**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]** | **Biuret reagent** | Incubate for 10 mins. | **Conc. [g/l]** |
| **Blank** | 2 | - | 3 ml | 0 |
| **1** | 1.6 | 0.4 | 1 |
| **2** | 1.2 | 0.8 | 2 |
| **3** | 1 | 1 | 2.5 |
| **4** | 0.8 | 1.2 | 3 |
| **5** | 0.6 | 1.4 | 3.5 |
| **6** | 0.4 | 1.6 | 4 |

|  |  |
| --- | --- |
| **Tube 8 [supernatant]** | |
| **supernatant** | 2 ml |

|  |  |
| --- | --- |
| **Tube 7 sample with resuspended pellet** | |
| **resuspended** | 2 ml |

-Add 3 ml of biuret reagent to tubes 7 and 8, then incubate for 10 mins.

- 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.

|  |  |  |
| --- | --- | --- |
| **Tube No.** | **Absorbance at 540 nm** | **Conc. of BSA** |
| **1** |  |  |
| **2** |  |  |
| **3** |  |  |
| **4** |  |  |
| **5** |  |  |
| **6** |  |  |
| **7 (Resuspended pellete)** |  | ……….? |
| **8 (Supernatant)** |  | ……….? |

* **Notice the absorbance of your sample, If it was high what should you do?**