CHAPTER 2

THE PREPARATION AND EVALUATION OF THE MIP COMPOSITE CELLULOSE MEMBRANE

This chapter explained about the experiment and results of the composite MIP membrane which was designed. In this plan, the experiment would be started by preparation of the polymers and composite MIP membranes, having effect to enantioselective of composite MIP membrane and investigation in vitro penetration using rat skin. For preparation of the MIP bead and composite MIP membrane, the technique of suspension polymerization to form MIP microparticle, phase inversion, grafting method to form composite MIP membrane, morphology and property of composite MIP membrane were studied. The factor media and ratio of polymer affect to enantioselective release of the target compound were studied in enantioselective. Finally, the in vitro percutaneous penetration test was studied. These experiments were designed show that whether the composite MIP membrane can be developed as transdermal drug delivery system of *S*-propranolol from racemic propranolol.

2.1 Chemicals and reagents

2.1.1 Standard chemicals

- *R*- and *S*-propranolol (Purity > 99.5%) was purchased from Sigma-Aldrich (Milwaukee, WI, USA)
- Racemic propranolol (Purity > 98.0%) was purchased from Sigma-Aldrich (Milwaukee, WI, USA)
- 1-pyrene butyric acid (Purity > 97.0%) was purchased from Sigma-Aldrich (Milwaukee, WI, USA)

2.1.2 Reagents and materials

- Methacrylic acid (MAA) was purchased from Sigma-Aldrich (Milwaukee, WI, USA). MAA was distilled under vacuum before use.
- Ethylene glycol dimethacrylate (EDMA) was purchased from Sigma-Aldrich (Milwaukee, WI, USA). EDMA was washed with 1 M aqueous sodium hydroxide, dried over Na_2SO_4 and distilled under vacuum before use.
- 2,2'-azobis-(isobutyronitrile) (AIBN) was purchased from Janssen (Geel, Belgium)
- 3-methacryloxypropyltrimethoxysilane (3-MPS) was obtained from Sigma-Aldrich (Milwaukee, WI, USA)
- Perfluoro(methylcyclohexane) (PMC) was obtained from Sigma-Aldrich (Milwaukee, WI, USA)
- 2-(*N*-Ethylperfluorooctane sulfonamide) ethylacrylate (PFA) was obtained from Janssen (Geel, Belgium)
- Polyethyleneglycol 2000 monomethyl ether (PEG2000MME) was obtained from Sigma-Aldrich (Milwaukee, WI, USA)
- The perfluoro polymeric surfactant (PPS) containing 95% acryloyl-2-*N*-ethylperfluoroalkylsulfonamide (PFA) and 5% acryloyl PEG2000 monomethyl ether (PEG2000MME) was prepared, according to the method described by Mayes and Mosbach (Mayes *et al.,* 1996).
- *N*-methylmorpholine-*N*-oxide (NMMO) was obtained from Sigma-Aldrich (Milwaukee, WI, USA)
- Poly (caprolactone triol) (PCL-T) was obtained from Sigma-Aldrich (Milwaukee, WI, USA)
- Membrane for phase inversion, bacterial cellulose (BC) sheet as membrane base was purchased from Thai nano cellulose Co.LTD., (Songkhla, Thailand). Membrane for grafted was kindly gift from Assist. Prof. Dr. Sanae Kaewnopparat. The cellulose membrane had a weight of 273.33 ± 32.14 μg/cm^2 (n =3) and thickness of 20-30 μm (as measured by the SEM method).

● Buffer pH 3 and 5.5 were prepared from place the specified amount of sodium acetated (pH 3 and 5.5 are 1.10 and 5.98 g, respectively) in a 1000 ml volumetric flask, add the specified volume of the acetic acid solution (pH 3 and 5.5 are 20 and 3 ml, respectively) , then add water to volume, and mix (USP 27 NF22).

● All solvents were analytical grades and dried with molecular sieves to eliminate traces of water before use.

2.2 Preparation of the composite MIP membranes by phase inversion and grafted method

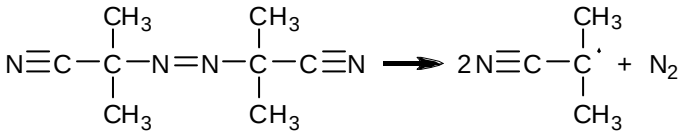
The aim of this experiment was to study the morphology and properties of composite MIP membrane prepared by phase inversion and a silanised coupler as an additional anchor for the MIP (grafted method). Polymer solution film casting and subsequent phase inversion, the main approach towards technical polymeric membranes can also be applied for molecular imprinting. The formation of porous MIM from a compatible blend of a matrix polymer—for adjusting a permanent pore structure—and a functional polymer—for providing binding groups—could provide even more alternatives. By grafting method, the structure of the base membrane is used as a means to adapt both pore size—permeability—as well as internal surface area—binding capacity—to the desired application. Using a thermal-initiated cross linking graft copolymerization yielded very thin MIP layers which were non-covalently anchored and covered the entire surface of the base membrane (Ulbricht, 2004).

2.2.1 Preparation of the MIP microparticles

The MIP microparticles were synthesized by radical suspension polymerization. The mechanism of free radical polymerization consists of three stages. The first is the initiation reaction is the attack of a monomer molecule by a primary radical originating from initiator. This process involves two reactions:

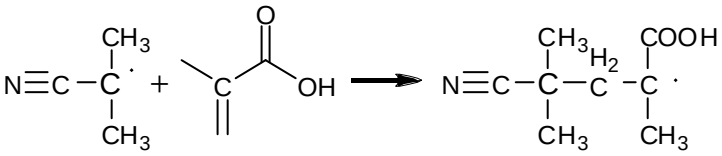
(1) Decomposition of the initiator to form primary radicals *i.e.,*

$$I \longrightarrow 2R^{\cdot} \quad \text{or}$$



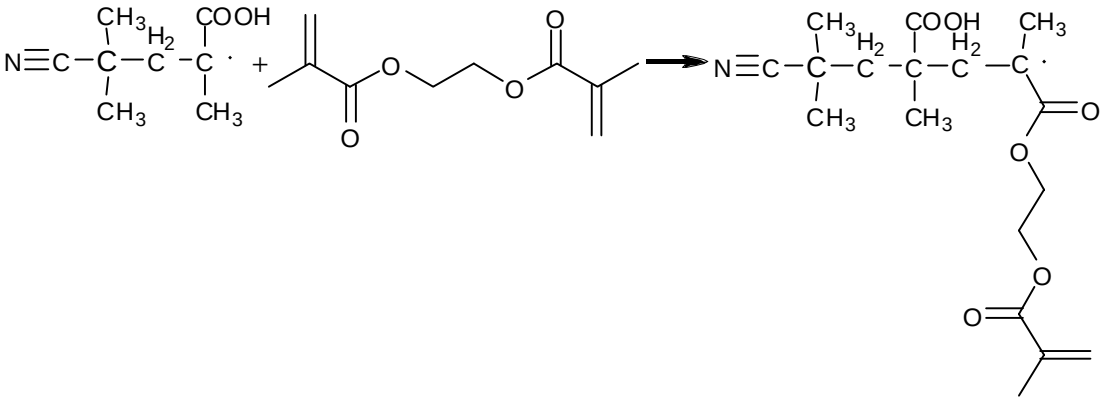
(2) The actual initiation reaction *i.e.,*

