Module ChE-311 Biochemical Engineering

Downstream processing
Exercices on
Protein precipitation

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Exercise 3.1 Salting-out with ammonium sulphate

A solution of ammonium sulphate at a concentration of 660.7 g/kg_{eau} is added dropwise to 100g of a protein solution at 15.0 mg/g_{eau}. After the addition of 83.1 g ammonium sulphate solution the mixture becomes turbid and a precipitate appears.

1. Which is the concentration of ammonium sulphate at this moment?
2. Which is the corresponding protein solubility?
3. Add this measuring point to the first line of the table below and determine the ß and K_s parameters of the Cohn equation for the precipitation of this protein with ammonium sulphate (please work with $log_{10}(S)$ and with ionic strength)

$[(NH_4)_2SO_4]$ [mol/kg_{eau}]	S [g/kg_{eau}]		
2.00	3.16		
2.50	0.56		
3.00	0.10		

NB : molecular weight of $(NH_4)_2SO_4$ is 132.14 g/mol

Exercise 3.2 Edwin J. Cohn strikes again

A protein solution has an initial concentration of 15 g/l. After addition of ammonium sulphate up to 0.5 and 1.0 M, the residual protein concentrations in the supernatant after centrifugation were 13.5 and 5.0 g/l respectively.

- Based on this information, determine the coefficients of the Cohn equation
- Which $(NH_4)_2SO_4$ concentration will I have to reach in order to precipitate 95% of the protein?

IMPORTANT : In the absence of more detailed information, assume the Cohn equation is defined on a $\log_{10}$ basis with salt concentrations (and not ionic strength) in mole/l

Exercise 3.3a Fractional precipitation

Four different proteins are in a solution together, each with its own set of Cohn parameters (base ln, Ks defined relative to salt concentration and not to the ionic strength). The initial concentration of each protein is also given in the table below.
Assuming all these parameters remain constant in the presence of other proteins:

1. Determine the highest possible recovery yield of protein 4 if it is to be collected at 100% purity
2. Which is the highest achievable recovery yield for protein 3 if it should represent at least 99% of the proteins present in the supernatant?

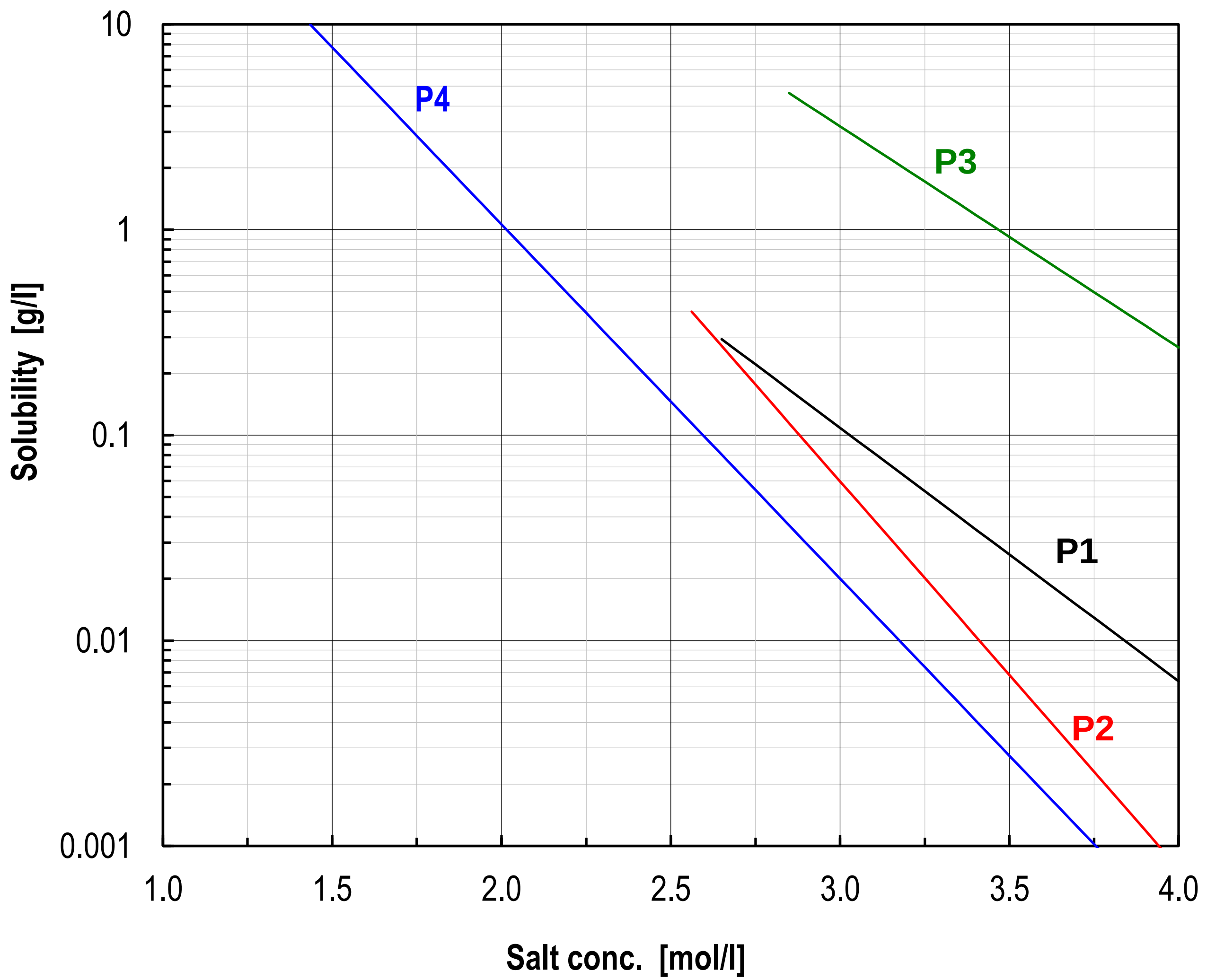
Protein	β	K_s [M^{-1}]	S_0 [g/l]
1	6.30	2.84	0.3
2	10.20	4.34	0.4
3	8.60	2.48	5.0
4	8.00	3.97	10.0

The graph on next slide integrates all the data contained in the table above

Exercise 3.3b

Fractional precipitation

(Appendix to exercise 3.3a)



Exercise 3.4a

Protein precipitation: working with solubility curves

The solubility of a protein in the presence of ammonium sulphate has been measured and a curve drawn. It is shown in the Appendix to this exercise.

We have 25 g of a 30.93 mg/g_{water} solution of protein, to which a 667 g/kg_{water} solution of ammonium sulphate is added dropwise.

1. Which quantity of salt solution will have to be added before a precipitation occurs?
2. How much salt solution will I have to add to the original protein solution to reach a 25% mass fraction of salt in the mixture?
3. What would the solubility of the protein be at this point?
4. What would the precipitation yield be?

NB: molecular weight of ammonium sulphate is 132.14 g/mol

Exercise 3.4b

Appendix to exercise 3.4 a