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MDPI

Proceedings

A Mini Literature Review on Current Advances in Protein Purification Techniques †

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Abstract: Protein purification is an ever-vital approach for academia and industry. This paper mainly reviews and discusses one of the core components of proteomics, the latest advances in separation technology for protein components, focusing on five different methods. The MCPA system is incredibly economical for protein treatment research to purify samples. Because of the excessive protease interest of Ulp1 toward SUMO fusion, excessive protein products may be made within 1/2 of an hour through this method. The magnetic separation strategies will offer a higher process of protein purification within the close to imminent because of a few advantages. The evaluation established that the ATPS technique is a cost-effective, time-saving (30 min), and high-recuperation approach that can be scaled up for commercial purposes. Herein, ATPS may be an approach for the purification of single-step separation and keep away from multi-step purification just like the chromatography technique. Therefore, our recent reviews provide a capable technique for competent protein purification.

Keywords: Review; MCPA system; SUMO fusion; magnetic separation; ATPS technique; chromatography technique

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1. Introduction

Proteins are commonly created in heterologous structures because it's tough to acquire high-quality products from herbal sources. Thoughtful expression and purification of recombinant proteins continue to be fundamental problems for biotechnology. Several practises were used to simplify the purification procedure, including the usage of affinity [1,2]. Because figuring out the structure of the relationship between a protein and a ligand is important for biochemistry and finding new drugs [3,4], protein purification is an important method for both academia and industry [5,6].

Isolation, separation, and purification of diverse proteins, peptides, and specific molecules are employed in nearly all branches of bioscience and biotechnology. For this reason, the separation of technological know-how and generation is a critical region important for, in addition, traits in bio-oriented studies and generation. "New separation techniques are needed that can treat dilute solutions or solutions with only small amounts

of target molecules in the presence of a large number of other compounds, even in the particulate matter" [7].

The improvement and alertness of proteomics are inseparable from separation and identity generation. Natural proteins are often mixtures, and many critical proteins are poor in biomaterials. Therefore, the isolation of proteins without affecting their shape, composition, and activity has continually been an issue in proteomics research [8]. With the improvement of generation, many new technologies have been implemented for protein separation, along with multi-column plate adapters (MCPA) [9], the cell-surface show primarily based on the SUMO-ulp1 system [10], aqueous 2-segment system (ATPS) [11], chromatography [11,12], and magnetic separation [7,13], etc. According to the residences of proteins, it's a growing fashion to split proteins to combine a couple of methods that can hold the hobby and shape of proteins higher and reap better resolution.

This paper mainly reviews and discusses one of the core components of proteomics, the latest advances in separation technology for protein components, focusing on five different methods.

2. Multi-column plate adapter (MCPA) system

Many protein structure and function studies require multiple protein purification techniques to contribute to protein purification methods. The case of therapeutic protein purification, such as antibodies, is referred to. Since then, technologies have been developed to meet the demand. However, higher protein yield and purity in one step come with a higher price, which is usually not feasible for small-scale research [14]. Immobilised metal affinity chromatography (IMAC), automated Fast Protein Liquid Chromatography (FPLC), and Protein Maker are the instruments that are used now.

Some researchers believe cheaper protein purification with higher purity and shorter time can be developed with a multi-well collection plate and simple gravity. This is the main idea behind the multi-column plate adapter (MCPA) system. The system can be set up in different ways and with various conditions, like other types of resin, buffer systems, wash buffer imidazole concentration, and lysate load volume [15].

The MCPA system consists of a series of long-drip filter plates with a sealing mat and other conventional laboratory supplies, as in Figures 1 and 2. The system can be reused repeatedly for protein purification after cleaning. The system uses affinity chromatography as a protein purification technique. Some researchers used ion-exchange column chromatography with the method [16].

