CHAPTER 2

EXPERIMENTAL METHODS

2.1 MATERIALS

2.1.1 Polymers

Polyethersulfone supplied, as a gift sample, by Solvay Advanced Polymers, L.L.C., USA, was used as received. Polyetherimide (UDEL^{R} 1000) supplied by GE plastics, India as a gift sample. It was dried at 150^{o} C for 4 h before used.

2.1.2 Solvents and other chemicals

Analar grade N-methyl -2-pyrrolidone (NMP) from SD Fine chemicals, India was used as such without purification. Analar grade Acrylic acid ,N-Vinyl pyrrolidone was procured from E.Merck Ltd., and used as monomers for grafting. Other solvents such as 1,2-dichloroethane (DCE), N,N-dimethyl acetamide (DMAc),acetone and sodium lauryl sulfate (SLS) of analar grades from Merck (I) Ltd., were used as such without further purification. Analar grade Sodium hypochlorite was purchased from E.Merck Ltd., and used without further purification as cleaning agent

2.1.3 Proteins

Proteins viz., Bovine Serum Albumin (BSA), $\overline{M}_w$ = 69 kDa, Pepsin, $\overline{M}_w$ = 35 kDa, Trypsin, $\overline{M}_w$ = 20 kDa and Egg Albumin (EA),

$\overline{M}_w$ = 45 kDa were purchased from SRL Chemicals Ltd., India and used as received.

Sodium mono basic phosphate anhydrous and sodium dibasic phosphate hepta hydrate were procured from SRL Chemicals Ltd., Mumbai, India and used for the preparation of phosphate buffer solutions.

2.2 MEMBRANE FORMULATIONS

2.2.1 Solution Blending of Polymers

Pure Polyethersulfone (100%) and blend Polyethersulfone - Polyetherimide with different composition, in N- Methyl Pyrrolidone (NMP) solvent were prepared by constant mechanical stirring in a round bottom flask for 4 h at 40^{o}C. The homogeneous solution of pure PES and PES/PEI blend solutions were tightly closed and placed in the chamber at room temperature for 6 h to get rid of air bubbles produced during stirring.

2.2.2 Casting of Membranes

All membranes were prepared by phase inversion method. The blend solutions were poured onto a glass plate and dragged through a Doctor's knife parallel to the plate. The thickness of the membranes was controlled by varying the thickness of the adhesive tapes at the two ends of the doctor's blade. After allowing the solvent to evaporate for a predetermined period at low humidity and appropriate temperature conditions, the plate was then immersed into a bath having solvent, nonsolvent and surfactant for final precipitation. Evaporation of solvent from the solution inside the chamber leads to the formation of top layer. Besides skin layer formation, evaporation can also leads to formation of macrovoids (Zeman and Fraser 1993). Prior to membrane casting, gelation bath was prepared by

mixing 2L distilled water (non-solvent), 2wt% NMP (Solvent) and 0.2 wt% SLS (Surfactant) at10 ^{o}C. Soon after evaporation the solution with the plate was immersed into the gelation bath, phase inversion occurs immediately and after few minutes, thin polymeric film separates out from the glass plate. After 30 minutes of gelation , polymeric membranes were removed from the gelation bath and thoroughly rinsed with deionized water to remove all solvent and surfactants. The thickness of the membrane was measured using a micrometer. The membranes were stored in water with 0.1% formalin solution in order to remove the bacterial and fungi reactions.

Table 2.1 Compositions and casting conditions of PES/PEI blend membranes

Blend Composition Polymer 17.5 wt%		wt %
PES	PEI	Solvent, NMP
100	0	82.5
90	10	82.5
80	20	82.5
70	30	82.5
Casting Solution Temperature = 40±2^{o}C Casting Temperature=34±2^{o}C Casting relative humidity= 20±2 % Solvent Evaporation time = 30s		

2.3 PHOTO CHEMICAL GRAFTING OF MEMBRANES WITH ACRYLIC ACID

5 wt % solution of acrylic acid in water was degassed by bubbling with nitrogen for 5 min and the prepared membrane samples were immersed

into the monomer solution in a Petri dish for 2 min. The immersed samples were covered by a glass plate to avoid contamination produced from air. The membrane samples were then subjected to UV irradiation for various time intervals. A UVA Print Chamber equipped with a high pressure mercury lamp with wavelength more than 345 nm, an intensity of 60±10 mW/cm^2 was used. After irradiation, the membranes were taken out and rinsed immediately with deionized water several times to remove the unreacted monomers and physically adsorbed polymer. The washing was done at room temperature for 30 min (Susanto et al 2000).

