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A REVIEW ON PROTEIN PURIFICATION TECHNIQUES

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ABSTRACT
Purification techniques are fundamental in biotechnology for isolating and refining biomolecules from complex mixtures. It plays a pivotal role in various scientific disciplines, including structural biology, enzymology, drug discovery, and industrial biotechnology. This review traces the historical development of purification methods, from traditional approaches to modern innovations. We discuss the principles, applications, and advancements of purification techniques, highlighting their impact on biotechnological research and industrial processes.

KEYWORDS: Protein purification, chromatographic techniques, multidimensional chromatography, electrophoresis.

INTRODUCTION
Proteins play vital roles in biological processes, making their isolation and purification essential for research and industrial applications. This article aims to provide a comprehensive review of the diverse techniques employed in protein purification, highlighting their strengths, weaknesses, and applications.[1]

Old techniques
Salt techniques
Biomolecule purification and separation are essential phases in a few procedures, from DNA extraction to protein isolation. One useful technique used in these procedures is called "salting out." By selectively precipitating target biomolecules from solution, salting out methods make use of the concept of preferred solvation to facilitate the purification and separation of these biomolecules. The fundamentals of salting out methods, their uses in biotechnology, and current developments in this area are all covered in this article.[2]

Principle of Salting Out
The theory behind salting out is that when the concentration of certain salts rises, the solubility of proteins and nucleic acids in aqueous solutions falls. When salts are present, the hydrophobic interactions and hydrogen bonding between water molecules and biomolecules are disrupted, which leads to these phenomena. The amount of water available for solvation is essentially decreased when salts like sodium chloride or ammonium sulphate are added to a solution containing biomolecules because they compete with the biomolecules for water molecules. As a result, the biomolecules tend to precipitate out of solution and lose their solubility.[3]

Types of Salting Out Techniques
Ammonium Sulfate Precipitation: Since ammonium sulphate is very soluble and may be employed to finely regulate precipitation, it is frequently used in salting out methods. Target biomolecules can be selectively precipitated while leaving impurities in solution by progressively adding ammonium sulphate to a protein or nucleic acid solution. Centrifugation can be used to collect and further purify the biomolecules that have precipitated.

Fractional Precipitation: Salt is progressively added to a solution step-by-step during fractional precipitation, raising the concentration until the desired biomolecule precipitates out. Complex mixtures may be separated using this approach, which enables the selective precipitation of various biomolecules according to their solubility properties.

Reverse Salting Out: When reverse salting out, the solution's salt content is high at first then progressively drops. When purifying biomolecules that are more soluble at greater salt concentrations, this approach is very helpful.

Applications of Salting Out Techniques
Salting out techniques find wide-ranging applications in biotechnology, including.
Protein Purification: Proteins from complicated mixtures, including cell lysates or culture supernatants, can be purified by salting out. Researchers can get rid of impurities and acquire a highly pure sample for further analysis or downstream uses by selectively precipitating the target protein.

Nucleic Acid Extraction: Nucleic acid extraction from biological materials, including the extraction of DNA and RNA, also uses salting out. Pure nucleic acid samples can be isolated by precipitating nucleic acids with salts to remove impurities like proteins and lipids.

Enzyme Purification: Enzymes are purified using salting out processes for use in a variety of industrial and scientific applications. Researchers can create very pure enzyme preparations with higher activity and stability by precipitating the desired enzyme selectively.[4]

Chromatography
Among the several chromatographic techniques, cellulose-based chromatography is particularly advantageous due to its environmental friendliness and versatility. But even with all of its benefits, this approach frequently has drawbacks like high processing times and low resolution. We examine the complexities of cellulose-based chromatography in this article, as well as its promise and difficulties.[5]

Understanding Cellulose-Based Chromatography
In cellulose-based chromatography, the stationary phase is cellulose, a common polysaccharide presents in plant cell walls. Its biodegradability, renewability, and availability make it a desirable substitute for traditional stationary phases. The term "cellulose-based chromatography" refers to a group of methods that include high-performance liquid chromatography (HPLC), paper chromatography, and thin-layer chromatography (TLC), each with special benefits and uses.[6]

