

# Cellulose membrane modified with molecularly imprinted polymer for the enantioselective-controlled release of non-steroidal anti-inflammatory drugs

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## Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) have been widely used for regional inflammatory disorders. The topical application of NSAIDs is a superior way to avoid the well-known gastrointestinal side-effects or other systemic side-effects induced by NSAIDs. The advantages of transdermal drug delivery of NSAIDs over other conventional routes of administration include enhanced therapeutic efficacy, better patient medication compliance and reduced systemic side-effects [1]. Among NSAIDs, 2-arylpropionic acids (profens) such as ibuprofen and naproxen exist as the racemic mixture of its enantiomeric forms. The pharmaceutical activity of these drugs is in the enantiomers of *S*, while the *R*-enantiomers are considerably less potent in inflammation and may residue in fatty tissue [2].

A molecularly imprinted polymer (MIP) is a synthetic polymer possessing selective molecular recognition properties because of recognition sites within the polymer matrix that are complementary to the analyte molecule in the shape and positioning of functional groups [3]. The use of MIPs as new sorbents for affinity membrane extraction is becoming more widespread, due to their potentially high selectivity. A number of composite membranes functionalized with thin MIP layers have been previously developed to attain adsorption specificity [4].

The aim of the study was to develop enantioselective-controlled release systems of NSAIDs by using the molecularly imprinted polymer (MIP) grafted on to cellulose membrane. Microporous cellulose membranes were generated by bacterial-cultivation and those were used as a matrix for a two-step grafting procedure to yield a MIP by *in situ* copolymerisation within the thin barrier layer of the base material. Using a silanisation procedure, supramolecular complexes of template molecules and suitable functional isopropenylates were grafted onto the surface of cellulose

membranes. MIP synthesis mixtures comprising 4-vinylpyridine (VPD) and acrylamide (ACM) as functional monomers, ethylene glycol dimethylmethacrylate (EDMA) and *N,N'*-methylenebisacrylamide (MBAA) as cross-linking monomers and *S*-ketoprofen or *S*-naproxen or *S*-ibuprofen as template, in the presence of the template enantiomer were successfully polymerized, which allowed the generation of MIP grafted membranes that might act as a selective barrier.

## 2. Experimental

### 2.1. Chemicals and reagents

Ethylene glycol dimethacrylate (EDMA), 4-vinylpyridine, acrylamide and 3-methacryloxypropyltrimethoxysilane (3-MPS) were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). Ketoprofen, naproxen, ibuprofen, *S*-ketoprofen, *S*-naproxen and *S*-ibuprofen were obtained from Aldrich Chemical Company (Milwaukee, WI, USA).. 2,2'-Azobis-(isobutyronitrile) (AIBN) was obtained from Janssen (Geel, Belgium). Yeast extract and peptone were obtained from Difco Laboratories, USA. Succinic anhydride and cyclopropanecarbonyl chloride were supplied by Fluka Chemie AG (Buchs, Switzerland). EDMA was purified by extraction with 10% (w/v) CaCO<sub>3</sub>, washing with water, drying over anhydrous sodium sulfate and subsequent distillation under reduced pressure. All solvents used were of analytical grade and were dried with molecular sieves before use. Working standard solutions were prepared daily. The stability of *S*-ibuprofen, *S*-ketoprofen and *S*-naproxen in pH 5.5 citrate and pH 7.4 phosphate buffer solutions was investigated at 4°C, room temperature (30°C) and 37°C, since these enantiomers were used under these conditions. The results showed that more than 99% content of both *S*-ibuprofen, *S*-ketoprofen and *S*-naproxen was found after 7 days incubation

at either temperature, indicating good stability of the enantiomers under these conditions.

## 2.2. Preparation of bacterial cellulose membranes

Cellulose membrane was obtained by incubating *Acetobacter xylinum* TISTR 975 in coconut juice supplemented with 4% sucrose (w/v) at pH 5.0. A stainless steel round shallow tray of 39 cm diameter was used to grow the cellulose-producing bacteria at 30°C at the surface of culture medium under static conditions. The culture volume was 500 ml and the effective area for membrane growth was 20 cm<sup>2</sup>. Buffered Schamm & Hestrin's medium (BSH medium) was employed as the pre-culture medium, composed of 2.0% (w/v) glucose, 0.5% (w/v) yeast extract, 0.5% (w/v) peptone, 0.033% (w/v) Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O and 0.011% (w/v) citric acid.H<sub>2</sub>O pH 5.0. *Acetobacter xylinum* TISTR 975 was grown in 50 ml of BSH medium for 3 days to use as a pre-culture. The pellicles of bacterial cellulose (0.01 mm thickness) formed on the surface of this medium surface were harvested aseptically.

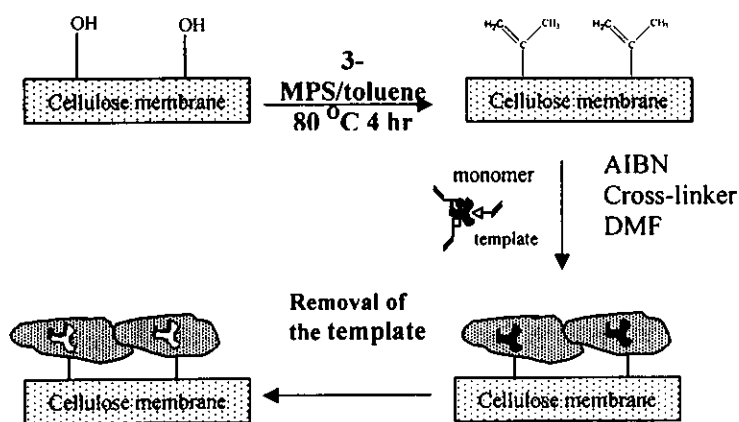
Cellulose membrane was prepared by placing 4 pieces of cut pellicle (1x1 cm) in the tray, approximately 10 cm from the edge of the tray and an equal distance from each other. For cellulose membrane production, the pre-culture of bacteria was inoculated into 500 ml of the culture media and the tray was covered with a linen cloth, followed by incubation for 1 day. The cellulose pellicle formed on the surface of the media was removed and discarded. The remaining culture was further incubated under the same conditions for a further day. The formed cellulose membrane was harvested and transferred to 1% (w/v) NaOH at 80°C for 24 h and then thoroughly washed with distilled water to remove any remaining associated



microorganisms and proteins. The pure cellulose sheets were dried at 37°C overnight and kept in a dust free atmosphere until required for use.

### 2.3. Preparation of MIP composite membrane

The grafting procedure of ethylene glycol dimethacrylate and 4-vinylpyridine copolymer onto the pore-containing surface of the bacterially-derived membrane is illustrated in Scheme 1. Since ketoprofen enantiomer is not soluble well in the solvent system used for synthesizing process, thus we used naproxen enantiomer as alternative for this study.



**Scheme 1.** Illustration of preparation of bacterial cellulose membrane grafted with molecularly imprinted polymer against S-naproxen or S-ibuprofen.

