CHAPTER 1

INTRODUCTION

1.1 Introduction

Interests in immobilized enzymes and their applications to bioprocessing, analytical system, and enzyme therapy have been exploited for many years. It is thus believed that the first attempt to prepare an immobilized enzyme was perhaps made in 1953, when Grubhofer and Schleith coupled such enzymes as carboxypeptidase, diastase, pepsin, and ribonuclease with diazotized poly(*p*-aminostyrene). Immobilized enzymes can often be used more advantageously than the corresponding free enzymes. With immobilized enzymes, the process can be run continuously because of the resulting stabilization of enzymes when subjecting to high temperatures, pH and inhibitor concentration [1]. Enzymes can be recovered and reused after reactions and enzymes can be formed into shapes such as membranes or beads for fitting to specific reaction processes.

Protease was the proteolytic enzyme that can be found in plants, animals and microorganisms. The protease used in this study was produced from a microorganism. The microorganism that can produce protease is found in fungi and bacteria. The use of microbial proteases in industrial applications have been used in detergent, tanning, photographic, textile industries, etc [2]. For the present reseach, we aim to stabilize

the alkaline protease enzyme on polyacrylamide, and poly(acrylamide-co-methacrylic acid) beads to make the immobilized enzyme.

Polymers based on acrylamide and its derivatives were among the most easily available and relatively inexpensive water-soluble materials that offer a unique combination of useful properties. These polymers were widely applied in various fields of technology such as flocculating, gelling, film-forming and hydraulic-friction-reducing agents, etc. The main synthesic method of acrylamide based polymers is by radical polymerization either in solution, inverse emulsion or suspension [3]. Acrylamide can be copolymerized with many vinyl monomers, for example, acrylic acid, methacrylic acid, styrenesulfonic acid, etc [4]. Acrylamide is widely used as the matrix polymer for immobilized enzymes. Several authors have reported properties of immobilized enzymes on the polyacrylamide and its derivatives with difference methods [5-10]. However, the immobilization of alkaline protease by inverse suspension polymerization by a redox initiation has not been yet published.

In this work, we have synthesized polyacrylamide and poly(acrylamide-co-methacrylic acid) as the matrix polymers for immobilizing alkaline protease enzyme from *Bacillus licheneformis* by inverse suspension polymerization.

1.2 Objective

1.2.1 To synthesize the polyacrylamide and poly(acrylamide-co-methacrylic acid) beads for immobilizing alkaline protease enzyme by inverse suspension polymerization.

1.2.2 To optimize immobilization condition of alkaline protease on polyacrylamide and poly(acrylamide-co-methacrylic acid) beads by inverse suspension polymerization.

1.2.3 To compare the activity and stability of alkaline protease after immobilization on polyacrylamide and poly(acrylamide-co-methacrylic acid) with the unimmobilized enzyme.

1.3 Scope and Research Plan

This research covers the work of synthesis of protease immobilized on polyacrylamide and poly(acrylamide-co-methacrylic acid) through investigations of reaction parameters and evaluation of the enzymatic activities on various substrates. The research is planned as follows:

1.3.1 Survey of the relevent literature.

1.3.2 The experimental work was carried out as follows:

1.3.2.1 Polymerization and immobilization of alkaline protease by inverse suspension polymerization of acrylamide monomer with the conditions shown below:

a) The optimum concentration of monomer of 3.14, 4.57, 6.28, 9.14 mM of the enzymatic activity was carried out.

b) The optimum concentration of enzyme of 0.25, 0.5, 1.5, 2.5, 5.0 mg/cm^3 of the enzymatic activity was carried out.

c) The optimum stirring rate during polymerization at 100, 200, 300 and 400 rpm of the enzymatic activity was carried out.

d) The optimum polymerization time at 1, 2, 3, and 4 h of the enzymatic activtiy was carried out.

e) The optimum polymerization temperature at 0, 10, 20, 30, 40°C of the enzymatic activity was carried out.

f) The optimum initiator (ammonium persulfate (APS)) concentration of 3.13, 6.56, 9.39, 12.52 mM of the enzymatic activity was carried out.

g) The optimum accelerator (N,N,N′,N′-tetraethylmethylene diamine (TEMED)) concentration of 47.75, 95.95, 143.25, 191.05 mM of the enzymatic activity was carried out.

h) The optimum crosslinker (N,N′-methylene-bis-acrylamide (MBA)) concentration of 15, 30, 60, 90, 120 mM of the enzymatic activity was carried out.

i) The optimum of surfactant (Pluronic PE 8100) concentration of 5.3, 10.6, 15.9, 21.2 mM of the enzymatic activity was carried out .

j) The optimum copolymer ratio (acrylamide with methacrylic acid) of 100/0, 97.5/2.5, 95/5, 90/10% W/W of the enzymatic activity was carried out.

1.3.2.2 The characterizations of polyacrylamide and poly(acrylamide-co-methacrylic acid) were investigated by FT-IR .

1.3.2.3 The thermal analyses of polyacrylamide and poly(acrylamide-co-methacrylic acid) were investigated by DSC.

1.3.2.4 The immobilized enzyme on the beads was determined for enzymatic activity, and percentage immobilizaton as well as percentage conversion was carried out. The opitimum concentration of immobilized enzyme as enzymatic activity on polyacrylamide and poly(acrylamide-co-methacrylic acid) were evaluated by protein digestion, pH and thermal stability, and storage stability comparing with the free one.