$$R^{\cdot} + M \longrightarrow RM^{\cdot} \quad \text{or}$$



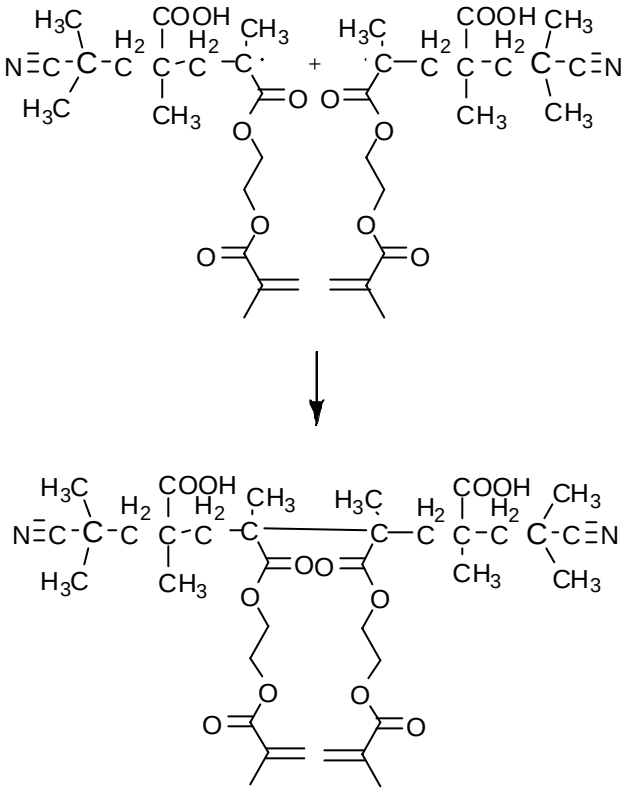
The second is propagation. This reaction is repeated hundred to many thousands of times for each chain formed, as already stated; it can be written as:

$$RM^{\cdot}_{n} + M \longrightarrow RM^{\cdot}_{n+1} \quad \text{or}$$

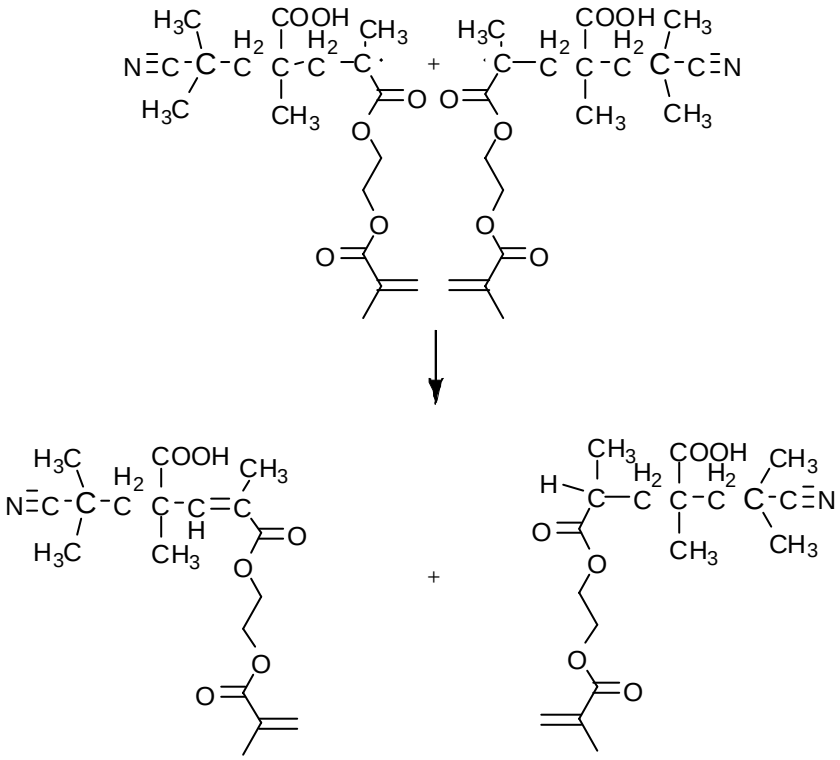


The third are termination. Growing radicals can react with each other in two different ways: (1) By recombination, a homopolar bond is formed by pairing the single electrons of the free radical sites of two chain:

$$R\text{-}M^{\cdot}_{n} + R\text{-}M^{\cdot}_{p} \longrightarrow R\text{-}M_{n+p}\text{-}R \quad \text{or}$$



(2) By disproportionation, whereby a hydrogen atom is transferred from one chain to the other; two molecules of "dead" polymer are formed, one of them bearing a double bond at chain end. The suspension polymerization was chosen due to the fact that it is desired to produce small spherical beads so that the size of particles can be controlled physical and chemical phenomena (Rempp *et al.,* 1991).



2.2.2 Synthesis of the MIP and NIP microparticles

MIP microparticles, selective to *S*-propranolol were prepared by suspension polymerization method, according to that described by Mayes and Mosbach (Mayes *et al.,* 1996), using perfluoro polymeric surfactant (PPS) as a surfactant and PMC as a dispersing phase. PPS was synthesized by heating a mixture of PFA (16.2 mmol), acryloyl PEG monomethylether (0.9 mmol), AIBN (0.2 mmol) and chloroform (20 ml) at 60^{o}C for 2 days in a water bath. The solvent was then gently removed by vacuum evaporation to yield a sticky yellow paste. The MIP beads were produced by the suspension polymerization method, so that the polymerizing mixture [template molecule (3 mmol *S*-or *R*- propranolol), MAA (12 mmol), EDMA (0.31 mol) and AIBN (0.36 mmol)] was reacted in the presence of PPS and PMC in chloroform under nitrogen with exposure to the UV light (366 nm) at room temperature for 4 hr. The polymer beads were collected and sequentially washed with 10% acetic acid in methanol and methanol for several times, until no residual template molecule was detected by HPLC in the rinse (see section 2.3.3). Finally, the polymer beads were dried under vacuum for 24 hr (Suedee *et al.*, 2000).

The non-MIP (NIP) that is used to verify the selectivity of MIP was prepared identically to the MIP, except that the polymerizing mixture is omitted of template molecule.

2.2.3 Evaluation of the recognition properties of the MIP particles

The objective of this section is to examine whether the MIP prepared under experimental (section 2.2.2) have recognition properties to the template. The ability of the MIP particles to selective rebind propranolol enantiomers was evaluated using a solid phase extraction procedure. In order to determine the recovery of bound drug enantiomers from aqueous solution, four different weights of MIP (2, 5, 10 and 15 mg) and corresponding NIP microspheres were incubated in 3 ml of pH 7.4 phosphate buffer saline (PBS) containing 60 μg/ml racemic propranolol at room temperature (30±1^{o}C) for 12 hr. The incubated dispersions were centrifuged at 3000 G for 5 min. The concentration of propranolol enantiomers in the supernatant was determined using the stereospecific HPLC method (see section 2.3.3). The amount of each enantiomer bound was calculated from the difference in concentrations before and after incubation. All experiments were run in triplicate.

