**BCH 333**

**Lab Sheet #3**

**Salting out and dialysis:**

**Materials:**

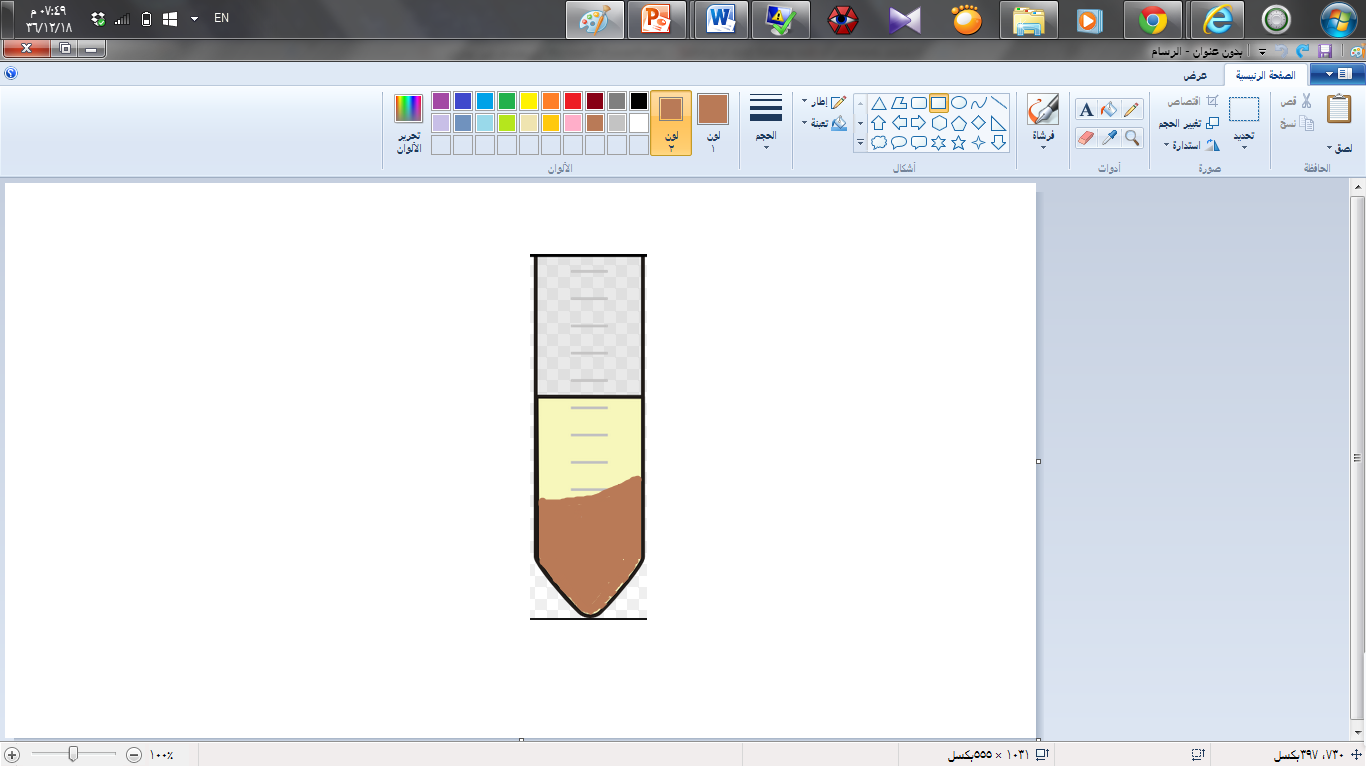
**Chemicals:** chicken skeletal muscle, homogenizing buffer, ammonium sulfate, bovine serum albumin (5g/l)"as standard protein", biuret reagent.

**Glassware:** 8 test tubes, 8 plastic cuvettes, centrifuge tubes, measuring cylinder, pipettes[1ml,2ml,5 ml], beakers and dialysis bag.

