**Lab sheet #3**

**-Proteins-II-**

* **Objective:**
1. Determination of an unknown concentration for protein sample using Biuret method.
2. Getting familiar with standard curve.
* **Methods:**

1. Set up 8 tubes as follows:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Biuret****reagent** | **Sample****[unknown concentration]****(ml)** | **Bovine serum albumin (BSA) Standard Concentration (5 g/L)****(ml)** | **Water (ml)** | **Tube** |
| 3 ml | - | - | 2 | **Blank** |
| - | 0.4 | 1.6 | **A** |
| - | 0.8 | 1.2 | **B** |
| - | 1 | 1 | **C** |
| - | 1.2 | 0.8 | **D** |
| - | 1.4 | 0.6 | **E** |
| - | 1.6 | 0.4 | **F** |
| 2 | - | - | **Unknown sample** |

2. Let the tubes stand at room temperature for 10 min.

3. Read absorbance at **540 nm** against the blank.

* **Results:**

|  |  |  |
| --- | --- | --- |
| **Absorbance at 540 nm****[Y- axis]** | **Protein concentration (5 g/L)****[X- axis]** | **Tube** |
|  | C1 X V1 = C2 X V25 x 0.4 = ? x 2🡺?= 1 | **A** |
|  |  | **B** |
|  |  | **C** |
|  |  | **D** |
|  |  | **E** |
|  |  | **F** |
|  | …………………………… | **Unknown sample** |

**Calculations:**

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* Plot a standard curve for absorbance at 540 nm against albumin std. concentration (g/L).
* From the standard curve find out the unknown protein concentration.