ too detailed, but several examples...? ..?

• Protein Chromatography – Jungbauer
↳ too detailed, rather for master thesis

In some examples: too much information → some data is not required to solve the problem

Ad 6) ~~100 000~~ ppm → μg/g ! ≙ 100 000 000 000 / 10   1 g / 10 $\frac{1}{10}$ g → 0.1 g ≙ 100 mg ≙ 10% purity of protein
~~1000 000~~   100 000 μg, 100 000 000 ng, 100 000 000 000 pg ↳ $1 \cdot 10^{11}$ pg ✓

• Assumption: max 10 pg / dose DNA

~~100 00~~ → 10^{11} → 10^{10} 10 pg reduction

1.) Clearance f. = 2 ≙ log(2) ≙ 100   clearance is log based!

$\frac{10^{11}}{100}$ → $\frac{100000000000}{100}$ → $1 \cdot 10^{8}$ first step! 2nd step!.. etc

$10^{11} \xrightarrow{1} 10^{9} \xrightarrow{2} 10^{7} \xrightarrow{3} 10^{5} \xrightarrow{4} 10^{3} \xrightarrow{5} 10^{1}$

↳ 5 ~~steps~~ reduction steps (≙ 10 log steps)

2.) 90% = step yield. → after every step: 90% remain
Wanted: overall yield?
↳ 5 steps → $y = 0.9^5 = 0.59$ → ~~(1 g/l · 0.59 → 0.59 g/l)~~
↳ 59%

3.) 70%
↳ $y = 0.7^5 = 0.17$ → 17%
↳ For an expensive product it is NOT economically viable; for an cheap product it is

Ad 1) Assumption: 10 pg / dose → 0.5 g/l ~~300~~ 150 g ~~300 L~~ not req.
↳ 10 pg / 250 mg dose ≙ 40 pg/g   30 mg/l
1) 30 μg / ml ~~300 L~~ → 30 mg / l   ~~[illegible]~~ mg / l → 60 mg per gram product

→ reduction from 60 mg/g → 40 pg/g
$6 \cdot 10^{10}$ pg → $4 \cdot 10^{1}$ pg → ~~log reduction factor~~

log reduction factor = $6 \cdot 10^{10} - 4 \cdot 10^{1} = (5.9 \cdot 10^{10})$
↳ log($6 \cdot 10^{10}$) to be on the safe side! ?!!

60.000 000 000

EXAM: NICE HANDWRITING ...
("If you have 3% error per step, what would be significance?")

1) 2.) $Y = 50\% \hat{=} 0.5$

~~reduction~~

$6 \cdot 10^{10}$ reduction

conc. fed:

$$\frac{C_f \cdot V_f}{C_H \cdot V_H}$$ f...feed, h...harvest

$V_f = 300\,l$
$C_{f_{DNA}} = 30\ \mu g/ml$

~~C_H = 10 pg/dose~~

300 l ... 0.5 g/l → 150 g
if Y = 50% → final = 75 g

$C_{H_{PROTEIN}} = 10\ g/l$ (→ final formulation) → $V_H = \frac{75\ g}{10\ g/l} \rightarrow 7.5\ l$

WANTED: $C_{H\,DNA}$ = 400 pg/l (calculate) $\hat{=}$ 0.4 pg/ml
via 40 pg/g and 10 g/l

$$\rightarrow \frac{3 \cdot 10^{7}\ pg/ml \cdot 300\ ml}{4 \cdot 10^{1}\ pg/ml\ 7500\ ml}$$

→ $= 3 \cdot 10^{9}$ is c ; WANTED: log

→ $\log(3 \cdot 10^{9}) = 9.5$

P2.) 5 steps, yield 50%

1.) yield of individual steps? Folien - Introduction (?)