The grafting was carried out in the presence of the propranolol enantiomer template. A cellulose membrane was reacted with 3-MPS (10% w/w in toluene) at 80°C for 5 h. The resulting membrane was then thoroughly washed in methanol and dried. The reacted cellulose membrane was then placed in crystallizing dish, 18 cm in diameter. A solution containing 12 mmol of MAA (or ACM) as a functional monomer, 0.05 mol of EDMA (or MBAA) as a cross-linking monomer, 2 mmol of S-naproxen (S-ketoprofen or S-ibuprofen) as template and 0.7 mmol of AIBN as a radical polymerization initiator in DMF (2 ml) was poured onto the surface of cellulose membranes. The dish was then purged with nitrogen for 2-3 min to remove oxygen

(which acts as a radical scavenger) before closure and the temperature maintained at 60°C for 18 h. After polymerisation, membranes were transferred to a Soxhlet extractor and extracted with THF for at least 72 h before further extraction with methanol for 72 h to remove any non-grafted polymer, monomer, residual initiator and template molecule. The prepared membranes were dried *in vacuo* overnight. The complete removal of the template molecule from the polymer was confirmed by its absence from the final rinse of the polymer, as verified by HPLC method. Blank composite membranes (non-imprinted polymer (NIP) cellulose membranes), for control experiments were prepared in the same manner as MIP modified cellulose membrane but with the template molecule omitted (these membranes are referred to as NIP membranes). The change in weight of the membrane induced by the grafting procedure was measured using a microbalance (Precisa 300 A, USA: capacity 305 g, readability 0.1 mg). 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.

## 2.4. Characterisation methods

### 2.4.1. Surface morphology and cross-section study

The membrane morphology of the initial and modified cellulose was studied by scanning electron microscope observation of the cross-section and inner and outer surfaces at an accelerating voltage of 15 kV with the samples being sputter-coated with gold before imaging (Jeol serie JSM 5800LV, CA, USA). The morphology was further examined with an atomic force microscope (AFM) using a Digital Instruments NanoScope IIIa scanning probe microscope (Veeco Instruments GmbH, Germany).

AFM observations were carried out at room temperature without any previous treatment, using rectangular silicon nitride cantilevers with pyramidal tips.

#### *2.4.2. Pore size measurements*

The pore size of membranes was estimated from surface pictures of the membrane obtained by SEM with the aid of a Carnoy computer imaging program (Lab of Plant Systemics, Belgium).

#### *2.4.3. IR spectral analysis*

IR spectroscopy (FT-IR 1600, Perkin-Elmer, CT, USA) was used to confirm the attachment of MAA/EDMA copolymer to the cellulose polymer. In addition, ATR-FTIR spectra of cellulose membranes and MIP-modified cellulose membranes were determined using an Equinox 55 FT-IR spectrometer (Bruker, Switzerland). One hundred scans were obtained at a resolution of  $\pm 2 \text{ cm}^{-1}$ . A ZnSe internal reflection element was used in the ATR at an incident angle of  $45^\circ$ , giving an IR penetration depth of 0.5 - 5  $\mu\text{m}$ .

#### *2.4.4. Electrical resistance measurements*

The degree of membrane fibrillation was assessed from the value obtained for the resistance of membrane. Also, impedance measurement was used to confirm the deposition of a MIP layer onto the surface of the cellulose membranes. Electrochemical resistance measurements of membranes were carried out by short-circuit current technique using a Revision G Voltage-Current Clamp, Model VCC 600 (Harvard Apparatus, CA, USA). Ussing chambers having effective area of  $1 \text{ cm}^2$  were

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 current in the circuit. A 60  $\mu\text{A}$  current was applied and the membrane potential difference,  $PD$  (mV) and the short circuit current,  $I_{sc}$  (A) were recorded simultaneously. The membrane resistance,  $R_m$  ( $\Omega\cdot\text{cm}^2$ ) was calculated from  $PD/I_{sc}$ , based on the Ohm's law. These were corrected by eliminating the offset voltage between the electrodes and solution resistance, which was determined prior to each experiment using identical bathing solutions. All experiments were carried out at  $25\pm 1^\circ\text{C}$ .

#### 2.4.5. Mechanical properties measurements

The mechanical strength of the membranes was measured using a Universal testing machine (Lloyd, UK) with an operating head load of 100 N. The membranes were then placed between the grips of testing machine. The grip length was 2.5 cm and the speed of testing was set at the rate of 30 mm/min. Tensile strength was calculated according to the equation: tensile strength ( $\text{kN}/\text{m}^2$ ) = max load (kN)/cross sectional area ( $\text{m}^2$ ).

#### 2.4.6. Thermogravimetric analysis

Thermogravimetric analysis (TGA) of membranes was carried out using a Perkin-Elmer DTA7 analyzer (Perkin Elmer, CT, USA). A portion of membrane (0.5 mg) was heated from 50 to  $800^\circ\text{C}$  at  $10^\circ\text{C}/\text{min}$  in nitrogen gas.

#### 2.4.7. Degree of swelling of membranes

The degree of swelling of cellulose and modified cellulose membranes was evaluated in pH 5.5 and pH 7.4 buffers, since for some applications membranes were used in these conditions. 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 tubes containing phosphate buffer pH 7.4 or citrate buffer pH 5.5 at room temperature (~30°C). The membranes were incubated in the medium until the weight of wet membranes remained stable, which usually occurred after approximately 7 h incubation. Before measuring the weight of the wet membrane, surface water was gently removed with a tissue. The wet films were dried under vacuum at room temperature to a constant weight (over a minimum of 2 days) and stored in a desiccator at room temperature before measurements. The degree of swelling of the membrane (%) was calculated from the equation:  $(W_{wet} - W_{dry}) / W_{dry} \times 100\%$  where  $W_{dry}$  and  $W_{wet}$  are the weights of dried and wet samples, respectively. Each test was carried out in sets of three.

#### 2.5. Measurement of partition coefficient

The membrane-pH 5.5 or pH 7.4 buffer partition coefficient was evaluated by equilibrating racemic ibuprofen (or naproxen) solutions with a membrane. The difference between the initial and equilibrium concentrations of each enantiomer in the aqueous phase was determined and hence the amount of enantiomer of NSAIDs sorbed to the membrane was calculated. In a typical binding assay, the membrane (1cm<sup>2</sup>) was added to 5 ml of an aqueous solution containing 100 µg.ml<sup>-1</sup> of racemic propranolol, and stirred overnight at room temperature for equilibrium to be established. The partition coefficient ( $K$ ) was calculated from the equation:  $K = C_p / C_s$ ,

where  $C_p$  is the concentration of the analyte associated with the membrane, and  $C_s$  is the concentration of the analyte in the solution. The selectivity factor representing the effect of the imprinting process was the ratio of  $K$  of the *S*-isomer to  $K$  of the *R*-isomer.

