**Lab Sheet 5**

**Protein isolation from animal and plant source**

**Salting out method**

 **Aim:**

To precipitate the target proteins which their precipitation occurs at 60% ammonium sulfate saturation.

**Protocol:**

1. **Salting out of protein A by 40% and 60% ammonium sulphate saturation:**

* 1. Measure the volume of your crude extraction and calculate the weight in g of ammonium sulphate needed to saturate the solution 40% using Table 1.
	2. Add the required salt to the solution slowly and gradually with small quantities and mix well continuously using magnetic stirrer while the sample is placed in ice.
	3. After the addition is completed and the salt is completely dissolved, centrifuge at 3500 rpm for 10 min.
	4. Discard the supernatant and dissolve the pellet in 10 ml of extraction buffer (0.1 M Tris-HCl, pH 7.4).
	5. Re do the steps to precipitate the target enzyme at 60% saturation.
1. **Removing of salts molecules by dialysis:**

* 1. Pre-wet the membrane by soaking the dialysis bag in the dialysis buffer for at least 30 min.
	2. Close the dialysis bag from one side and load the sample.
	3. Close the other side and place the bag in a beaker filled with 0.1 M Tris-HCl, pH 7.4 buffer or phosphate buffer 0.1 M, pH 7.0.
	4. Dialyze for 1 to 2 h at room temperature.
	5. Change the dialysis buffer and dialyze for another 1 to 2 h.
	6. Change the dialysis buffer and dialyze overnight at 4°C.