CHAPTER I
INTRODUCTION

Proteins and antibiotics are the most important chemicals used in pharmaceutical and biological industries. They usually present at extremely 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 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 scaled up easily and operated continuously is liquid-liquid extraction, but it is difficult to find a solvent having desired selectivity and being gentle to protein and antibiotic structures. Reverse micellar system or water in oil microemulsion is the extractant which possesses these properties. This method is considered to be more suitable for separating proteins and antibiotics than regular liquid-liquid extraction or other methods of separating. The transferring of proteins into solvents frequently results in irreversible denaturation or loss of biological activity. It has been known that many proteins and antibiotics can be solubilized in microemulsions based on apolar solvents such as aliphatic hydrocarbons without minimum denaturation or loss of function (Pires *et al.*, 1996).

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 mainly 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, cosurfactant type and concentration, and the incorporation of bioaffinity ligands.

AOT is the most often-used surfactant in protein extraction study due to the fact that AOT can form reverse micelles without a cosurfactant. However it has a number of serious limitations: once proteins are extracted into AOT reverse micellar phase, it is difficult to separate proteins from the surfactant and the phase separation of AOT system takes a long time. These problems might be overcome by using other surfactant systems such as sodium bis(2-ethylhexyl) phosphate (NaDEHP), an anionic surfactant which has the same hydrocarbon tail as AOT but a different polar head and can form water in oil microemulsion or reverse micellar solution under certain conditions. Eventhough forming of NaDEHP reverse micelle is more difficult than AOT in the absence of cosurfactant because of high salinity needed but its reverse micelle can be easily broken by converting the sodium salt (NaDEHP) to a non-surface-active divalent metal salt [$M(DEHP)_2$] and the surfactant can be readily recycled, also the phase separation is much faster than AOT system (Hu and Gulari, 1996). In addition, NaDEHP commonly used as the organophosphorus extractant in hydrometallurgy and nuclear industry has received more interest in the field of surface science. Water solubilized in such aggregates is thought to mimize the water confined in biological membranes since NaDEHP molecules have a similar phosphate headgroup to that phospholipids (Li *et al.*, 1997).

In our previous work, we have studied the extraction of α-chymotrypsin from aqueous using the reverse micellar system of NaDEHP/isooctane/brine. The results have shown that α-chymotrypsin can be quantitatively extracted and extraction efficiency was strongly affected by pH and salt concentration in the aqueous phase. At near neutral pH and low salt concentration, high extraction efficiency was obtained. At pH above isoelectric point (pI) of the protein (pH 8.5), extraction of the protein into the reverse micelles decreased dramatically. Increasing salt concentration resulted in a decline in the proteins transferred into the micellar phase due to lessening attractive interaction. It was also found that cosurfactant strongly affected the activity of extracted enzyme which is believed to be due to the

modification of interfacial properties of reverse micelles and their water capacity. This should result in a more favorable environment for hosting enzymes which, in turn, leads to increase the activity of enzyme in reverse micelles. Therefore, in this study the extraction and the activity of α-chymotrypsin using NaDEHP reverse micellar system was further investigated with a focus on the effect of bile salt cosurfactant in comparison with tributylphosphate (TBP) on extraction efficiency and activity of recovered α-chymotrypsin.