## 2.6. Permeation determination

The enantioselective transport of the cellulose and modified cellulose membranes was evaluated by a dialysis method using a vertical Franz-type diffusion cell. A membrane (exposed area  $0.78 \text{ cm}^2$ ) was mounted between the two chambers of the diffusion cells, the volumes of the donor and receptor chambers being 1.0 ml and 2.5 ml, respectively. The required amount of racemic ibuprofen (or naproxen) was dissolved in either pH 5.5 or 7.4 phosphate buffer solutions ( $\mu = 0.2$ ) to obtain the donor solutions. Buffer (pH 7.4) was introduced into the receiver compartments of the diffusion cells. Drug release was measured by the removal of samples ( $250 \mu\text{l}$ ) from the receiving chamber at appropriate time intervals over 6 h. The volume of the sample withdrawn was replaced by the same volume of the medium. Each test was carried out in sets of six.

The diffusion of each enantiomer of NSAIDs was determined using the chiral-HPLC analytical method outlined in section 2.8. The cumulatively permeated amounts ( $\mu\text{g}$ ) were calculated and plotted as a function of time. The flux  $J$  ( $\mu\text{g cm}^{-2} \text{ h}^{-1}$ ) is defined by:

$$J = Q \cdot A^{-1} \cdot t^{-1}$$

where  $Q$  ( $\mu\text{g}$ ) is the amount of analyte permeated,  $A$  ( $\text{cm}^2$ ) is the effective membrane area and  $t$  (h) is the time. The selectivity of the membrane was defined as the ratio of the permeation flux of *S*-isomer to that of the *R*-isomer.

The paired *t*-test was employed to compare the amounts of the two enantiomers released at every time point. The significance level was set at  $p < 0.05$ .

### 2.7. *In vitro* percutaneous penetration study

Racemic naproxen (or ibuprofen) was dissolved in phosphate buffer saline (PBS pH 7.4) to produce drug concentration of  $100 \mu\text{g}\cdot\text{ml}^{-1}$ .

The *in vitro* percutaneous penetration study of the MIP-grafted membrane was performed with a Franz-type diffusion cell (see section 2.6). Male mice (8-9 weeks), weighing 28-32 g were sacrificed by snapping the spinal cord at the neck. Rectangular sections of dorsal skin were shaved and excised from the animal using surgical scissors. Adhering fat and other visceral debris were removed from the undersurface with tweezers. The excised skin was immediately mounted between the half-cells, with and without a coupled test membrane, such that the dermal surface was in contact with the receptor fluid and the epidermal side in contact with the test membrane (if present). Drug solution (1 ml) was applied to the membrane surface and the cell was maintained at  $37^\circ\text{C}$  by an external circulating water-bath. The receptor phase was stirred constantly at 250 rpm with a magnetic bar. An aliquot (250  $\mu\text{l}$ ) of receptor fluid was collected at set time intervals over 2 days and replaced with the same volume of fresh PBS. The concentration of NSAIDs in the collected sample was determined by HPLC (Section 2.8).

## 2.8. HPLC analysis

The amounts of enantiomers were analyzed by enantiospecific HPLC using chiral columns. The HPLC system consisted of a Waters 600 HPLC (Bedford, USA) with a Waters 717 plus autosampler, equipped with a Waters 486 Tunable absorbance detector. Chromatograms were recorded using a Waters 746 integrator. A flow rate of  $1.0 \text{ ml}\cdot\text{min}^{-1}$  was used throughout.

HPLC analyses of either ibuprofen enantiomers or ketoprofen enantiomers required a chiral-AGP protein column (150 mm x 3.1 mm I.D., particle size  $5 \mu\text{m}$ , ChromTech, Hägersten, Sweden), a mobile phase system of 100 mM dioctylamine in phosphate buffer pH 7.0 and UV detection at 273 nm. Typical retention times for *R*-ibuprofen, *S*-ibuprofen, *R*-naproxen and *S*-naproxen were 4.0, 6, 3.5 and 8 min, respectively. Correlation coefficients for the calibration curves in the range  $2\text{-}25 \mu\text{g}\cdot\text{ml}^{-1}$  for *R*- and *S*-enantiomers of ibuprofen and  $0.5\text{-}5 \mu\text{g}\cdot\text{ml}^{-1}$  for *R*- and *S*-enantiomers of naproxen were greater than 0.999. The sensitivity of detection was  $1.9 \mu\text{g}\cdot\text{ml}^{-1}$  for ibuprofen enantiomers and  $0.06 \mu\text{g}\cdot\text{ml}^{-1}$  for naproxen enantiomers and the reproducibility of the peak areas of both enantiomers of either naproxen or ibuprofen was more than 95%.



### 3. Results and discussion

IR spectra of both the parent cellulose and either VPD-EDMA or ACM-MBAA copolymerized cellulose membranes are shown in Figure 1. In comparing these spectra, an absorption peak at  $1720\text{ cm}^{-1}$ , was apparent in the MIP grafted polymers which was assigned to the stretching of the introduced C=O of acid groups.

