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Comparison of Chemical and UV Photo-Grafting Modification on Polyamide Microfiltration Membrane for the Preparation of Membrane Chromatography

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Membrane chromatography has been used widely as an alternative to the conventional packed bed chromatography for protein separation. Membrane chromatography used an adsorptive membrane that carried specific chromatography functionality. In the current study, a membrane chromatography was prepared by modification of commercial polyamide microfiltration membrane with acrylic acid monomer. Two modification methods were compared which are UV photo grafting and chemical grafting *via* redox reaction. Modification parameters studied were initiator concentration (1-50 mM), monomer concentration (0.2-5 M) and reaction time (5-60 min). The highest lysozyme binding capacity achieved was 0.175 mg of lysozyme/cm^2 membrane for the membrane prepared *via* UV photo grafting using 10 mM of photo-initiator, 0.1 M of acrylic acid and 15 min of reaction time.

Keywords: Protein separation, Membrane chromatography, UV grafting, Chemical grafting, Polyamide membrane.

INTRODUCTION

Increasing growth in biotechnology industries such as pharmaceutical and food industries requires reliable and efficient methods to purify the commercial-scale quantities of proteins [1]. Various type of separation can be used to capture the desirable protein of interest. The most common technique is by using packed bed chromatography. However, conventional packed bed chromatography shows several short comings such as high pressure drop, limited diffusional of solute to the binding site and low flow rate capability [2-4]. Membrane chromatography is used as an alternative to the packed bed chromatography for protein separation. Membrane chromatography uses an adsorptive membrane that carried specific chromatography functionality. The production of chromatographic membranes involve a chemical modification of the microfiltration membrane with specific type of functional groups to produce different types of chromatography interactions inside the membrane [5].

Various types of functional group could be introduced to the membrane according to their application and functionality. UV grafting is one of the common methods used for modification of ultrafiltration (UF) membrane for fouling control. UV grafting is simple, useful and versatile in improving the surface properties of the polymers [6]. The membrane was exposed to the UV light at certain wavelength and intensity in specified time of radiation. The monomer is grown continuously on the radical groups generated during the UV grafting process. Commercial polyethersulfone (PES)UF membrane was grafted by Taniguchi and Belfort [7] at 300 nm wavelength with various types of monomer such as N-2-vinyl pyrolidinone (NVP), 2-hydroxyethyl methacrylate (HEMA), acrylic acid (AA), 2-acrylamidoglycolic acid (AAG), 3-sulfopropyl methacrylate (SPMA) and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) in order to produce membrane that has low protein fouling. Abuhabib *et al.* [8] combined two monomers which are acrylic acid and ethylenediamine dihydrochloride during grafting to improve the fouling resistance of PES nanofiltration membrane for brackish water desalination. Mansourpanah and Momeni [9] studied the effect of UV-irradiation time and acrylic acid concentration on the performance and morphology of modified polyamide thin film composite (TFC) membranes.

Another approach for monomer grafting is through chemical grafting *via* redox reaction. The concept is quite similar to UV photo-grafting which produced free radical on the membrane surface for monomer attachment, but it is assists by redox initiator pair. Different redox initial pairs were used during grafting such as potassium persulfate ($K_2S_2O_8$)-potassium thiosulfate ($K_2S_2O_3$) [10], $K_2S_2O_8$–sodium sulfite (Na_2SO_3) [11], $K_2S_2O_8$–$Na_2S_2O_5$ [12] and potassium methabisulfite ($K_2S_2O_5$)–$Na_2S_2O_8$ [13].

Much of the membrane modification in the previous works, mainly aim for producing fouling resistant membrane

by introducing new functionality of the membrane. In the current study, negatively charged functional group is required for binding to the protein through ionic interaction for preparing membrane chromatography. Polyamide membrane was grafted with acrylic acid monomer using two modification approaches which are chemical grafting using redox initiator and UV photo-grafting. The effects of modification parameters on the protein binding capacity were studied such as initiator concentration, monomer concentrations and time of reaction.

