4.1 Materials

4.1.1 Membranes

The commercial polysulfone (PS) and polyethersulfone (PES) membranes used in this work are listed in Table 4.1. Figure 4.1 presents the polymer chemical structure.

![Figure 4.1 Chemical structure of PS and PES](image)

4.1.2 Feed

4.1.2.1 Sugarcane juice

Fresh sugarcane mixed juice (from mill house) and clarified juice (from clarifier) was collected from a local sugar mill located about 100 km from New Delhi. Untreated sugarcane juice was obtained from a local handmill. The mill samples were stored in a freezer at approximately -4°C. Required amounts of the sample were thawed to room temperature prior to membrane filtration. Samples once used were discarded and a fresh lot used for each experiment.
4.1.2.2 Polysaccharide fraction

The polysaccharide fraction was isolated following the protocol described by López-Barajas et al. 1998). Required quantity of the clarified or untreated juice was thawed and the polysaccharide constituents were precipitated by addition of 0.5 ml of 0.1M HCl (Merck (India) Limited, Bombay, India) followed by around 23 ml of 96% ethanol (Bengal Chemicals and Pharmaceuticals Ltd., Kolkata, India or Changshu Yangyuan Chemical, China) per 10 ml of juice. The ethanol volume was experimentally adjusted to ensure maximum precipitation. The sample was left overnight at ambient temperature to allow the precipitate to settle. The supernatant was decanted and the precipitate was filtered through a 0.45μm MF membrane (Millipore, U.S.A.) and dried in an oven at 45-50˚C until it reached constant weight. To obtain the soluble fraction, the sample was dissolved in MilliQ water and filtered through a 0.45μm MF membrane (Gelman Sciences, U.S.A.). The filtrate was frozen and then lyophilized (Christ-Alpha 1-4 freeze drier, Osterode am Harz, Germany). The freeze-dried samples were stored in a vacuum desiccator. All polysaccharide UF experiments were carried out with 1.5 g/L freshly reconstituted solution of the freeze-dried fraction.

Table 4.1. Membranes investigated

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Material*</th>
<th>Supplier</th>
<th>NMWCO* (kD)</th>
<th>PWP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFUF15</td>
<td>Surface modified PES</td>
<td>Permionics, India</td>
<td>15</td>
<td>NA</td>
</tr>
<tr>
<td>HFUF30</td>
<td>Surface modified PES</td>
<td>Permionics, India</td>
<td>30</td>
<td>NA</td>
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<td>PES</td>
<td>Permionics, India</td>
<td>50</td>
<td>NA</td>
</tr>
<tr>
<td>P010F</td>
<td>Permanently hydrophilic PES</td>
<td>Microdyn-Nadir, Germany</td>
<td>10</td>
<td>150-250</td>
</tr>
<tr>
<td>P020F</td>
<td>Permanently hydrophilic PES</td>
<td>Microdyn-Nadir, Germany</td>
<td>20</td>
<td>200-400</td>
</tr>
<tr>
<td>UF-PES-030H</td>
<td>Permanently hydrophilic PES</td>
<td>Microdyn-Nadir, Germany</td>
<td>30</td>
<td>100-250</td>
</tr>
<tr>
<td>UF-PES-050H</td>
<td>Permanently hydrophilic PES</td>
<td>Microdyn-Nadir, Germany</td>
<td>50</td>
<td>250-500</td>
</tr>
<tr>
<td>UF-PS-100H</td>
<td>Permanently hydrophilic PS</td>
<td>Microdyn-Nadir, Germany</td>
<td>100</td>
<td>300-600</td>
</tr>
<tr>
<td>P150F</td>
<td>Permanently hydrophilic PS</td>
<td>Microdyn-Nadir, Germany</td>
<td>150</td>
<td>2100</td>
</tr>
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<td>PES</td>
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<td>10</td>
<td>NA</td>
</tr>
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</tr>
<tr>
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<td>PS</td>
<td>Alpha-Laval, Denmark</td>
<td>100</td>
<td>NA</td>
</tr>
</tbody>
</table>

* Suppliers’ data, **Test condition: 3bar, 20°C, 700rpm
4.1.2.3 High molecular weight (HMW) component in polysaccharide fraction