2.2.4 Preparation of the composite MIP particle membrane by phase inversion technique

In this experiment, the ready-made MIP particulate was loaded in a bacterial cellulose membrane along with racemic propranolol, using phase inversion method. A hybrid approach of in situ polymerization and phase inversion is realized. The polymerization of functional monomers had been performed in the presence of the template, and the resulting solution of linear copolymers with the associated template had then been directly used for film casting/immersion precipitation towards porous molecular imprinted membrane. The MIP site and membrane morphology showed be formed in the same step from the same building blocks, either monomer or polymers. For this, the composite MIP cellulose membrane (total weight 0.2 g) was prepared by mixing an appropriate polymer particulate with racemic propranolol (10 mg). Each membrane contained 10 mg of racemic propranolol, MIP (100, 75, 50 mg), and 0.096 g of

cellulose membrane. The drug and the polymer were mixed together at each ratio of drug: polymer. Then, the mixture was poured in warm solutions of 50 wt% of cellulose membrane in NMMO, and was gently stirred. Afterwards, the suspension was poured in a glass plate (10 cm diameter), and the resulting membrane was immersed in a water bath maintained at 32^{o}C. After 5 minutes, the composite MIP particle membrane was taken from the washing water and then dried and stored under room temperature until use (Mayes *et al.,* 1996). A composite NIP cellulose membrane was prepared using the same procedure as that for the MIP membranes. The blank composite MIP particle membrane and the match membrane (NIP membrane) were prepared using the procedure described above but omitting of propranolol. The entrapment of racemic propranolol in the obtained composite membrane was found to be about the same in each case (2.1 mg/cm^{2}) as examined by quantifying the drug in the rinse of casting with the use of HPLC as analysis method, and the amount of drug entrapped was obtained by subtracting the amount of drug in the rinse from the amount of drug formulated.

2.2.5 Preparation of the composite MIP membrane by grafting method

For preparation of MIP grafted composite cellulose membrane, the grafting was carried out in the presence of the EDMA as cross-linking monomer, MAA as functional monomer, AIBN as initiator and propranolol enantiomer as template. A cellulose membrane was reacted with 3-MPS (10% w/w in toluene) at 80^{o}C for 5 hr to change –OH of cellulose membrane as C=C for reaction with MIP mixture. The resulting membrane was washed in methanol and dried. The reacted cellulose membrane was placed in glass dish, 18 cm in diameter.

Table 2.1 Different formulations of MIP grafted membrane

Formula	Composition	Ratio
S-MIP# 1	template:MAA:EDMA	1:3:8
S-MIP# 2	template:MAA:EDMA	1:3:5
S-MIP#3	template:MAA:EDMA	1:3:2

Each formula (Table 2.1) was added 0.7 mmol of AIBN in DMF (2 ml) and it was poured onto the surface of cellulose membranes. The dish was purged with nitrogen for 5 minute to remove oxygen before closure and the temperature maintained at 60^{o}C for 18 h. After polymerization, membranes were transferred to a Soxhlet apparatus to extract the template with 10% (w/v) acetic acid in methanol for 72 h before extraction with methanol for 72 h. Finally, the membranes were dried in vacuum overnight (see Figure 2.1). The non-imprinted polymer (NIP) cellulose membrane (control) was prepared using protocol same as that for the MIP cellulose membrane but no template molecule added in the polymerizing mixture. The degree of modification (DM) was calculated from the difference in weight between the sample modified with a deposited MIP layer and the initial unmodified sample.

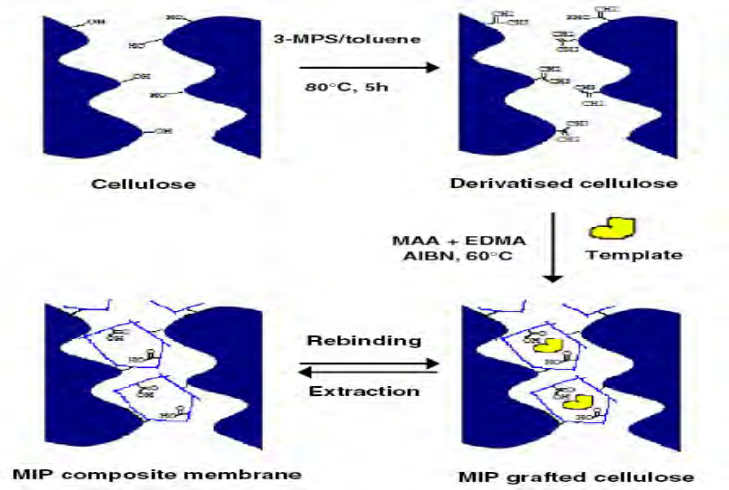


Figure 2.1 Overview of MIP composite membrane prepared by grafting method (Bodhibukkana *et al*., 2006)

2.2.6 Characterization methods

2.2.6.1 Surface morphology

The membrane and polymer morphology were studied by scanning electron microscope (SEM) at an accelerating voltage of 15 kV with the samples beging sputter-coated with gold before imaging with a Jeol series JSM 5800 LV SEM (CA, USA).

2.2.6.2 Electrical resistance measurements

Impedance measurement was used to inspect the deposition of MIP microsphere and MIP thin layers into the cellulose membrane as well as porosity of the membrane. Electrochemical resistance measurements of the composite MIP cellulose membrane were carried out by short-circuits current technique using a Revision G Voltage Current Clamp, Model VCC 600 (Harvard Apparatus, CA, USA). The test membrane was mounted in the measuring cell and a current was established across it using a potentiostat via an amplifier with high-resistance inputs. The four-electrode potentiostat assured a passage of current between the two calomel electrodes in such a manner as to hold constant amplitude of voltage between the two identical reversible silver-silver chloride electrodes and the intensity and phase of the current in the circuit. A 60 μA current was applied, and the membrane potential difference (PD; mV) and the short circuit current (I_{sc} ; A) were recorded. The membrane resistance, R_m (Ω cm^2) was calculated as PD/ I_{sc}. All experiments were carried out in three replications using different batches of membranes at 30±1^oC

2.2.6.3 Pore size measurements

The pore size of membrane was estimated from surface picture of membrane by SEM (n=10).

2.2.6.4 Mechanical properties measurements

The mechanical strength of three different batches of membranes was measured using a Universal testing machine (Lloyd, UK) with an operating head load of 100 N. Tensile strength was calculated according to the equation: tensile strength (Pa) = max load (N)/ cross-sectional area (m^2).

2.2.6.5 Degree of swelling of the composite MIP cellulose membrane

The degree of swelling of the composite MIP cellulose membrane was evaluated in pH 7.4 buffer. All polymer membranes were vacuum-dried at room temperature for at least 3 days before testing. The membrane samples were weighed and then soaked in individual chambers containing phosphate buffer pH 7.4 at room temperature (~30°C). The membranes were incubated in the medium until the weight of wet membranes remained stable, which occurred after approximately 6 h of incubation. Before measuring the weight of a wet membrane, the surface water was gently removed with a tissue. The wet membranes were dried under vacuum at room temperature to a constant weight before measurements. The degree of swelling of the composite MIP membrane (%) was calculated by the equation 4:

$$\%swelling = \frac{(W_{wet} - W_{dry})}{W_{dry}} * 100 \qquad \text{equation (4)}$$

where W_{dry} and W_{wet} are the weights of a dried and a wet sample, respectively. Each test was carried out in sets of three.

2.2.7 Results and discussion

2.2.7.1 The MIP particle composite cellulose membrane

The MAA-based polymer beads prepared under experimental conditions were rather uniform, smooth surface microspheres and had particle size less than 50 μm (Figure 2.2).

