

*M = M<sub>0</sub> e<sup>-k<sub>d</sub>t</sup>*

### Problem 1

Your task is to develop a purification process of a therapeutic recombinant protein for parenteral application. Your starting DNA concentration is 30 µg DNA/ml. You start from a 300 L solution with a protein concentration of 0.5 g/L. The final formulation is 10g/L and the dose is 250 mg.

1. Calculate the required log reduction factor of DNA of the purification process in order to meet the requirements for medicinal application.
2. Calculate the individual reduction factor for a overall yield of protein of 50% for the entire process sequence.
3. Recalculate the same problem for a dose of 3 mg.

You have to make assumptions and justify these assumptions.

Points 3 + 2 + 1

### Problem 2

For the same product as defined in problem 1 you have established a purification sequence consisting of 5 individual steps. For simplicity the losses in each step are identical.

1. Calculate the yield of each individual step
  2. Recalculate the yield of each individual step if the total yield is only 35%
  3. Calculate the yield of process 3, 4, and 5 step if the first step has a yield of 90% and the second 75%. The overall yield is still 50%. Assume identical yield in process step 3, 4, and 5. How many doses can you produce from the 300 L starting solution.
- 5 step process total; calculate  $y_3, y_4, y_5$*

Points: 1+2+3

### Problem 3

Your task is to scale up a protein precipitation, which is performed in a stirred tank reactor. At laboratory scale the process has been optimized in a 300 ml stirred vessel with a shear rate of 3000 s<sup>-1</sup>. The large scale will be performed at 2000L.

1. Predict the required time to complete precipitation at small scale
2. In the large scale vessel it is not possible to reach a shear rate of 3000 s<sup>-1</sup>, and you operate at 1000 s<sup>-1</sup>. How much time is required for completion of precipitation at large scale?
3. How big is the power input in the large and small scale vessel? Discuss the energy consumption in relation to house hold equipment, car or railway.

Which assumptions are required? Justify the assumptions.

Points 2+2+2

4) PEG 6000 is used for precipitation of proteins.

M 1. Calculate the hydrodynamic radius of PEG 6000. Justify the assumptions, which you have made.

M 2. Calculate the selectivity (slope of the solubility line) of PEG precipitation with PEG 6000 for separation of IgG.

(Sort of)

→ take 20 because we take a value between PEG 4000 or 10000 interpolation in a plot is also possible

5) Scale up the virus inactivation by UV-radiation in a tubular reactor and you need at least 4 LVR. In laboratory scale you have found out that with a residence time of 45 min is sufficient to reach 4 LVR.

1. Design a reactor to process 2000 liter of a protein solution in reasonable time; explain what a reasonable time is.
2. Explain the variables, which you can change during scale up.
3. After scale up you have found out that the transmission has been halved in large scale due to slight precipitation. How can you compensate this problem by changing the reactor configuration.

M6) You have to purify a recombinant protein for therapeutic purposes which are administered parenteral with a single dose of 1 g. Your starting concentration is 1 g/l of protein and the DNA contamination 100 000 ppm. The step yield for the protein is 90%. The clearance factor for DNA of each step is 2.

not required.

TM: "nasty" XD

- M 1. How many purification steps do you need to reach the required maximal DNA level?
- M 2. What is the overall yield of the process?
- M 3. Is the process economically viable for a step yield of 70%?

Q7) You have measured a Zeta-potential of a particle in the range of + 10 mV.

"easy one"

1. Calculate the electrophoretic mobility for a solution with a viscosity similar to water
2. Justify why you are not able to deduce the size of the a particle from this experiment.

8) Somebody has developed a new cell line based on human origin. The cell line has excellent production properties and makes perfect human glycosylation. After development of the cell the scientist found out that the genome carries a lot of viral genes and oncogenes. In order to save their cell line they redefine the residual DNA allowed per dose of final product.

1. Calculate the safety factor for 20 oncogens per genome.
2. What would be the residual DNA per dose to have the same safety factor of  $10^{-8}$  in in-vitro assays or  $5 \times 10^{-13}$  in tumor assays in chicken?
3. Argue that there is no need for a redefinition, because with the original criteria the product is safe and argue that it is impossible to detect the residual DNA in a dose containing 500 mg antibody at a concentration of 50mg/ml.

9) You have an average protein of 10 nM diameter with an average stability in a concentration 10 mg/ml. Your task is to select an analytical method to detect the denatured form. You must make certain assumptions to solve this problem

1. Calculate the % of denatured form which is present at room temperature in a physiological buffer.
2. What is the limit of detection required for an analytical method to detect the denatured form?

10) Find out the initial size of a protein aggregate after the first phase of precipitation. Your protein has a relative molecular mass of 20 000 and you assume that the diffusivity of the protein in the solution with the precipitant is 1/10 of the diffusivity in water. You will start precipitation with 20mg protein /ml and after 1 min you observe only 0.2 mg/ml in the supernatant.

1. Calculate the size of the protein aggregate after initial precipitation phase
2. Estimate how many proteins have aggregated to one particle

11) *E.coli* broth is a Non-Newtonian fluid.

1. Calculate the apparent viscosity of a *E.coli* broth with a dry mass of 15g/l at a shear rate of  $10 \text{ s}^{-1}$ .
2. How high is the increase in apparent viscosity after homogenization?
3. Is the shear rate of  $10 \text{ s}^{-1}$  meaningful?



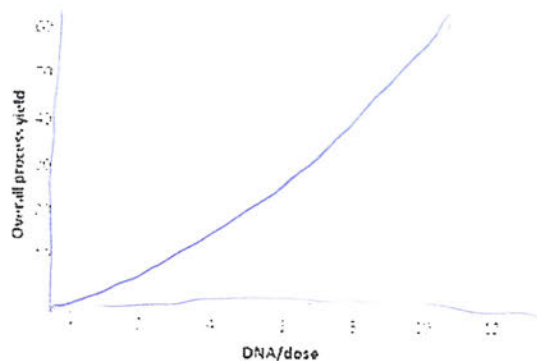
12) Your task is to scale up a virus inactivation procedure by UV irradiation using a plug flow reactor. In the process development a virus clearance of 4.5 log step was observed in 1 cm tubular reactor with a residence time of 45 min.

$\rightarrow r = 1 \text{ cm}!$

1. Design a process which is capable of virus inactivation of 100 L in 24 hours. You can choose any reactor configurations, but the 4.5 log steps must be warranted.
2. Justify your assumptions for the process
3. Redesign the process for a tubular reactor when the length is restricted to 15 m and justify your redesign.

13) In light of the current hypocrisy of Ebola a politician requested that the DNA level in biopharmaceuticals must be reduced by a factor 100. Explain him why the current level is high enough and what is the likelihood of suffering from a side effect when 80 million patients (approximately 10 times the population of Austria) are treated with a biopharmaceutical having a DNA contamination of 10 pg/dose.

1. Calculate the risk of a side effect by DNA contamination!
2. Calculate the increase in production using assuming a pareto function as shown below (yield  $= (\text{DNA/dose})^2 / 2$  ! Make the assumption that the production costs directly correlate with the yield.



3. Discuss the risk benefit ratio based on this scenario!

14) You have designed a PEG precipitation of antibodies with PEG 6000 at room temperature. The solubility without PEG is 180 mg/ml. You have added 10% to a solution containing 5 g antibodies /liter and observed a solubility of 01.g/l. You are not satisfied with the yield.

1. Improve yield by increasing PEG! How much PEG do you need for 99% yield?