CHAPTER 2

EXPERIMENTAL METHODS

2.1 MATERIALS

2.1.1 Polymers

In the study described in this thesis, cellulose acetate (CA), aminated polyethersulfone (APES), and carboxylated polyethersulfone (CPES) are being used. Commercial grade Polyethersulfone (Radel PES, Solvay). Commercial grade MYCEL cellulose acetate CDA 5770 (Acetyl content 39.99 wt %) procured from Mysore Acetate and Chemicals Company Limited, India.

Polyethylene glycol 600 was procured from Merck (I) Ltd., and was used as such, as a non-solvent additive. Polyethyleneimine, ($\overline{M}_w$ = 6,00,000 to 10,00,000) 50% aqueous solution was procured from Fluka Chemie AG, Steinheim, Switzerland and used as 1 % aqueous solution for the studies.

2.1.2 Solvents and Other Chemicals

Analytical grade N,N'-Dimethylformamide (DMF) and DCM from M/s. Qualigens Fine Chemicals, Glaxo India Limited was procured, sieved through molecular sieves (Type-4Å) for removing moisture and stored in dried condition prior to use. Other solvents of analar grade such as acetone, 1,2-dichloromethane and methanol from Qualigens Fine Chemicals Ltd., India were used. Sodium lauryl sulphate (SLS) of AR grade was obtained

from Qualigens Fine Chemicals Ltd., India and used as surfactant. Hydrochloric acid (97-99%), was obtained from Qualigens Fine Chemicals, Glaxo India and used as amination process.

2.1.3 Proteins

Proteins viz., Bovine Serum Albumin (BSA), M_w=69 kDa, Pepsin, M_w=35 kDa, Trypsin, M_w=20 kDa were purchased from SRL Chemicals Ltd., India and used as received. Egg Albumin (EA), M_w=45 kDa was obtained from CSIR Bio Chemical Centre, New Delhi, India.

2.1.4 Metal Salts

Analar grades of Copper sulfate, Zinc sulfate and Nickel sulfate were received from E-Merck (India) Limited and used as such in the preparation of aqueous metal ion solutions. Sodium monobasic phosphate anhydrous and sodium dibasic phosphate hepta hydrate were procured from CDH Chemicals Ltd., India and used for the preparation of phosphate buffer solutions.

Deionized and distilled water was used for the preparation of metal, protein, 1 wt % polyethyleneimine aqueous solution and also for the gelation bath.

2.2 AMINATION OF POLYETHERSULFONE

The amination of PES was prepared via chloromethylation followed by reaction with trimethylamine according to the following procedure.

2.2.1 Preparation of Aminated Polyethersulfone (APES)

The 20 g of PES was dissolved in 50 ml of dichloromethane (DCM) with constant stirring using mechanical stirrer. 10 ml of concentrated hydrochloric acid with 10 g of trioxane and 5 g of zinc chloride is added slowly using a dropping funnel. Then the viscous solution was stirred for 6 h at room temperature. The viscous polymer solution was precipitated using ethanol and precipitate was thoroughly washed until pH becomes 7. Then 20 ml DMF and trimethylamine were added to it. The white precipitate was obtained by the addition of methanol. The aminated polyethersulfone was dired for 24 h in vacuum oven. The dried highly crystalline aminated polyethersulfone was used for the preparation CA/APES blend membranes.

2.2.2

Preparation of Carboxylated Polyethersulfone (CPES)

The 20 g of polyethersulfone was dissolved in 50ml DMF it was cooled to -50°C by immersion in a dry-ice/alcohol bath. N-butyllithium was injected dropwise at a rate of about 30 ml for one hour using a syringe pump. The mixture initially turned green, then later developed a red – brown coloration and become very viscous. Following the addition, the solution was stirred for 15 min and then the stirrer was removed. Several blocks of dry-ice were freshly prepared from CO_2 and these were promptly added to the lithiated polyethersulfone and mixed vigorously with a large spatula. During this addition, the flask was swept with a fast stream of nitrogen to prevent moisture condensation. The resulting thick whitish polymer precipitate was left overnight to warm to room temperature and then residual DMF was decanted off. Carboxylated polymer was recovered in the lithium salt form by agitating the precipitate with ethanol and drying it in an oven. The dried

highly crystalline carboxylated polyethersulfone was used for the preparation CA/CPES blend membranes.

2.2.3 Characterization of Aminated, Carboxylated Polyethersulfone

The aminated and carboxylated sample was characterized for functional group determination by FT-IR Spectroscopy and Nuclear magnetic resonance (NMR) spectra. FT-IR spectra were recorded on a Perkin-Elmer, model-Spectrum RX1 Fourier transform spectrometer either with powder samples inside a diamond cell by using KBr pellets composed of 50 mg of IR spectroscopic grade KBr and 1mg polymer sample was used to prepare pellets.