$Y_i = \sqrt[5]{0.5} = 0.87 \rightarrow 87\%$ (because $0.5^5 = y$)

$\sqrt[n]{Y_T} = Y_i$

RESULTS in orange

3.) $Y_1 = 90\% \hat{=} 0.9$; $Y_2 = 0.75$; $Y_T = 0.5$

a) 5 steps $Y_1 \cdot Y_2 \cdot Y_3 \cdot Y_4 \cdot Y_5 = Y_T$
4 steps
3 steps

↳ $Y_T = Y_1 \cdot Y_2 \cdot Y_3 \cdot Y_4 \cdot Y_5$

$0.5 = 0.9 \cdot 0.75 \cdot$

↳ 0.5

|| WAS CORRECT!! XD

a) $\frac{0.5}{0.9 \cdot 0.75} = 0.74$

b) $\sqrt[2]{\frac{0.5}{0.9 \cdot 0.75}} =$

c) $\sqrt[3]{\frac{0.5}{0.9 \cdot 0.75}} = 0.9$

300 l starting solution: ferm:
0.5 g/l → 150 g $\xrightarrow{50\%}$ 75 g

$\rightarrow \frac{75\ g}{0.25\ g/dose} = 300$ doses

5. andere Zettel →

(B)

a) $0.9 \cdot 0.75 \cdot Y_3 = 0.5$ — 3 step
b) $0.9 \cdot 0.75 \cdot Y_3 \cdot Y_4 = 0.5$ — 4 step
c) $0.9 \cdot 0.75 \cdot Y_3 \cdot Y_4 \cdot Y_5 = 0.5$ — 5 step

$Y_3 \cdot Y_4 \cdot Y_5 \rightarrow Y^3$

$Y_3 = Y_4 \rightarrow Y^2$

a) $\frac{0.5}{0.9 \cdot 0.75} = Y_3 = 0.74$ $\qquad Y_4 =$

b) $\sqrt{\frac{0.5}{0.9 \cdot 0.75}} = Y = \sqrt{0.74} = 0.86$

c) $\sqrt[3]{\frac{0.5}{0.9 \cdot 0.75}} = Y = \sqrt[3]{0.74} = 0.9$!

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4) PEG 6000: ~~Assumptions~~

(Eg.: $r_h = \frac{k \cdot T}{6 \cdot \pi \cdot \eta \cdot D_0}$) — k: Boltzmann constant.

OR:

$r_{h\,PEG} = \left(\frac{3 \cdot [\eta] \cdot M_{r,PEG}}{4 \cdot \pi \cdot 2.5 \cdot N_A}\right)^{\frac{1}{3}}$ — cubic root; N_A: Avogadro

↳ SLIDE; mass examples

NOTE: PEG: hydrodynamic radius, NOT mass influences precipitation!! Hence, selectivity!

1.) Assumptions // lookit up:

- $[\eta] = 20$ for 4000-10000 PEG mass

↳ $r_h = \left(\frac{3 \cdot 20 \cdot 6000}{4 \pi \cdot 2.5 \cdot 6.022 \cdot 10^{23}}\right)^{\frac{1}{3}} =$

Juckes Theory: ($\beta = k \cdot r_h^{0.211}$)

2.) • β can be predicted

CAREFUL!! [cm²]

Diffusivity of a protein can be gest by Tyn (?) & Gusek (?)

↳ $D \sim \left(\frac{2.7 \cdot 10^{-5}}{M_R^{1/3}}\right)$ — M_R: protein mass

D protein →

→ $\beta = (0.0076 \cdot r_{h\,PEG}^{0.211} - 0.45) \cdot r_{h\,prot}$

↳ β

$r_h = \frac{k \cdot T}{6 \pi \cdot \eta \cdot D_0}$ — ad η: either assume (water) or, better: calculate as 10% PEG in water

• $M_R = 150\ kDa$ ASS. ≙ 150 000 Da e.g. antibody

→

10) Aggregation / precipitation → perikinetic / orthokinetic → formation (??) phase → agg. break up   see slides

1)
Perikinetic particle growth: $\frac{1}{c} = \frac{1}{c_0} + K(t)$ 1 min

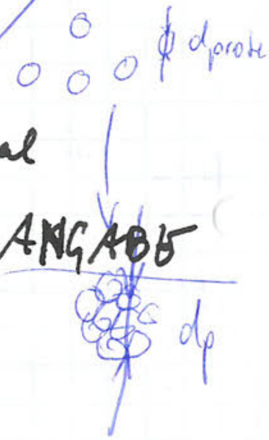
$K = 8\pi \cdot D_s \cdot d_p \cdot N_A V$ ✓

MW = 20 000

↳ ~~$K = \frac{c_0}{c \cdot t} = \frac{20}{0.2 \cdot 1} \frac{mg/l}{g/l \cdot min} =$~~

$K = \left(\frac{\frac{1}{c} - \frac{1}{c_0}}{t}\right) = 0.083\ sec^{-1}$

$d_p = \frac{K}{8\pi \cdot D_s \cdot N_{Avogadro}}$ again $D_s \approx$ ~~$\left(\frac{2.7 \cdot 10^{-5}}{M_w}\right)^{\frac{1}{3}}$~~ $=$ (20 000) $D_s \approx \frac{2.7 \cdot 10^{-5}}{\sqrt[3]{M_w}}$



↳ d_p = ...