**Instruments:** Vortex, centrifuge device, blender, and spectrophotometer.

**A-Salting out:**

**Method:**

****

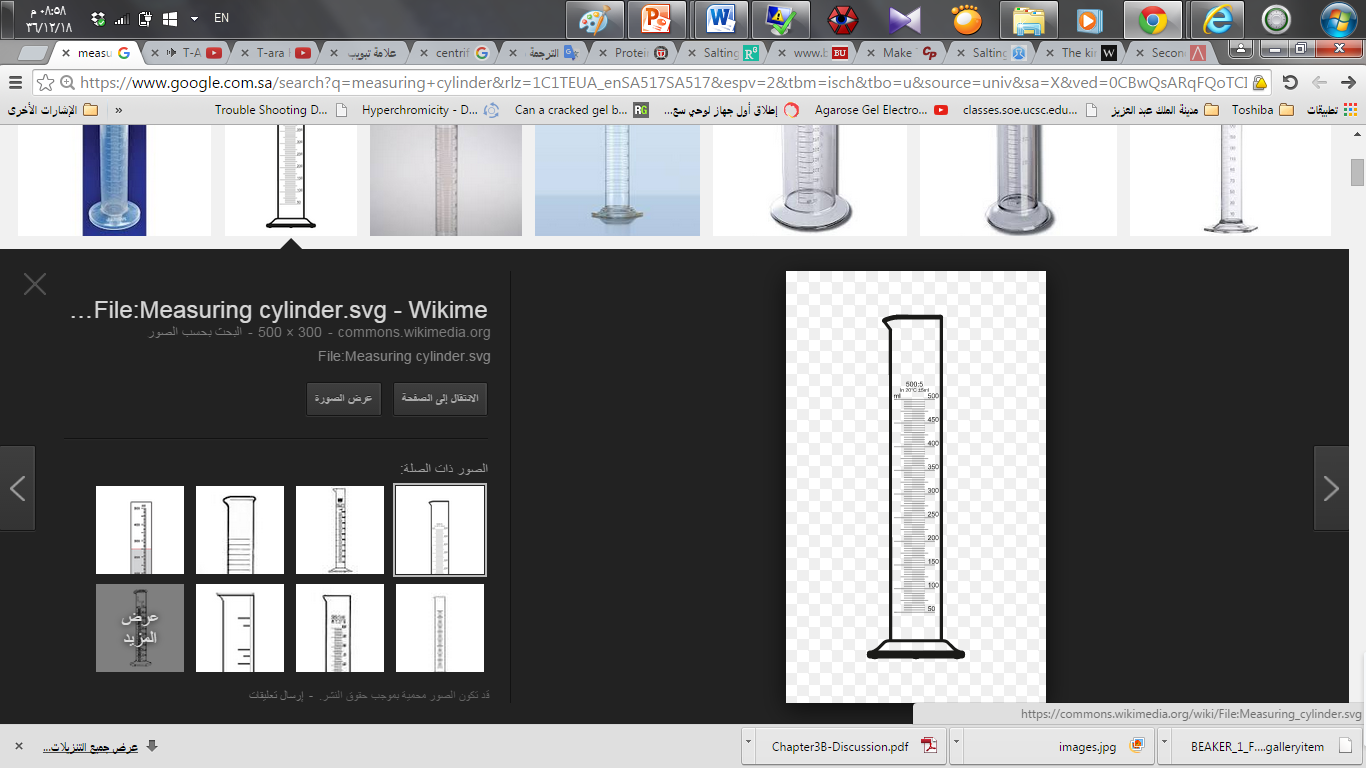
**(2)**

Centrifuge the homogenate

at 3000 rpm, for 10 min. at 4˚C.

**(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.

****

**(4)**

Measure the volume of the "supernatant".

