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Precipitation of proteins by ammonium sulphate

Proteins can be precipitated from aqueous solutions by adding ammonium sulfate to the protein solution. The following table shows the weight (g) of ammonium sulfate needed for one liter of solution to produce a desired change in the concentration (% saturation) of ammonium sulfate at 25°C. To avoid possible denaturation of proteins, precipitation is conventionally carried out at 0°C. Precipitation of proteins depends on several factors including: the number and position of polar groups, molecular weight of the protein, pH of the solution, and temperature at which the precipitation is performed. The concentration at which antibodies precipitate varies among species; most rabbit antibodies precipitate with a 40% saturated solution, whereas mouse antibodies require 45-50% saturation. Protocol: 1. Allow serum or ascitic fluid to thaw. 2. Determine total volume and centrifuge at 3000g for 30 minutes. 3. Transfer sample to a beaker containing a stir bar and place on a magnetic stirrer. 4. While the sample is stirring, slowly add saturated ammonium sulfate to bring the final concentration to 50% saturation. 5. Once the total volume of ammonium sulfate is added, move the beaker to 4°C for 6 hours or overnight. 6. Transfer the precipitate to a conical tube and centrifuge at 3000g for 30 minutes. 7. Carefully remove and discard the supernatant. 8. Invert the conical tube and drain well. 9. For serum or ascites, resuspend the pellet in 30%-50% of the starting volume in 1XPBS. 10. For monoclonal antibody tissue culture supernatants, resuspend the pellet in 10% of the starting volume in 1X PBS. Note: The table shows the weight (g) of ammonium sulfate needed for one liter of solution to produce a desired change in the concentration (% saturation) of ammonium sulfate. To separate proteins using salt precipitation, mix an antibody solution with dialysis tubing and dialyze against three changes of 1XPBS/0.08% Sodium Azide. Allow enough space for the antibody solution to expand during dialysis - doubling its volume is usually sufficient. Remove excess antibody solution from the tubing and spin it down to eliminate remaining debris. Measure the concentration and store at -80°C for long-term preservation. Ammonium sulfate, a reliable salt precipitant, can efficiently purify proteins by precipitation. Its high solubility in water and ability to interact with multiple water molecules make it an ideal choice. In practice, ammonium sulfate is added either as a solid or a saturated solution to separate desired proteins. Initially, adding more salt increases protein solubility due to 'salting-in', but excessive ionic strength leads to 'salting-out' as the dissolved salt competes with proteins for water molecules, causing them to aggregate and precipitate out. By carefully controlling salt concentration, specific proteins can be isolated by gradually increasing ammonium sulfate levels to precipitate non-desirable proteins, recovering the supernatant, and then adding more salt to isolate the desired protein. Since salt precipitation doesn't denature proteins, recovered fractions can be stored in a salt solution for extended periods without worrying about bacterial contamination. Ammonium sulfate can also be used to concentrate proteins by removing excess salt, resolubilizing the pellet in standard buffers or lower ammonium sulfate concentrations, and further purifying it using hydrophobic interaction chromatography or gel filtration chromatography. Additionally, ammonium sulfate can be used to guide some proteins back into their native conformations by gradually increasing its concentration after unfolding them with denaturants like urea. When working with ammonium sulfate, it's essential to break up clumps and grind the salt using a mortar and pestle, adding only small amounts at a time and waiting for dissolution before proceeding. The process of salt precipitation is a common method for concentrating proteins, but it also has its disadvantages. To minimize the acidifying nature of ammonium sulfate, use a buffer such as 50 mM HEPES or Tris. Analytical grade ammonium sulfate should be used due to potential heavy metal contamination in lower grades. However, this process requires prior knowledge of the protein's solubility and may not purify it. The salt must be removed from the protein sample before further processing through dialysis or chromatography can occur. salting out proteins from solution was discovered over 120 years ago by Franz Hofmeister. he found that adding different salts caused precipitates to form in solutions of egg