Exercise 3.1 – Salting-out with ammonium sulphate

A 660.7 g/kg_{water} solution of ammonium sulphate is added dropwise to 100 g of a 15.0 mg/g_{water} protein solution. After 83.1 g of the electrolyte solution have been added, the mixture becomes turbid, indicating that the protein solubility limit has been reached and precipitation has started.

1. What is the salt concentration at this point?

The 100 g of 15.0 mg / g_{water} protein solution contains:

$$\mathrm{m_{p,l}} = 100 * \frac{15}{(15+1000)} = 1.478 \ \ g \ \ for \ \ protein$$

$$\mathrm{m_{water,l}} = 100 * \frac{1000}{(15+1000)} = 98.522 \ \ g \ \ for \ \ water$$

The 83.1 g of ammonium sulfate solution at 660.7 g / kg_{water} contains:

$$\mathrm{m_{s,l}} = 83.1 * \frac{660.7}{(660.7+1000)} = 33.06 \ \ g \ \ for \ \ ammonium \ \ sulfate$$

$$\mathrm{m_{water,l}} = 83.1 * \frac{1000}{(660.7+1000)} = 50.04 \ \ g \ \ for \ \ water$$

The ammonium sulfate concentration at this time is therefore:

$$c_{s,water} = \frac{33.06}{132.14} * \frac{1}{(0.09852+0.05004)} = 1.682 \ \ mol/kg_{water}$$

2. What is the protein solubility under these conditions?

The protein concentration in this mixture corresponds to its solubility. It is:

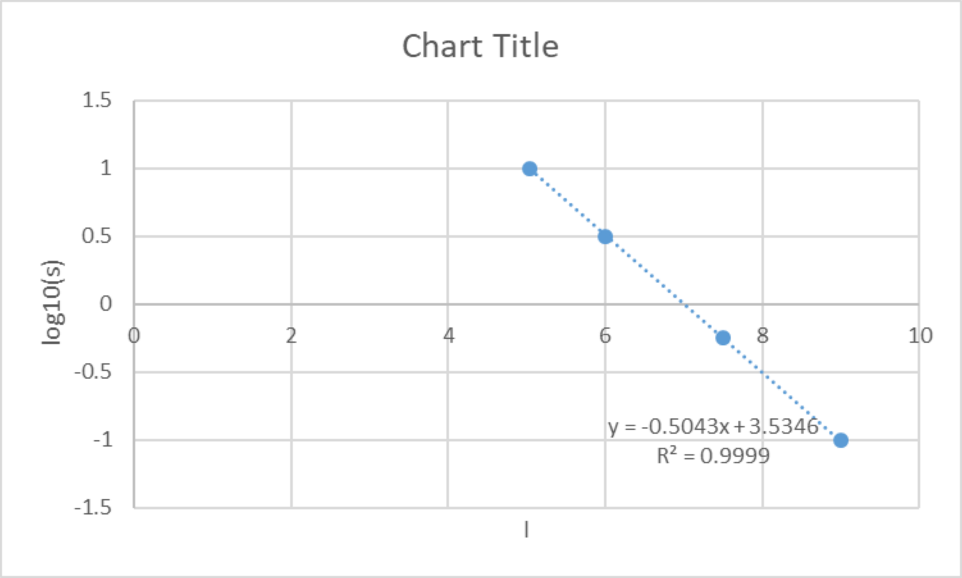
$$c_{protein,water} = \frac{1.478}{(0.09852+0.05004)} = 9.95 \ \ g/kg_{water}$$