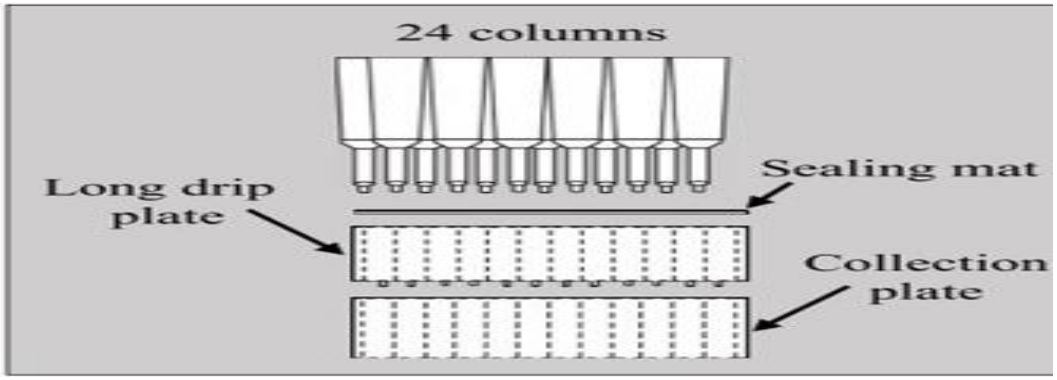

Figure 1. Schematic assembly of the MCPA system [9].

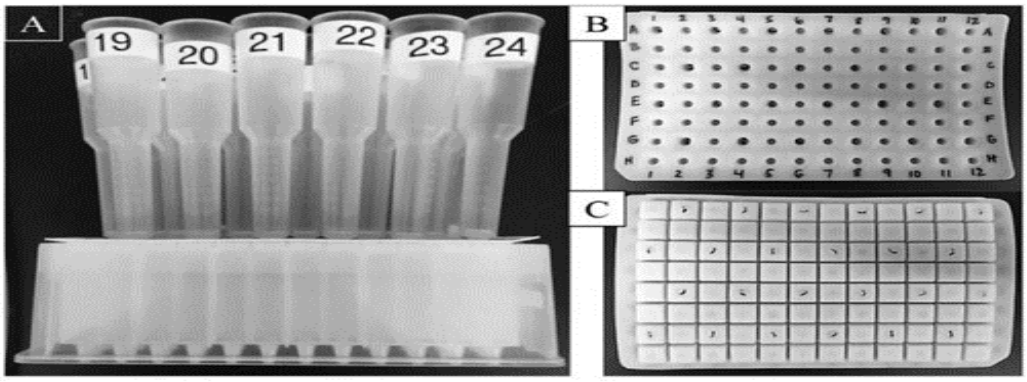

Figure 2. The MCPA system has 24 columns and six evenly spaced holes per row over alternating lines for parallel protein purification [9].

A study of an MCPA system with affinity chromatography without vacuum that cost only $45 worked well to purify proteins, as summarised in Figure 3. In Figure 4, vacuum manifolds greatly assisted the purification process by completing it in less time than gravity.

The study successfully purified yeast AbpSH3 mutants using Nickel (Ni) resins under denaturing and native purification conditions. This was indicated by SDS-PAGE analysis. Under native purification conditions, the samples with common contaminants at 25 kDa that were found under denaturing conditions [9] would be cleaned up.

The protein purification method has been scaled up from one column per protein for 24 different samples to 12 columns per protein for two different samples. The samples were high purity with the same contaminants as in small-scale protein purification. [9] found that the MCPA system for protein purification also made enough protein for biophysical analysis, such as circular dichroism (CD) spectroscopy.