The HMW component in the polysaccharide fraction (prepared in section 4.1.2.2) was isolated as follows. 1.69 g of the water soluble polysaccharide fraction was dissolved in 50 ml MilliQ water. To this solution, 0.315 g of NaN₃ (Jansen Chimica, Belgium) was added. The solution was diafiltered using 10kD Hydrosart UF membrane (Sartorius AG, Göttingen, Germany). Prior to diafiltration, the membrane was rinsed with 100% ethanol for 2 h followed by water rinsing for 30 minutes. The diafiltration lasted 72 h. The isolation of the HMW component was monitored by size exclusion chromatography (SEC) (Jasco PU-2080 Plus, Japan) by analyzing the permeate collected at regular time intervals. The HMW component was retained by the membrane while the lower molecular weight constituents passed into the permeate. The dialysis was considered to be completed once no low molecular weight constituents were detected in the permeate. The retentate was freeze-dried (Christ-Alpha 1-4 freeze drier, Osterode am Harz, Germany). This freeze-dried fraction was re-dissolved in water and any insolubles were allowed to settle down. The supernatant was decanted and again freeze dried under the same conditions, to obtain the HMW isolate.

4.2 Methods

4.2.1 Feed characterization

4.2.1.1 Protein analysis

4.2.1.1.1 Kjeldahl method

The total nitrogen content in sugarcane juice was determined by Kjeldahl method (AOAC Method #920.152). 1 ml of sample was mixed with 0.35 g mercuric oxide (HiMedia Laboratories Limited, Mumbai, India), 3.5 g anhydrous sodium sulfate (Qualigens Fine Chemicals, Mumbai, India), 10 ml concentrated sulfuric acid (Qualigens Fine Chemicals, Mumbai, India) and digested for 1-2 h till a clear solution was obtained. The digested mixture was cooled to room temperature and 12.5 ml of 8% sodium thiosulfate solution (Qualigens Fine Chemicals, Mumbai, India) was added to precipitate the Hg. The supernatant volume was made up to 100 ml. 35 ml of strong NaOH solution (450 g in 1 L water) was added to 5 ml of this diluted sample and the mixture was distilled in a Wagner-Parnas apparatus with the tip of the condenser...
immersed in a conical flask containing 0.1 N sulfuric acid with 3 drops of methyl red indicator. Once the NH$_3$ was completely distilled, 5-6 ml of condensate was collected and the excess standard acid in the conical flask was titrated with standard NaOH solution. For each set of experiments, blank correction was also performed. The % protein was calculated as follows:

\[
\%N = \frac{[(\text{ml of standard acid} \times \text{normality of acid}) - (\text{ml of standard NaOH} \times \text{normality of NaOH})] \times 1.4007}{(1/100) \times 5}\]

\[
\% \text{ protein} = \%N \times 6.25
\]

4.2.1.2 UV spectroscopy

This was used for estimating the protein content in the sugarcane juice polysaccharide fraction. Samples were analyzed at 280 nm using a 1 cm path length quartz cuvette in a UV spectrophotometer (Varian, CARY-50Probe, Germany). The solution concentration was 1.5 mg/ml.

4.2.1.3 BCA protein microassay

Protein content of juice polysaccharide fraction was also estimated by the bicinchoninic acid (BCA) microassay (Smith et al., 1985). Standard solution of the macromolecular polysaccharide fraction was prepared in phosphate buffer (pH 7). Micro BCA reagents A, B, and C were obtained from Pierce, Rockford, U.S.A. The reaction mixture (1:1 sample to reagent) was incubated for 2 h at 37°C after which it was allowed to stand for 20 minutes to cool down to ambient temperature. Absorbance was measured at 562 nm in a 92 well microplate spectrophotometer (µQuant, BIO-TEK Instruments Inc., U.S.A) and the protein content was determined from the standard calibration curve prepared with BSA (bovine serum albumin). For feed solutions prepared in water, 50 µl of the sample was diluted to 150 µl by adding phosphate buffer before analysis.