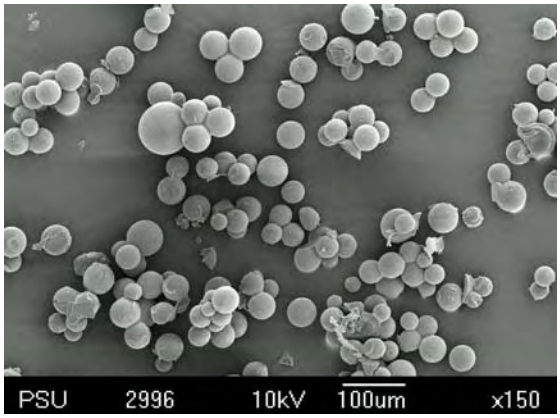


Figure 2.2 SEM images of molecular imprinted polymer particles

The selective extraction of enantiomer of a fixed concentration of racemic propranolol in pH 7.4 phosphate buffer by increasing amounts of MIP particles was evaluated at room temperature. The enantioselectivity of the binding of propranolol was measured as the amount of each enantiomer bound to the particles expressed as a percentage of the original concentration and this was plotted as a function of polymer content (Figure 2.3).

The binding of both propranolol enantiomers to either the MIP bead microparticles was markedly greater than to the corresponding NIPs. In addition there was clear evidence of enantioselectivity when the polymer content was 10-15 mg for the MIP bead microspheres. In the case of polymer bead microspheres as the polymer content was increased then so the percentage of enantiomers bound increased significantly. This suggests that the binding sites may be more accessible in the particles produced by emulsification. The enantioselectivity of binding to either the MIP bead microspheres appeared to increase as a function of increasing in the polymer content.

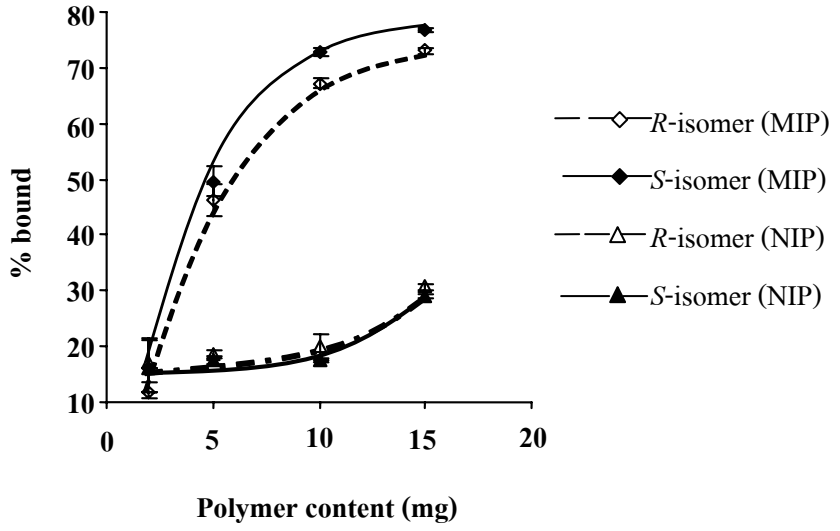


Figure 2.3 The recovery of rebinding of propranolol enantiomers with imprinted and non-imprinted polymer of microsphere at various polymer contents in pH 7.4 phosphate buffer solution and room temperature; (mean ± S.D., n = 3)

2.2.7.2 The MIP grafted cellulose membrane and its characteristics

The composite MIP cellulose membrane obtained from a MIP integrated with a cellulose membrane by phase inversion was shown in Figure 2.4 and 2.5. The physical and mechanical properties of the composite MIP cellulose membrane, and the composite NIP cellulose membrane at various polymers loading, were determined. The data were presented in Table 2.2. It was found that the mean electrical membrane resistance of the MIP loaded cellulose membrane was higher than that of the NIP loaded cellulose membrane. This indicates the differences in the distribution of MIP and NIP particles in the cellulose membrane. No difference in the tensile strength and the degree of swelling of the MIP-and NIP-loaded cellulose membrane was found. The results indicated that the composite cellulose membrane swelled greatly in an aqueous solvent (>70%). The composite MIP grafted cellulose membrane prepared by reactive pore filling of bacterial cellulose membrane with MIPs having recognition sites for the propranolol enantiomer at three different formulation were obtained. Such formulation would be expected to lead to an alteration in the density of the grafted MIP layers. The DMs of the three modified membranes were similar with an average DM of 0.80 mg/cm^2. The variation in the DM

of individual membranes was found to be less than 10%. It can be explained that the MIP mixture can interact with cellulose membrane base become MIP thin layers into membrane base. It was found that the MIP graft membrane formulation 1 gave higher polymer network for MIP membrane as compare to formulation 1 and 2. SEM surface images of three formulations for MIP grafted cellulose membrane and membrane base show the different MIP network from into the membrane pore (Figure 2.6-2.9). From SEM cross-section, MIP mixture formulation 1 generate polymer matrix into the cellulose membrane more than formulation 2, 3 and cellulose membrane base as shown in Figure 2.10-2.13. Because of the formulation 1 compared higher content of cross-linking monomer (EDMA) than formulation 2 and 3. The physical and mechanical properties of the MIP grafted cellulose membrane were determined. No differences of mean electrical membrane resistance of MIP grafted cellulose membranes of three formulations were obtained and this value increased upon modification from cellulose membrane. This indicates that these three formulations gave no difference in distribution of MIP layers inside cellulose membrane. An increase in the tensile strength and degree of swelling of composite membrane after modification were obtained. It was found that the value of tensile strength and swelling of MIP grafted cellulose membrane was higher than that these of formulation 2 and 3, because the formulation 1 has EDMA cross-linker more than formulation 2 and 3. The average pore diameters obtained by SEM, proved to be very similar, within the range 0.05-0.15 μm (see the value for each type of membrane in Table 2.3).

Table 2.2 Characteristics of composite MIP cellulose membrane prepared by phase inversion method (mean±SD, n=3)

Membrane loaded with MIP beads	Tensile strength (MPa)	Impedance (Ωcm^2)	Pore size (μm)	Degree of swelling (%)
cellulose membrane	6.70±1.10	1.23±0.10	30.05±0.15	62.35±0.51
NIP	7.20±0.40	1.96±0.09	35.00±0.04	72.76±0.78
R-MIP	7.50±0.30	3.07±0.06	37.50±0.06	73.25±0.71
S-MIP	7.60±0.30	3.11±0.09	37.50±0.05	73.98±0.65

Table 2.3 Characteristic of composite MIP cellulose membrane prepared by grafted method (mean±SD, n=3)

Membrane grafted with *S*-MIP	Tensile strength (MPa)	Impedance (Ωcm^2)	Pore size (μm)	Degree of swelling (%)
cellulose membrane	23.50±1.30	1.56±0.11	0.15±0.08	32.25±0.47
S-MIP #1	58.00±1.80	1.69±0.10	0.06±0.09	41.62±0.76
S-MIP #2	42.00±1.00	1.92±0.13	0.07±0.06	37.66±0.88
S-MIP #3	25.60±0.50	1.78±0.13	0.13±0.05	35.11±0.96

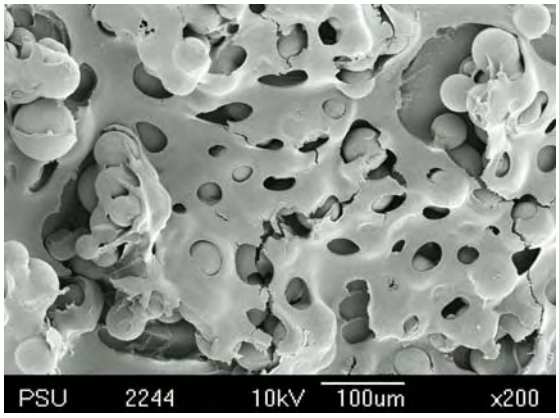


Figure 2.4 The SEM image of the composite MIP cellulose membrane prepared by phase inversion method