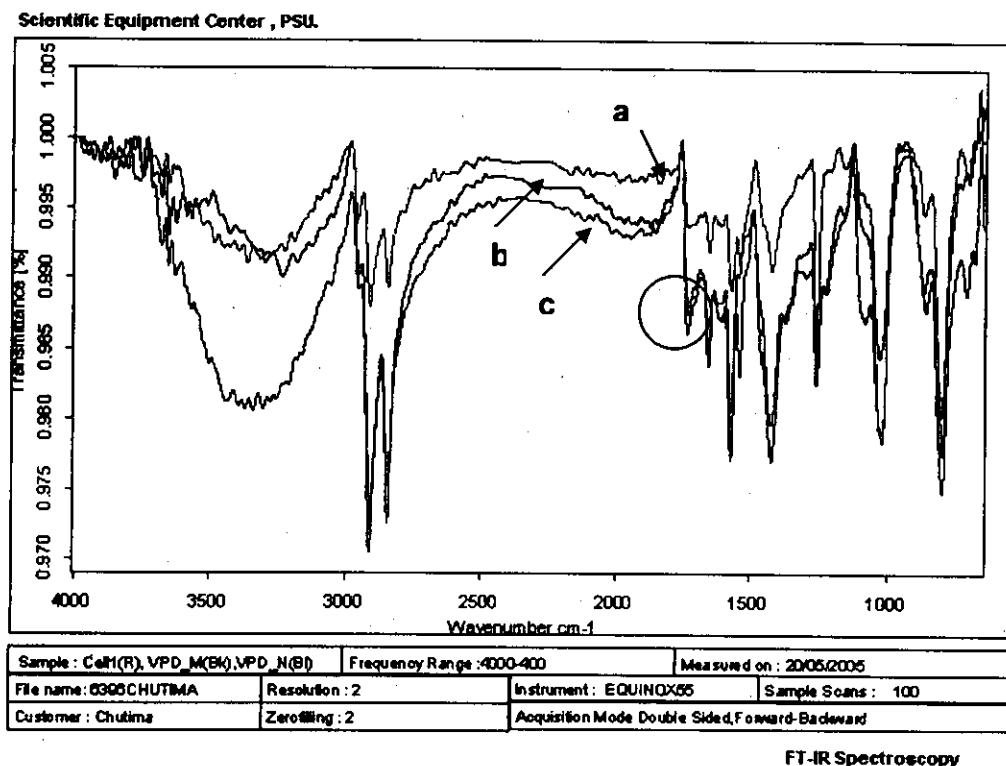
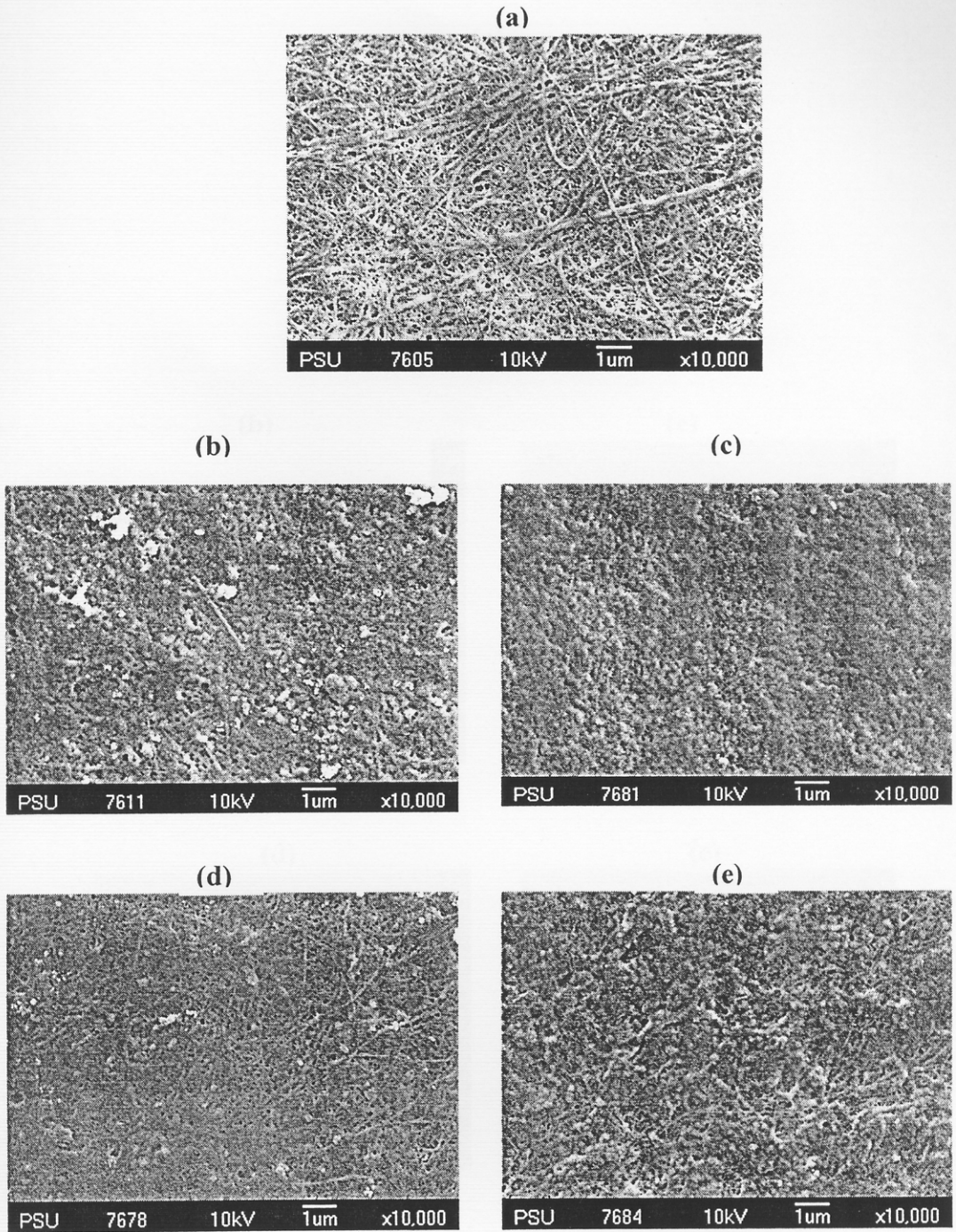
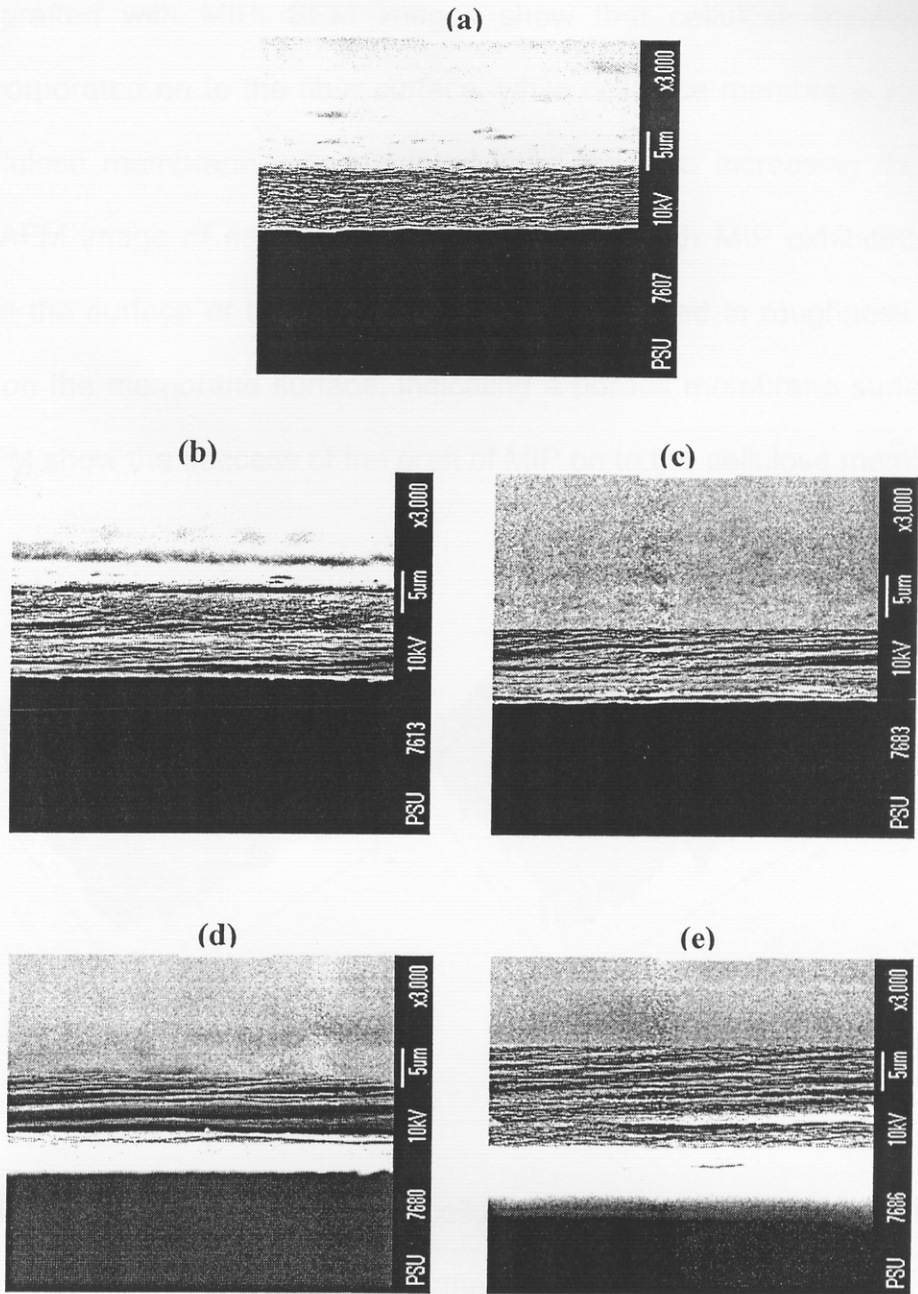


Figure 1: ATR-FTIR spectra of (a) cellulose membrane (b) MIP- and (c) NIP- membranes prepared with VPD-EDMA copolymer. The peak at  $1720\text{ cm}^{-1}$  correspond to the interaction of C=O groups in the grafted polymers.