Time Consumption
Time-consuming nature is one of the main problems with cellulose-based chromatography. Generally, the analytes diffuse through the stationary phase throughout the separation process, which might take a while, particularly in types of chromatography that use paper or TLC. Extended analysis periods can result from factors that affect the rate of diffusion, including temperature, solvent content, and thickness of the stationary phase In order to address this problem, academics have looked at a number of approaches. One strategy is to optimize the chromatographic conditions by modifying the stationary phase composition, chromatographic method, and the kind and concentration of the mobile phase. Furthermore, shorter analysis times have been made possible by quicker data processing and capture because of developments in automation and instrumentation.[7]

Limited Resolution
Cellulose-based chromatography has limited resolution, particularly when trying to separate closely related molecules. This is due to its porous nature, which can result in broad peak shapes and low peak capacity. Consequently, chromatographic peaks may overlap, and the separation process's effectiveness may be reduced. This shortcoming is especially noticeable when analyzing complex mixtures containing several components with similar physicochemical properties.

There are several strategies that can be used to improve the resolution in cellulose-based chromatography. Some of these strategies include introducing specific functional groups into the stationary phase through derivatization, developing new stationary phase materials with improved chromatographic performance, and incorporating modifiers or additives in the mobile phase to increase selectivity. Utilizing multidimensional chromatographic methods and combining mass spectrometry with cellulose-based chromatography can also enhance resolution and aid in peak identification.[8]

Application
Environmental Monitoring
Researchers use cellulose-based materials to analyze environmental samples. Cellulose’s flexibility enables effective separation of complex mixtures, making it useful for monitoring contaminants, evaluating water quality, and analyzing pesticide residue.

Pharmaceutical Research and Development
Cellulose-based stationary phases are frequently used in pharmaceutical labs for drug analysis and quality assurance. Scientists use cellulose columns to separate chiral drug enantiomers, which are mirror-image molecules, to ensure the safety and effectiveness of pharmaceuticals.

Food and Beverage Industry
Cellulose-based chromatography is used to examine food additives, tastes, and pollutants, enabling the identification and measurement of substances such as colorants, sweeteners, and preservatives. The use of cellulose columns in the food industry helps ensure quality and legal compliance.

Natural Product Isolation
Cellulose columns are a useful tool for separating natural compounds from plant extracts. These columns use stationary phases made of cellulose, which allows researchers to isolate and purify bioactive molecules with great precision. The ability to comprehend plant metabolites and discover new drugs both rely heavily on this application.[9]

Electrophoresis

Gel Electrophoresis

Gel electrophoresis is a technique that uses a gel matrix to separate molecules. Two common types of gel used in this process are polyacrylamide and agarose. Polyacrylamide is used for small molecules, like proteins, while agarose is used for larger molecules, like DNA.[10] The gel acts as a filter and slows down the migration of molecules based on their size. Larger molecules move more slowly through the gel's pores than smaller ones do. As a result, when an electric current is applied, molecules are separated into bands based on their size.[11]

Applications of Electrophoresis

Electrophoresis finds application in diverse areas of research and diagnostics.

DNA Analysis: DNA sequencing, PCR product analysis, restriction mapping, and DNA fingerprinting all rely on gel electrophoresis to sort DNA fragments based on size. This allows scientists to examine gene expression patterns, detect genetic abnormalities, and assess genetic variations.

Protein Analysis: Protein electrophoresis is widely used in drug development, proteomics, biochemistry, and research on protein purity, isoform identification, and post-translational modifications.[12]

Clinical Diagnostics: In clinical laboratories, electrophoresis is critical for diagnosing hemoglobinopathies and cystic fibrosis, analyzing serum proteins, and identifying genetic mutations.[13]

Environmental Monitoring: In environmental science, electrophoresis methods are used to analyze DNA from environmental samples, identify contaminants, and evaluate microbial diversity.[14]

Intermediate

Affinity chromatography

Affinity chromatography is a process that relies on the selective and reversible binding interactions between a ligand immobilized on a solid support matrix and a target molecule. This technique is based on the lock-and-key mechanism, where other molecules can pass through without any hindrance while the target molecule (the "key") interacts preferentially with the immobilized ligand (the "lock"). To ensure the success of affinity chromatography, a strong ligand with high affinity and specificity for the target molecule is essential. Depending on the target biomolecule, the ligand can be anything from small compounds like medications or cofactors to antibodies, enzymes, receptors, and nucleic acids.[15]

The Affinity Chromatography Process

The process of affinity chromatography typically involves several key steps.

