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# Applications of Aqueous Two-Phase Systems for the Recovery of Recombinant Proteins

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### Introduction

Recent advances in molecular biology have vastly increased the scope of bioprocess technology. A combined knowledge of molecular biology and bioprocess technology is an urge to strengthen biotechnology industry into revolutionary new products. The production of purified proteins from genetically modified organisms depends on the effectiveness of the separation processes used. These purified proteins have widespread application in many areas of biotechnology such as biocatalysts, diagnostics and therapeutics. Hence, a critical step in the preparation of recombinant proteins is purification. The conventional techniques such as chromatography, electrophoresis and precipitation have been widely employed in purification of recombinant proteins. In recent, one of the most economical downstream processing for recombinant proteins purification is aqueous two phase system. Aqueous two-phase system has become a proven tool used in separation and purification technology. The rapid development of aqueous two phase systems in partitioning, separation, and purification of recombinant proteins is mainly due to its advantages like low toxicity to environment, gentle environmental condition to recombinant proteins and inexpensive cost. This method is able to give high recovery yield and high purity in a single step, ensuring the process is economically viable and the price of products become competitive.

The polymer/salt aqueous two phase systems have been used to purify the recombinant human immunodeficiency virus-like particles from Chinese hamster ovary cells supernatant. Viruslike particles is an important candidate for a new generation of biopharmaceuticals. Polyethylene glycol-ammonium sulfate aqueous two phase system demonstrated effective purification of the recombinant human immunodeficiency virus-like particles in a single step strategy [1]. Inclusion of alcohol as phase component in aqueous two phase system can make the recombinant protein to be easily recovered by removing the alcohol using evaporation [2]. Besides the application of alcohol/salt aqueous two phase system, the ionic liquid-based aqueous two phase system has been successfully used in the purification of recombinant lipase in the latest development. Ionic liquids are potential green solvents to replace the existing volatile organic solvents. A high purification factor value (4.05-fold) indicates that 1-ethyl-3methylimidazolium tetrafluoroborate/potassium phosphate is capable of attaining an excellent degree of recombinant lipase

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purity, suggesting that the ionic liquid-based aqueous two phase system is suitable for implementation in a large scale process for the recombinant lipase purification [3]. An aqueous two-phase flotation, which is a combination of aqueous two phase system with solvent sublation, has also been employed as a purification method for recombinant proteins by eliminating the high shear forces exerted during mixing and protein precipitation at the interfacial phase that occurred in aqueous two phase system. An aqueous two-phase flotation composed of polyethylene glycol and sodium citrate revealed its potential for direct purification of thermostable lipase 42 from recombinant *Escherichia coli* BL21(DE3) pLysS [4]. The capacity of aqueous two-phase flotation as a promising separation and purification method in biotechnological processes was demonstrated as it is able to increase the purity degree of the recombinant lipase.

Based on the above information, it seems that the use of aqueous two phase system is very prospective to recover the proteins from recombinant organisms. Research and development in this approach shall be extended to various downstream processes for better understanding. The separation and purification capacity of recombinant proteins by aqueous two phase system is relied on the characteristics of phase components, in which, some information on these shall be determined. A model that can be used to generate molecular understanding of processes related to the purification of proteins in aqueous two phase system is also required. The technical feasibility of the scaling-up of aqueous two phase system shall be identified. Publication in this area of research and development in Open Access Journal of Molecular

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Sciences will help molecular biologist and bioprocess technologists to work together to make repositories of research findings, which can ensure continued access to scholarly publications into the distant future. Publication in open access also helps libraries in undeveloped areas around the world to have greater access to the scholarly resources. Views and opinions from researchers worldwide on the published articles can also be discussed and shared.

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