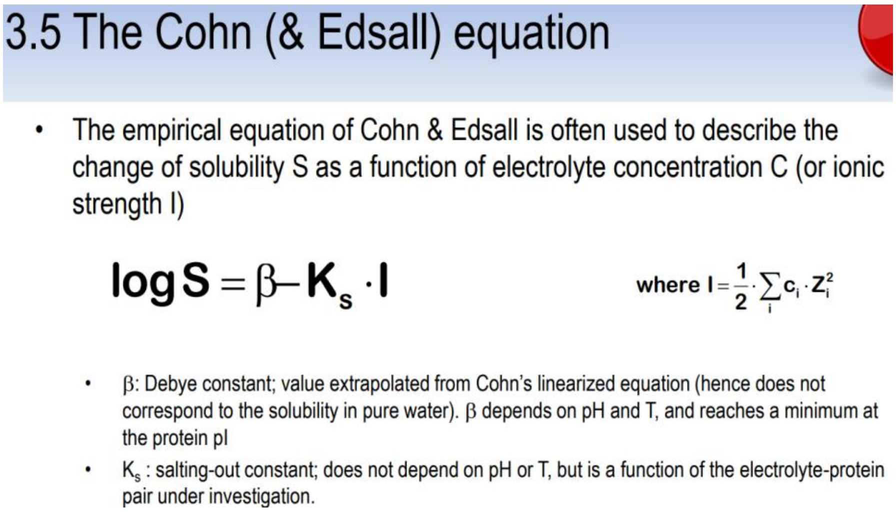
3. Use the data you have just calculated to complete the table below and determine the parameters of the Cohn equation (β and Ks). Please work with logS and ionic strengths.
NB: The molecular weight of ammonium sulphate is 132.14 g/mol.

The salt and protein concentrations are then added to the table and the data linearized according to the Cohn equation to yield the β and Ks coefficients

$[(NH_4)_2SO_4]$ $[mol/kg_{eau}]$	S $[g/kg_{eau}]$	log10(s)	I
1.682	9.95	0.997823081	5.046
2.00	3.16	0.499687083	6
2.50	0.56	-0.251811973	7.5
3.00	0.10	-1	9

The result of the linear regression is shown below:





3.5 The Cohn (& Edsall) equation

- The empirical equation of Cohn & Edsall is often used to describe the change of solubility S as a function of electrolyte concentration C (or ionic strength I)

$$\log S = \beta - K_s \cdot I \qquad \text{where } I = \frac{1}{2} \cdot \sum_i c_i \cdot Z_i^2$$

- β: Debye constant; value extrapolated from Cohn's linearized equation (hence does not correspond to the solubility in pure water). β depends on pH and T, and reaches a minimum at the protein pI
- K_s : salting-out constant; does not depend on pH or T, but is a function of the electrolyte-protein pair under investigation.

β=3.5346, Ks=0.5043

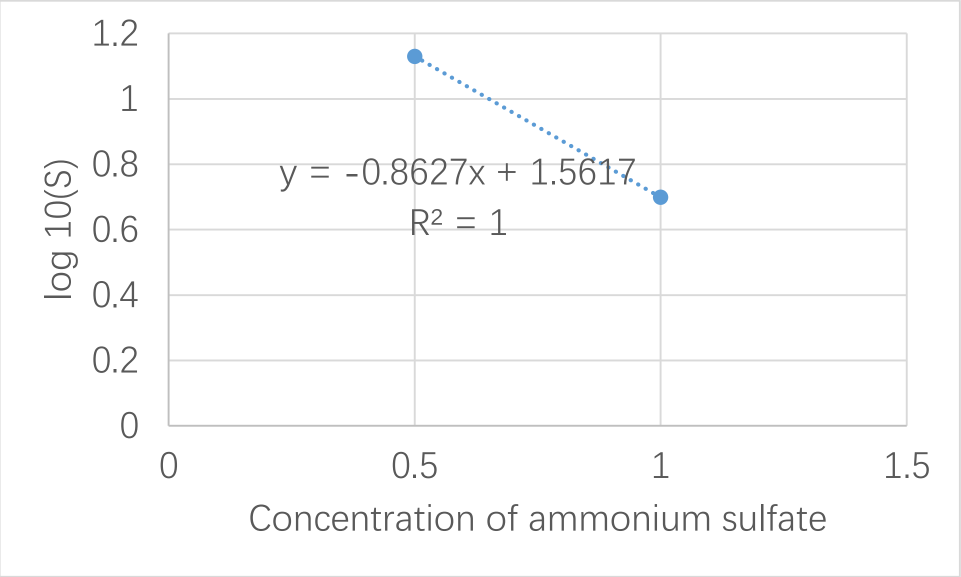
Exercise 3.2 – Edwin J. Cohn strikes again

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 log10 basis with salt concentrations (and not ionic strength) in mole/l

$[(NH_4)_2SO_4]$ [mol/L]	S [g/l]	log10(s)
0.5	13.5	1.130333768
1	5	0.698970004
1.955069823	0.75	-0.124938737



β=1.5617, Ks=0.8627, on a log10 basis with salt concentrations (and not ionic strength) in mole/l.