EXPERIMENTAL

Commercial microfiltration polyamide membrane, 47 mm diameter and 0.45 μm pore size, were purchased from Sartorius Stedim Biotech (Goettingen, Germany). Acrylic acid was used as a monomer and bought from Acros Organics (Geel, Belgium) and benzophenone as photoinitiator from Merck's (Darmstadt, Germany). In binding experiment, lysozyme (LZY) (Sigma) was dissolved in sodium phosphate buffer pH 7. The binding buffer was 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.

UV photo-initiated grafting: The membrane was modified according to the method developed by Ulbricht and Yang [14]. Acrylic acid was used as a monomer and benzophenone 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 benzophenone on the membrane surface. The membrane was presoaked in benzophenone solution dissolved in methanol for 15 min and then immersed in the methanol solution for 1 min. The membrane was took out from the solution and wiped with filter paper to remove any excess methanol on the membrane. Dried membrane was equilibrated in acrylic acid monomer solution for 15 min and was exposed to 365 nm UV light (UVP Model B-100AP, LLC, US) at different time for grafting. Grafted membrane was rinsed several times with fresh water and dried overnight in the oven at 60ºC.Three grafting parameters were investigated which are benzophenone initiator concentration (1-50 mM), monomer concentration (0.02-5 M) and grafting time (5-60 min).

Chemical grafting *via* redox reaction: A similar method by Belfer *et al*. [15] was followed for the redox grafting. Grafting solution was prepared by mixing appropriate amount of redox initiators (mol ratio of $K_2S_2O_8$:$K_2S_2O_5$ is 1:1) into acrylic acid monomer solution. Polyamide membrane was immersed into 30 mL of this mixture in 50 mL centrifuge tube. The reaction was carried out on tube rotator for better mixing and reaction at specific grafting time. The membrane was removed from the tube at the end of grafting and washed 3 times with water. Washing steps is necessary in order to remove unreacted monomer and homopolymer on the membrane. The membrane was then dried in oven at 60 °C for overnight. Similar to UV photo grafting, three grafting parameters were investigated which are redox initiator concentration (1-50 mM), monomer concentration (0.02-5 M) and grafting time (5-60 min).

Protein binding: Modified membrane about 2 cm^2 rectangular size was equilibrated in sodium phosphate pH 7 buffer solution for 1 h. The membrane was removed from the equilibration buffer and wiped with tissue to remove any residual buffer. Equilibrated membrane was immersed in 1.5 mL solution of 0.25 mg/mL of lysozyme for overnight. The remaining protein solution was analyzed by measuring the protein absorbance at 280 nm using UV-visible spectrophotometer (Shimadzu, UV 1800). All the steps involve in binding experiment were done on tube rotator for effective mixing between the solution and membrane. The binding capacity was calculated using the eqn. 1.

$$\text{Binding capacity} = \frac{V(C_0 - C_f)}{A} \quad (1)$$

where C_0 and C_f represent the initial and final protein concentration respectively in mg/L. V is the total volume of protein solution in mL and A is the area of the membrane in cm^2.

RESULTS AND DISCUSSION

Effect of initiator concentration on membrane grafting: Grafting on polyamide membrane requires an initiator to generate free radicals for both chemical and UV grafting method. In chemical grafting, $K_2S_2O_8$ and $K_2S_2O_5$ were used as the redox initiator. Meanwhile, benzophenone was used as the photo initiator in the UV photo grafting. Fig. 1 showed the lysozyme binding capacity for the membrane grafted using different initiator concentration using chemical and UV photo grafting methods. The UV grafting was performed at 5 min grafting time using 5 M of acrylic acid. In chemical grafting, 60 min grafting time using 0.1 M of acrylic acid was used.

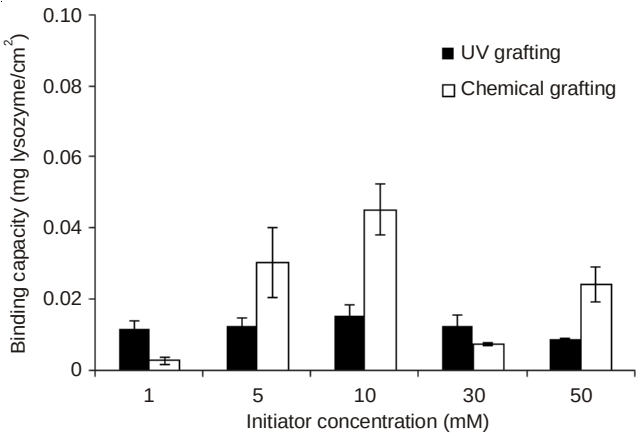

Fig. 1. Effect of initiator concentration during grafting process on the lysozyme binding capacity. Polyamide membrane was grafted using 5 M of acrylic acid for 5 min in UV photo grafting. The concentration of acrylic acid for chemical grafting is 0.1 M with 60 min grafting time