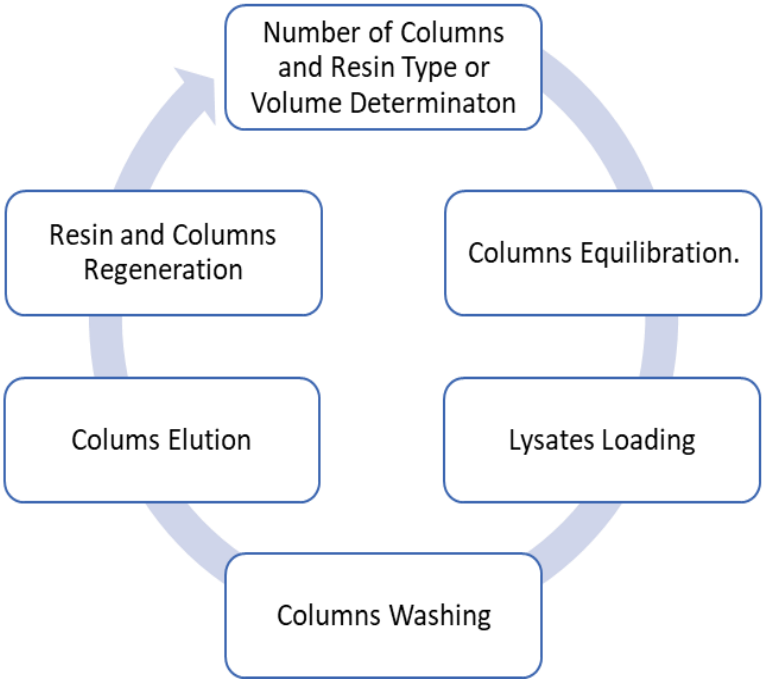

Figure 3. Purifying Yeast AbpSH3 mutants using affinity chromatography with Ni resin and MCPA under vacuum process summarised protocols [9].

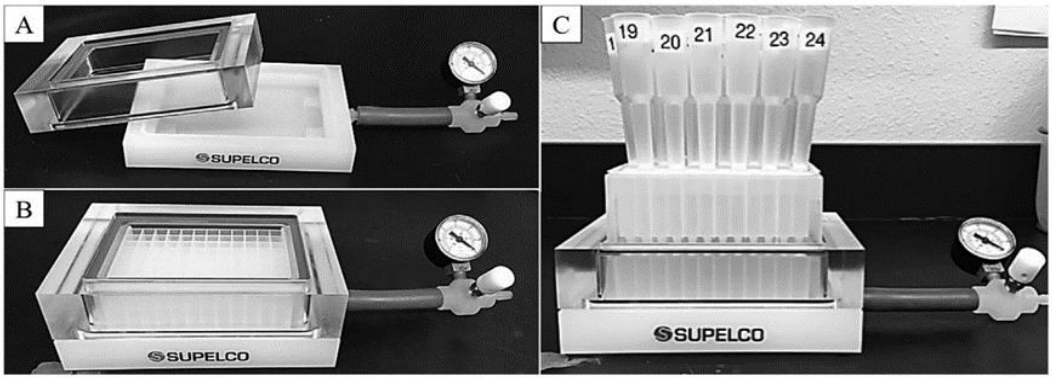

Figure 4. Column chromatography with MCPA and vacuum manifold assembly [9].

3. Cell-surface display based on SUMO-Ulp1 system

The cell-surface display offers an opening to target proteins on the surface of microbial cells, and the fusion with an anchoring motif completes the cell-surface display. This technique has been known as a practical method for numerous applications, including in the development of vaccines, environmental bio-adsorbents, and whole-cell biocatalysts [17]. Currently, this technique has been suggested as able to simplify protein purification. [10] reported the application of cell-surface display in protein purification founded on the cleavage of a SUMO-fused target protein by Ulp1 protease revealed on the surface of Escherichia coli cells as described above (Figure 5). SUMO, a ubiquitin-like protein, has been used to improve target protein stability and solubility through N-terminal fusion [18,19]. Ulp1 protease can cut the SUMO tag, which breaks the SUMO tertiary configuration and makes a native target protein with no extra amino acids [20].

The effectiveness of the SUMO-Ulp1 structure in protein purification can be archived using two different vectors: (i) the expression of SUMO-fused target protein on cell surfaces and (ii) the expression of Ulp1 protease on cell surfaces [10]. In this system, the N-terminal of a SUMO-fused target protein will be cleaved by the surface-displayed Ulp1 protease. This leads to the release of native target proteins in the buffer solution. Surface-displayed SUMO and Ulp1 protease can be removed together with the cells by centrifugation. As a result, the target protein can be collected in the form of a supernatant after centrifugation and further improved in its purity by simple ultrafiltration. For example, it has been reported that the purity of the target protein obtained was recorded at more than 80% using the SUMO-Ulp1 system. The target protein purity was improved by more than 90% using simple ultrafiltration [10]. Overall, this technique is a simple way to purify the target protein because it only needs the cleavage and centrifugation steps.

