**Material:**

**Reducing sugar estimation in milk using dinitrosalysalic acid**

* Dinitrosalicylic acid reagent (DNS) (1 g of dinitrosalicylic acid + 200 mg of crystalline phenol + 50 mg of sodium sulphite in 100 ml of 1% NaOH)
* 40% sodium potassium tartrate.
* Working glucose standard 100 mg/ 100 ml (by dilute 10 ml of stock solution to 100 ml )

**Sample preparation** : 1. -Homogenize 1g of sample (honey , skimmed milk) in 50 ml of 80% hot ethanol . 2. Centrifuge the content and and collect the supernatant . 3. Evaporate alcohol by heating the contents over water bath. (Don’t heat directly, alcohol is highly inflammable). 4. Dissolve the contents left over in 100 ml of distilled water.

**Method:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Tube | Glucose solution | sample | water | DNS reagent |  | Soduim potasuim tartarate |
| **B** | **--** | **--** | **1** | **3** | **Cover the tubes (with aluminuim foil)**  **And heat for 5 min. in a boiling water bath** | **1** |
| **1** | **0.1** | **--** | **0.9** | **3** | **1** |
| **2** | **0.2** | **--** | **0.8** | **3** | **1** |
| **3** | **0.3** | **--** | **0.7** | **3** | **1** |
| **4** | **0.4** | **--** | **0.6** | **3** | **1** |
| **5** | **0.5** | **--** | **0.5** | **3** | **1** |
| **6** | **0.6** | **--** | **0.4** | **3** | **1** |
| **7** | **0.7** | **--** | **0.3** | **3** | **1** |
| **S1** | **--** | **1** | **---** | **3** | **1** |
| **S2** | **--** | **0.6** | **0.4** | **3** | **1** |

* **Mix the contents.**
* **Cool by immersing in cold water and read at 510 nm.**
* **Plot the standard curve and calculate the amount in the sample from standard curve and calculate the contents.**

**Result:**

|  |  |  |
| --- | --- | --- |
| Tube | Absorbance | CHO content (mg/dl) |
| **B** | **--** | **--** |
| **1** |  |  |
| **2** |  |  |
| **3** |  |  |
| **4** |  |  |
| **5** |  |  |
| **6** |  |  |
| **7** |  |  |
| **8** |  |  |
| **9** |  |  |
| **10** |  |  |
| **S1** |  |  |
| **S2** |  |  |

**- Calculation:**

**……………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………**