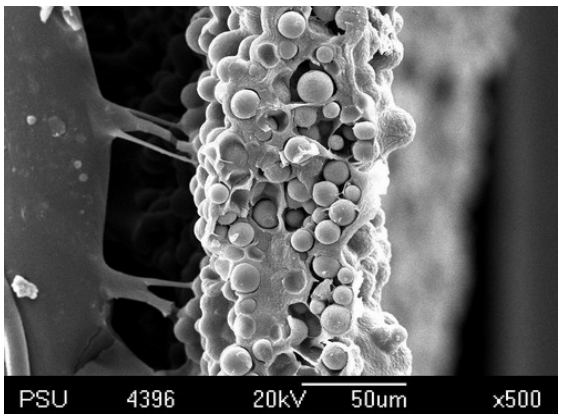


Figure 2.5 Cross-section image of the composite MIP cellulose membrane prepared by phase inversion method

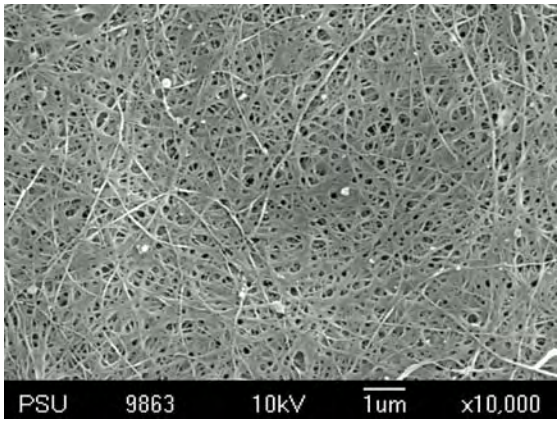


Figure 2.6 The surface morphology of the cellulose membrane base

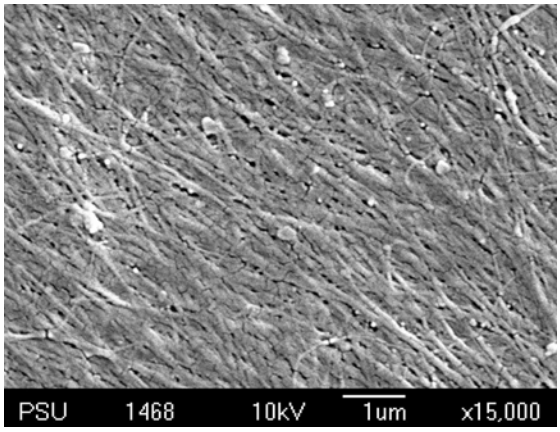


Figure 2.7 The surface morphology of the composite MIP cellulose membrane prepared by grafting method for formulation 1

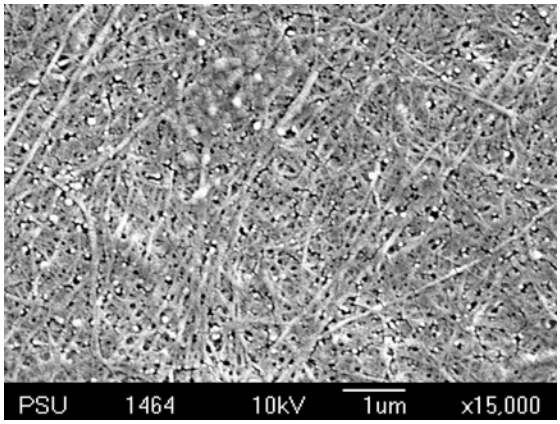


Figure 2.8 The surface morphology of the composite MIP cellulose membrane prepared by grafting method for formulation 2

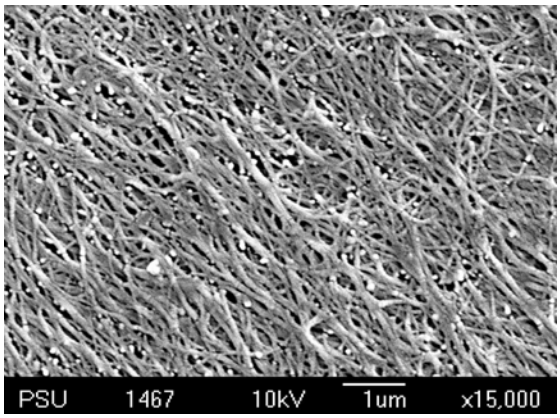


Figure 2.9 The surface morphology of the composite MIP cellulose membrane prepared by grafting method for formulation 3

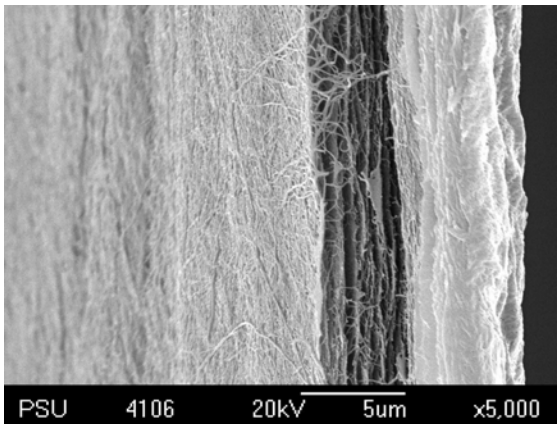


Figure 2.10 Cross-section image of the cellulose membrane base

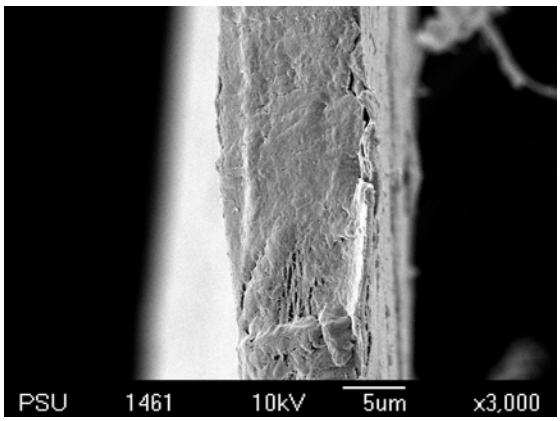


Figure 2.11 Cross-section image of the composite MIP cellulose membrane prepared by grafting method for formulation 1

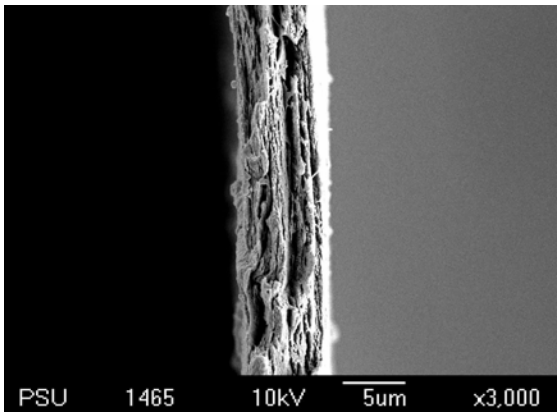


Figure 2.12 Cross-section image of the composite MIP cellulose membrane prepared by grafting method for formulation 2

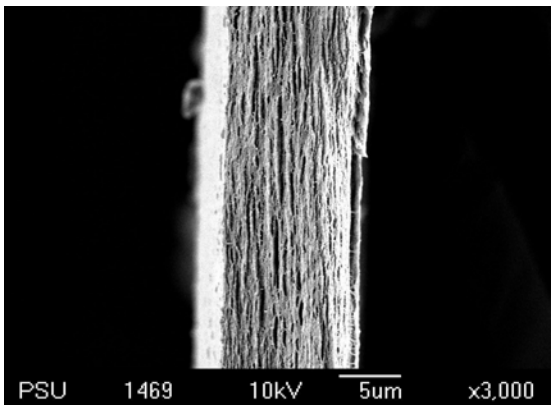


Figure 2.13 Cross-section image of the composite MIP cellulose membrane prepared by grafting method for formulation 3

2.3 Optimization of some parameter in composite MIP membrane synthesis and experiment condition

The objective of this study was to identify the parameter that can affect to the enantioselective transport of the composite MIP membrane. For this purpose, citrate buffer pH 3, 5.5, phosphate buffer 7.4 and chloroform were selected as media. Since, chloroform was used for polymerizing process, whist pH 7.4 and 5.5 represented physiological pH of blood and skin surface, respectively, as well as pH 3 represented acid condition. Moreover, the effect of polymer content on enantioselective transport of composite MIP membrane was investigated.