4.2.1.2 Carbohydrate analysis

4.2.1.2.1 Total carbohydrate

The total carbohydrate in sugarcane juice was estimated by phenol-sulfuric acid method (Dubois, 1956). 1 ml of juice sample, diluted appropriately with RO water, was treated simultaneously with 1 ml of 5% phenol solution (Qualigens Fine Chemicals, Mumbai, India) and 6 ml of concentrated sulfuric acid (Qualigens Fine Chemicals, Mumbai, India). The solution was mixed in a vortex mixer and then allowed to stand for at least 1 h for complete color development. The absorbance was measured at 480 nm in a spectrophotometer (Spectronic
20D, Milton Roy Company, U.S.A.). The total carbohydrate content was calculated from the standard curve for glucose.

4.2.1.2.2 Dextran

Dextran was analyzed using Roberts’ copper method (Altenburg, 1993). 1 ml of juice sample was treated with 0.3-0.4 g of bentonite powder (HiMedia Laboratories Limited, Mumbai, India), 0.1 ml of 10% trichloroacetic acid (Qualigens Fine Chemicals, Mumbai, India) and 4 ml of absolute ethyl alcohol (Bengal Chemicals and Pharmaceuticals Ltd., Kolkata, India). The precipitate obtained was washed with 80% ethyl alcohol (Bengal Chemicals and Pharmaceuticals Ltd., Kolkata) in a Whatman 42 filter paper, dissolved in 3 ml water and then filtered again through Whatman 42 filter paper. 2 ml of the filtrate was mixed with 0.4 ml 2.5 N NaOH (Qualigens Fine Chemicals, Mumbai, India), 0.4 ml copper reagent and 0.03-0.04 g bentonite and heated in a boiling water bath for 5 minutes. The copper reagent was prepared by dissolving 3 g of CuSO₄·5H₂O (HiMedia Laboratories Limited, Mumbai, India) and 30 g of sodium citrate (Central Drug House (P) Limited, Mumbai-New Delhi, India) in 1 L RO water. The copper-dextran complex after heating was precipitated on the bentonite filter aid. The solution was cooled to room temperature and the precipitate was filtered through sintered glass filter (grade 2) and washed with a solution prepared by mixing 50 ml water, 10 ml copper reagent solution and 10 ml 2.5 N NaOH. The precipitate was placed on a vacuum filter and washed successively with 0.8 ml sulfuric acid, followed by 0.2 ml water. The filtrate was collected and its volume was made up to 3.5 ml with water.

The filtrate was used to conduct the phenol-sulfuric acid test as described in section 4.2.1.2.1. Each sample was tested in triplicate. The standard curve was prepared using standard glucose solution. Since dextran is anhydroglucose with 90% molecular weight equivalent of glucose, the concentration of dextran was estimated by multiplying the concentration of glucose by a factor of 0.9. The dextran concentration was calculated as follows:

Dextran, mg = [glucose concentration from standard curve × 0.9 × final volume of copper-dextran complex × solution of alcohol precipitate] / [aliquot taken for Cu precipitation × aliquot taken for alcohol precipitation]

= [glucose concentration from standard curve × 0.9 × 3.5 × 3]/ [2 × 1].............(3)
4.2.1.3 Elemental analysis
The carbon, nitrogen, hydrogen and oxygen content of the juice polysaccharide fraction was determined using the CHNS-O analyzer (CE-Instruments- EA1110, Italy). The iron (Fe^{2+}) and calcium (Ca^{2+}) content was determined by atomic absorption spectrophotometer (Unicam 939, Germany) after sample digestion in 1:1 HCl-water mixture.

4.2.1.4 Size exclusion chromatography (SEC)
The feed, permeate & retentate samples obtained from the UF of the juice polysaccharide fraction were analyzed by size exclusion chromatography (SEC) using a Jasco PU-2080 Plus HPLC system (Japan) with a Waters RI R401 refractive index detector (U.S.A.). A PSS Suprema linear column (particle size: 10 µm, dimension: 600/8 mm) was used. The column was equilibrated with MilliQ water containing 0.01 M sodium azide and calibrated in the range of 180 to 300,000 D with standard dextran calibration kit (Polymer Standard Service, Mainz, Germany). The detector temperature was set at 40°C and the eluent flow rate was 1 ml/min. The time axis of the chromatogram was converted into molecular weight by using the dextran calibration curve with PSS software (Mainz, Germany).

