**BCH 333**

**Salting out and dialysis**

**Method:**

1) Cut skeletal muscle (100g) into small pieces and homogenize it for 10 minutes in 300 ml of homogenizing buffer (pH 6) in a blender.

2) Centrifuge at 2000 rpm for 10 min. at 4˚C.

3)Discard the residue (palette) and adjust the pH of supernatant to pH6.0).

5) Measure the volume of the "supernatant".

-The volume of the supernatant is = ……………………… ml.

6) Calculate the required amount of ammonium sulfate salt needed to saturate the solution 40% using ammonium sulfate in grams.

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-Amount of ammonium sulfate salt needed to saturate the solution with 40% is = ……………………….g.

7) Add the required salt to the solution slowly with small quantities and mix well continuously after each addition.

8) After the addition is completed and the salt is completely dissolved, centrifuge at 3500 rpm for 10 min.

9) Discard the pellet and measure the volume of the supernatant.

-The volume of the supernatant is= ……………………… ml.

10) Calculate the required amount of ammonium sulfate salt needed to saturate the solution 60% using ammonium sulfate in grams.

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-Amount of ammonium sulfate salt needed to saturate the solution with 60% is = ……………………….g.

11) Add the required salt to the solution slowly with small quantities and mix well continuously after each addition.

12) Centrifuge for 10 min. at 3500 rpm.

13) Dissolve the pellet in 10 ml homogenizing buffer and place it and the supernatant in separated dialysis bag for 30 min. (**B-Dialysis**)

