



**BCH 462**

**Lab (3)**

# **Extraction and Determination of Bacterial Proteins.**

# Isolation of bacterial proteins involves several steps :

Growth and induction of bacterial cultures.

Lysis of cells in a suitable buffer containing a detergent

DNase and RNase treatment for the removal of the nucleic acids.

Determine the protein concentration using suitable method.

Passage of the extract through an affinity resin and finally elution of proteins.

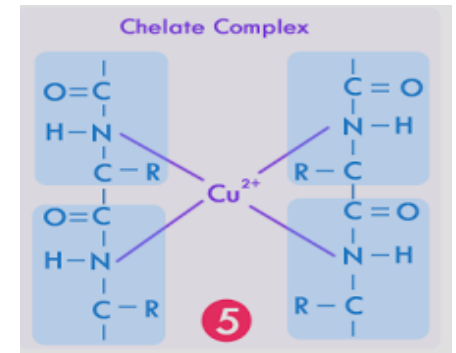
Or subject bacterial cells to ultrasonic vibration to fragment the cells, macromolecules, and membranes using **Sonicate**



# Determination of Bacterial Proteins by Biuret Method

-Biuret method, is used to determine the protein concentration, using standard curve of concentrations..

-It is based on copper ions binding to peptide bonds of protein under alkaline condition to give a violet (purple) color.



-The intensity of the color resulting from the ( $\text{Cu}+\text{protein}$ ) complex is linearly proportional to the concentration of protein present in the solution.

# Review

Steps:	Lab #1 [Plasmid Isolation]	Lab #2 [Transformation of Competent Cells]	Lab #3 [Estimation of Protein Concentration]
1	Growth of the bacterial culture.		
2	Harvesting of the bacteria by centrifugation		
3	-Lysis of the bacteria -Purification of plasmid DNA	Using CaCl <sub>2</sub> solution and brief heat shock to transform the competent cells.	-Lysis of the bacteria -Estimation of protein concentration using Biuret method.