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Quantitative methods for protein determinations

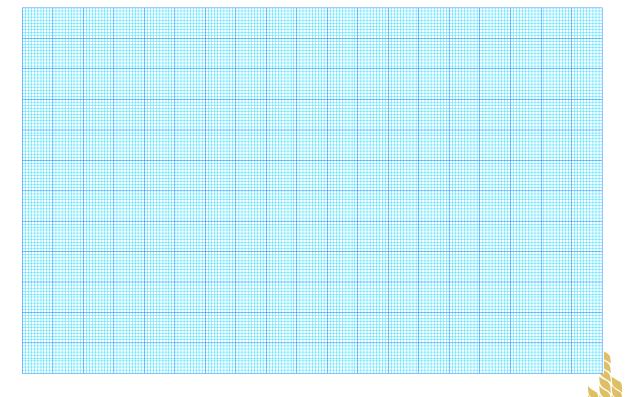
Task 1. Quantitative determinations of protein concentration by use of biuret method - the preparation of standard curve

Protocol: The preparation of standard curve

Prepare different dilutions of standard solution of protein (Casein) in accordance to table:

| No of tube | Casein (1 mg/cm ³) | H ₂ O dest | Concentration | Absorbance |
|------------|-----------------------------------|-----------------------|---------------|------------|
| 0 | _ | 1 cm^3 | 0,000 | |
| 1 | $1,0 \text{ cm}^3$ | _ | 1% | |
| 2 | $0,8 \text{ cm}^3$ | 0,2 cm ³ | 0,8% | |
| 3 | 0,6 cm ³ | 0,4 cm ³ | 0,6% | |
| 4 | 0,4 cm ³ | $0,6 \text{ cm}^3$ | 0,4% | |
| 5 | 0,2 cm ³ | 0,8 cm ³ | 0,2% | |

Add 4 $\rm cm^3$ of copper reagent to each tube and incubate in room temperature for 25-30 min. Measure the absorbance against blank (tube 0) at wave length 545nm. Prepare the plot of dependencies between the concentration of casein and absorbance.







Task 2. Quantitative determination of protein in unknown sample

The aim: quantitative determination of protein content in unknown sample by use of biuret method and previously prepared standard curve.

Protocol: Add 1 cm³ of sample to 4 cm³ of copper reagent. After 25-30 min of incubation in room temperature measure the absorbance twice at wave length 545 nm against blank (1cm³ of H₂O dest. and 4 cm³ of copper reagent). Calculate the concentration of protein in unknown sample by use of standard curve.

| Measurement | Absorbance at 545 nm | Concentration of protein [%] |
|-------------|----------------------|------------------------------|
| 1 | | |
| 2 | | |
| Mean | - | |

Attention! Standard curve will be used again in further classes and semesters. Please keep it in your records

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