Table 2.2 Grafting condition of AA- g- PES/PEI blend membranes

Membranes		**Acrylic Acid(Monomer) Wt %**	**UV Irradiation time, min**
PES	**PEI**		
100	0	5	2
90	10	5	2
80	20	5	2
70	30	5	2
100	0	5	4
90	10	5	4
80	20	5	4
70	30	5	4
100	0	5	6
90	10	5	6
80	20	5	6
70	30	5	6
Grafting Temperature = 30±2^oC			

2.4 GRAFTING OF MEMBRANES WITH N-VINYL PYRROLIDONE

5 wt % solutios of N-Vinyl pyrrolidone in water were degassed by bubbling with nitrogen for 10 min. the membrane samples were immersed into the solution in a Petri dish. A UV print chamber equipped with a high pressure mercury lamp with wavelength more than 345 nm, an intensity of 60±10 mW/cm^2. After grafting, the membranes were rinsed immediately with deionized water several times to remove any unreacted monomer or physically adsorbed polymer. The washing was done at room temperature for 30 min as mentioned above. The same procedure was adopted for grafting.

Table 2.3 Grafting condition of NVP- g - PES/PEI blend membranes

Membranes		**N-Vinyl Pyrrolidone (Monomer), Wt %**	**UV Irradiation time, min**
PES	**PEI**		
100	0	5	2
90	10	5	2
80	20	5	2
70	30	5	2
100	0	5	4
90	10	5	4
80	20	5	4
70	30	5	4
100	0	5	6
90	10	5	6
80	20	5	6
70	30	5	6
Grafting Temperature = 30 ± 2 ^oC			

2.5 EXPERIMENTAL SET UP

The ultrafiltration (UF) experiments were carried out in a batch type, dead end cell fitted with a Teflon coated magnetic paddle.

The flow sheet of the experimental apparatus is illustrated in Figure 2.1.

The following are the specifications of ultrafiltration test cell used.

UF Cell	:	Amicon model 8400 Millipore Ltd., Bangalore, India
Capacity, ml	:	450.0
Membrane dia, mm	:	76.0
Filtration area, cm^2	:	38.5
Minimum volume for operation, mL	:	10.0
Height, cm	:	23.0
Diameter, cm	:	12.0
Weight, kg	:	0.9
Maximum pressure	:	483 kPa (4.8 atm)
Temperature (max) °C	:	60.0
Hold up volume, ml	:	10.0
Tubing	:	1/8” (3.175 mm) ID
Materials		
Top and Base	:	Polyacetal (Dextrin)
Pressure relief valve	:	Polyacetal

Chamber reservoir	:	Polycarbonate
Magnetic stirrer bar	:	Teflon coated
Porous membrane support disc	:	Polypropylene
Pressure cum reservoir tank capacity	:	7.5 Litres

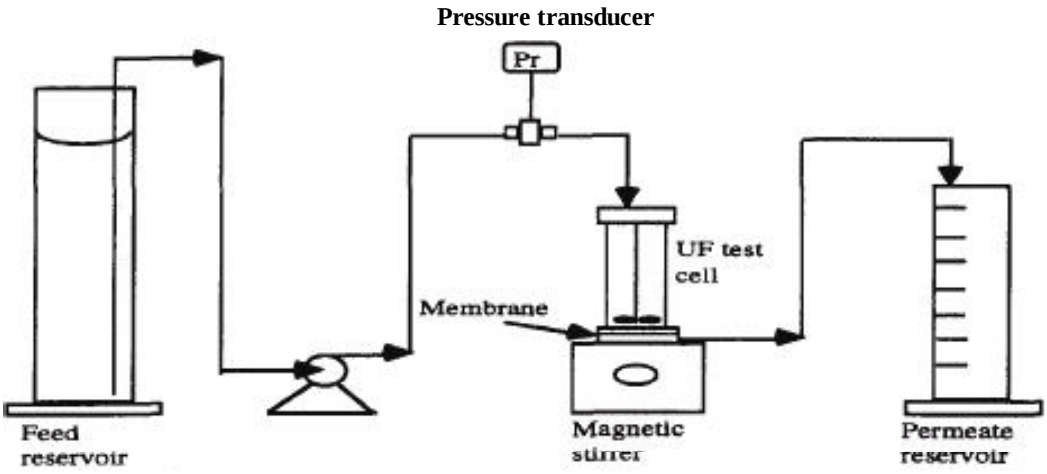

Figure 2.1 Flow sheet of the experimental apparatus

2.6 MEMBRANE CHARACTERIZATION

The thicknesses of the prepared membranes were measured using a micrometer (Mityutoyo, Japan), at various parts of the membrane. The thickness of the membranes maintained in the present studies was 0.22 ± 0.02 mm. The prepared membranes were cut into the desired size needed for fixing it up in the ultrafiltration cell. The characterization experiments were performed with prepared membranes in the stirred-cell ultrafiltration kit. The feed employed with agitation under pressure to minimize concentration polarization effect (Long and Anderson, 1984). The membranes were initially pressurized with distilled water at 414 kPa for 5 h

and compacted to attain steady-state flux. These pre-pressurized membranes were subsequently characterized and utilized for further studies.