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

**(3)**

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

**(5)**

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

…………………………………………………………………………………………………………………………………………………………………………

…………………………………………………………………………………………………………………………………………………………………………

-Amount of ammonium sulfate salt needed to saturate the solution with 40% is

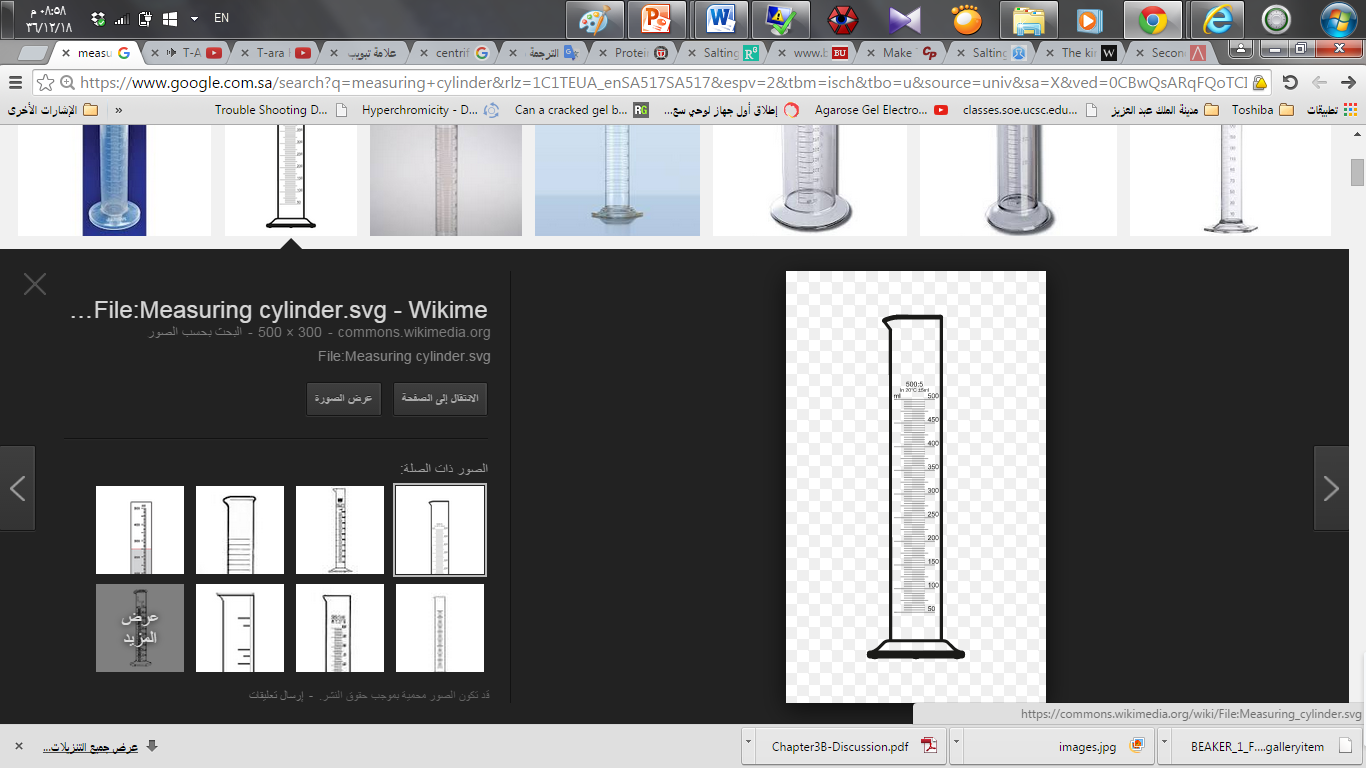
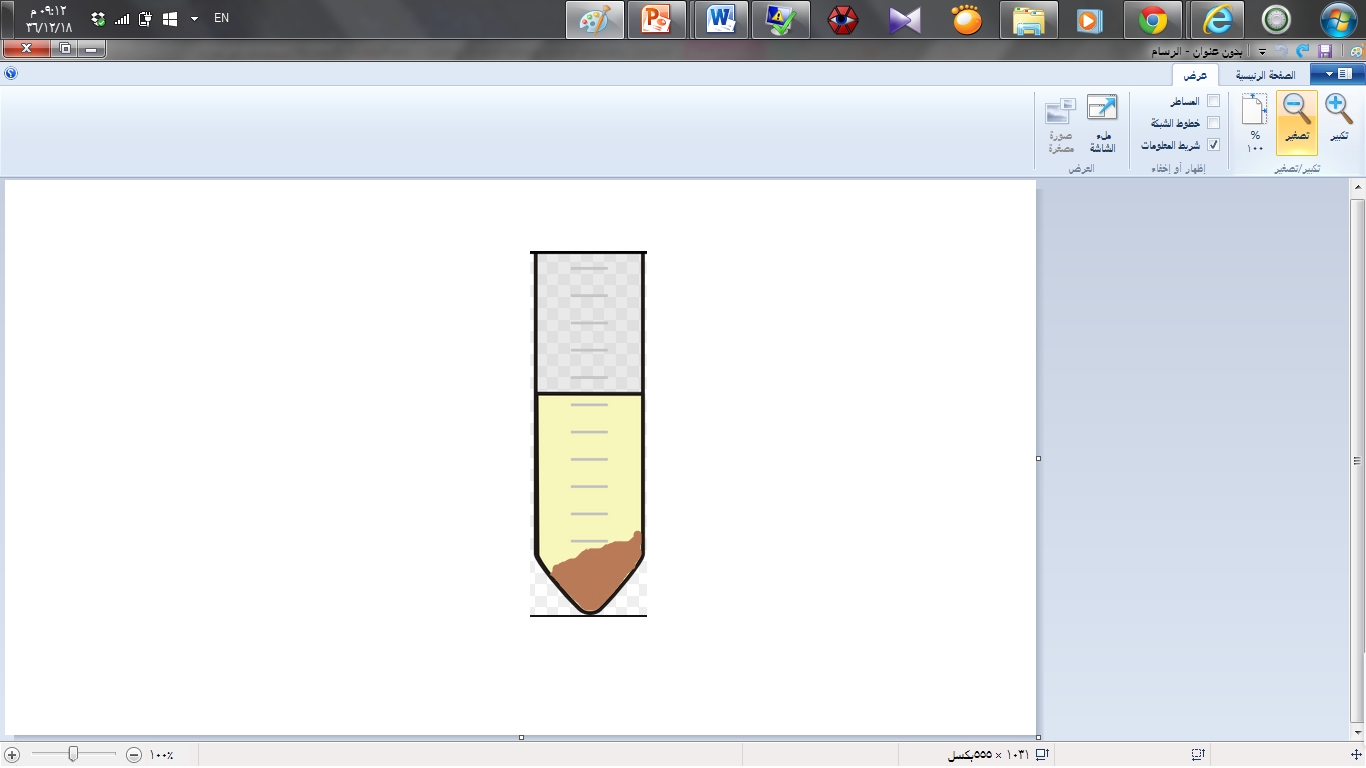
= ……………………….g.