4.2.1.5 Fourier Transform Infrared - Attenuated Total Reflectance (FTIR-ATR) analysis
Fourier Transform Infrared - Attenuated Total Reflectance (FTIR-ATR) spectroscopy of the juice polysaccharide sample and the HMW fraction was performed using an Equinox 55 IR spectrophotometer (Bruker Optics, U.S.A.). A total of 64 scans were performed at a resolution of 4 cm^{-1} with a diamond crystal at temperature 21 ± 1 °C. A program written for the Opus software from Bruker was used to record the spectra and for the selection of the corresponding backgrounds.

4.2.1.6 Nuclear Magnetic Resonance (NMR) spectroscopy
NMR spectroscopy was conducted for the HMW component in the juice polysaccharide fraction (section 4.1.2.3). The sample was prepared in D2O and the NMR spectrum was obtained at 500MHz (Bruker Avance DRX-500, U.S.A.).

4.2.1.7 Mass spectroscopy
The low molecular weight components during dialysis (section 4.1.2.3) were analyzed in a mass spectrophotometer (Bruker Bio TOF III, U.S.A.) equipped with a chromatography system (Agilent 1100 HPLC with Gerstel /CTC MPS3...
auto sampler). The mass range of the instrument was 100-3000 amu which could be extended to 30000 amu at reduced accuracy. The instrument was operated in ESI (electron spray ionization) mode.

4.2.2 Membrane characterization

4.2.2.1 Molecular weight cutoff (MWCO)

The MWCO and pore size distribution of both pristine and fouled membranes was determined using dextran sieving test. Dextrins of five different molecular weights viz. dextran T10 (8 to 12 kD), T40 (35 to 50 kD) ,T70 (55 to 65 kD), all from Pharmacia Biotech AB (Uppsala, Sweden) and dextran 15 (15 to 20 kD), dextran 100 (100 to 200 kD), both from Serva Feinbiochemica GmbH & Co.(Heidelberg, Germany) were mixed to prepare a test solution with a total dextran concentration of 1 g/L. 0.01 M sodium azide was added to the solution as preservative.

The membrane disc to be characterized was assembled in a stirred cell (Amicon 8050) with a non-woven microporous polypropylene support underneath. Stirring was carried out at a fixed speed of 400 rpm with a magnetic stirrer (IKA-Labortechnik, Janke & Kunkel GmbH & Co. KG, Germany). Pristine membranes were first tested with pure water (MilliQ) at pressures of 1, 0.5 and 0.2 bar. Thereafter, the water was drained out, the cell dried and the membrane tested with 50 ml dextran solution. Fouled membranes were directly tested with dextran solution. At least 5 to 7 ml filtrate was collected in a single pass. To minimize concentration polarization, the applied pressure was maintained at 0.2 bar during dextran UF. The feed, permeate & retentate samples were analyzed by SEC using a Jasco PU-2080 Plus HPLC system (Japan) with a Waters RI R401 refractive index detector (U.S.A.) as described earlier (section 4.2.1.4). The sieving coefficient at each molecular weight was determined as follows:

\[ S_{\text{MolWt}} = \frac{C_{\text{Permeate}}}{C_{\text{Feed}}} \]  \hspace{1cm} (4)

where \( S \) is sieving coefficient at a definite molecular weight and \( C \) is the concentration.
4.2.2.2 Fourier Transform Infrared - Attenuated Total Reflectance (FTIR-ATR) analysis

FTIR analysis was carried out for select pristine and fouled membrane samples. The pristine membranes were initially cleaned to remove any preservative. The membrane disc was immersed in 100% ethanol in a beaker and stirred continuously for 2h. It was then removed and washed with RO water before immersing in water and stirring for 30 minutes. The fouled membranes were used as is. Both the ethanol cleaned pristine membrane and the fouled membrane discs were dried overnight in an oven at 30ºC and the FTIR spectrum recorded (Equinox 55 IR spectrophotometer, Bruker Optics, U.S.A.) as described earlier (section 4.2.1.5).

4.2.2.3 Scanning Electron Microscopy (SEM)

SEM was conducted for select pristine, fouled and chemically cleaned membranes. Two different electron microscopes were used since the analysis was done in two laboratories. All images for UF-PES-030H membrane were obtained using FEI QUANTA 400F (Czech Republic). For UF-PES-050H membrane, Leica Stereoscan 440 (U.K.) was used. The images for the pristine and fouled UF-PS-100H membrane were captured by FEI QUANTA 400F and rest was obtained with Leica Stereoscan 440.