whites. biochemistry has used this trick for 120 years to purify proteins while arguing about how it works. the current accepted mechanism says that salting proteins out occurs when water molecules are taken away from protein solvency to solvent shells around ions in the salt. salts high in the hofmeister series are most efficient at protein precipitation due to stable solvent shells that increase surface tension and hydrophobic effect, stabilizing protein structure. proteins with more hydrophobic surface character precipitate at lower salt concentrations. ammonium sulfate is commonly used for purification or concentration due to anion and cation both being high in the hofmeister series. it has a solubility of around 4m, with most proteins precipitating by 3.2m. Given article text here As with other salts like ammonium sulfate, TCA induces precipitation through hydrophobic aggregation. However, TCA also disrupts the solvation layers around proteins and partially denatures them, increasing their hydrophobic surface exposure to the solvent. This makes TCA effective at lower concentrations than other solvents, allowing for smaller sample volumes without significantly reducing protein concentration. Nonetheless, TCA can coagulate proteins, making them less soluble in aqueous buffers after denaturation. As a result, these methods are typically used with samples that don't require functional enzymes, such as SDS-PAGE or mass spectrometry analysis. When using TCA, the acid nature of the compound necessitates washing the protein pellet with acetone to remove it or adding base to neutralize its pH. At high salt levels, proteins typically lose solubility, leading to a phenomenon known as salting-out (Green and Hughes, 1955). This effect also enhances the stability of a protein's native conformation. Conversely, certain ions can denature proteins by promoting solubility. The mechanism behind salting-out involves preferential solvation due to the exclusion of salt from water closely associated with a protein's surface layer, known as its hydration layer (Rupley et al., 1983). The hydration layer plays a crucial role in maintaining solubility and the correct native conformation. Protein-water interactions occur through ion hydration between charged side chains, hydrogen bonding between polar groups and water, and hydrophobic hydration involving apolar residues. Hydrophobic hydration reduces the configurational freedom of water molecules near apolar residues, resulting in decreased entropy and unfavorable energy conditions. When salt is added to a solution, it increases the surface tension of water, enhancing hydrophobic interactions between proteins and water. This leads to protein folding and self-association, ultimately causing precipitation as the system gains entropy and becomes energetically favorable (Timasheff and Arakawa, 1997). The increase in surface tension is often described by the Hofmeister series (Parsegian, 1995), where salts that favor salting-out raise the surface tension the most. Ammonium sulfate ((NH4)2SO4) is commonly used for this process due to its high solubility and ability to effectively increase the surface tension of water. When working with solid ammonium sulfate, it's recommended to use a mortar and pestle to break up any lumps and analytical-grade material free from contaminants like heavy metals. Addition of ammonium sulfate can acidify the solution, so a buffer such as HEPES or Tris should be used at a concentration of 50 mM or higher. When calculating the amount of solid ammonium sulfate needed, online tools or equations provided in this appendix (EnCor Biotechnology Inc., can be utilized to determine the correct amounts. Alternatively, saturated ammonium sulfate solution at 25 °C can be purchased from suppliers like Sigma-Aldrich. Note: In literature, 'sulphate' is sometimes spelled as 'sulfate,' particularly in UK English. Pelleted proteins can be easily dissolved in standard buffers without denaturation, making them suitable for gel filtration and buffer exchange. Alternatively, the protein can be dissolved in a non-precipitating concentration of ammonium sulfate (e.g., 1 M) and then applied to a hydrophobic interaction matrix. Selective precipitation is often used for low-molecular-weight proteins like interleukin-1β, which require higher salt concentrations than larger molecular proteins. For example, IgG can be precipitated from blood sera with 40-45% ammonium sulfate, followed by anion exchange chromatography. Ammonium sulfate has been widely used to fractionate membrane proteins due to its ability to stabilize and precipitate proteins bound to lipids or detergents. However, the resulting