Washing: After removing unbound molecules with a buffer solution, only the molecules that are bound to the target remain attached to the column Selection of Ligand: Choosing the appropriate ligand is crucial and depends on the target molecule and the desired level of purification Immobilization: The ligand is attached to a solid support matrix via adsorption or covalent bonding to ensure stability during chromatography.[16]

Sample Loading: The sample containing the target molecule is loaded into a chromatography column filled with an immobilized ligand. The target molecule selectively binds to the ligand while non-target molecules pass through the column Finally; To remove the target molecule from the column, a competing ligand can be used, or the buffer's parameters can be altered to break the binding relationship. The purified target molecule is carefully recovered by optimizing the elution conditions to the highest possible extent.[17]

Applications of Affinity Chromatography

Affinity chromatography finds wide-ranging applications in various scientific disciplines.

Protein Purification: Using affinity chromatography is a widely adopted method to purify recombinant proteins, enzymes, antibodies, and other biomolecules due to its high specificity and yield.

Drug Discovery: Affinity chromatography is a crucial tool in drug development that enables drug target identification, characterization, lead compound screening, and research on drug-receptor interactions.[18]

Biological Research: Affinity chromatography is a technique used by researchers to study biological processes, such as ligand-receptor binding kinetics, DNA-protein and protein-protein interactions.

Clinical Diagnostics: Clinical diagnostics use tests based on affinity chromatography to track illness development, identify biomarkers, and examine protein-protein interactions.[19]

Ion exchange chromatography

"Ion exchange" refers to a chemical process that can be reversed, in which ions that are attached to a solid resin with a similar charge can be replaced with ions from a solution. This process occurs because of the electrical attraction between ions that have opposing charges. "Ion exchange chromatography" is a popular biotechnology technique that makes use of this phenomenon to separate and purify biomolecules based on their net charge.[20]

Applications in Biotechnology

Protein Purification: Protein purification methods extensively employ ion exchange chromatography. Depending on the pH of the solution and the amino acid composition of the protein, various net charges are present. By selecting the appropriate resin and adjusting the pH of the buffer solution, certain proteins can be coupled to the ion exchange resin, while contaminants are eliminated. The purified proteins can then be retrieved in a subsequent step by elution using a gradient of pH or salt concentration.[21]

Drug Delivery Systems: Ion exchange resins are used to create drug delivery systems that can regulate the pace of medication release. By incorporating medications into ion exchange matrices and adjusting the pH or ion concentration, the release of drugs can be controlled. This enables medications to be released gradually and under control, leading to improved therapeutic efficacy and reduced adverse effects.

Environmental Remediation: Ion exchange technology is used to remove heavy metals and other contaminants from wastewater and polluted soils. This technology uses ion exchange resins that have a specific affinity for the target ions, which helps in the removal of impurities from the aqueous phase. This process plays a crucial role in restoring ecosystems and mitigating environmental contamination.[22]

Gel filtration chromatography

One effective way of separating molecules according to their size is by using gel filtration chromatography. This method involves size exclusion chromatography (SEC) which uses porous gel matrices to differentially exclude molecules, allowing biomolecules to be purified with high resolution.[23]

Principles of Gel Filtration Chromatography

Gel filtration chromatography is based on the principle of size-dependent partitioning of molecules. The method involves introducing a mixture of molecules to the top of a chromatographic column packed with porous beads made of cross-linked polymers such as dextran or agarose. These beads form an interconnected network of holes of varying diameters, allowing molecules of different sizes to be separated based on their ability to penetrate the pores.

When a sample passes through a column containing a gel matrix, smaller molecules can deeply enter the pores, causing a slower elution time. In contrast, larger molecules cannot fit through the pores and move through the column more quickly. The process of differential migration based on size or molecular weight results in the separation of molecules.[24]

Applications of Gel Filtration Chromatography

Gel filtration chromatography finds widespread application in various fields, including biochemistry, molecular biology, pharmaceuticals, and biotechnology. Some common applications include Protein Purification: Proteins can be extracted using gel filtration chromatography, which isolates target proteins according to their size and separates them from smaller contaminants like salts and nucleic acids. This technique can be used for complicated mixtures such as cell lysates or culture supernatants.

