CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Protein extraction and solubilization in surfactant containing organic solvents is becoming an attractive methodology in the field of bioseparations. In this study, the extraction of α-chymotrypsin using the reverse micellar system of sodium bis(2-ethylhexyl) phosphate (NaDEHP)/isooctane/brine was reported. Influence of factors affecting the extraction efficiency such as pH, salt concentration, protein loading, and type of cosurfactants was examined. From the experimental results, conclusions can be drawn as follows:

1. This study clearly demonstrate that α-chymotrypsin can quantitatively be extracted using the reverse micellar system of NaDEHP. At near neutral pH and low salt concentration, more than 90% of the protein could be extracted from the aqueous solution into the reverse micelles in the organic phase. The experimental results have shown that the extraction efficiency is strongly dependent on pH and salt concentration in the aqueous phase.

2. For pH effect, pH of the aqueous solution determines the surface net charge of α-chymotrypsin and therefore affects electrostatic interactions between the protein and NaDEHP head groups which can favor the transfer of protein into the organic phase. At pH above pI of α-chymotrypsin (8.5), the percentage of protein transferred into the reverse micelles decreased dramatically due to the reduced attraction.

3. For the effect of salt concentration in aqueous phase, increasing salt concentration resulted in a decline in the protein transferred into the micellar phase due to shielding of the electrostatic interaction or Debye screening effect.

4. For the recovery of the extracted protein, backward extraction was performed by contacting the micellar phase with a divalent cation aqueous solution ($CaCl_2$) which caused the reverse micelles to destabilize, thus releasing the protein into the second aqueous solution. In general, the protein recovery was found to be in a range of 70% using this backward extraction technique.

5. When different initial protein concentrations were used, the optimal protein concentration was found to be 0.5 mg/ml, which gives the highest extraction percentages in both forward and backward steps. It is speculated that ratio of surfactant forming the micelles to protein being transferred may play important role in the extraction which requires further study.

6. After backward extraction, the enzymatic activity of the recovered proteins was examined using a simple hydrolysis reaction. The highest activity of the recovered enzyme was found to be approximately 60% of the fresh one's. This can be attributed to the inhibitory effect of the salt present in the micelles and the possible adsorption of surfactant onto the enzyme.

7. Among three types of cosurfactants used in this study, TBP shows the best performance both in terms of extraction and recovery percentages. It is interesting to note that the other two cosurfactants (2-ethyl-1-hexanol and 1-heptanol) have tremendous adverse effect on the activity of the recovered enzyme. Only about 20% or less of its original activity was observed.

5.2 Recommendations

Further investigation should be done in order to improve the extraction technique in terms of recovery percentage and activity of the recovered enzyme. Other backward extraction techniques such as using counterionic surfactant should be explored as well as the use of other types of cosurfactants which may result in higher enzymatic activity of the recovered protein than the activity observed in this study. In addition, purification of the NaDEHP surfactant should be considered. On the other hand, while not being optimized yet the process can potentially be applied to the extraction of valuable enzymes from various natural sources.