For images with FEI QUANTA 400F, the pristine membranes were cleaned with absolute ethanol for 2 h followed by water wash for 30 minutes. Membrane fouling was done in the dynamic mode (as described in section 4.2.3.1.2). Samples of pristine and fouled membranes were dried overnight at 30ºC in an oven. The dried samples were cut into 3x3mm pieces and coated with silver adhesive in a sputter coater (K550, EMITECH Limited, Kent, England) before SEM analysis. For images with Leica Stereoscan 440, pristine, fouled and cleaned membrane samples were prepared following the method described in section 4.2.3 and 4.2.4.

4.2.3 Adsorption and Ultrafiltration (UF) experiments

4.2.3.1 Sugarcane juice UF

Preliminary membrane assessment with sugarcane juice was carried out in a stainless steel SEPA ST cell (Osmonics, U.S.A.) with a filtration area of 16.9 cm2. The experiments were performed at ambient temperature (24-28ºC), at a constant pressure of 1 bar in a dead-end filtration mode and a concentration
factor of 1.7. A fresh membrane was used for each experiment and the membrane washing and water flux measurements, both before and after UF, were performed using RO water. Flux was determined volumetrically by measuring the time required to collect a known volume of permeate.

Prior to UF, the clarified juice was centrifuged at 6000 rpm for 18 minutes and the mixed juice at 9000 rpm for 15 minutes to remove suspended solids. Limited experiments were also conducted without any suspended solids removal.

The flux and fouling data was analyzed using the following parameters.

Flux \( (L/m^2 \cdot h) = \text{permeate volume} / (\text{time} \times \text{membrane filtration area}) \) \( \cdots \) \( (5) \)

Normalized pure water flux (PWF) \( (-) = J_1 / J_0 \) \( \cdots \) \( (6) \)

where \( J_0 \) & \( J_1 \) are the PWF \( (L/m^2 h) \) of the pristine and fouled membranes respectively. The \( J_1 / J_0 \) ratio is also an inverse measure of membrane fouling, with higher \( J_1 / J_0 \) values indicating lower fouling.

\[
D_i = C_{i,f} V_{i,f} - C_{i,p} V_{i,p} - C_{i,r} V_{i,r}
\]

\( \cdots \) \( (7) \)

where \( D \) is deposition in mg of the component \( i \) on the membrane, \( C \) is concentration of foulants \( (mg/ml) \) and \( V \) is volume \( (ml) \). The subscripts \( f, p, r \) stand for feed, permeate and retentate respectively.

\[
R_i(\%) = \left(1 - \frac{C_{i,p}}{C_{i,f}}\right) \times 100
\]

\( \cdots \) \( (8) \)

where \( R \) is the rejection of component \( i \), \( C_p \) and \( C_f \) are concentration of component \( i \) in permeate and feed respectively.

4.2.3.2 Polysaccharide fouling - static mode

Static fouling experiments were conducted with a concentration of 1.5 mg/ml juice polysaccharide solution. The solution was transferred into a 10 ml capacity stirred UF cell (Amicon 8003 cell, Millipore, Bedford, U.S.A.) assembled with a membrane disc of approximately 3.8 cm\(^2\) active filtration area. The backside of the membrane was sealed with a polymeric adhesive tape to prevent any permeation through the pores. The filtration cell was placed on a magnetic stirrer operated at 400 rpm. The membrane disc was kept in contact with the fouling solution for 65 minutes, after which it was removed from the cell and rinsed twice by dipping in MilliQ water. The seal from the backside was then removed. The fouled disc was then used for subsequent analyses like water flux.
measurement and MWCO determination. The samples of fouling solution (initial) and after the adsorption experiment (final) were analyzed further for MW profile, protein content and UV sensitivity.