precipitates may have lower density than protein-only precipitates, causing them to float during centrifugation. Crystallization is another traditional method of protein purification that involves extracting ammonium sulfate-precipitated protein with successive dilute solutions at low temperature. Ammonium sulfate can also be used to remove contaminating nucleic acids from protein solutions by applying the protein to a small anion exchange column equilibrated with 0.4M ammonium sulfate, where the nucleic acids bind to the column and the protein is collected in the flow-through. In addition to its uses in purification, ammonium sulfate can stabilize proteins by preferential solvation, inhibiting bacterial growth and contaminating protease activities. It can also induce native conformations in unfolded proteins, such as those denatured by urea. For example, recombinant HIV-1 Rev expressed in E. coli was folded using 0.5-1.0 M ammonium sulfate after being solubilized with urea and purified by ion-exchange chromatography. Finally, the text provides some technical details about calculating the weight of solid ammonium sulfate and its specific volume (sp. vol.) at different temperatures. To calculate the amount of ammonium sulfate (NH4)2SO4 needed to add to 1 liter of solution at a certain temperature, you can use two equations. First, if you want to increase the saturation from S1 to S2, you can use the equation: volume(ml)=100(S2−S1)1−S2. For example, to raise a 20% saturated solution to 70%, you would add 166.66 ml of saturated solution to reach a total volume of 266.66 ml. Alternatively, if you want to calculate the weight (in grams) of NH4)2SO4 needed to add to 1 liter of solution at a certain temperature, you can use the equation: weight(g)=Gsat(S2−S1)1−(PS2), where Gsat is the amount of NH4)2SO4 in 1 liter of saturated solution at that temperature. The data provided includes tables with values for various temperatures, including: * Grams of NH4)2SO4 needed to add to 1 liter of solution * Molarity of the resulting solution * Density and specific volume of the solution These tables can be used as a reference to calculate the amount of NH4)2SO4 needed to achieve a desired concentration. **Calculations for Adding Ammonium Sulfate Solution to Water at 0°C** To calculate the amount of 3.8 M Ammonium Sulfate solution needed to achieve various molarities in a 1-liter water solution at 0°C, we need to consider the initial and final volumes of the solution. **Initial and Final Volumes** The table shows that when 3.8 M Ammonium Sulfate is added to a 1-liter water solution at 0°C: * For every 0.2 M increase in molarity, the volume increases by approximately 4-5 mL. * The final volume after adding the solution ranges from 1000 mL to 1017 mL for different initial molarities. **Relationship between Initial and Final Volumes** The table illustrates that there is a positive correlation between the initial and final volumes of the solution. As the initial molarity increases, so does the final volume. **Implications of Adding Ammonium Sulfate Solution** These calculations demonstrate how adding a concentrated solution of Ammonium Sulfate to water at 0°C affects the volume and molarity of the resulting solution. The results have implications for various scientific applications, such as preparing solutions with precise concentrations or determining the effects of solute addition on solution properties. Let me know if you'd like me to simplify or expand on this paraphrased version! The text appears to be a collection of references and data related to biochemical research, specifically on protein purification and stability. It includes tables and charts that detail the solubility of proteins in various salt concentrations and organic solvents. The references cited include studies on protein crystallization, solubility, and stability, as well as techniques for membrane protein purification. Some of the specific topics covered include: * Protein solubility in aqueous solutions of salts and organic solvents * Crystallization as a purification technique * Contribution of surface free energy perturbations to protein-solvent interactions * Effect of sulphate and urea on the stability and reversible unfolding of β-lactamase * Stabilization of protein structure by solvents The references include works from various authors and journals, such as Methods Enzymol, Biochemistry, Journal of Molecular Biology, Nature, Trends in Biochemical Sciences, and Analytical Biochemistry.

Purpose of ammonium sulfate precipitation. Why ammonium sulphate is used for protein precipitation. Ammonium sulfate precipitation. What is ammonium sulphate precipitation.