**(7)**

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

**(6)**

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



**(8)**

Discard the pellet and measure the volume of the supernatant.

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

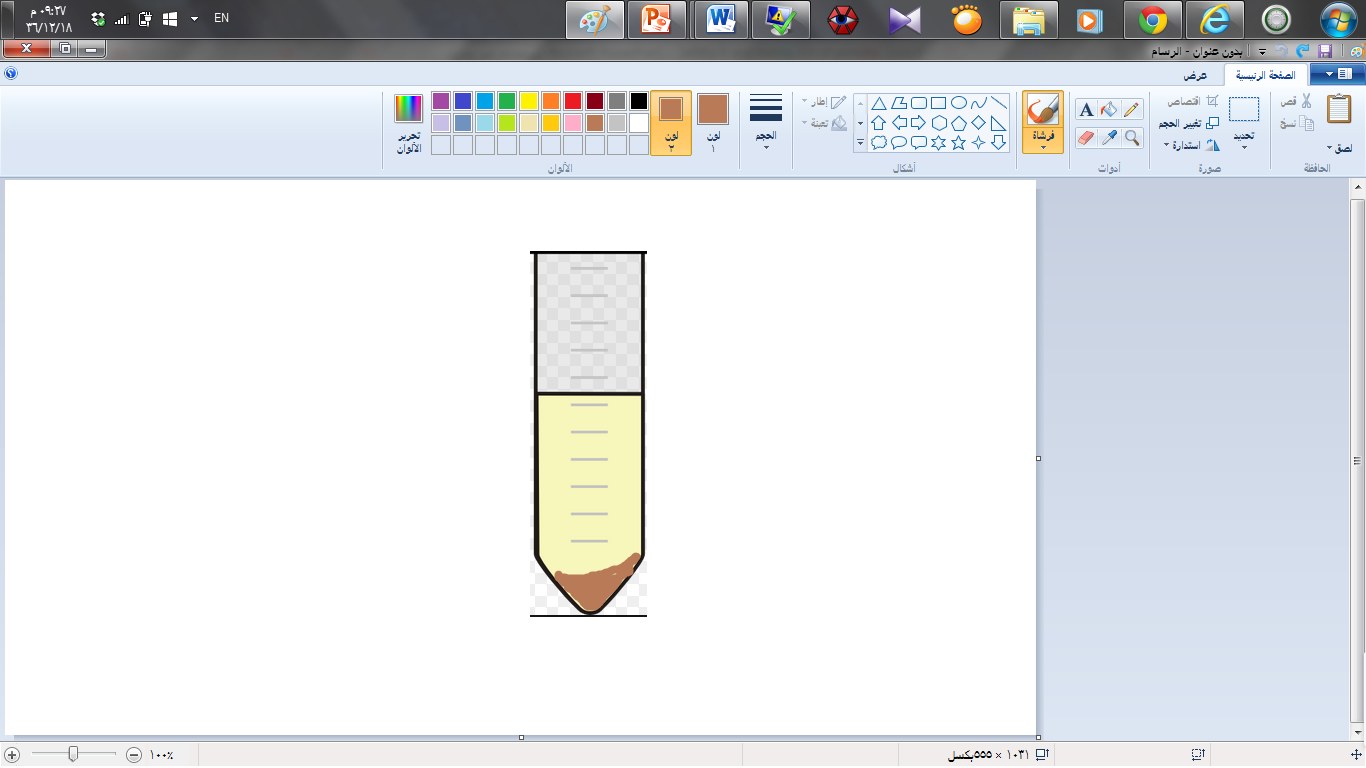
**(9)**

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

…………………………………………………………………………………………………………………………………………………………………………

…………………………………………………………………………………………………………………………………………………………………………

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



**(11)**

Centrifuge for 10 min.

at 3500 rpm.