Nuclear magnetic resonance (NMR) spectra were recorded on a BRUKER – NMR operating at a resonance frequency of 300 MHz for ^{1}H NMR. For this analysis, 40 mg of polymer solution was prepared in deuterated dimethyl sulfoxide. This method is normally used to measure the intensity of the appropriate signal (Zaidai et al 2003) from recovered and washed APES, CPES samples dissolved in deuterted DMSO.

A DSC 200 PC differential scanning calorimetry (DSC) was employed to study the thermal transition behavior of APES and CPES samples. The samples 10 mg were preheated under nitrogen with heating rate of 10°C/min. A METLER TA 4000 system thermogravimetric analyzer (TGA) was employed to study the thermal stability behavior of APES and CPES samples. The samples 10 mg were preheated in air from room temperature to 100°C to remove moisture, cooled at 90° C, then reheated from that temperature to 800°C at 10°C/ min in air.

2.3 MEMBRANE FORMULATIONS

The polymer blends based on, cellulose acetate, aminated polyethersulfone and carboxylated polyethersulfone (17.5 wt %) were prepared by individually blending the two polymers in different compositions (Table 2.1) in presence and absence of additive, pore former, PEG 600 in a polar solvent, DMF, under constant mechanical stirring in a round bottom flask for 3 h at 40°C. The homogeneous solution was allowed to stand for 1 h in airtight condition to get rid off the air bubbles.

2.4 PREPARATION OF MEMBRANES

2.4.1 Solution Blending of Polymers

Pure cellulose acetate, aminated polyethersulfone and carboxylated polyethersulfone with different concentrations of pore former, PEG 600, in solvent DMF were prepared by mechanically stirring at 40°C for 4 h (Table 2.1). Pure cellulose acetate and carboxylated polyethersulfone (Table 2.2) casting solutions with different concentrations of additive, PEG 600, in solvent DMF were also prepared by mechanically stirring at 60°C for 4 h.

2.4.2 Preparation of Membranes

All membranes were prepared by the "diffusion induced phase separation " method, namely, casting a thin film of the polymeric solution on a glass plate and, after allowing the solvent to evaporate for a predetermined period at the desired humidity and temperature conditions, immersing it into a bath of non-solvent (water, solvent, surfactant) for final precipitation. Prior to membrane casting, a gelation bath of 2L of distilled water (non-solvent), containing 2% DMF (Solvent) and 0.2% SLS (Surfactant) was prepared and cooled to 10°C.

The homogeneous solution of CA,CA/APES and CA/CPES prepared both in presence and absence of additive PEG 600 were placed in

the controlled casting room at a temperature of 34 ± 2°C. The relative humidity in the casting room was maintained at 20 ± 2%.

Table 2.1 Compositions and casting conditions of CA/APES blend membranes

Blend composition (%) Polymer		**Wt %**	
CA %	**APES %**	**Additive, PEG 600**	**Solvent, DMF**
100	0	0	82.5
90	10	0	82.5
80	20	0	82.5
70	30	0	82.5
100	0	2.5	80.0
90	10	2.5	80.0
80	20	2.5	80.0
70	30	2.5	80.0
100	0	5.0	77.5
90	10	5.0	77.5
80	20	5.0	77.5
70	30	5.0	77.5
100	0	7.5	75.0
90	10	7.5	75.0
80	20	7.5	75.0
70	30	7.5	75.0
100	0	10.0	72.5
90	10	10.0	72.5
80	20	10.0	72.5
70	30	10.0	72.5

Table 2.2 Compositions and casting conditions of CA/CPES blend membrane

Blend composition (%) Polymer		Wt %	
CA %	**CPES %**	**Additive, PEG 600**	**Solvent, DMF**
100	0	0	82.5
90	10	0	82.5
80	20	0	82.5
70	30	0	82.5
100	0	2.5	80.0
90	10	2.5	80.0
80	20	2.5	80.0
70	30	2.5	80.0
100	0	5.0	77.5
90	10	5.0	77.5
80	20	5.0	77.5
70	30	5.0	77.5
100	0	7.5	75.0
90	10	7.5	75.0
80	20	7.5	75.0
70	30	7.5	75.0
100	0	10.0	72.5
90	10	10.0	72.5
80	20	10.0	72.5
70	30	10.0	72.5

Total weight percentage of polymer = 17.5 wt %.

Casting solution temperature = 85 ± 2°C.

Casting temperature = 34 ± 2°C

Casting relative humidity = 20 ± 2 %,

Solvent evaporation time = 30 s.

Total weight percentage of polymer = 17.5 wt %.

Casting solution temperature = 42 ± 2°C

Casting temperature = 25 ± 1°C

Casting relative humidity = 50 ± 2%

Solvent evaporation time = 30 s.