2.3.1 In vitro release evaluation of composite MIP membrane

Release studies were carried out in a borosilicate glass bottle which had a 5 ml of dissolution media, buffer pH 3, pH 5.5, pH 7.4 or chloroform (30±1^{o}C). A 1×1 cm MIP membrane prepared at different ratio of drug: polymer (i.e. 1:5, 1:7.5, 1:10) was soaked in the medium between 0 and 6 h and stirred at 180 rpm. Samples (300 μl) were drawn from the dissolution medium at appropriate time intervals to determine the amount of drug enantiomer release by using the stereoselective HPLC method (see section 2.3.3). In each test, the mean amount of each enantiomer release from three samples was used to characterize the drug release.

The cumulative amount (μg) was calculated and plotted as a function of time. The permeate flux (J_s, μg $cm^{-2}h^{-1}$) was calculated by equation 5:

$$J_s = Q * A^{-1} * t^{-1} \quad \text{equation (5)}$$

where Q (μg) is the amount of analyte permeated, A (cm^2) is the area of effective membrane and t (hr) is the time. The diffusion coefficient (D, cm^2h^{-1}) was calculated from equations 6:

$$D = \text{flux} * h^2 * C^{-1} \quad \text{equation (6)}$$

where h (cm) is thickness of membrane as measured by SEM and C (μg/ml) is concentration of propranolol enantiomer applied (Martin *et al.,* 1993). The selectivity of the composite MIP membrane was defined as a ratio of the diffusion coefficient of the *S*-isomer to the *R*-isomer releases.

2.3.2 Diffusion determination

In this part, the enantioselective diffusion of the composite MIP cellulose membrane and the corresponding NIP composite cellulose membrane was evaluated by the dialysis method using a vertical Franz diffusion cell. The membrane (exposed area 0.80 cm^2) was mounted between the donor and the receiving phase, their volumes being 1 and 2.5 ml, respectively. The required amount of racemic propranolol (100 μg/ml for phase inversion and 40 μg/ml for grafted method) was dissolved in pH 7.4 phosphate buffer solutions to obtain a donor solution (0.5 ml). Buffer pH 7.4 was into the receiving phase. The drug diffusion was measured by sampling of the sample (250 μl) from receiving phase at time intervals over 6 hr. The volume of a sample withdrawn was replaced by the same volume of a medium. Each test was carried out in set of three. The diffusion of propranolol enantiomer obtained from the sampling was measured by using the chiral-HPLC analytical method (see section 2.3.3). The cumulative amount (μg) was calculated and plotted as a function of time. The flux (J_s; μg cm^{-2} h^{-1}) and the diffusion coefficient (D, cm^2h^{-1}) were calculated by equation 5 and 6, respectively. The selectivity of the composite

MIP membrane was defined as a ratio of the diffusion coefficient of the *S*-isomer to the *R*-isomer releases.

2.3.3 Stereospecific HPLC method

A 100×4.6 mm 5 μm AGP chiral column (ChromTech Ltd, U.K.) was used. The chromatographic system comprised a Waters 600 HPLC system (Bedford, USA) with Waters 717 plus autosampler (20 μl loop), equipped with a 486 variable wavelength set at 290 nm and a Waters 746 integrator. A 1 ml/min flow rate of 2-propanol in 55 mM ammonium acetate buffer pH 4.1 was pumped to HPLC. The retention times were 4.5 min and 7.6 min for *R*-propranolol and *S*-propranolol, respectively. Correlation coefficients for the calibration curves in the range of 2–25 μg ml^{-1} for *R*- and *S*-propranolol enantiomers were greater than 0.999. The sensitivity of detection was 1.3 μg ml^{-1} and the reproducibility of the peak areas of both enantiomers was more than 95%.

2.3.4 Statistical analysis

The results of the drug release, permeation and penetration in this experiment were expressed as a mean±S.D. Paired *t*-test was used on the data, and the significance limit of 95% confidence intervals was applied.

2.3.5 Results and discussion

2.3.5.1 In vitro release study

The release profile of *R*- and *S*-propranolol from the MIP and NIP composite membranes was studied at various pHs by using Franz diffusion cell, as shown in Figure 2.14. The entrapment of *R*-propranolol (106.90±10.00 μg/cm^2) and *S*-propranolol (106.40±5.8 μg/cm^2) in the composite membranes were similar, using the measurement protocol same as that for propranolol. At pH 3, the release of both *R*- and *S*-propranolol enantiomers was faster than that at

pH 5.5, pH 7.4 but the enantioselectivity was found to decrease with decreasing pH. And, the maximum *S/R* selectivity was found at pH 7.4 (Figure 2.15). The solubility of propranolol in acidic pH is higher than in basic pH and this leads to acceleration in the release of the drug into more acidic media. At pH 7.4 the difference in release was the highest, and the pKa value of propranolol was 9.5 which propranolol was protonated when pH of medium was reduced. Additionally, the MAA-EDMA polymer was a weak acid which had a pKa value at 8-9. The polymer was not dissociated at low pH and the membrane surface carried a slight negative charge. Thus, protonated propranolol may not be able to bind with a MIP. At pH 7.4, the degree of ionization of propranolol would not change significantly. On the other hand, the MIP could have exhibited the enantioselective ability for propranolol base, if a stereoselective interaction had occurred at a selective site.

The release of propranolol enantiomers from the MIP composite cellulose membrane when chloroform was used as donor solvent was depicted in Figure 2.14. The release rate of both *R*- and *S*-propranolol enantiomers was greater in chloroform, compared to the pH 7.4 buffer However, the enantioselectivity was more pronounced in buffer pH 7.4 than that in chloroform (see Figure 2.15). This can be explained by that in aqueous solvent the propranolol enantiomers interact to the recognition more greatly than in chloroform which allow the acceleration of the drug in transport (Spivak *et al.,* 2005).

The release profiles of propranolol enantiomers for different ratios of drug: polymer showed that increasing the polymer content of composite membrane resulted increment in stereoselectivity of release propranolol enantiomers and the ratio of drug and polymer at 1:10 gave the release of *S*-propranolol faster than that of the *R*-propranolol, and the difference of *R*- and *S*-enantiomer releases appeared increase at 1:5 and 1:7.5 (see Table 2.4). This may be related to the affinity of the MIP in the cellulose membrane. And, the faster release of enantiomers may be affected by the reduction of interaction of the drug and MIP particles in the membrane. When the drug and polymer ratio was increased, the flux of *S*-propranolol was decreased, but the enantioselectivity was more pronounced (see Table 2.4).