1.3.2.5 Water absorption of polyacrylamide and poly(acrylamide-co-methacrylic acid) in deionized distilled water and saline solutions were investigated.

1.3.2.6 The polyacrylamide and poly(acrylamide-co-methacrylic acid) were investiaged for morphology and particle size by scanning electron microscopy.

1.3.2.7 The immobilized enzyme on the polyacrylamide and poly (acrylamide-co-methacrylic acid) was qualitatively analysis by protein staining to localize the sites of enzyme entrapment.

1.3.3 The data were recorded , discussed and concluded.

1.3.4 The thesis was written.

1.4 Application and Advantage of the Work

1.4.1 The polyacrylamide and poly(acrylamide-co-methacrylic acid) that were synthesized by an inverse suspension polymerization can be applied to many chemical fields such as papermaking, water treatment, mining industries, oil recovery, etc. The immobilized alkaline protease on the polyacrylamide and poly(acrylamide-co-methacrylic acid) beads can be applied to biotechnological areas, for example, detergent industry, degumming of natural silk, improvement of rubber recovery by digestion of the undesired protein in latex skin, etc.

1.4.2 The immobilized alkaline protease on the polyacrylamide and poly (acrylamide-co-methacrylic acid) beads are more stable than the free enzyme.

1.5 Experimental Scheme

Figure 1.1 shows the condensed experimental scheme of this research.

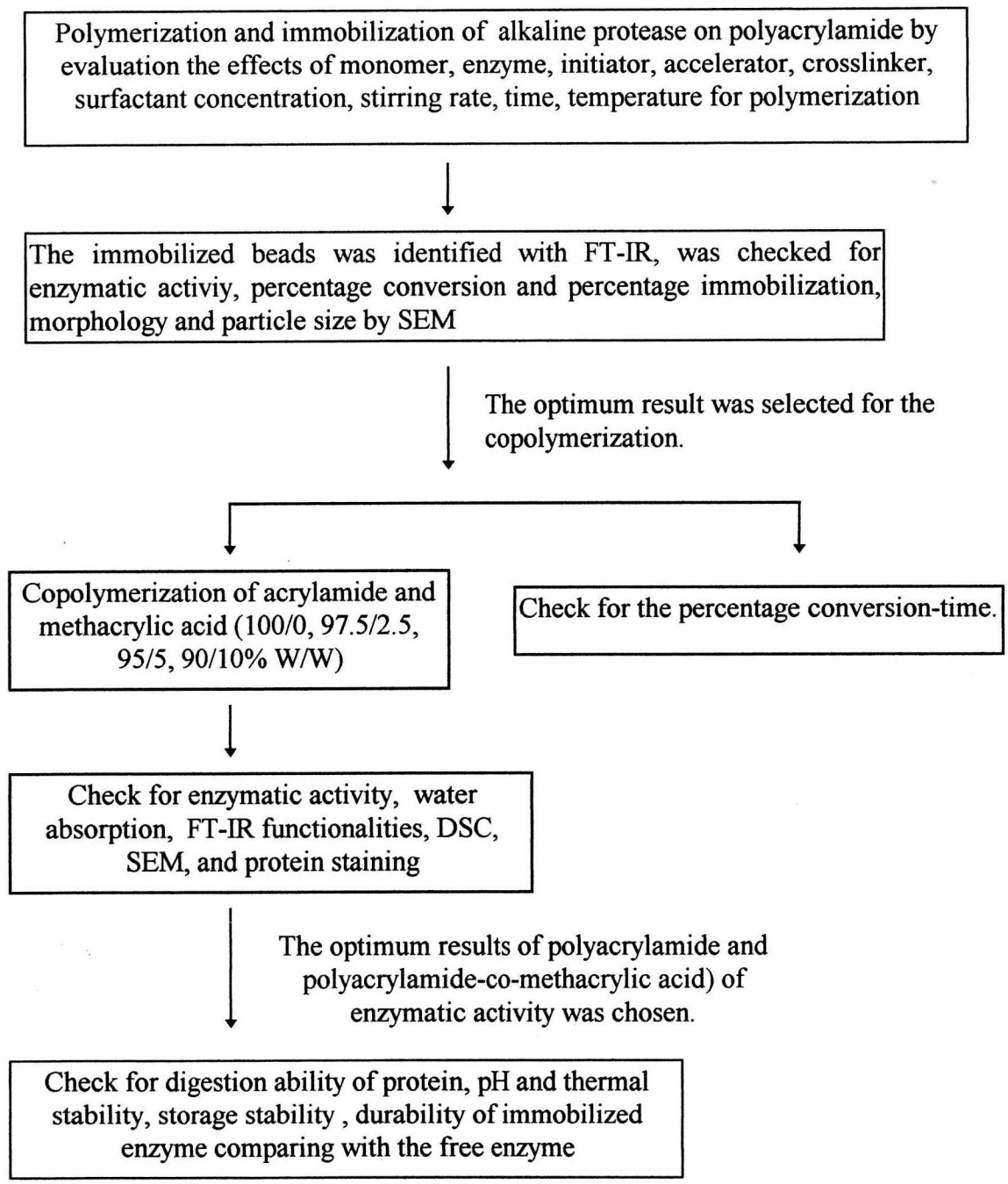

Figure 1.1 The experimental scheme of this study