The homogeneous solutions were spread over a smooth glass plate with the help of a knife-edge. The thickness of the membranes was controlled by varying the thickness of adhesive tapes at the sides of the glass plate. The glass plate was kept in an environment of controlled temperature and humidity during membrane casting as specified. Solvent present in the casting solution was allowed to evaporate for 30 s.

An evaporation step prior to immersion can lead to top layer formation. A point of interest is to investigate the conditions necessary to give rise to top layer formation. Besides top layer formation, evaporation can also induce macrovoid formation (Zeman and Fraser 1993) The glass plate was subsequently immersed in a gelation bath, which is generally maintained at a known temperature of 10°C. Immediately phase inversion starts and after few minutes thin polymeric film separated out from the glass. After 30 minutes of gelation, the polymeric film (membrane) was removed from the gelation bath and thoroughly rinsed with demineralized water to remove all solvent and surfactant. Then the actual thickness (approximately 0.22 mm) of the membranes was measured by using a micrometer. The membrane was always wet stored in 0.25% formaldehyde solution.

2.5 ULTRAFILTRATION SET UP

The ultrafiltration (UF) experiments were carried out in a batch type, dead end cell (ultrafiltration cell - S76-400-Model, Spectrum, USA) fitted with a Teflon coated magnetic paddle.

The flow sheet of the experimental apparatus is illustrated in Figure 2.1. The schematic diagram of ultrafiltration test kit cell used in the present study is illustrated in Figures 2.2.

The following are the specifications of ultrafiltration test cell used.

UF Cell	:	Model No. S76-40 from Spectrum Inc., USA
Capacity	:	450.0 ml
Membrane dia, mm	:	76.0 mm
Filtration area	:	38.5 cm^2
Minimum volume for operation	:	10.0 ml
Height	:	23.0 cm
Diameter	:	12.0 cm
Weight	:	0.9 kg
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
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	:	5 Litres

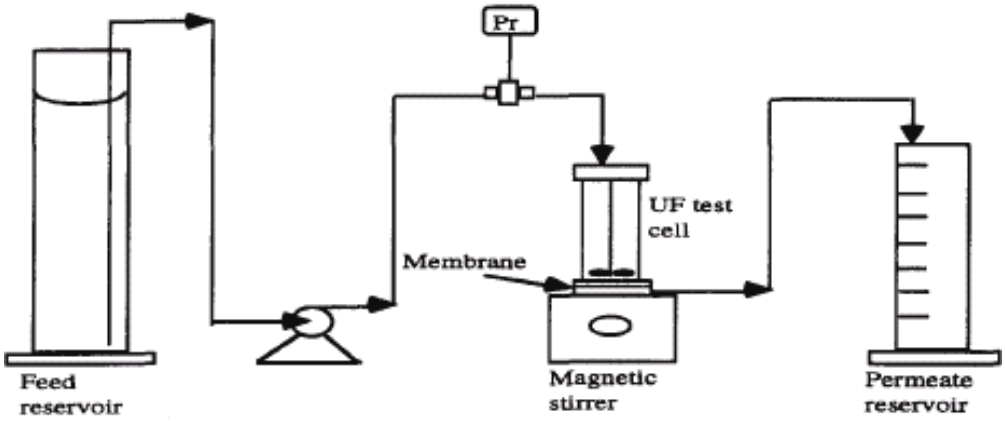

Figure 2.1 Flow sheet of the experimental apparatus

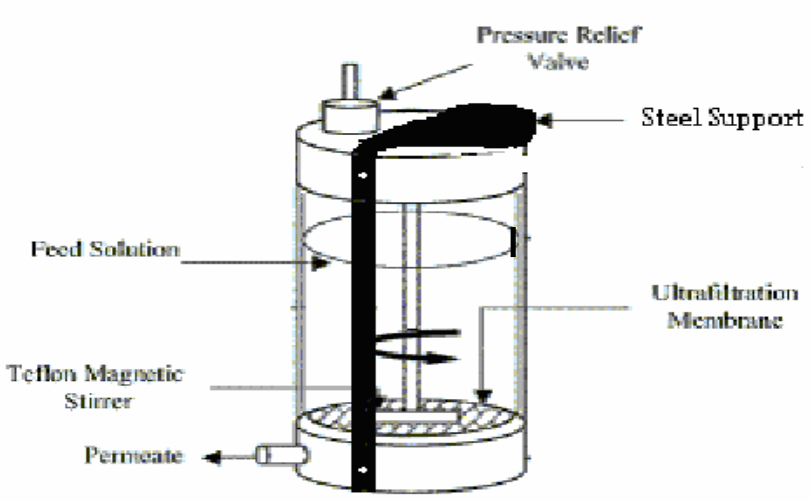

Figure 2.2 Schematic Diagram of Ultrafiltration test Kit cell

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 ±0.02mm. The membranes prepared 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 5h 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 Stirred UF test cell with efficient filtration area of 38.5 cm^2. The cell was placed on magnetic stirring table and connected to an auxiliary pressurized reservoir containing a deionized water. The water in reservoir was pressurized using nitrogen gas from cylinder and pressurized water into the cell. The initial flux of the membrane 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. This was in agreement with the published work (Kutowy and Sourirajan, 1975).