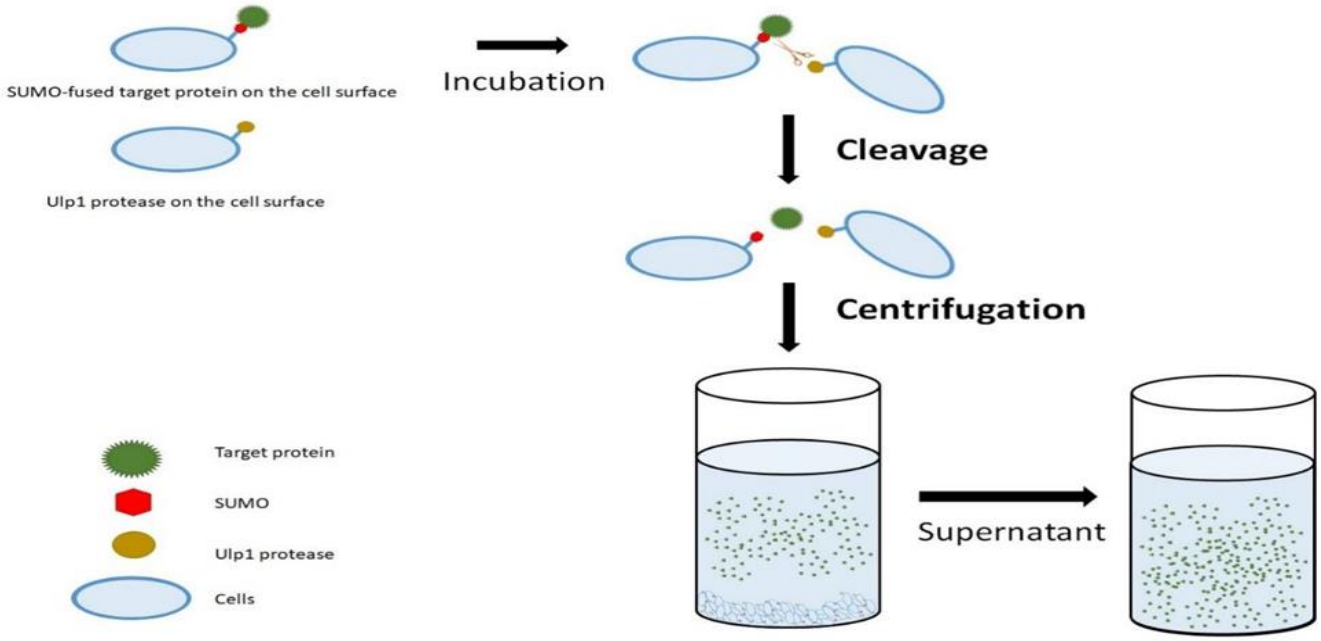

Figure 5. The diagram shows the protein purification approach established on a cell-surface demon stration using the SUMO-Ulp1 system.

4. Cell-surface display based on SUMO-Ulp1 system

In protein purification, the magnetic separation technique offers a simple approach to obtaining the target protein's high purity level by applying magnetic beads. Magnetic beads are magnetic carriers and affinity ligands [7]. The target protein is bound explicitly to the specific ligands on the magnetic beads' surface, creating a magnetic complex during incubation (Figure 6). Surprisingly, a magnetic separator can easily and quickly extract the target protein from the magnetic complex. After the removal step, the washing step can be used to further separate the target protein from the other things that aren't what they should be.

Magnetic separation techniques, including cost-effectiveness, offer several advantages, including high yield and binding capacity, sensitivity, as well as reproducibility ([21,22]. In this technique, the separation procedure can be conducted openly in primary samples, which shortens the total purification period. Furthermore, this technique could be helpful for large-scale operations because of its strength and efficiency. As a part of the motion described above, magnetic separation also offers a very gentle process for the purification of the target protein. Using a magnetic separation method to clean up large protein complexes has been shown to make a more stable protein than the traditional column chromatography method [23].