The lysozyme binding capacity of photo grafted membrane was not influenced by the initiator concentration increased from 1 to 50 mM. The lysozyme binding capacity was slightly changed and showed a capacity within the range of 0.009 mg/cm^2 to 0.015 mg/cm^2. It is believed that high concentration of acrylic acid monomer (5 M) used during UV photo grafting gives an extreme condition for the formation of free radical

on the membrane. According to Schwark and Ulbricht [16], mild condition is preferable for the growth of radicals during UV photo grafting. Less number of radicals generated influences the subsequent monomer attachment on the membrane, which will result in low protein binding capacity. It is observed that photo grafting at low acrylic acid concentration of 0.1 M gives the best protein binding capacity. Another possible explanation for low protein binding of UV photo grafted membrane at different initiator concentration is related to competing homopolymerization, especially when high hydrogen donor medium such as methanol was used [17]. Competing homopolymerization could reduce the formation of the radical on benzophenone since the hydrogen remains replacing from the methanol molecule.

Fig. 1 shows the increment of redox initiator pairs of $K_2S_2O_8$ and $K_2S_2O_5$ up to 10 mM increased the protein binding of the grafted membrane. The highest protein binding chemical grafted membrane was achieved at this concentration which gives the value of 0.045 mg lysozyme/cm^2. Initiator concentration higher than 10 mM may produced excessive amount of radicals generated. This will accelerate the reaction of initiators with the radicals and hence interrupted the monomer propagation reaction [18].

Effect of monomer concentration on membrane grafting: The second grafting parameter investigated was acrylic acid monomer concentration. The acrylic acid monomer concentration varied from 0.02 to 5 M with fixed value of initiator concentration and grafting time. The best initiator concentration at 10 mM was used. The grafting time for UV grafting and chemical grafting was fixed at 5 and 60 min, respectively. The effect of monomer concentration during grafting on the lysozyme binding capacity was shown in Fig. 2. Both techniques showed the same binding pattern in which the lysozyme binding capacity increased with increasing acrylic acid monomer concentration up to 0.1 M. At monomer concentration higher than 0.10 M, the binding capacity was reduced. The binding capacity for UV grafted membrane was higher than chemical grafted membrane at all acrylic acid monomer concentration used. The lysozyme binding capacity of UV grafted and chemical grafted membrane were in the range of 0.011 mg/cm^2 to 0.143 mg/cm^2 and 0.008 mg/cm^2 to 0.040 mg/cm^2, respectively.

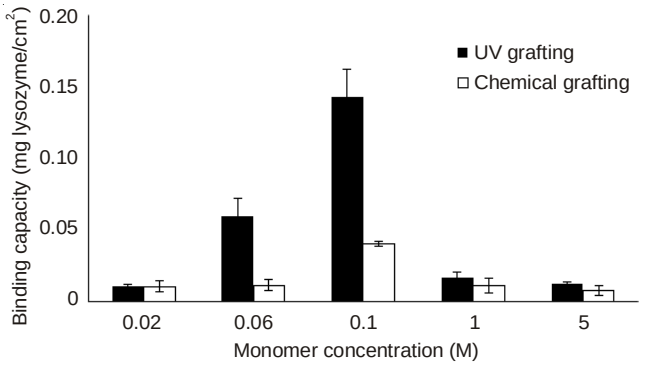

Fig. 2. Effect of acrylic acid monomer concentration during grafting process on the lysozyme binding capacity. Polyamide membrane was grafted using 10 mM of photo-initiator for 5 min in UV grafting technique. The concentration of initiator for chemical grafting is 10 mM with 60 min grafting time