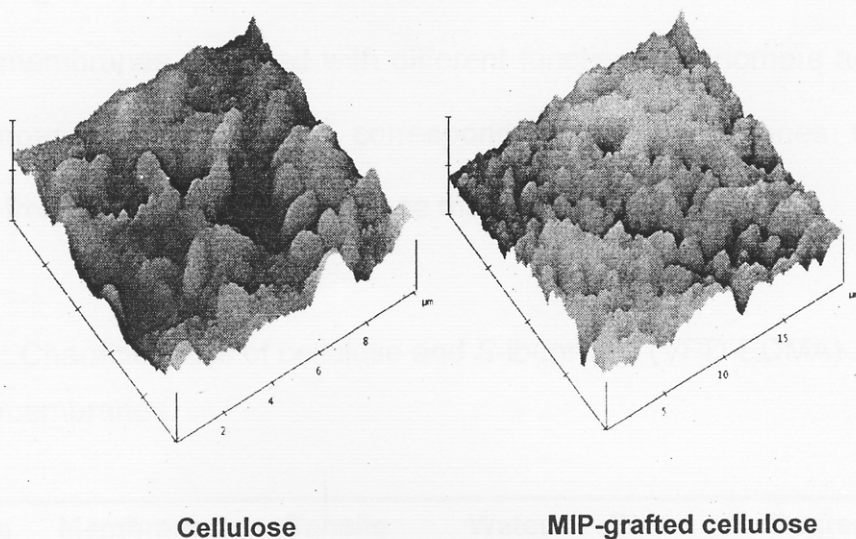


**Figure 3a:** SEM images showing surface morphology of (a) the initial cellulose membranes and the MIP modified membranes (bottom), (b) S-ibuprofen (VPD-EDMA)-, (c) S-ibuprofen (ACM-MBAA)-, (d) S-naproxen (VPD-EDMA)-, and (e) S-naproxen (ACM-MBAA)- MIP grafted cellulose membranes.



**Figure 3b:** SEM-cross-section images of (a) the initial cellulose membranes and the MIP modified membranes (bottom), (b) S-ibuprofen (VPD-EDMA)-, (c) S-ibuprofen (ACM-MBAA)-, (d) S-naproxen (VPD-EDMA)-, and (e) S-naproxen (ACM-MBAA)-MIP grafted cellulose membranes.

SEM and AFM were used to visualize the surface of bacterial cellulose membrane grafted with MIP. SEM images show that cellulose membrane had additives incorporated on to the fiber surface, while cellulose membrane (reference) did not. Cellulose membrane with imprinted layer leads to increasing the surface roughness. AFM image of cellulose membrane grafted with MIP exhibited a rough surface while the surface of the reference slightly decreased in roughness and had many fibrils on the membrane surface, indicating a porous membrane surface. Both SEM and AFM show the success of the graft of MIP on to the cellulose membrane.



**Figure 4.** AFM image showing bacterial cellulose membrane (left) and S-ibuprofen (VPD-EDMA) MIP grafted cellulose membranes (right).

Fibers of bacterial cellulose membrane were still apparent after modification with MIP but when the MIP was applied to this cellulose membrane, it did not penetrate as effectively into the tighter meshed polymer chains but accumulating at the surface. The SEM micrographs would suggest that a great amount of MIPs is coupled to the outer surface of the cellulose. In comparison, the copolymer of ACM/MBAA covers the outer surface of the cellulose more efficiently than the



copolymer of VPD/EDMA does. SEM studies revealed that modified cellulose membranes have larger pore at membrane surface than cellulose membrane, indicating occlusion of the pores by modification with the copolymer. The SEM micrographs indicated increase of the thickness of membrane bases (10-14  $\mu\text{m}$ ) about 1-2  $\mu\text{m}$  after grafting with a MIP. The modified membranes have an average degree of modification (DM) of 1  $\text{mg}/\text{cm}^2$ .

### 3.2. Characterisation of MIP-grafted cellulose membranes

The general properties of either S-ibuprofen- or S-naproxen-MIP grafted cellulose membranes prepared with different functional monomers and cross-linkers were compared with those of corresponding NIP membranes which were all produced from the same basic cellulose membrane (Table 2.1-2.4).

**Table 2.1:** Characteristics of cellulose and S-ibuprofen (VPD-EDMA)-MIP grafted cellulose membranes.

Membrane	Membrane resistance ( $\Omega.\text{cm}^2$ )	Tensile strength ( $\text{kN}/\text{m}^2$ )	Water content (%)	Pore size (nm)*	Degree of swelling (%)**	
					pH 5.5	pH 7.4
Cellulose	1.88	10.73 $\pm$ 4.13	3.10	70	91.66 $\pm$ 7.21	70.83 $\pm$ 7.21
NIP	3.76	12.70 $\pm$ 2.04	4.02	56	60.60 $\pm$ 18.92	48.48 $\pm$ 10.49
MIP	3.76	15.05 $\pm$ 7.74	2.07	56	78.78 $\pm$ 5.24	51.51 $\pm$ 13.88

\* Refer to mean pore size estimated from surface pictures of membrane obtained by SEM ( $\pm$  SE, n=10).

\*\* mean  $\pm$  SE, n=3.

**Table 2.2:** Characteristics of cellulose and S-ibuprofen (ACM-MBAA)-MIP grafted cellulose membranes.

Membrane	Membrane resistance ( $\Omega \cdot \text{cm}^2$ )	Tensile strength ( $\text{kN/m}^2$ )	Water content (%)	Pore size (nm)*	Degree of swelling (%)**	
					pH 5.5	pH 7.4
Cellulose	1.88	10.73 $\pm$ 4.13	3.10	70	91.66 $\pm$ 7.21	70.83 $\pm$ 7.21
NIP	3.13	4.81 $\pm$ 4.71	4.88	42	85.18 $\pm$ 6.41	51.85 $\pm$ 6.41
MIP	3.13	6.06 $\pm$ 2.53	3.33	42	88.88 $\pm$ 11.11	59.21 $\pm$ 6.41

Refer to mean pore size estimated from surface pictures of membrane obtained by SEM ( $\pm$  SE, n=10).

\*\* mean  $\pm$  SE, n=3.

