DEPROTEINIZATION OF BLOOD

1. Introduction

- Deproteinization of blood refers to the process of removing proteins from blood samples, typically to analyze other components like metabolites, small molecules, or drugs.
- Proteins, especially large ones like albumin, can interfere with certain assays, making their removal necessary for accurate analytical results.

2. Purpose of Deproteinization

- **Improve Analysis Accuracy**: Proteins can bind to or interact with small molecules, affecting the analysis of non-protein compounds.
- **Prevent Interference**: In biochemical assays, proteins may precipitate, block equipment, or alter assay results.
- **Sample Preparation for Mass Spectrometry**: Deproteinization is often required before mass spectrometry to focus on metabolites, drugs, or other low-molecular-weight compounds.

3. Common Methods of Deproteinization

1. **Chemical Precipitation**: Involves using chemicals to precipitate proteins, leaving the desired components in the supernatant.
    - **Trichloroacetic Acid (TCA)**: Precipitates proteins by lowering pH.
    - **Perchloric Acid (PCA)**: Commonly used for deproteinization in clinical settings.
    - **Organic Solvents**: Solvents like acetone or ethanol denature proteins, causing them to precipitate.
2. **Ultrafiltration**: A mechanical method where blood plasma is passed through a membrane with a specific molecular weight cutoff, retaining proteins while allowing smaller molecules to pass through.
3. **Centrifugation**: Often combined with chemical precipitation, centrifugation helps separate the precipitated proteins from the liquid containing the target molecules.
4. **Dialysis**: A membrane-based method where the sample is placed in a semipermeable membrane that allows small molecules to diffuse out, leaving proteins behind.
5. **Heat Treatment**: Heating the sample to around 60-70°C can denature proteins, which then precipitate out of solution.

4. Deproteinization Using Organic Solvents

- **Acetonitrile**: Commonly used in combination with high-performance liquid chromatography (HPLC) for drug analysis. It denatures and precipitates proteins effectively.
- **Methanol**: Used for protein precipitation in various analytical techniques.

- **Ethanol**: Often used due to its ability to precipitate proteins without denaturing small molecules.

5. Deproteinization for Clinical and Laboratory Applications

1. **Plasma and Serum Analysis**: Deproteinization is necessary when analyzing components like glucose, cholesterol, or drug levels, where proteins might interfere with the results.
2. **Pharmacokinetic Studies**: Drug metabolism and pharmacokinetic studies rely on deproteinization to measure drug concentrations accurately without protein interference.
3. **Metabolomics**: Deproteinization is critical in metabolomics to focus on small molecules like amino acids, nucleotides, and sugars, which are obscured by the presence of proteins.
4. **Toxicology**: In forensic toxicology, deproteinization helps isolate and identify toxic substances in the blood.

6. Challenges in Deproteinization

- **Loss of Small Molecules**: Some methods can also cause the loss of small molecules, affecting the final analysis.
- **Incomplete Protein Removal**: Inefficient deproteinization can leave residual proteins, which may still interfere with assays.
- **Protein Denaturation**: Certain chemical methods can denature proteins irreversibly, making it difficult to recover them if needed for further analysis.

7. Advances in Deproteinization Techniques

- **Automated Systems**: Advances in laboratory automation have led to more efficient deproteinization techniques that reduce human error and improve consistency.
- **Nanotechnology**: Nanoparticles are being explored to selectively bind and remove proteins from blood samples, improving the precision of deproteinization.