The lysozyme binding capacity increase with the increasing of monomer concentration at low range of acrylic acid monomer concentration (*i.e*. 0.02 to 0.10 M) as shown in Fig. 2. At this stage, the number of new carbonyl group attached to the membrane was increased, which providing more space for the positively charged protein to bind to the membrane [19]. An increasing monomer concentration in dilute monomer concentration allows thicker polymer functional structure to be grown in the membrane [20,21]. However, increasing the acrylic acid monomer concentration higher than 0.10 M will decrease the lysozyme binding capacity. At high monomer concentration, due to the high rate of the propagation and termination reaction, the formation of homopolymer can be occurred which limit the attachment of carbonyl group in the membrane [20,21].

Effect of reaction and UV exposure time during membrane grafting: The effect of reaction or exposure time during the membrane modification at 0.10 M monomer concentration and 10 mM of initiator was studied in the range of 5 to 60 min. As shown in Fig. 3, no significant influence on the lysozyme binding capacity with the increment of grafting time for the membrane modified *via* chemical grafting. The binding was in the range of 0.011 to 0.014 mg/cm^2. A similar pattern was obtained by Littunen *et al*. [22] who grafted nanofibrillated cellulose with acrylic monomers at grafting time from 60 to 90 min using redox-initiated free radical method.

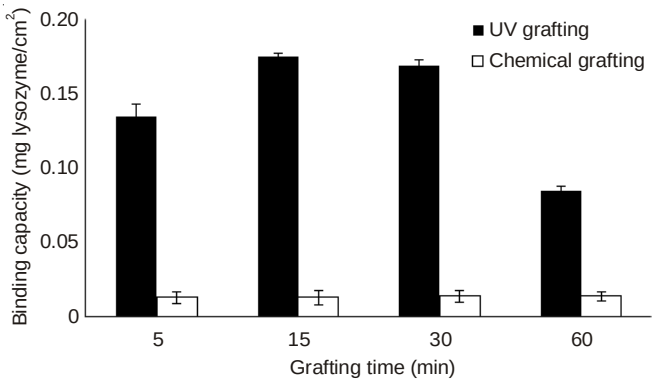

Fig. 3. Effect of reaction time during grafting process on the lysozyme binding capacity. Polyamide membrane was grafted using 10 mM of photo-initiator and 0.1 M acrylic acid in UV grafting technique. The concentration of initiator for chemical grafting is 10 mM with 0.1 M acrylic acid monomer concentration

UV photo grafting was effected by the grafting duration as shown in Fig. 3. The amount of lysozyme binding capacity was increased as the UV exposure time increased from 5 min to 15 min, then start decreasing beginning 30 to 60 min grafting time. The highest lysozyme binding capacity was 0.175 mg/cm^2 at 15 min of UV exposure time. Before reaching optimum grafting exposure time, the monomer kept grown on the membrane by reacting with the free radical generated in the membrane. However, after optimum time achieved, excessive UV light exposure could damage the membrane. The polymer backbone is broken at this stage and will degrades the membrane [23]. Degradation of the membrane will limits the availability of the active site for the protein binding. Over polymerization time can also causes the formation of the monomer brushes

on the membrane pore and becoming more crowded and less accessible for protein adsorption. In extreme cases, longer brushes may even block the protein assessment to the some part of the binding sites inside the pores and decrease the overall binding capacity [24,25].

Conclusion

The comparison between UV photografting and chemical grafting *via* redox reaction to produce negatively charged membrane chromatography for protein separation was reported in this study. The effect of initiator concentration, monomer concentration and reaction time on the lysozyme protein binding capacity was studied for both techniques. Chemical grafting *via* redox reaction was effected by the monomer concentration and initiator concentration. Grafting time showed less influence in chemical grafting. Monomer concentration and grafting time gave significant influences on the lysozyme binding capacity for the UV photo grafted membrane. The highest lysozyme protein capacity of 0.175 mg lysozyme/cm^2 membrane was achieved by UV photo grafted membrane using 0.1 M acrylic acid monomer concentration, 15 min of UV exposure and 10 mM of benzophenone. The current results will be used for optimization of UV photo grafting process using response surface methodology for future study.

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