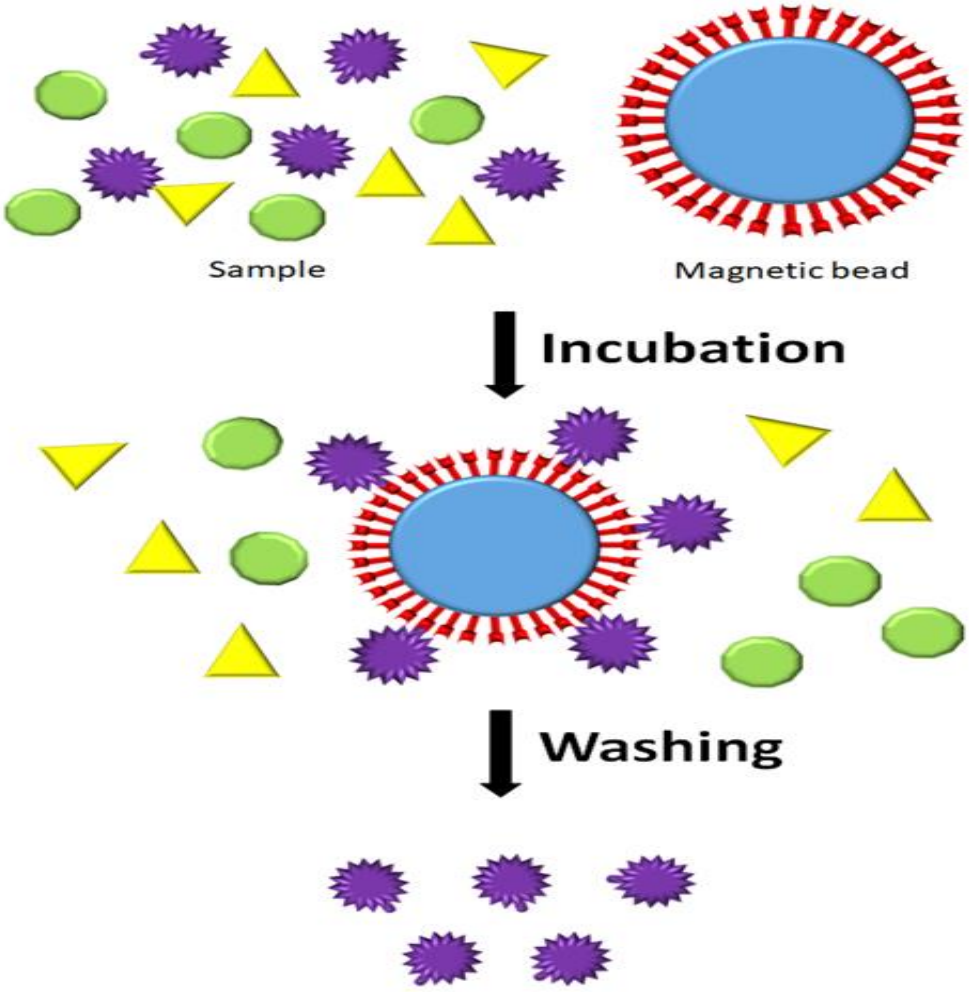

Figure 6. The principle of magnetic separation techniques.

5. Aqueous Two-Phase System (ATPS)

"Aqueous two-phase system (ATPS) is a liquid-liquid separation technique that has shown great potential for the extraction, recovery, and purification of a great variety of biological compounds" [24]. It is a technique commonly used to separate and purify enzymes and proteins. The advantages of ATPS in protein purification include the simplicity and speed of the separation with minimal denaturation of the enzymes [25]. The most important part of both phases is water (80 to 90%), and most polymers stabilise protein structure [26]. The ATPS process is shown in Figure 7.

There are several types of ATPS, such as polymeric aqueous two-phase systems (ATPS-P) and micellar aqueous two-phase systems (ATPS-M). Additionally, reverse aqueous two-phase micellar systems (ATPS-RM) and ionic liquid-based aqueous two-phase systems [27].