**Table 2.3:** Characteristics of cellulose and S-naproxen (VPD-EDMA)-MIP grafted cellulose membranes.

Membrane	Membrane resistance ( $\Omega \cdot \text{cm}^2$ )	Tensile strength ( $\text{kN/m}^2$ )	Water content (%)	Pore size (nm)*	Degree of swelling (%)**	
					pH 5.5	pH 7.4
Cellulose	1.88	10.73 $\pm$ 4.13	3.10	70	91.66 $\pm$ 7.21	70.83 $\pm$ 7.21
NIP	3.13	8.20 $\pm$ 0.08	3.52	56	107.4 $\pm$ 12.83	59.25 $\pm$ 23.12
MIP	3.13	4.52 $\pm$ 2.70	2.77	56	85.18 $\pm$ 12.83	66.66 $\pm$ 11.11

Refer to mean pore size estimated from surface pictures of membrane obtained by SEM ( $\pm$  SE, n=10).

\*\* mean  $\pm$  SE, n=3.

**Table 2.4:** Characteristics of cellulose and S-naproxen (ACM-MBAA)-MIP grafted cellulose membranes.

Membrane	Membrane resistance ( $\Omega \cdot \text{cm}^2$ )	Tensile strength ( $\text{kN/m}^2$ )	Water content (%)	Pore size (nm)*	Degree of swelling (%)**	
					pH 5.5	pH 7.4
Cellulose	5.02	8.20 $\pm$ 0.88	3.02	70	73.80 $\pm$ 4.12	64.28 $\pm$ 7.14
NIP	6.27	9.08 $\pm$ 2.79	3.44	42	92.85 $\pm$ 0.01	80.95 $\pm$ 4.12
MIP	6.27	4.75 $\pm$ 0.85	3.83	42	92.85 $\pm$ 7.14	78.57 $\pm$ 7.14

\* Refer to mean pore size estimated from surface pictures of membrane obtained by SEM ( $\pm$  SE, n=10).

\*\* mean  $\pm$  SE, n=3.

In general, the values obtained for the electrical resistance of membranes provided an indication of the leakage of membrane and it was found that the electrical resistance of the cellulose membrane increased upon modification. This was presumably a consequence of the potential occlusion of the pores by modification with the copolymer. An increase in the tensile strength of the membranes after modification was found, possibly as a consequence of the introduction of a rigid copolymer of VPD/EDMA within the pores of cellulose membrane, leading to a decrease in chain movement. Modifying cellulose membrane with MIP comprising of ACM/MBAA copolymer also allows a reduction in the tensile strength of cellulose membrane, suggesting that copolymer of VPD/EDMA or ACM/MBAA cover onto the surface of cellulose membrane rather than fill into the pore of cellulose membrane. It was found that both cellulose and modified cellulose membranes swelled to a greater extent in pH 5.5 than in pH 7.4 buffer.

Thermogravimetric analysis indicated that the parent cellulose was a thermostable polymer, with 80% of the weight loss occurring at temperature of 460°C. This was the only sharp weight loss that occurred and it was ascribed to the



decomposition of polymer main chain. When the MIP and NIP membranes were analysed by TGA, two sharp transitions of loss in weight (occurred in two separate temperature ranges). The first, at around 100°C was attributed to desorption of water from the membrane, and this allowed the water content of the membranes to be determined and this was found to be about 2-4%. The second large weight loss occurred at approximately 460°C, corresponding to the composition of the cellulose membrane.

### *3.3. Enantiomer uptake and imprinting effect*

#### *3.3.1. Effect of type of monomer on imprinting effect of membrane*

The partition coefficient of enantiomers of NSAIDs from aqueous solutions into the MIP membranes prepared using different functional monomers and cross-linkers was compared in order to determine the effect of type of monomer on imprinting efficiency (Table 3.1-3.2). The selectivity of the membranes, expressed as the ratio of the partition coefficient of the *S*-isomer expressed as a ratio of that of the *R*-isomer was found markedly higher for the *S*-MIP membrane in comparison that achieved in the control membrane. This difference indicates that the molecular imprinting procedures have produced cavities with a higher affinity for the *S*-enantiomer of NSAIDs in MIP. The selective sorption of the MIP grafted cellulose membrane prepared with ACM/MBAA copolymer was greater than that prepared with VPD/EDMA copolymer. In general, *S*-ibuprofen-MIP grafted cellulose membranes adsorbed template enantiomers more efficiently than *S*-naproxen-MIP grafted cellulose membranes.



**Table 3.1:** Partition coefficient ( $K$ ) of ibuprofen enantiomers and diffusion coefficient ( $D$ ,  $\mu\text{m}\cdot\text{min}^{-1}$ ) of *S*-ibuprofen MIP grafted cellulose membranes prepared with different type of monomer (mean  $\pm$  SE,  $n=3$ ) for pH 5.5 buffer.

Solute*	VPD-EDMA Membrane		ACM-MBAA Membrane	
	$K$	$D$	$K$	$D$
<i>R</i>	110.02	6.21	68.91	1.58
<i>S</i>	353.22	8.21	332.95	3.13
<i>S/R</i> ratio	3.21	1.32	4.83	1.98

\* Refer to *R*- or *S*-isomer of ibuprofen.

**Table 3.2:** Partition coefficient ( $K$ ) of naproxen enantiomers and diffusion coefficient ( $D$ ,  $\mu\text{m}\cdot\text{min}^{-1}$ ) of *S*-naproxen MIP grafted cellulose membranes prepared with different type of monomer (mean  $\pm$  SE,  $n=3$ ) for pH 5.5 buffer.

Solute*	VPD-EDMA Membrane		ACM-MBAA Membrane	
	$K$	$D$	$K$	$D$
<i>R</i>	35.63	2.97	56.02	3.92
<i>S</i>	9.70	3.66	9.21	12.45
<i>S/R</i> ratio	3.67	1.23	6.08	3.17

\* Refer to *R*- or *S*-isomer of naproxen.

Sequential diffusion measurements with enantiomers of NSAIDs indicate an initial delay for diffusion (higher sorption) and an overall slightly faster transport of the template for MIP grafted cellulose membranes. Ratio of effective diffusion coefficients (ideal separation factor) for MIP grafted cellulose membranes indicates a slightly facilitated transport of the template enantiomers determined by imprinting. The selective diffusion of the MIP grafted cellulose membrane prepared with ACM/MBAA copolymer was greater than that prepared with VPD/EDMA copolymer.

### 3.3.2. Effect of amount of monomer on imprinting effect of membrane

The effect of monomer on the controlled release of membrane has been investigated for cellulose membrane grafted with MIP that was prepared by using ACM as the functional monomer and MBAA as the cross-linking monomer. It was found that increasing of MBAA content in polymer layer results in a decrease of flux of both enantiomer as this is due to an increase in the thickness of barrier. The selectivity of the membrane for ibuprofen enantiomer was optimal at monomer content of 1.6 mM.

