PROTEIN FRACTIONATION AND ISOLATION

Principle:

When high concentrations of various salts (*e.g.* $(NH_4)_2SO_4$, Na_2SO_4, NaCl) are added to protein solution, precipitation of proteins ensues. This phenomenon is reversible and is caused both by neutralization of protein particle charges and by protein molecule dehydratation.
Selecting the proper salt concentration, it is possible to quantitatively separate a single protein while leaving others in solution. Globulins are precipitated by half-saturated $(NH_4)_2SO_4$ solution while albumins only by the nearly saturated solution.

Problem:

Decide which of the two unknown samples contains only albumins and which contains mixture of albumins and globulins.

Procedure:

Test tubes A and B both contain 3 ml of a sample solution (either albumins or mixture of albumins and globulins).

1) Add 3 ml of saturated $(NH_4)_2SO_4$ solution to each test tube and shake it. You get solutions half saturated with $(NH_4)_2SO_4$.
2) Wait for 5 minutes. The precipitate appears in the test tube containing globulins.
3) Add solid $(NH_4)_2SO_4$ (in small quantities) into the test tubes to achieve full saturation (it stops dissolving). Albumin is precipitated giving rise to the turbidity also in the tube B and intensifying it in the tube A.

TOTAL PROTEINS IN SERUM

Principle:

Peptide bond reacts with alcaline solution of copper sulphate forming cyan-bluish complex.

Reagents:

- biuret reagent (solution of $CuSO_4$ in 0.1M NaOH)
- standard solution of a protein (its concentration is written on the blackboard)

Procedure:

Prepare the samples according to the following table and shake them immediately.

	sample	**standard**	**blank**
serum (ml)	0.1	–	–
standard (ml)	–	0.1	–
distilled water (ml)	–	–	0.1
biuret reagent (ml)	3.0	3.0	3.0

Allow the samples to incubate for 12 minutes at room temperature. Then measure the absorbance (A_{sample} and A_{standard}) at 545 nm of both the sample and the standard against the blank.
Calculate the results and fill in the final table.

Calculation:

$$c_{\text{sample}} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \cdot c_{\text{standard}} =$$

Normal values: 65–87 g/l

Results:

	serum	**standard**
absorbance		
CONCENTRATION		

TOTAL PROTEINS IN AMNIOTIC FLUID

Principle:

Peptide bonds react with alcaline solution of copper sulphate forming cyano-bluish complex (biuret reaction). By adding Folin-Ciocâlteu reagent, the coloration is enhanced due to the oxidation of tyrosine and tryptophane residues by phosphomolybdate and phosphotungstate ions.

Reagents:

- working solution ($CuSO_4$ in 0.1N NaOH)
- Folin reagent
- standard solution of a protein (6.75 g/l)

Procedure:

Prepare the samples according to the following table and shake them immediately.

	sample	**standard**	**blank**
working solution (ml)	5	5	5
amniotic fluid (ml)	0.05	–	–
standard (ml)	–	0.05	–
distilled water (ml)	–	–	0.05

Wait for 15 minutes and then add 0.5 ml of the Folin reagent to each test tube and mix them well.
Allow the samples to incubate for 30 minutes at room temperature. Then measure the absorbance (A_{sample} and $A_{standard}$) at 720 nm of both the sample and the standard against the blank.
Calculate the results and fill in the final table.

Calculation: $$c_{sample} = \frac{A_{sample}}{A_{standard}} \cdot c_{standard} =$$

Normal values: 4–9.5 g/l (depends on the gestation week)

Results:

	amniotic fluid	**standard**
absorbance (*A*)		
CONCENTRATION		