2.6.1 Compaction

The thoroughly washed membranes were loaded in the UF test cell connected to the pressurized reservoir filled with distilled water. The initial flux of the membranes was measured after fixing the membrane in the UF cell and pressurizing it at a transmembrane pressure of 414 kPa. The water flux was measured at an interval of one hour. It was observed that the flux declined sharply in the earlier hours and reached a steady state after 4-5 h (Kutowy and Sourirajan 1975).

The initial flux was measured 10 minutes after the pressurization of a test cell. The water flux declined sharply in the beginning and reached steady state after approximately 3-4 h. The compacted membranes were then used for further characterizations.

2.6.2 Pure Water Flux

After compaction, permeability of pure water through the membranes were measured using the experimental apparatus shown in Figure 2.1. The compacted membranes were then subjected to a transmembrane pressure of 345 kPa. The pure water flux was measured for every 1 h for 4-5 h. The PWF was determined from the following equation (Osada and Nakagawa 1992).

$$J_w = \frac{Q}{A.\Delta t} \tag{2.1}$$

where, J_w the water flux (l m^{-2} h^{-1}); Q is the quantity of water permeated (l); Δ t is the sampling time (h); and A is the membrane area (m^2).

2.6.3 Degree of grafting

Degree of grafting was gravimetrically determined as the weight increase per outer surface area. (Susanto et al 2007)

$$DG = \frac{M_{gr} - M_0}{A} \quad (2.2)$$

where M_0 = initial membrane sample weight

M_{gr} = grafted membrane sample weight

A = outer surface area of the membrane sample

DG = degree of grafting

2.6.4 Contact Angle

The contact angle is a measure of the ability of a liquid to spread on a surface. The method consists to measure the angle between the outline tangent of a drop deposited on a solid and the surface of this solid. The contact angle is linked to the surface energy and so one can calculate the surface energy and discriminate between polar and apolar interactions. When a drop is deposited on a planar solid surface, the angle between the outline tangent of the drop at the contact location and the solid surface is called contact angle (θ). Generally contact angle measurements give three important informations. Determination of hydrophilic and hydrophobic of nature the surface, Surface free energy calculation and non homogeneity of the surface by measuring the hysteresis between advancing angle and recessing angle.

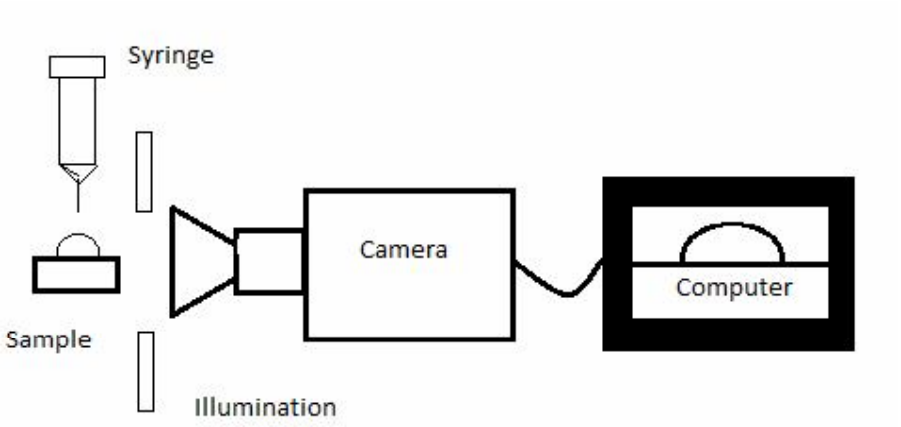

Figure 2.2 Schematic representation of

Three parameters such as Solid-Liquid interfacial tension γSL, Solid-Vapour interfacial tension γSV (γS), Liquid-Vapour interfacial tension γLV (γL) influence the shape of drop at solid surface .These three parameters are linked with the contact angle θ by the Young equation.