Analysis of Protein Complexes: This method utilizes gel filtration chromatography to reveal the stoichiometry and oligomeric state of protein complexes in solution by sorting proteins based on their size, making it a useful tool for determining the composition of protein complexes and exploring protein interactions.

Determination of Molecular Weight: Gel filtration chromatography is a reliable method for determining the molecular weight of biomolecules. By comparing the elution volumes of target molecules with those of established standards of varying molecular weights, researchers can accurately determine the size of their sample molecules.[25]

Advance techniques

HPLC

High Performance Liquid Chromatography (HPLC) is a commonly used analytical method in various industries including forensics, food and beverage, pharmaceuticals, and environmental analysis. It is used for the precise separation, identification, and quantification of compounds. This page aims to provide an overview of the fundamentals of HPLC, its applications in different sectors, and helpful resources for further research.[26]

Principles of HPLC

In the HPLC chromatographic process, a liquid mobile phase is forced through a stationary phase at high pressure. Once the mixture to be studied is injected into the system, its components are isolated based on their interactions with the stationary phase. Key principles include.

Stationary Phase: Consists of a solid support material (commonly silica or polymers) coated with a thin layer of liquid. The choice of stationary phase depends on the properties of the analytes and the separation goal.

Mobile Phase: Typically, a mixture of solvents such as water, acetonitrile, or methanol, which elutes the analytes through the stationary phase. The composition and gradient of the mobile phase can be adjusted to optimize separation.

Detection: Various detectors, like UV-Vis, fluorescence, or mass spectrometry, are employed to detect separated analytes based on their physical or chemical properties.

Column: The column containing the stationary phase is where separation occurs. Columns can vary in length, diameter, and particle size, affecting resolution and efficiency.[27]

Applications of HPLC

Pharmaceutical Analysis: In quality control and drug development, HPLC is widely used to analyze medication purity, detect contaminants, and quantify active pharmaceutical ingredients.[28]

Environmental Monitoring: In environmental samples, HPLC is useful for detecting and quantifying contaminants such as pesticides, herbicides, and heavy metals in water, soil, and air.[29]

Clinical Diagnostics: High-performance liquid chromatography (HPLC) is an essential tool in clinical labs for examining proteins, amino acids, vitamins, and hormones in biological samples to diagnose and track diseases.[30]

Food and Beverage Industry: HPLC is an analytical tool used in food analysis to detect pollutants, additives, preservatives, and nutritional content. It plays a vital role in identifying the quality and authenticity of beverages such as fruit juices, wine, and beer.

Forensic Science: The use of HPLC aids in the study of drugs of abuse, toxicological screening, detection of chemical residues in forensic materials, and measurement of blood alcohol content.[31]

Fusion Tags

In the dynamic realm of biotechnology, fusion tags stand out as versatile tools, offering researchers an array of possibilities for protein engineering, purification, and visualization. These molecular markers, often small peptides or proteins are fused to target proteins to facilitate their manipulation and analysis. From enabling protein purification to aiding in the study of protein interactions, fusion tags have become indispensable in modern biotechnological research.

Protein Purification: Streamlining the Process

One of the most common applications of fusion tags is in the purification of recombinant proteins. By fusing a target protein with a known affinity tag, such as Poly histidine (His-tag), glutathione S-transferase (GST), or maltose-binding protein (MBP), researchers can selectively bind the fusion protein to a corresponding affinity resin. This allows for efficient purification of the target protein from complex cellular lysates or culture supernatants. Additionally, fusion tags can facilitate the removal of contaminants, ensuring a high degree of purity in the final product.[32]

Enhancing Solubility and Stability

Protein solubility and stability are critical factors in biotechnological applications, particularly in protein expression and crystallization studies. Fusion tags can significantly improve the solubility and stability of target proteins, thereby enhancing their functionality and utility. For instance, fusion with solubility-enhancing tags like maltose-binding protein (MBP) or small ubiquitin-like modifier (SUMO) can promote proper folding and prevent aggregation of the target protein, especially when expressed in heterologous host systems.[33]

Facilitating Protein-Protein Interactions

Studying protein-protein interactions is fundamental to understanding cellular processes and signaling pathways. Fusion tags play a pivotal role in elucidating these interactions by enabling the affinity purification of protein complexes. By fusing interacting proteins with different tags, such as the tandem affinity purification (TAP) tag or the FLAG-tag, researchers can isolate protein complexes under native conditions, preserving their native structure and function. This approach has revolutionized the study of protein interaction networks and has led to insights into various biological processes.[34]