↳ and $\frac{D_s}{10}$ = actual D_s because ANGABE

Thün Gussek is for water, but we have $\frac{1}{10} \cdot D_{water}$

2) How many proteins have aggregated into one particle
= FRACTAL DIMENSION ... $D_f \approx 2.2 - 2.5$

$\left(\frac{d_p}{d_{protein}}\right)^{2.3}$ = ~ no. of proteins in the aggregate

→ Thün and Gussek AGAIN + (d_h) prev. pg.   ← previous page →

CAREFUL: SI UNITS!!!

$d_h = 2 \cdot \left(\frac{k \cdot T}{6 \cdot \pi \cdot \eta \cdot D_0}\right)$

12) $LVR = k_v \cdot \frac{D_{uv}}{V_t} = 4 \cdot k_v \cdot \frac{T_m \cdot I_0}{d} \cdot \left(\frac{L}{u}\right)$ residence time

a) 1 cm reactor calculation first   ~~d = 1 cm~~ ~~u = ?~~ ~~t = 45 min~~

→ ~~$u = \frac{L}{t} = \frac{1\ (cm)}{45\ (min)} =$~~   d = 1 cm ?! $\frac{L}{u} = 45$ min

Volumetric flow = area · velocity !! $\dot{V} = u \cdot A$ nicht i.d. Unterlagen!

→ $A = r^2 \cdot \pi = 3.14\ cm^2$ → $\hat{=}\ 0.000314\ m^2$??? $cm^2 = 10^{-4}\ m^2$

AND 100 l in 24 h → $\frac{100}{24} \frac{l}{h} = 4.16\ l/h$ =

↳ ~~$\frac{100\,000}{24 \cdot 3600} \frac{m^3}{sec} = 1.157 \frac{m^3}{sec}$~~

100 l ≙ $100 \cdot 10^{-3}\ m^3$!!   Bunsen Roscoe law

↳ $\dot{V} = \frac{100 \cdot 10^{-3}\ m^3}{24 \cdot 3600\ sec} = 2.7 \cdot 10^{5}\ m^3/sec$

→ $u = \frac{\dot{V}}{A} = 0.085$ → $\frac{L}{u} = 45$ min → $L = 45 \cdot 60 \cdot 0.085 = 9.9$ m

9.9 m is plausible.
Redesign to 15 m

: ?? ? __ ?

13) Ebola risk ....

Factor 100. Currently: 10 pg / dose $\xrightarrow{fac 100}$ $\frac{10}{100}$ → (0.1 pg/dose)

Risk assessment: Safety factor: $5 \cdot 10^{-13}$ → 1 effect in $5 \cdot 10^{13}$ | cam

1.+ 2.) Explain = ~~8 000 000 / 5·10^13~~ or ~~8·10^6 / 5·10^13~~ 80 mill = $8 \cdot 10^7$ → 100 femto gram

↳ 1 in $5 \cdot 10^{13}$ / x in $8 \cdot 10^7$?!

↳ $\frac{8 \cdot 10^7}{5 \cdot 10^{13}}$ =

2.) $y = \frac{(DNA/dose)^2}{2}$

$y_1 = \frac{(10)^2}{2} = 50\ \%$

$y_2 = \frac{(0.1)^2}{2} = \frac{0.01}{2} = 0.005\ \%$

(% ?? why ??)

→ $\frac{50}{0.005}$ → costs would explode!! 10 000x more!

" You can do exam until SS 2017 "

→ scale down of prod. process of coag. factor
BAXALTA master thesis

~~$1 / 5 \cdot 10^{13} = 8 \cdot 10^7$, $\frac{1}{5 \cdot 10^{13}} = \frac{x}{8 \cdot 10^7}$~~