$$- \gamma SV + \gamma SL + \gamma LV \cos \theta = 0$$

2.6.5 Membrane Hydraulic Resistance (R_m)

The pure water flux of the membranes at different transmembrane pressures, viz., 69, 138, 207, 276, 345 and 414 kPa were measured. The hydraulic resistances of the membranes (R_m) were evaluated from the slope of the plot of pure water flux Vs transmembrane pressure difference (ΔP) using the following equation (Bhattacharyya et al 1974).

$$J_w = \frac{\Delta P}{R_m} \qquad (2.3)$$

2.6.6 Molecular Weight Cut-off (MWCO)

Molecular weight cut-off of a membrane is determined by identifying an inert solute, which has the lowest molecular weight and has solute rejection (SR) of 80 - 100% in steady-state ultrafiltration experiments (Sarbolouki 1982). Thus, proteins such as trypsin, pepsin, egg albumin and bovine serum albumin were chosen and the concentration of the proteins in the feed and permeate were determined using Total Organic Carbon Analyser. (Kang et al 2007) Dextrans of different molecular weights were used for the determination of MWCO of polyurethane and sulfonated polysulfone ultrafiltration blend membranes (Malaisamy et al 2002).

2.6.7 Morphological Studies

The top surface and cross-sectional morphology of the PES, PES-PEI, AA-(PES-PEI) and NVP-(PES-PEI) membranes were studied using Scanning Electron Microscopy (S-3400, SI.No.340745-05,India). The membrane samples were air dried to remove the surface water and fractured under cryogenic conditions using liquid nitrogen and were dried at 21 ± 1ºC. The sample was dipped into a water bath for 1s before freeze fracturing. Water dipping allowed facile fracturing of the membrane (Han and Bhattacharyya, 1995). The membranes were cut into pieces of varied sizes, mopped with filter paper and immersed in liquid nitrogen for few seconds to fracture the membranes. These dried membrane samples were stored in desiccators and used for SEM studies.

The samples were mounted on Gold-sputtered sample called ‘studs’ to provide electrical conductivity to very thin layers of polymeric membranes and photomicrographs were taken in very high vacuum conditions operating at 15-25 kV depending upon the physical nature of the sample (Brink et al

1993). Scanning Electron Microscopy (SEM) were recorded to study the surface and cross-sectional morphology of the polymeric membrane samples.

2.6.8 FT- IR STUDIES

In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for identification of unknown compounds and confirmatory tool for organic compounds.

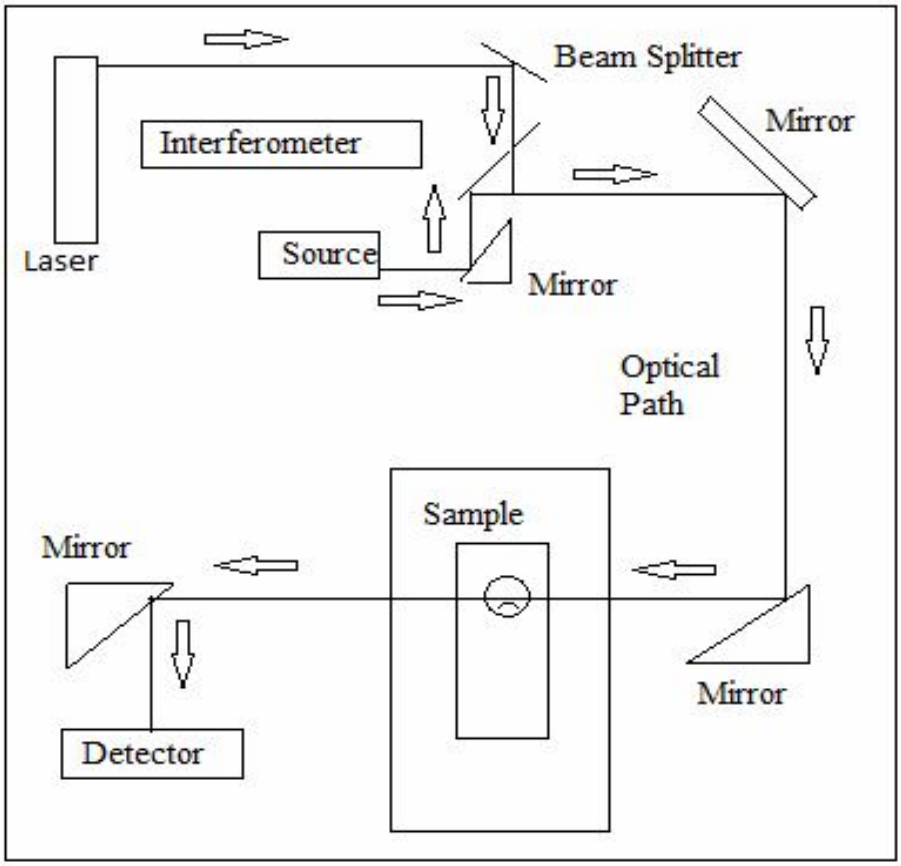

Figure 2.3 Schematic representation of FT-IR spectroscopy

Fourier Transform –Infrared studies were carried out using PerkinElmer Spectrum Version 10.02.00. The sample compartment is continuously purged with by a nitrogen flux.