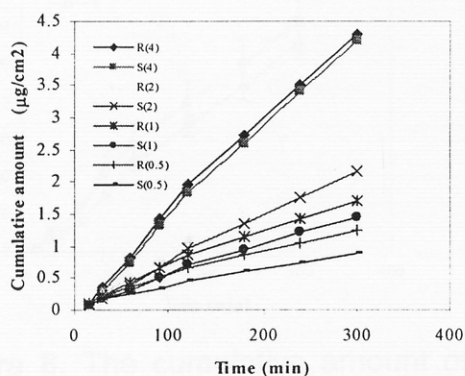
**Table 4:** Partition coefficient ( $K$ ) of ibuprofen enantiomers for S-ibuprofen MIP grafted cellulose membranes prepared by copolymerization of ACM and MBAA (mean  $\pm$  SE, n=3).

Amount of Monomer (M)	Solute*	$K$			
		pH 5.5		pH 7.4	
0.8	R	303.70		179.50	
	S	335.59	1.1	235.49	1.31
1.6	R	68.91		127.22	
	S	332.95	4.83	297.38	2.33
3.2	R	53.72		107.36	
	S	240.89	4.48	267.31	2.48
6.4	R	57.02		96.23	
	S	219.59	3.85	200.36	2.08

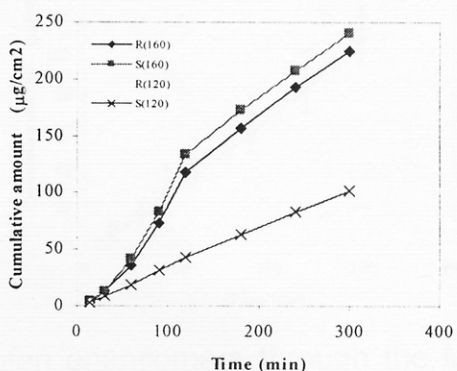
The selective sorption of the composite MIP membrane proved sensitive to the pH of the sample solution as the binding affinity of *S*-propranolol to cellulose at pH 5.5 was greater than at pH 7.4.

### 3.4. Selective transport of composite MIP membranes

From the results obtained in binding studies, bacterial cellulose membrane (blank membrane) seems to adsorb the *R*-naproxen greater than the *S*-naproxen. In contrast, cellulose membrane similarly adsorbed two enantiomers of ibuprofen. The partition coefficient ( $K$ ) decreased with the modification of surface of cellulose membrane. The enantioselectivity for binding of naproxen on the cellulose membrane modified with VPD-EDMA copolymer was greater than that on cellulose and corresponding NIP grafted cellulose, in both pH 5.5 and 7.4. In all the cases the binding of *R* and *S*-ibuprofen on the membranes was not significantly different.



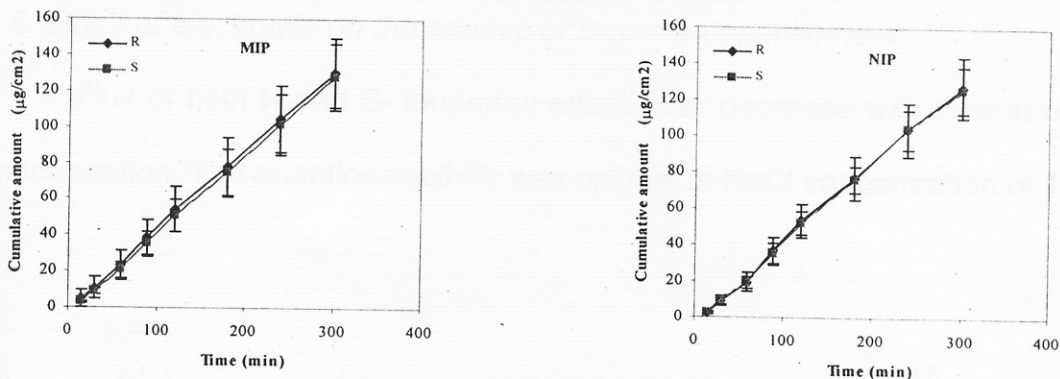
**Figure 5:** The cumulative amount of **naproxen** enantiomers in variety of concentration ( $\mu\text{g/ml}$ ) in donor phase through the **bacterial cellulose**



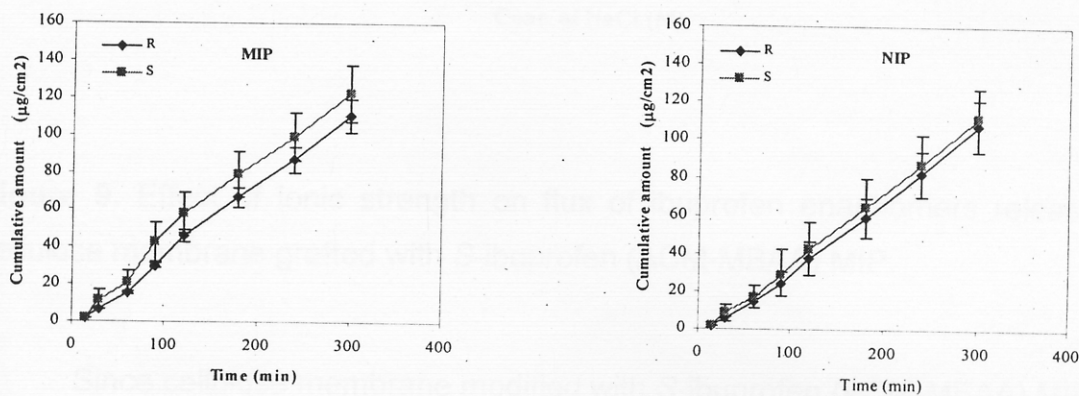
**Figure 6:** The cumulative amount of **ibuprofen** enantiomers in variety of concentration ( $\mu\text{g/ml}$ ) in donor phase through the **bacterial cellulose**

Subsequent release study of bacterial cellulose membrane (membrane base) for racemic naproxen and ibuprofen revealed that bacterial cellulose membrane retarded the release of *S*-naproxen especially at low concentration, and it did not show any difference in release of ibuprofen enantiomers, both at high and low

concentrations(see Figure 5). Bacterial cellulose tended to release more of the S-ibuprofen than the R-ibuprofen (see Figure 6).



**Figure 7.** The cumulative amount of ibuprofen enantiomers through the MIP- and NIP-cellulose membrane (VPD-EDMA polymer).



**Figure 8.** The cumulative amount of ibuprofen enantiomers through the MIP- and NIP-cellulose membrane (AM-MBA polymer).