Visualizing Proteins *In Vivo*

In addition to aiding in protein purification and interaction studies, fusion tags are invaluable tools for visualizing proteins in living cells. Fluorescent protein tags, such as green fluorescent protein (GFP) and its variants, can be fused to target proteins to track their localization and dynamics in real-time. This non-invasive approach allows researchers to monitor protein trafficking, organelle dynamics, and protein-protein interactions within living cells, providing invaluable insights into cellular function and behavior.[35]

Membrane-based techniques

Biotechnology, the interdisciplinary field merging biology with technology, has revolutionized various industries, from healthcare to agriculture. Within this vast domain, member-based techniques have emerged as powerful tools, enabling researchers to explore the intricate mechanisms of biological systems with unparalleled precision and depth. These techniques, which involve the manipulation and analysis of individual biological molecules or cells, hold immense promise for advancing our understanding of life processes and developing innovative solutions to pressing challenges. In this article, we delve into the realm of member-based techniques in biotechnology, exploring their applications, significance, and future directions.[36]

Understanding Member-Based Techniques

Member-based techniques encompass a wide array of methods that focus on studying individual biological components, such as molecules or cells, in isolation or within their natural context. These techniques leverage advanced instrumentation, molecular biology principles, and computational tools to probe the structure, function, and interactions of biological entities at a microscopic scale.

Single-Molecule Imaging: This technique allows researchers to visualize and track individual molecules in real-time, offering insights into their dynamics, behavior, and interactions. Fluorescence microscopy and superresolution imaging are commonly employed methods in this field.

Single-Cell Analysis: With the advent of high-throughput sequencing and microfluidic technologies, researchers can dissect the heterogeneity within cell populations and characterize individual cells based on their genomic, transcriptomic, or proteomic profiles. Single-cell analysis has profound implications for understanding cellular development, disease progression, and therapeutic responses.[37]

Protein Engineering and Directed Evolution: By manipulating individual amino acids within proteins or screening vast libraries of variants, researchers can design novel proteins with tailored functions or enhanced properties. This approach has numerous applications in drug discovery, enzyme optimization, and biocatalysis.

Single-Particle Cryo-Electron Microscopy (cryo-EM): Cryo-EM enables the visualization of biomolecular structures at near-atomic resolution, providing crucial insights into the architecture and function of macromolecular complexes. Recent technological advancements have significantly enhanced the speed and resolution of cryo-EM, making it an indispensable tool in structural biology.[38]

Applications and Significance

Member-based techniques have revolutionized various fields within biotechnology and beyond. In medicine, these techniques are driving advancements in personalized therapy, diagnostics, and regenerative medicine. For instance, single-cell analysis allows clinicians to profile tumor heterogeneity and identify therapeutic targets tailored to individual patients. Similarly, protein engineering facilitates the development of biopharmaceuticals with improved efficacy and reduced side effects.

In agriculture, member-based techniques are revolutionizing crop improvement and sustainable farming practices. By elucidating the molecular mechanisms underlying plant-microbe interactions or stress responses at the single-cell level, researchers can develop crops with enhanced resilience, productivity, and nutritional value. Moreover, single-molecule imaging techniques offer new insights into the dynamics of soil microbial communities and their roles in nutrient cycling and ecosystem health.

Beyond healthcare and agriculture, member-based techniques are driving innovation across diverse sectors, including environmental monitoring, bioenergy production, and materials science. For example, single-particle cryo-EM has facilitated the design of novel nanomaterials with applications in drug delivery, energy storage, and catalysis. Similarly, single-cell analysis is shedding light on microbial diversity in natural ecosystems and its impact on biogeochemical cycles, climate change, and ecosystem functioning.[39]

Multi-dimensional chromatography

Chromatography has long been a cornerstone technique in biotechnology, enabling the separation, identification, and purification of complex mixtures of biomolecules with unparalleled precision.[40] In recent years, multidimensional chromatography has emerged as a powerful tool, offering enhanced resolution and sensitivity for the analysis of intricate biological samples.[41] This article explores the principles, applications, and advancements of multidimensional chromatography in biotechnology, highlighting its transformative potential in various fields.[42]

Principles of Multidimensional Chromatography

Multidimensional chromatography involves the sequential coupling of two or more chromatographic separation techniques, each exploiting different physicochemical properties of the analytes. By combining multiple separation mechanisms, multidimensional chromatography offers superior resolving power compared to traditional single-dimensional techniques.

