CHAPTER 5

CONCLUSION AND SUGGESTION

Alkaline protease can be entrapped on polyacrylamide and poly(acrylamide-co-methacrylic acid) by inverse suspension polymerization. The effects of concentrations of monomer, crosslinker, enzyme, initiator, accelerator, and surfactant were carried out for the polymeric beads and enzymatic activities. The stirring rate, time and temperature polymerization were also investigated. The research show interesting results of enzymatic activity, percentage immobilization and percentage conversion for industrial applications. Polyacrylamide and poly(acrylamide-co-methacrylic acid) can be applied in many fields such as paper making, mining, water treatment, oil recovery, and other systems. This immobilized enzyme possesses a higher thermal stability than the free enzyme. This novel immobilized enzyme on polyacrylamide and poly(acrylamide-co-methacrylic acid) can also be applied in biotechnology industry, such as detergent industry, improvement of rubber recovery by digesting the undesired protein in latex skin.

5.1 Conclusion

The findings can be summarized as follows:

5.1.1 The enzymatic activity of immobilized alkaline protease on polyacrylamide beads were decreased with increasing the concentration of

acrylamide, MBA, and APS. The enzymatic activity of the immobilized alkaline protease was increased with increasing the concentration of enzyme.

5.1.2 The optimum condition for immobilization of alkaline protease on polyacrylamide by inverse suspension polymerization comprised: the concentrations of AM (3.14 mM), alkaline protease (1.5 mg/5 cm^3), MBA (15 mM), APS (6.56 mM), TEMED (47.75 mM), Pluronic PE 8100 (10.6 mM), at stirring rate 300 rpm, time 2 h and temperature 30°C. The enzymatic activity was 178 units, with 42% immobilization and 93% conversion.

5.1.3 The condition suitable for immobilization of alkaline protease on poly(acrylamide-co-methacrylic acid) at 97.5/2.5, 95/5, 90/10% W/W, was consisted the concentrations of materials the same as mentioned in 5.1.2 except the concentration of TEMED, being increased to 95.5 mM. The enzymatic activity was decreased with increasing the methacrylic acid concentration. The enzymatic activity of homopolymer of acrylamide was carried out to compare with the copolymer of acrylamide and methacrylic acid. The enzymatic activity of polyacrylamide those of was higher than poly(acrylamide-co-methacrylic acid).

5.1.4 The water absorption of polyacrylamide was higher than poly (acrylamide-co-metahcrylic acid)s. For the copolymers of acrylamide and methacrylic acid, the water absorption was increased with increasing methacrylic acid concentrations. The absorption of polyacrylamide and poly(acrylamide-co-methacrylic acid)s in saline solutions was less than those in the water.

5.1.5 The free and immobilized enzyme can be digested many substrates such as, casein, BSA, and blood. The free and immobilized enzyme can not digest

gelatin. The free enzyme has a higher ability for digestion protein than the immobilized enzyme.

5.1.6 The free and immobilized enzyme show the optimum enzymatic activity at the same temperature of 45°C. The optimum enzymatic activity of free enzyme is found at pH 10.0 while the optimum enzymatic activity for immobilized enzyme was shifted to pH 10.5. The immobilized enzyme was stable than the free enzyme at high alkaline solutions. The free enzyme can be kept in -20 to 4°C for one month without losing enzymatic activity. The free enzyme lose its enzymatic activity 51% while the immobilized enzyme on polyacrylamide and poly(acrylamide-co-methacrylic acid) lose their enzymatic activity 37% and 44%, respectively, after being kept in the 60°C environment for one month. The temperature and storage stability on the enzymatic activity profile reveal that the stability of immobilized enzyme higher than the free enzyme.

5.1.7 The polyacrylamide and poly(acrylamide-co-methacrylic acid) showed porosity on the supports's surface. Polyacrylamide and poly(acrylamide-co-methacrylic acid) showed intramolecular reaction of imidization that lead to increase insoluble support.

5.1.8 The enzyme was entrapped in the polymer beads and no leakage under washings could be observed.

5.1.9 Different of blue colour after staining the immobilized enzyme indicated that the enzyme was entrapped on the polyacrylamide and poly(acrylamide-co-methacylic acid)s and could be used as the primary screening of the quantity of the enzyme before determining their enzymatic activity.

5.2 Suggestion for Further Work

Based on the current facilities at Chulalongkorn University, the researchers can pursue the following aspects:

5.2.1 To expand the scale of production of the immobilized enzyme on polyacrylamide or poly(acrylamide-co-methacrylic acid) for testing in biotechnology industries.

5.2.2 To increase the immobilized enzymatic capacity on the polymer support by either changing the kind of monomers, or the method of immobilizations.

5.2.3 To improve the polymer support properties such as copolymerization of acrylamide and N-isopropylacrylamide which can swell and shrink at a transition temperature of 32°C, can be applicable to drug delivery system. Copolymerizing acrylamide with other monomers such as, acrylic acid, sodium acrylate to improve the water absorption.

5.2.4 To reduce the cost for polymerization and immobilization of enzyme by changing the contineous phase to a cheaper one, such as vegetable oil (the most preferred one as soybean oil) instead of the expensive paraffin wax.

5.2.5 To apply this method for immobilizing other biocatalysts.