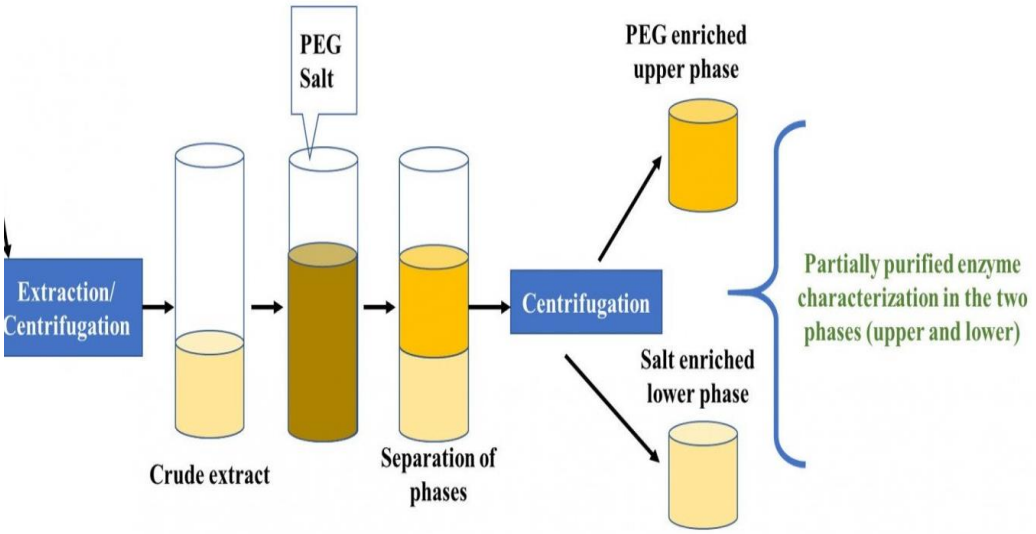

Figure 7. Aqueous Two-Phase System (ATPS) in the separation and purification process [28].

6. Types of ATPS

6.1. Polymeric Aqueous Two-Phase System

"Polymeric aqueous two-phase system (ATPS-P) is a liquid-liquid purification technique using polymers and salt solutions or polymer/polymer solution mixtures" [29]. Two water-soluble solutes separate into two immiscible aqueous-rich phases based on polymer-polymer, polymer-salt, or salt-salt solute combinations [29]. The advantage of this structure is that it is simply due to the combination of polymer and salt, even though the price of polymers can be high compared to the cost of salt.

6.2. Micellar Aqueous Two-Phase System

"Micellar aqueous two-phase systems (ATPS-M) are formed by surfactant solution. Surfactants are amphiphilic molecules containing a hydrophilic (the head) and a hydrophobic (the tail). "The surfactant head can be charged either anionic or cationic, dipolar (zwitterionic), or non-charged (non-ionic)" [30]. Surfactants form micelles solutions, and the system functions based on the property that some micelle solutions present to phase separate into a micelle-rich and a micelle-poor phase, with conditions such as temperature, pH, and ionic strength [31].

6.3 Reverse Aqueous Two-Phase Micellar Systems

"The reverse systems (ATPS-RM) are surfactant-based, using nanometer-sized water pools. These water pools are formed by a monolayer of surfactant molecules entrapping water "[32]. These aggregates can solubilise the different molecules depending on their hydrophobicity, size, and charge [33].

6.4 Ionic Liquid-Based Aqueous Two-Phase Systems

Ionic liquids are also considered green solvents. Ionic liquids have been applied in bio-catalysis, electrochemistry, and bio-separations [34]. "Ionic liquids have been investigated as novel aqueous two-phase systems (ATPS-IL)" [35]. Ionic liquids are usually comprised of inorganic or organic cations and anions. Most of the time, they are liquids at room temperature and have low vapour pressure and a wide range of structures.

7. Chromatography Techniques

Chromatography discusses a cluster of separation systems that involve molecular retardation regarding the solvent front that develops over the measurable [36]. Chromatography techniques in separating protein mixtures are the most effective and have been widely used to purify individual proteins [37]. The purifying process depends on the protein size, charge, hydrophobicity, and bio-specific interaction. Figure 8 shows the particular properties of the protein [38].

The first stage of the chromatography procedure is typically a capturing phase, where the product will bind to the adsorbent while the impurities do not [39]. Further, weakly bound proteins will be washed away so we can elute the target protein [40]. There are several types of chromatography methods. The types of chromatography methods are reviewed in the following sections.