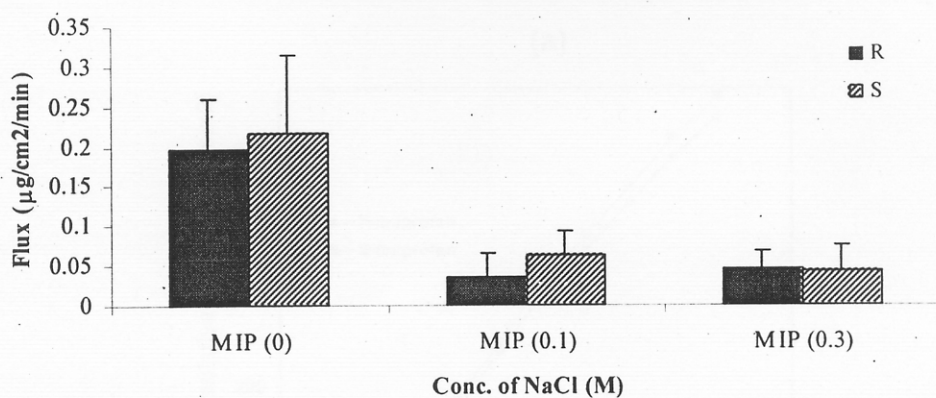
Cellulose membrane grafted with S-naproxen MIP layer that was prepared with the copolymer of ACM/MBAA and its corresponding NIP grafted cellulose membrane were studied for release of naproxen enantiomers. It was found that the release of naproxen enantiomers from both MIP- and NIP-grafted cellulose membranes was not different, as shown in Figure 7. In contrast, cellulose membrane grafted with S-ibuprofen MIP layer comprising of the copolymer of ACM/MBAA gave



a difference in release of *S*-ibuprofen and *R*-ibuprofen at every time points (see Figure 8). This difference was found to be statistically significant ( $P < 0.05$ ).

### 3.5. Effect of electrolyte on the release of ibuprofen enantiomers.

Flux of both *R*- and *S*-ibuprofen enantiomer decrease with increasing the salt concentration. The enantioselectivity was optimal at NaCl concentration of 100 mM.

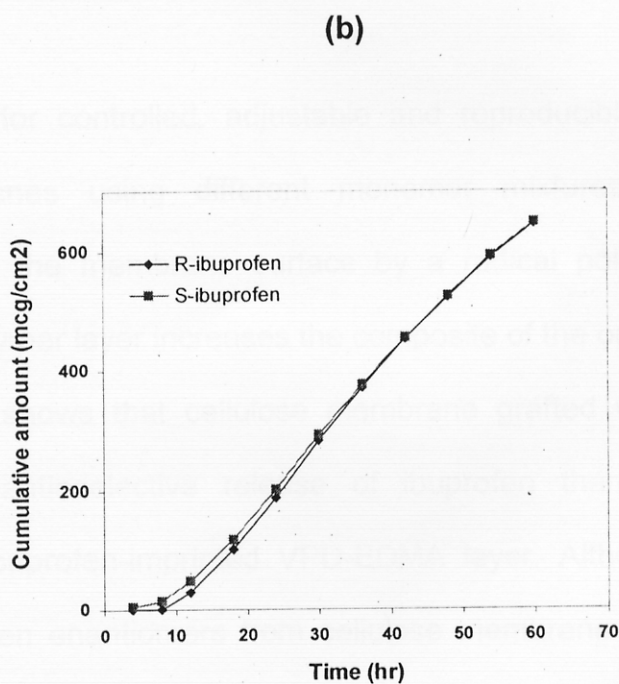
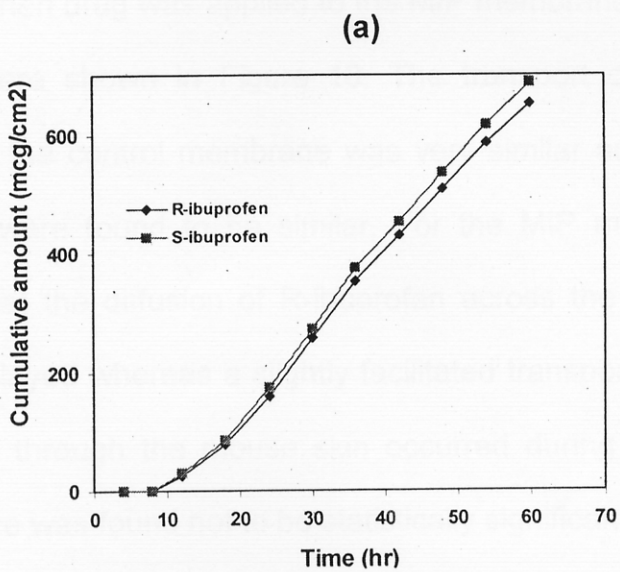


**Figure 9.** Effect of ionic strength on flux of ibuprofen enantiomers released from cellulose membrane grafted with *S*-ibuprofen (ACM-MBAA) MIP.

Since cellulose membrane modified with *S*-ibuprofen (ACM-MBAA) MIP shows selectivity for releasing of ibuprofen enantiomer, and also the preferred enantiomer for diffusion is eutomer, thus the cellulose membrane modified with *S*-ibuprofen (ACM-MBAA)-MIP was used as selective membrane for enantioselective-controlled release of racemic ibuprofen.

### 3.6. Transdermal enantioselective-controlled release of *S*-ibuprofen MIP-grafted cellulose membranes

The *in vitro* percutaneous permeation study of ibuprofen enantiomers when passing through S-ibuprofen MIP-grafted cellulose membranes was investigated using mouse excised skin. The selective transfer of the MIP membrane was performed with the concentration of racemic ibuprofen in the donor phase being  $250 \mu\text{g}\cdot\text{ml}^{-1}$ . Control experiment determined the percutaneous transport of drug across NIP grafted cellulose membrane placed on mouse skin.



**Figure 10:** The permeation of propranolol enantiomers from pH 7.4 buffer solution across full-thickness mouse skin at 37 °C in the presence of (a) S-ibuprofen(ACM-MBAA) MIP grafted cellulose membrane or (b) NIP grafted cellulose membrane placed on the surface of the skin (mean  $\pm$  SE, n=6).

The cumulative amounts of ibuprofen transported across mouse skin as a function of time, when drug was applied to the MIP membrane positioned in place on the skin surface are shown in Figure 10. The transport of both enantiomers of ibuprofen through the control membrane was very similar and the flux rates of the two enantiomers were found to be similar. For the MIP membrane, the resulting curves indicate that the diffusion of R-ibuprofen across the S-MIP membrane-skin layer is slightly delayed whereas a slightly facilitated transport of S-ibuprofen across S-MIP membrane through the mouse skin occurred during 20-60 h of transport. Also, this difference was found not to be statistically significant ( $P > 0.05$ ).

## Conclusion

Conditions for controlled, adjustable and reproducible grafting of bacterial cellulose membranes using different monomer mixtures are established. A premodification of the membrane surface by a radical polymerization towards a ACM-MBAA copolymer layer increases the composite of the outer surface.

The result shows that cellulose membrane grafted with ACM-MBAA layer shows higher enantioselective release of ibuprofen than cellulose membrane modified with S-ibuprofen-imprinted VPD-EDMA layer. Although the difference of release of ibuprofen enantiomers from cellulose membrane grafted with AM-MBA-based MIP layer is statistically significant the enantioselectivity shown is modest.

However, there is no enantioselectivity of the diffusion of ibuprofen across the MIP grafted cellulose membrane placed on the mouse skin. A development of the preparation method of combining MIP into cellulose membrane allows an increase in the enantioselective release of ibuprofen. Bacterial cellulose membrane with a suitable controlled surface and pore functionalization may yield a membrane with appropriate transport properties for the target compound.

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