

**Properties of aminoacids and proteins – laboratory class worksheet**

**Aim: Preparing Ammonium sulfate solutions to test the effect of increasing ionic strength on albumin precipitation.**

Protein solubility is strongly influenced by ionic strength. At low salt concentrations, ions can stabilize proteins in solution, but at higher concentrations they compete for water molecules and disrupt the hydration layer around the protein, leading to aggregation and precipitation a process known as salting-out. In this on line laboratory exercise, you will virtually prepare a series of ammonium sulfate solutions of increasing molarity and use them to examine how rising ionic strength affects the precipitation of serum albumin, a highly soluble plasma protein.

First calculate mass of ammonium sulfate for preparation of tested solutions:

**Complete the table below:**

ammonium sulphate (mol/l)	ammonium sulphate (g)	water (ml)
1		10
2		10
3		10
4		10
5		10

*Molar mass of ammonium sulfate: 132.1 g/mol*

**Experimental Procedure**

- Prepare an albumin solution: 15 g albumin in 100 mL water.
- Pipette 5 × 10 mL aliquots into labelled tubes S1-S5.
- Add prepared ammonium sulfate solutions (10 mL) to each tube.
- Mix and incubate for 10–15 minutes. Centrifuge. Remove supernatant and weigh the precipitated albumin (after drying).
- Record the mass of precipitate (data are shown below).

Fill in the table:

Initial $(\text{NH}_4)_2\text{SO}_4$ concentration (M)	$(\text{NH}_4)_2\text{SO}_4$ concentration (M)	Albumin added (g)	Albumin in supernatant (g)	Albumin precipitated (g)	% precipitated
1				0.02	
2				0.28	
3				1.08	
4				1.26	
5				1.45	

**Plot a graph:**

x-axis:  $(\text{NH}_4)_2\text{SO}_4$  concentration (M)

y-axis: albumin in supernatant (g)

Attach the graph here:

**Conclusion:**

1. At which ammonium sulfate concentration does albumin begin to precipitate rapidly?
2. At which ammonium sulfate concentration precipitate albumin completely?
3. Explain why albumin precipitates at high ionic strength (describe the salting-out mechanism).

**Review structure and function of albumin**

- a) Describe the structure of serum albumin.
- b) What is the normal concentration of albumin in blood plasma?
- c) List three major physiological functions of albumin.