CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

Chapter 3 consists of the experiment methodology in this study. Figure 3.1 illustrated the major research methodology involves during this study. The experiment can be divided into three major parts. The first part involved the screening of the membrane modification route, which are UV photo grafting and chemical grafting via redox reaction. Three parameters were investigated using One Factor at Time (OFAT) which is initiator concentration, monomer concentration and time of grafting. The performance of the modified membrane was evaluated in term of LZY protein binding capacity.

Based on the highest protein binding capacity obtain in OFAT study, UV photo grafting was selected for optimization in second part of the study. Response Surface Methodology (RSM) involving Central Composite Design (CCD) was used for the optimization process. Three factors were optimized which are initiator concentration, monomer concentration and time of grafting in order to produce membrane chromatography with high protein binding capacity.

In the last part, the optimized membrane chromatography was characterized and evaluated it performances by several method including degree of grafting, contact angle, Scanning Electron Microscope (SEM), Fourier Transform Infra-Red (FTIR), pure water flux test.

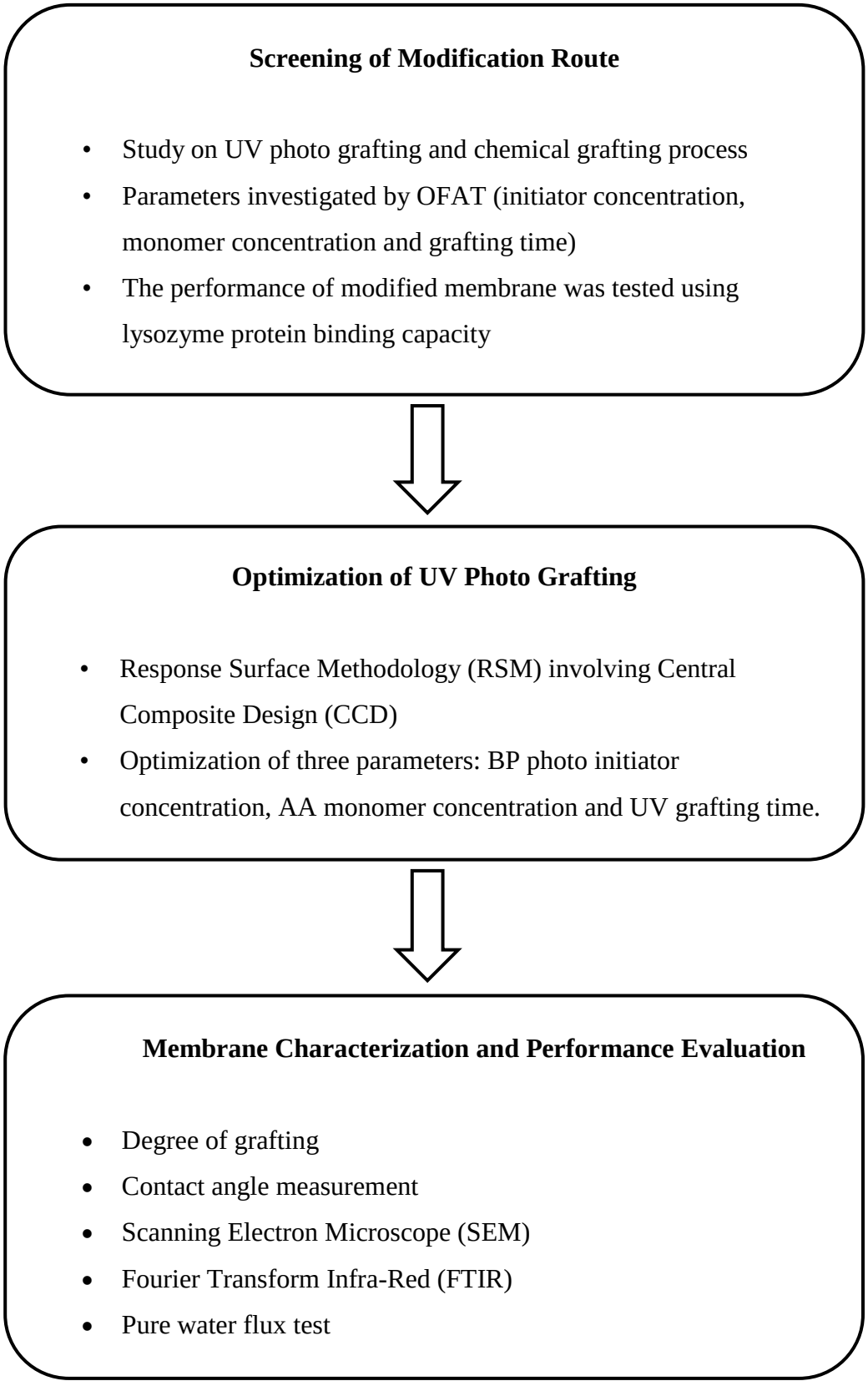

Figure 3.1: Flowchart of the research methodology

3.2 CHEMICALS

Commercial polyamide (PA) microfiltration (0.45 µm, 47 cm diameter) membrane was purchased from Sartorius Stedim Biotech (Goettingen, Germany). Acrylic acid (AA) was brought from Acros Organics (Geel, Belgium) and used as a monomer. The initiators used in this research are benzophenone (BP), potassium persulfate ($K_2S_2O_8$) and potassium metabisulfite ($K_2S_2O_5$) were brought from Merck (Darmstadt, Germany). In binding experiment, lysozyme (Sigma) was dissolved in sodium phosphate buffer pH 7, freshly prepared by dissolving appropriate amount of sodium dihydrogen phosphate and sodium dihydrogen phosphate heptahydrate in ultrapure water. All solvents and chemicals were reagent grade and were used as received.

3.3 EXPERIMENTAL PROCEDURE

3.3.1 One Factor at Time (OFAT)

Polyamide membrane was modified using two different grafting technique which are UV-initiated grafting and chemical grafting via redox reaction. During the modification process, One Factor at Time (OFAT) approach was performed in order to study the parameter that influence the modification and to select the low value and the high value of parameter for optimization process. OFAT is a conventional method which varies only one factor or variable at a time while keeping others fixed.

3.3.2 UV Photo-Initiated Grafting

The membrane was modified according to method developed by Ulbricht and Yang, (2005). AA was used as a monomer and BP as the photo-initiator. The function of photo – initiator is to generate free radicals on the membrane surface. The photo-initiated graft polymerization was performed by adsorption of BP on the membrane surface. The membrane was presoaked in BP solution dissolved in methanol for 15 minutes and then immersed in the methanol solution for 1 minute. The membrane was took out from the solution and wiped with filter paper to remove any excess methanol on the membrane.