CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In this research, the extraction and activity of α-chymotrypsin using two different microemulsion systems of sodium bis (2-ethylhexyl) phosphate (NaDEHP)/isooctane/brine were studied with an emphasis on the effect of cosurfactant. Two cosurfactants used in this study were tributylphosphate (TBP) and a bile salt cosurfactant, 3-[(3-cholamidyl-propyl)dimethylammonio]-1-propane sulfonate (CHAPS). The conclusion can be drawn as following:

1. The study of NaDEHP microemulsion formation using TBP and CHAPS as cosurfactants demonstrated that tributylphosphate (TBP) promoted the formation of water in oil microemulsion even at low salt concentration (e.g. 0.2 M NaCl). In contrast, the NaDEHP microemulsion with CHAPS was formed only low CHAPS concentration and high salt concentration. Therefore, TBP was clearly superior to CHAPS in terms of facilitation of microemulsion formation.

2. In forward extraction when the microemulsion with TBP was used, about 71% of α-chymotrypsin could be extracted into the micellar phase whereas only approximately 32% was extracted by the microemulsion with CHAPS.

3. In this work the backward extraction using divalent metal ion was shown to be efficient for the recovery of extracted enzyme from the NaDEHP microemulsion system with TBP, around 74% recovery was observed. But this technique was almost ineffective when applied to the system with CHAPS, as only 4% of protein was released.

4. The activity of α-chymotrypsin was determined by hydrolysis reaction of *N*-Glutaryl-L-phynyl-*p*-nitroanilide (GPNA) to produce *p*-nitroaniline. When compared to the activity of fresh protein, the recovered α-chymotrypsin from the system with TBP presented approximately 24% of the activity of the fresh protein. In the case of using microemulsion with CHAPS the recovered protein showed apparently 40% of the activity of the fresh protein which was much higher than the system with TBP.

5.2 Recommendations

Further study should be considered on the improvement of the extraction process to get high extraction efficiency and activity of recovered enzyme. Other backward extraction techniques such as use of counterionic surfactant should be used to increase the recovery. In order to enhance the enzyme activity, more suitable conditions or surfactant system for CHAPS cosurfactant should be investigated as well as the detailed study of protein denaturation. An exploration of the use of mixed cosurfactant between TBP and CHAPS is also interesting due to the possible synergistic effect on extraction and activity of protein.