# CHAPTER I
# INTRODUCTION

Proteins and antibiotics are known for their importance in the fields of pharmaceutical, food industries and biotechnology. Proteins and antibiotics are usually present at very low concentrations in a complex biomedium such as fermentation broth. This makes the product separation not only difficult but also expensive. Therefore downstream separation is usually the dominant cost in the production of pharmaceutical products. About 90% of total cost comes from separation and purification, so improving separation has considerable economic benefits.

In recent years, many techniques have been developed in biotechnology to achieve a highly efficient and economical separation process. One novel separation technique with the ability to be scale up easily, to be operated continuously, and to be highly selective is liquid-liquid extraction using microemulsion. This is remarkable in view of the fact that this method is more suitable for separating proteins and antibiotics than regular liquid-liquid extraction or other methods of separating that used in the past because the transferring of proteins into solvents frequently results in irreversible denaturation and loss of biological activity. For microemulsion many proteins and antibiotics can be solubilized in microemulsions based on apolar solvents such as aliphatic hydrocarbons without denaturation or loss of function.

Microemulsions are a thermodynamically stable mixture of oil, water and surfactant that form spontaneously upon mixing. They can be classified into 3 types based on the solution microstructure, namely Winsor I, II, and III. Winsor I surfactant is soluble in water and oil-in-water microemulsions are formed. In the oil phase there is no surfactant aggregates that exist in this region. Winsor II microemulsions are oil-continuous phases that contain a dispersion of water droplets stabilized by surfactant molecules at the droplet interface. Winsor III microemulsions have a structure that is not well defined, but display physical properties, which suggest that the phase is continuous in both oil and water. Reverse micelles are a subset of the broader class of Winsor II microemulsions. The hydrophilic core of these aggregates consists of the waters of hydration of the

surfactants head group. They are typically 60 to 100 Å in diameter. Reverse micelle separations with ionic surfactants have been used to extract proteins based on the net charge of the molecule.

It is widely accepted that the primary driving force for solubilization of protein molecule into the reverse micelle water-pool is the attractive electrostatic interaction between the protein molecule and the reverse micelle inner charge layer. For example, using a negatively charged surfactant such as bis (2-ethylhexyl) sodium sulfosuccinate (AOT), proteins and antibiotics that exhibit a net positive charge can be extracted into the organic phase. The potential for separating and purifying proteins by microemulsion depends mainy on their ability to transfer a target protein or antibiotic from an aqueous solution to a reverse micelle-containing organic phase and to be subsequently recovered in a second aqueous phase. The competence of reverse micelle system is not fully understood yet, but is known to be influenced by a number of factors, particularly pH, salt type and concentration, solvent type, temperature, surfactant type and concentration, and the incorporation of bioaffinity ligands. Understanding the role of the system properties and using the knowledge to enhance selectivity is crucial if this technique is to be used at an industrial scale.

The present work focused on the study of the extraction of the protein α-chymtrypsin using reverse micelle system of sodium bis(2-ethylhexyl) phosphate (NaDEHP) and isooctane. Effects of various system parameters such as pH, salt concentration, type of cosurfactant, and protein concentration on the extraction efficiency were investigated. In addition, the efficiency of recovery step by backward extraction and the activity of recovered protein were also studied.