The primary objective of multidimensional chromatography is to resolve complex sample mixtures with high efficiency and selectivity. This is achieved by fractionating the sample in one dimension and then subjecting individual fractions to further separation in subsequent dimensions. Common multidimensional chromatography configurations include 2D liquid chromatography (LC-LC), 2D gas chromatography (GC-GC), and combinations thereof.[43]

Applications in Biotechnology

In proteomics, multidimensional chromatography plays a pivotal role in the comprehensive characterization of complex protein mixtures. By coupling techniques such as reversed-phase chromatography with ion exchange chromatography or size exclusion chromatography, researchers can achieve unparalleled resolution for the identification and quantification of proteins in biological samples.

Multidimensional chromatography finds widespread applications across various domains of biotechnology, including proteomics, metabolomics, pharmaceuticals, and environmental analysis.

Similarly, in metabolomics, multidimensional chromatography facilitates the separation and analysis of metabolites from diverse biological matrices. By integrating chromatographic methods based on different separation mechanisms, metabolomic studies can achieve comprehensive coverage of the metabolome, enabling insights into cellular metabolism, disease mechanisms, and biomarker discovery.

In the pharmaceutical industry, multidimensional chromatography is employed for the purification of therapeutic biomolecules, such as monoclonal antibodies (m Abs), peptides, and nucleic acids. By combining chromatographic modes with complementary selectivity, manufacturers can purify biotherapeutics to unprecedented levels of purity, ensuring product safety and efficacy.[44]

Automated systems

In the dynamic realm of biotechnology, the purification of biomolecules is a crucial step in various processes, including drug discovery, therapeutic protein production, and genetic engineering. Traditional purification methods often involve time-consuming manual labor, are prone to errors, and lack scalability. However, with the advent of automated purification systems, these challenges are being overcome, leading to increased efficiency, reproducibility, and throughput in biotechnological workflows.

The Need for Automation in Purification

Purification of biomolecules such as proteins, nucleic acids, and peptides is essential for obtaining high-quality samples for downstream applications. Manual purification methods, although widely used, suffer from several limitations:

Inconsistent Results: Human variability can result in inconsistencies in purification outcomes, affecting the reliability of experimental data.

Labor-Intensive: Manual purification requires skilled personnel to perform repetitive tasks, leading to labor inefficiencies and potential human errors.

Time-Consuming: Traditional purification methods are often time-consuming, limiting the speed of research and development processes.

Limited Scalability: Manual purification methods are not easily scalable to accommodate high-throughput applications or large-scale production needs.[45]

Advantages of Automated Purification Systems

The adoption of automated purification systems offers several advantages over traditional manual methods:
Increased Efficiency: Automated systems significantly reduce purification times and enable parallel processing of multiple samples, leading to higher throughput and faster results.
Improved Reproducibility: By minimizing human intervention and standardizing purification protocols, automated systems ensure consistent and reproducible results across experiments.
Enhanced Quality Control: Real-time monitoring and control capabilities enable strict quality control, ensuring the purity and integrity of purified biomolecules.
Scalability: Automated purification systems can be easily scaled up or down to accommodate varying sample volumes and throughput requirements, making them suitable for both research and industrial applications.[46]

Applications in Biotechnology

Automated purification systems find wide-ranging applications in various fields of biotechnology, including:
Drug Discovery: Accelerating the purification of target proteins and screening of drug candidates.
Biopharmaceutical Production: Streamlining the production of therapeutic proteins, monoclonal antibodies, and vaccines.
Genetic Engineering: Purification of recombinant DNA constructs and gene editing tools such as CRISPR-Cas9.
Diagnostic Assays: Preparing purified biomolecules for use in diagnostic tests and assays.[47]

CONCLUSION

The evolution of purification techniques in biotechnology has been marked by a progression from traditional methods to advanced technologies that offer higher efficiency, purity, and scalability. As the demand for purified biomolecules continues to grow, ongoing research and innovation in purification techniques are essential for meeting the needs of diverse applications in biotechnology and beyond.

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