4.2.3.3 Polysaccharide fouling - dynamic mode
30 ml of the 1.5 mg/ml juice polysaccharide solution was transferred into a 50 ml capacity stirred UF cell (Amicon 8050 cell, Millipore, Bedford, U.S.A.) assembled with a membrane disc of about 11 cm2 active filtration area. Stirring was carried out with a magnetic stirrer operating at 400 rpm. The cell was pressurized with nitrogen and 6 ml of permeate was collected at an applied pressure of 1 bar. Thereafter, the membrane was removed from the cell, rinsed twice by dipping in MilliQ water and used for subsequent analysis. The feed, retentate and permeate samples were analyzed further for MW profile, protein content and UV sensitivity.

4.2.4 Membrane cleaning
All experiments were conducted in a stainless steel SEPA ST cell (Osmonics, U.S.A.) with 16.9 cm2 filtration area. The UF was performed at ambient temperature, at a constant pressure of 1 bar in a dead-end filtration mode. The various steps involved in membrane chemical cleaning are described in the following sub-sections.

4.2.4.1 Pristine membrane washing
Prior to use the pristine membranes were cleaned thoroughly to remove any preservative prior to use. The membrane disc was dipped in 100 ml RO water (1.2-1.9 µS) and sonicated for 3 minutes (Toshiba, 1.5L50, India). The pH, conductivity and total dissolved solids (TDS) of the water were measured after the wash. All measurements were made using probes from Eutech, CyberScan (Singapore). The procedure was repeated till the properties of the wash water were observed to be the same as that of the fresh water. The PWF of the cleaned pristine membrane (Jo) was measured with RO water.

4.2.4.2 Membrane fouling
Membrane fouling, both with polysaccharide fraction and sugarcane juice, was carried out following the method described in section 4.2.3.1. A concentration ratio of 1.5 was maintained in these experiments.
4.2.4.3 Fouled membrane cleaning

Known concentrations of the reagents (150-200 ppm NaOCl, 0.5%, 1.2% and 2% w/v NaOH, 0.5 M citric acid) were examined alone, in sequence and in combination. The fouled membrane disc was immersed in 50 ml of the appropriate cleaning solution for a known time period with intermittent mixing. The disc was then removed from the cleaning solution and washed repeatedly with fresh RO water. Washing was considered to be complete once the values were within ±0.5 unit of the fresh RO water. Subsequently, the PWF of the chemically cleaned membrane (Jc) was measured.

All the fouling and cleaning experiments were done at least in duplicate. The normalized PWF of the fouled and chemically cleaned membranes were calculated as follows:

Normalized PWF (-) fouled membrane = \( \frac{J_1}{J_0} \) ..............................(9)

Normalized PWF (-) chemically cleaned membrane = \( \frac{J_c}{J_0} \) ..............................(10)

4.2.5 Membrane surface modification

For each membrane type selected for surface modification, several 44 mm diameter discs were cut using a die. The discs were rinsed with absolute ethanol for 2h in a mechanical shaker followed by a water wash for 30 minutes to remove the preservative layer from the membrane. They were then compacted for 30 minutes at 3 bar pressure in an UF cell (Amicon 8050). Pure water flux was determined at 1 bar pressure and ambient temperature and discs having similar flux were chosen for modification. The method developed by Susanto et al. (2007) was used. The chosen membrane discs were immersed in a petri-dish containing 40 g/L standard solution of PEGMA poly (ethyleneglycol) 400 monomethacrylate monomer (Polyscience Inc., Warrington, PA, U.S.A). The discs were then exposed to UV light (\( \lambda \): 315-380nm, intensity 36mW/cm² for petri-plate covered samples and 41mW/cm² for uncovered sample). The intensity was calibrated for UV-A by UV meter (Dr. Hönle AG, Gräfelfing, Germany). After exposure to UV for a given time period, the membranes were immediately dipped in a beaker containing MilliQ water and rinsed for 30 minutes by gentle stirring using a magnetic stirrer. This was followed by a warm water (~65°C) rinse for 2 h and then with RO water at ambient temperature for another 30 minutes. The PWF of the modified membrane was then measured.
To estimate the degree of grafting (DG), the base membrane disc before modification was dried overnight at 30°C and weighed. The disc was then modified and its weight was determined again after drying. DG for a membrane disc with area A was calculated from the weight difference before ($W_{unmod}$) and after grafting ($W_{mod}$) of the membrane following the equation:

$$DG(\mu g/cm^2) = \frac{W_{mod} - W_{unmod}}{A}$$

...(vi)