2.6.9 Thermo Gravimetic Analysis

Thermal degradation behaviors of the PES, PES/PEI, Grafted membranes were investigated by a thermogravimetric analyzer (TGA Q500 V20.10, model) from room temperature to 800^{o}C with a heating rate of 20 ^{o}C/min under N_2 atmosphere. The measurements were conducted using 2–3 mg samples. The plots of weight (%) versus temperature were recorded. Thermogravimetric analysis mainly reveals the thermal stability of the membranes. It is used as an important tool in present study to confirm the attachment of monomers on to the surface.

2.7 SOLUTE REJECTION STUDIES

The % Solute Rejection was studied with the help of the following formula (Sarbolouki 1982).

$$\% \text{ SR} = 1 - \frac{C_p}{C_f} \times 100 \qquad (2.4)$$

where C_p = permeate concentration and C_f = feed concentration

2.7.1 Protein Rejection

The compacted membranes were mounted in the ultrafiltration cell, the feed reservoir was filled with the individual protein solution and pressurized to 345 kPa and maintained constant throughout the run. Different molecular weight of proteins such as trypsin, pepsin, egg albumin and bovine serum albumin were dissolved (0.1 wt%) in phosphate buffer (pH 7.2) and used as standard solutions. The concentrations of feed and the permeate

collected over measured time intervals were determined by Total organic Carbon Analysis, TOC V_{CPH},Shimadzu. The percentage protein rejections were evaluated from the concentration of the feed and permeate using equation (2.4). On completion of an each run, the membranes were removed from the UF kit and washed with distilled water to remove adhering proteins and then subjected to pure water flux measurement.

2.8 FOULING STUDIES

In part to address fouling resistance of the grafted membranes, membranes are systematically evaluated with two different hydrophilic monomers for their ability to increase membrane wettability and reduce fouling by protein, one is a neutral monomer, *N*-vinyl-2- pyrrolidinone and other one is weakly acidic (carboxylic) monomers, acrylic acid. These monomers have also been evaluated for their ability to mitigate protein fouling (Taniguchi et al 2004). Modified membranes were characterized in terms of contact angle, degree of grafting (DG), surface morphology and filtration performance, including flux, protein rejection, and fouling characteristics .

BSA was taken as a model for fouling studies due to its higher molecular weight (69 KDa). The membrane was compacted until the flux reaches a steady state. The pure water flux was measured at 345 KPa. After measuring the pure water flux, (J0) the reservoir was filled with (0.1 wt%) BSA solution of pH 7. After 60 minutes of filtration the Protein flux (Jp) was measured. The cell and the solution reservoir were fully emptied and refilled with deionized water. The membrane was cleaned in the stirred cell with deionized water for 20 min, and the water flux (*J1*) was measured again. After measuring the flux, the membrane sample was taken out and washed with 2 wt % of sodium hypochlorite followed by deionized water. Again the pure water fluxJ2 was measured for the same membrane The concentration of

protein in the permeate and in the feed were measured using TOC.Pure Water Flux (PWF)(J0),Protein flux after 60 min of filtration(Jp), PWF after hydraulic washing (J1),PWF after chemical Cleaning(J2)

Total Flux loss, Flux recovery, Reversible Fouling, Irreversible Fouling can be calculated using the following formula (14),15(Taniguchi et al 2003 and Kaeselev et al 2001)

$$\% \text{ Total flux loss, TLF} = 1-\left(\frac{Jp}{J0}\right)\times 100 \quad (2.5)$$

$$\% \text{ Flux recovery after hydraulic washing, FRW} = 1-\left(\frac{J1}{J0}\right)\times 100 \quad (2.6)$$

$$\% \text{ Flux recovery after chemical cleaning, FRC} = 1-\left(\frac{J2}{J0}\right)\times 100 \quad (2.7)$$

$$\text{Reversible fouling after hydraulic washing, RFW} = \left(\frac{J1-JP}{J0}\right) \quad (2.8)$$

$$\text{Reversible fouling after chemical cleaning, RFC} = \left(\frac{J2-JP}{J0}\right) \quad (2.9)$$

$$\text{Irreversible fouling, IRF} = \left(\frac{J0-J2}{J0}\right) \quad (2.10)$$