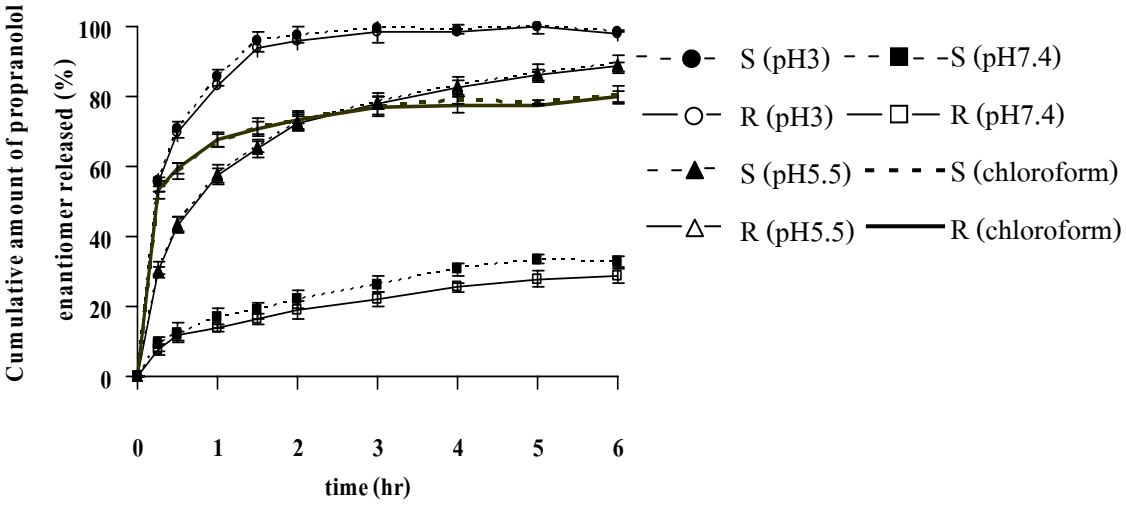


Figure 2.14 Time profiles of propranolol enantiomers at different medium (mean±SD, n=3)

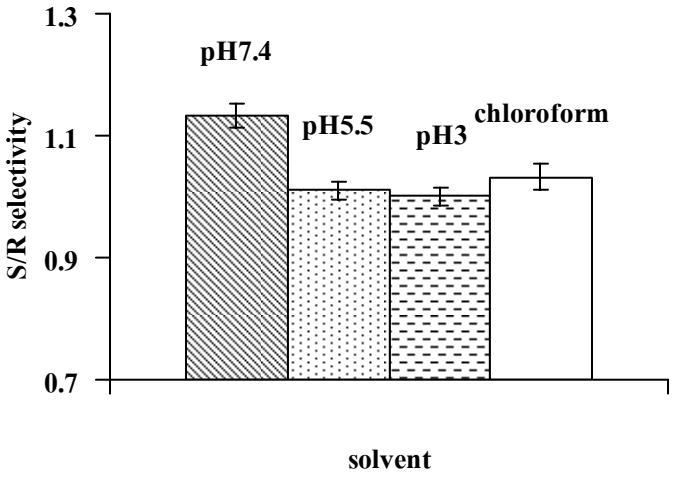


Figure 2.15 The selectivity of composite MIP cellulose membrane when incubate in buffer pH 3, 5.5, 7.4 and chloroform (mean±SD, n=3)

Table 2.4 In vitro release and diffusion measurement data of propranolol from the composite MIP particle membrane when prepared at different ratio of drug to polymer after application of pH 7.4 phosphate buffer in donor phase at 37±1^{o}C (mean±SD, n=3, *$P \leq 0.05$ of diffusion coefficient between *S*- and *R*-isomer)

Formula	Diffusion coefficient(*D*) of *R*-isomer (*10^{-7}; cm^2h^{-1})	Diffusion coefficient(*D*) of *S*-isomer (*10^{-7}; cm^2h^{-1})	*S/R* ratio	Significant *P*-value*
1:5 drug:*S*-MIP	17.90±0.45	18.10±0.10	1.01±0.07	0.008
1:7.5 drug:*S*-MIP	13.60±0.17	14.30±0.72	1.05±0.12	0.001
1:10 drug:*S*-MIP	8.25±0.82	9.40±0.87	1.13±0.56	0.001
1:5 drug:NIP	9.52±0.35	9.60±0.34	1.01±0.02	0.055
1:7.5 drug:NIP	9.34±0.38	9.42±0.37	1.02±0.02	0.005
1:10 drug:NIP	6.10±0.46	6.54±0.49	1.06±0.02	0.006

2.3.5.2 Enantiomeric release of the MIP composite membrane

The MIP composite cellulose membranes prepared with different polymeric contents were incubated in pH 7.4 phosphate buffer at room temperature, the *R*- and *S*-propranolol enantiomers diffused faster across the 35% MIP loading membrane than 50% and 75% MIP loading membrane. At 50% and 75% polymer loadings, the *S*-enantiomer was diffused faster than the *R*-enantiomer, but no difference between the 50% and 75% MIP loadings in terms of the enantioselectivity. The difference in the diffusion of *S*- and *R*-propranolol enantiomers was clear at the polymer loading of either 50% or 75%, but it was not the case with the polymer loading of 35%. This was because of an increase in the number of selective sites and was related to the capacity interaction between the drug and selective sites. In overall, increasing in MIP loading of the membrane increased the selectivity of release for propranolol enantiomers but decrease the diffusion coefficient of the enantiomers of propranolol (see Table 2.5).

For the MIP grafted cellulose membrane prepared at different compositions either two propranolol enantiomers of the MIP grafted membrane formulation 3 diffused faster than that of the MIP grafted membrane formulation 1 and 2. The MIP grafted membrane of formulation 1, 2 and 3 gave the *S*-enantiomer diffuse faster than the *R*-enantiomer but *S/R* selectivity of formulation 1 was higher than that of formulation 2 and 3 (see Table 2.6). This may cause by higher content of EDMA cross-linking monomer produces more polymer network which gives a rigid for imprint site. The enantioselectivity was from to decrease at above 30 mol% of total mass polymer. This can be explained by two reasons, *i.e.,* (1) an excess of MAA increases the amount of non-specific binding (2) there is a minimum amount of cross-linker necessary to form a rigid enough polymer network that will maintain the fidelity of the binding site (Spivak *et al.,* 2005).

Table 2.5 In vitro release and diffusion measurement data of propranolol from the composite MIP particle membrane when prepared at different polymer content after application of pH 7.4 phosphate buffer in donor phase at 37±1^{o}C (mean±SD, n=3, *$P \leq 0.05$ of diffusion coefficient between *S*- and *R*-isomer)

Formula	Diffusion coefficient(*D*) of *R*-isomer (*10^{-7}; cm^2h^{-1})	Diffusion coefficient(*D*) of *S*-isomer (*10^{-7}; cm^2h^{-1})	*S/R* ratio	Significant *P*-value*
35% *S*-MIP	2.95±0.69	2.41±0.19	0.98±0.16	0.670
50%*S*-MIP	1.05±0.46	1.84±0.59	1.41±0.13	0.002
75%*S*-MIP	1.04±0.44	1.28±0.73	1.51±0.06	0.002
35% NIP	1.75±0.16	1.79±0.12	1.01±0.30	0.018
50% NIP	1.16±0.01	1.15±0.02	0.95±0.04	0.004
75% NIP	1.17±0.16	1.18±0.19	1.00±0.07	0.002