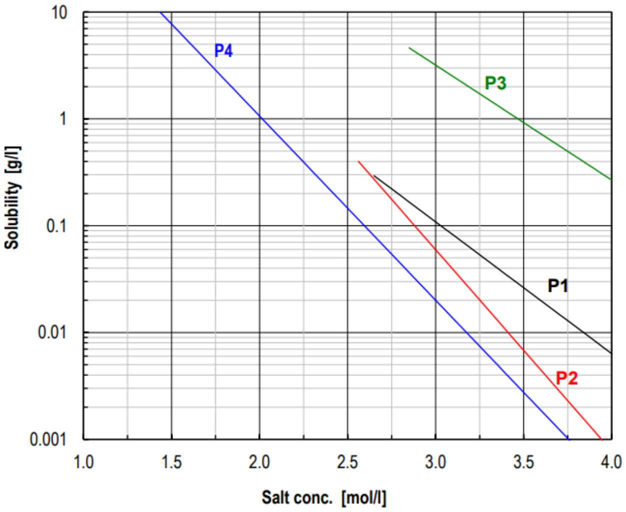
1M $(NH_4)_2SO_4$ concentration need to be reached in order to precipitate 95% of the protein

Exercice 3.3 – 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:

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 below integrates all the data contained in the table above



1. Determine the highest possible recovery yield of protein 4 if it is to be collected at 100% purity

The resolution of this problem involves a graphical examination of the Cohn lines for the four proteins studied. They are represented in the graph below. It is important that the upper value of the concentration for each of the proteins corresponds to their initial concentration S_0, which makes it easier to identify which of the components of the mixture will precipitate first when the salt concentration increases.

ATTENTION, the values of K_s and β are given on an ln base and the graph is of the log-lin type in base 10 for the vertical axis.

In this case the graph clearly shows that it is protein 4 which will precipitate first, followed by protein 2 from 2.56 mol/l, protein 1 from 2.65 mol/l then protein 3 from 2.85 mol/l ammonium sulphate.

This means that between 0 and 2.56 mol/l of salt, only protein 4 is precipitated. Its residual concentration when protein 2 begins to precipitate is:

ln(S4) = 8.00-3.97*2.56 = -2.163, hence S4 = 0.115 g/l

Starting from 10 g/l and assuming that the addition of salt does not change the volumes, this means we have precipitated (10 - 0.115)/10 = 0.989 = 98.9% of protein 4 between 0 and 2.56 mol/l of salt.

We can therefore recover almost 99% of pure protein 4 in the precipitate before it is contaminated by protein 2 when it starts to precipitate as well.

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?

The graph also shows that protein 3 is the last to precipitate and that its concentration in the supernatant remains much higher than those of the others as the salt content increases, so that one would expect it to represent a large majority (99%) of the proteins still present in solution. This amounts to looking for the salt concentration for which we have:

$$\frac{s_3}{s_1 + s_2 + s_3 + s_4} \geq 0.99$$

To solve this problem, we have:

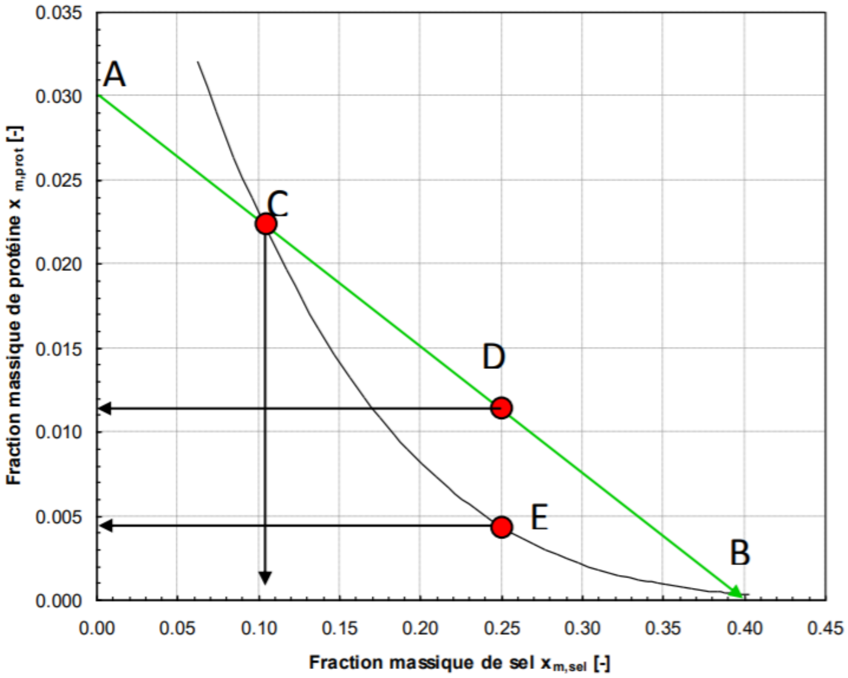
$$C_{salt} \geq 6.40 \ \ mol/l$$

This result leads to a comment: at 6.40 mol/l of precipitating agent, the residual concentration of protein 3 in the supernatant is of the order of 7*10-4 g/l, which represents only 1.4*10-2 % of the initial quantity. This approach to recovering a relatively pure protein 3 is hence of no interest.

Exercice 3.4 – 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?



Mass fraction of protein in the solution:

$$P_0 = \frac{30.93}{30.93+1000} = 0.03\text{, point A on the graph}$$

Mass fraction of salt in the solution:

$$S_0 = \frac{667}{667+1000} = 0.40\text{, point B on the graph}$$

Question 1

When adding the 40% salt solution to the 3% protein solution, we move along the straight line connecting points A (0.0 ; 0.003) and B (0.40 ; 0.0), as shown on the figure above (green arrow). As soon as we cross the solubility curve (Point C), the mixture will become turbid and precipitation will occur. This happens at a salt mass fraction of ca. 0.105.

The mass M_{AmSul} of salt solution that has been added up to this point can be calculated, but it can also be deduced by a graphical method: the lever rule. To do so one needs to measure the lengths of segments AC and CB:

25 x AC = M_{AmSul} x CB

With AC = 28 mm and AC = 82 mm, one finds M_{AmSul} = 8.54 g

Or as AC/CB=0.105/(0.40-0.105), from the X axis, you can also calculate it, and we get M_{AmSul} = 8.898305085

Question 2

To obtain a 25% mass fraction of salt in the mixture one has to keep adding salt solution until point D is reached. Using the lever rule again with segments AD and BD, one can determine that the total amount of salt solution that has to be added to the 25 g of protein solution is 42.1 g.

Or we can use the x axis instead of AD and BD, we know (0.40 – 0.25) * M_{AmSul} = 0.25 * 25, and we can get M_{AmSul} = 41.67 g

Question 3

The solubility of the protein at such a salt concentration can be read from the graph at point E and is approximately 0.0043 [-]

Question 4

The protein precipitation can be calculated as follows: the mixture at point D weighs a total of 25+42.1=67.1 g.

Its composition can be read from point D and allows the calculation of the amounts of protein (0.75 g), salt (16.78 g) and water (49.57).

However, only 67.1x0.0043 = 0.29 g of that protein is soluble. That means the rest (0.46 g) has been precipitated.

The precipitation yield is then 0.46/0.75 = 61%.

If you use M_{AmSul} = 41.67 g, you can get total weight at point D, 25+41.67=66.67 g. Its composition can be read from point D and allows the calculation of the amounts of protein (0.75 g), salt (16.68 g) and water (49.24). However, only 66.67 x 0.0043 = 0.29 g of that protein is soluble. That means the rest (0.46 g) has been precipitated. The precipitation yield is then 0.46/0.75 = 61%.