**(10)**

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

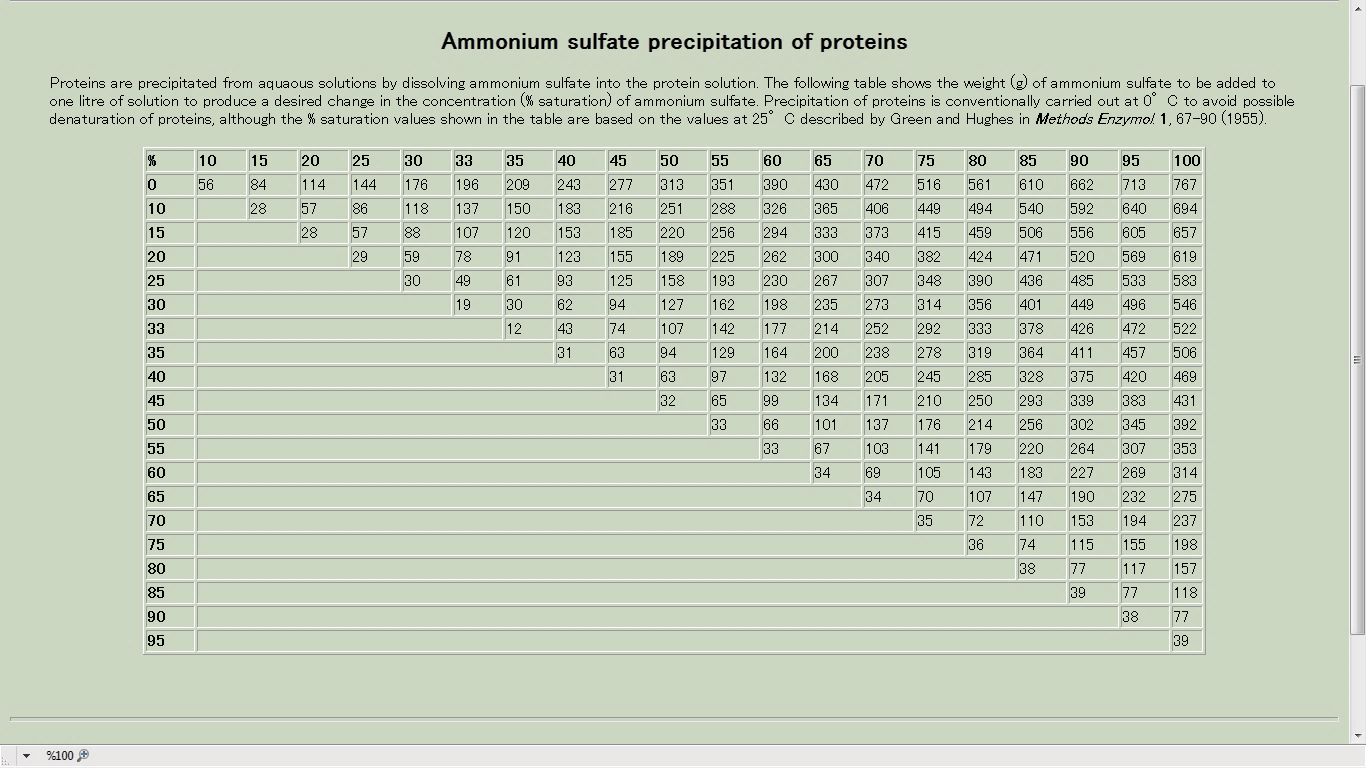
**(12)**

Dissolve the pellet in 10 ml homogenizing buffer and place it and the supernatant in separated dialysis bag for 30 min.

**Step** (**B-Dialysis**)

* **salt's fractionation table; indicate the grams of the salts to be added to one liter of solution.**

**Desired salts concentration**



**Initial salts concentration**

**C-Determination of protein by Biuret Method:**

**Standard stock of bovine serum albumin[BSA]=5g/l.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Tube No.** | **d.H2O** | **Std. of BSA**  **[5g/l]** | **Sample with unknown concentration** | **Biuret reagent** | Incubate for 10 mins. |
| **Blank** | 1 | - |  | 4 ml |
| **1** | 0.9 | 0.1 |  |
| **2** | 0.8 | 0.2 |  |
| **3** | 0.6 | 0.4 |  |
| **4** | 0.4 | 0.6 |  |
| **5** | 0.2 | 0.8 |  |
| **6** | 0 | 1 |  |
| **7 (supernatant)** |  |  | 1 | 4 ml |
| **8 (resuspended pellet)** |  |  | 1 | 4 ml |

- read at 540 nm.

-Prepare a standard graph using BSA[5g/l].

-Calculate the protein concentration of tube 7 and 8 from the standard graph, after determine the concentrations of slandered solution.

|  |  |  |
| --- | --- | --- |
| **Tube No.** | **Absorbance at 540 nm** | **Conc. of BSA [g/l]** |
| **1** |  |  |
| **2** |  |  |
| **3** |  |  |
| **4** |  |  |
| **5** |  |  |
| **6** |  |  |
| **7** |  | ……….? |
| **8** |  | ……….? |