Table 2.6 In vitro release and diffusion measurement data of propranolol from the MIP grafted cellulose membrane when prepared at different formular after application of pH 7.4 phosphate buffer in donor phase at 37±1^{o}C (mean±SD, n=3, *$P \leq 0.05$ of diffusion coefficient between *S*- and *R*-isomer)

Formula	Diffusion coefficient(*D*) of *R*-isomer (*10^{-7}; cm^2h^{-1})	Diffusion coefficient(*D*) of *S*-isomer (*10^{-7}; cm^2h^{-1})	*S/R* ratio	Significant *P*-value*
S-MIP #1	2.40±0.70	3.64±0.34	1.54±0.06	0.009
S-MIP #2	2.66±0.58	4.05±0.54	1.13±0.04	0.021
S-MIP #3	3.12±0.61	4.38±0.61	1.05±0.03	0.287
NIP #1	3.50±0.39	3.66±0.39	1.05±0.01	0.010
NIP #2	3.49±0.56	3.51±0.55	1.00±0.02	0.018
NIP #3	3.22±0.62	3.30±0.64	1.01±0.01	0.161

S-MIP #1 as formulation 1; template:MAA:EDMA (1:3:8)

S-MIP #2 as formulation 2; template:MAA:EDMA (1:3:5)

S-MIP #3 as formulation 3; template:MAA:EDMA (1:3:2)

2.4 In vitro evaluation of transdermal delivery

The intention of this experiment was to study the enantioselective penetration of propranolol of the prepared composite MIP membranes by in vitro percutaneous penetration using excised rat skin. The highlight of in vitro experimentation in transdermal delivery is to understand and predict the delivery and penetration of a molecule from the skin surface into the body via the skin of a living animal. Human skin is for many investigators not readily available. Such samples obtained are taken from a variety of body sites. The vast majority of in vitro experiments are conduct on animal skin, in particular the hairless mouse. Throughout the history of transdermal

delivery investigators have sought to find a predictive correlation between the penetration of molecules through animal and human skin. Although there exists a number of similarities, there is as yet no animal skin that completely mimics the penetration characteristics of human skin. Skin is usually removed from the animal by blunt dissection; principle barrier to penetration is the stratum corneum. Therefore, these investigators argue that only intact stratum corneum need be used. The lower skin layers have little effect on penetration and it is often desirable to reduce skin thickness without disturbing the barrier properties. Physical separation of the skin is best undertaken with an electric dermatome offering controllable thickness of section. The lack of sample width, ~2-3 cm, does not create a problem as more emphasis is placed upon small diffusion cells. Of principal concern is the abundance of skin appendages, e.g., hair follicles and sweat glands, which reach from surface level deep into the dermis. During sectioning all of these are severed, in essence creating a series of pores into the skin. The storage method of choice is usually chilling or freezing. If the principal barrier to penetration is the stratum corneum, then low temperatures would be expected to have little effect owing to its comparatively low water content (Hadgraft *et al.,* 1989).

2.4.1 In vitro percutaneous penetration study

The in vitro percutaneous penetration study of the composite MIP cellulose membrane was performed with a vertical Franz diffusion cell in a parallel experiment with a composite NIP cellulose membrane. The membrane positioned in place on the surface of excised skin of an adult male wistar rat. The donor phase was loaded with phosphate buffer pH 7.4 (300 μl for membrane prepared phase inversion with loaded racemic propranolol in membrane) or racemic propranolol solution (300 μg/ml 500 μl for membrane prepared phase inversion and graft method that without the loaded racemic propranolol in membrane) and receiving phase was loaded with 2.5 ml of pH 7.4 phosphate buffer. The cell was maintained at 37^{o}C by an external water bath. The receiving phase was gently stirred at 180 rpm. Sampling (250 μl) of a medium from receiving phase at time intervals over 2 days and replacing with the same volume of a fresh medium were carried out (A=0.8 cm^2). The stereospecific HPLC method was employed for enantiomer assay (see section 2.3.3). The mean amount of each penetration from three samples

was presented. The cumulative amounts of propranolol enantiomer released (μg) were calculated and plotted as a function of time. The lag time (hr) was investigated from the straight line cross time axis. The flux (J_s; μg cm^{-2} h^{-1}) was calculated by equation 5. The selectivity of the composite MIP membrane was defined as a ratio of the flux of the *S*-isomer to the *R*-isomer releases.

2.4.2 Results and discussion

2.4.2.1 In vitro percutaneous penetration study

From Table 2.7, the composite MIP particle membrane with racemic propranolol in membrane indicated that the penetration of the *R*-isomer across the MIP composite membrane was delayed. The selectivity of the MIP-loaded membrane was higher than that of the corresponding NIP-loaded membrane. The enantioselectivity exhibited by the composite MIP cellulose membrane in situ the excised rat skin is related to the affinity of propranolol imprinted microparticles for the *S*-propranolol by the imprinting process.

For composite *S*-MIP microparticle cellulose membrane without racemic propranolol in membrane, it was found that the *S*-isomer was more penetrate than the *R*-isomer. *S/R* selectivity of MIP cellulose membrane was higher than that of NIP membrane. From the result, this indicated that the enantioselectivity is related to the affinity of MIP microparticles (see Table 2.7).

The MIP grafted cellulose membrane gave the penetration of *S*-propranolol higher than that of *R*-propranolol. The *S/R* selectivity of the MIP grafted cellulose membrane was higher than the NIP grafted membrane due to the affinity of the MIP with template molecule (see Table 2.7). The *S/R* selectivity of the MIP grafted membrane and the MIP particle composite membrane is not significantly different (see Table 2.7). The percutaneous permeation of both *R*- and *S*-enantiomers of propranolol across NIP membranes applied to the skin was initially delayed, which is possibly due to the MAA-EDMA steric hindrances which might occur in the pores of the modified basic cellulose membrane. NIP membranes allowed slightly faster *S*-propranolol enantiomer transport than for the *R*-propranolol enantiomer. There is little facilitated *S*-propranolol enantiomer transport across NIP membranes**.** The lag time of *S*-isomer was lower

than that of the *R*-isomer in each formulation. The lag time of composite MIP membrane, prepared phase inversion load racemic propranolol in membrane, was higher than the other formulation because the solvent presumably diffuse into the membrane for dissolve the drug before penetrate through skin (see Table 2.7).

Table 2.7 In vitro rat skin permeation data of propranolol from composite MIP membrane after application of pH 7.4 phosphate buffer at 37±1^{o}C (mean±SD, n=3, *$P \leq 0.05$ of Flux between *S*- and *R*-isomer)

Formula	Flux of *R*-isomer ($\mu g\ cm^{-2}h^{-1}$)	Flux of *S*-isomer ($\mu g\ cm^{-2}h^{-1}$)	*S/R* ratio	Lag time of R-isomer (hr)	Lag time of S-isomer (hr)	Significant *P*-value*
Casting						
50%*S*-MIP	3.30±0.14	3.98±0.16	1.21±0.03	10.95±0.11	9.75±0.15	0.025
50% NIP	3.77±0.50	3.95±0.48	1.05±0.10	9.65±0.10	9.05±0.07	0.048
1:10 drug:*S*-MIP	1.10±0.13	2.12±0.21	1.93±0.15	21.35±0.15	20.15±0.24	0.010
1:10 drug:NIP	0.96±0.08	1.02±0.01	1.06±0.08	15.04±0.21	14.40±0.19	0.034
Grafting						
S-MIP #1	1.08±0.16	1.99±0.13	2.00±0.13	11.85±0.11	10.25±0.09	0.030
NIP #1	1.41±0.09	1.69±0.02	1.19±0.07	8.75±0.08	8.46±0.12	0.080