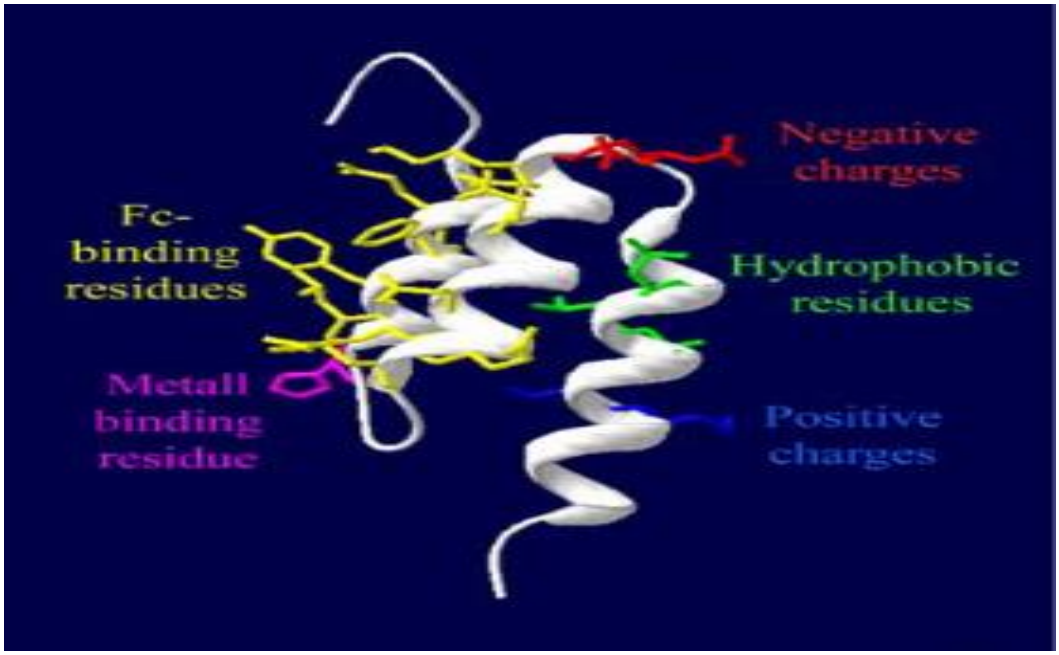

Figure 8. An example of properties that are used to separate proteins from one another [38].

7.1. Ion Exchange Chromatography

Ionic interactions are the basis for protein purification in the Ion Exchange Chromatography method [41]. Proteins with different surface charges compete with each other for clusters with opposite directions on an ion exchanger adsorbent. This makes the separation go further.

7.2 Affinity Chromatography

Affinity chromatography works based on the principle of an interacting protein. A protein has binding positions with harmonising surfaces to its ligand. "The binding is a

mix of van der Waals forces, electrostatic or hydrophobic interactions, and hydrogen bonds" [42].

8. Conclusion

With the further advancement of biotechnology and in-depth research on the structure and function of various proteins, protein separation and purification technologies, the MCPA system is an amazingly economical way for protein treatment research to purify samples. The new method for purifying proteins helps researchers improve their column chromatography. For example, it helps them figure out what kind and how much resin to use to clean up specific proteins and whether to clean up proteins on a large scale.

The cells harbouring the surface-immobilised SUMO and Ulp1 can be removed by modest centrifugation, with the purified protein confined to the supernatant. Because of the excessive protease interest of Ulp1 toward SUMO fusion, excessive protein production may be done within 1/2 of an hour through this method. Overall, the cell surface through the SUMO-Ulp1 approach gives an easy process for protein purification in its local shape that wishes the best cleavage and centrifugation steps.

Regular liquid column chromatography is the most commonly used approach for keeping apart and purifying goal proteins and peptides. Magnetic separation strategies are highly new and, nevertheless, under strict improvement. Magnetic affinity debris is presently usually used in molecular biology (especially for nucleic acid separation), cell biology and microbiology (separation of goal cells), and as elements of the techniques for the dedication of absolute analytes, the use of magnetic ELISA and associated strategies (exclusively for the commitment of medical markers and environmental impurities). Until now, separations on a small scale have succeeded, and consequently, the full capability of those strategies has no longer been completely oppressed. Overall, magnetic separation strategies will offer a higher method of protein purification within the close to imminent because of a few advantages.

The ATPS and chromatographic techniques for downstream processing of recombinant bromelain have been investigated. The evaluation established that the ATPS technique is a cost-effective, time-saving (30 min), and high-recuperation approach that can be scaled up for commercial purposes. Herein, ATPS may be an approach for the purification of single-step separation and keeping away from multi-step purification, just like the chromatography technique.

Therefore, it should be pointed out that the purity of the protein obtained in this review is still insufficient. Consequently, advanced study and hard work are needed to develop the purity of the protein in different ways since intracellular proteins are transported mainly by bacterial cells through the cell membrane. In carrier proteins [43], genetic engineering deletion of these proteins will decrease the secretion of non-target proteins; chemicals that prevent protein emission can also recover protein purity. Even though more research needs to be done before this protein purification method is widely used, our recent work gives us an excellent way to purify proteins.

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