The initial flux was measured 20 s after the pressurization of a test cell. The water flux declined sharply in the beginning and reached steady state after approximately 5-7 h. The pressure compacted membranes were the used characteristics experiments in ultrafiltration kit.

2.6.2 Pure Water Flux

After compaction, permeability of pure water through the membranes was measured using the experimental apparatus shown in

Figure 2.1. The feed solution flowed at constant pressure (345 kPa) through the inside of the flat sheet membrane in UF cell. The filtrate from the UF cell was collected in measuring cylinders. The time required for permeation of a prescribed amount of water was measured. After the permeation experiment, calculate the flux based on the inner surface area. From the measured values the PWF was determined using Equation (2.1).

$$J_w = \frac{V}{A\,t} = \frac{Q}{A.\Delta t} \qquad (2.1)$$

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

2.6.3 Water Content

The membrane samples were removed from the water and weighed immediately after blotting the free surface water. Then they were dried for over 7 h at 100 ± 5°C. The percentage of water content of the membranes was calculated using equation (Prabhakar et al 1986).

$$W_R = \frac{(W_1 - W_2)}{W_2} X\ 100 \qquad (2.2)$$

Where W_1 = wet weight of membrane

W_2 = dry weight of membrane

W_R = % water content

2.6.4 Membrane Hydraulic Resistance (R_m)

The performance of ultrafiltration membranes is dominated by the phenomenon of concentration polarization, which is caused by a build- up of rejected solute adjustment to the membrane surface. Flux in pressure-driven

membrane processes can be expressed by the resistance- in-series relationship (Fane et al 1981).

$$J_w = \left(\frac{dv}{dt}\right)\frac{1}{A} = \frac{\Delta P}{R_m + R_s} \tag{2.3}$$

where J_w is the water flux; v is the total volume of permeate; t is the filtration time; A is the membrane surface area; ΔP is the pressure difference; R_m is the membrane resistance (kPa/l m^{-2} h^{-1}) and R_s is the resistance due to the solute. R_m is obtained from the pure water run, since R_s can be neglected. 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 modified above Equation (2.3) (Bhattacharyya, 1974).

$$J_w = \frac{\Delta P}{R_m} \tag{2.4}$$

2.6.5 Molecular Weight Cut-off (MWCO)

Molecular weight cut-off of a membrane is determined by identifying an inert solute, which has the lowest MW 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 UV-visible spectrophotometer (Mahendran et al 2004).

2.6.6 Morphological Studies

The top surface and cross-sectional morphology of the CA/APES, CA/CPES membranes were studied using Scanning Electron Microscopy (LEICA Stereoscan, Cambridge, UK). 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. The dried bits of membranes were stored in desiccators and used for SEM studies.

The sample 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 Micrographs (SEM) at various magnifications were recorded to study the surface and cross-sectional view of the membrane samples.

2.6.7 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 a polar 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 nature of the

surface, surface free energy calculation and non homogeneity of the surface by measuring the hysteresis between advancing angle and recessing angle.

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.

$$- \gamma SV + \gamma SL + \gamma LV \cos \theta = 0 \qquad (2.5)$$

2.7 SOLUTE REJECTION STUDIES

Concerning the output or permeate concentration (C_p), it is convenient to give it in terms of the input or feed concentration (C_f), through the so called % solute rejection (Mahendran et al 2004).

$$\%SR = 1 - \frac{C_p}{C_f} \times 100 \qquad (2.6)$$

2.7.1 Protein Rejection

The characterized 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 (0.1wt %) in phosphate buffer (0.5m, pH 7.2) and used as standard solutions. The concentration of the feed solution was maintained constantly for all experiments. The permeate from the cell was collected over measured time intervals in graduated tubes and the tube contents were analyzed to determine the protein concentration by spectrophotometry at λ max of 280 nm using Hitachi U-2000

Spectrophotometer. The % protein rejection was evaluated from the concentration of the feed and permeate using equation (2.6). 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.

2.7.2 Metal-Ion Rejection

Aqueous solutions of Cu^{2+}, Ni^{2+} and Zn^{2+} with an approximate 1000 ppm concentration were prepared in 1wt % solution of Polyethyleneimine (PEI) in deionized water. The pH of these aqueous solutions were adjusted to 6.25 by adding small amount of either 0.1M HCl or 0.1M NaOH. Solutions containing PEI and individual metal ions or metal chelates were thoroughly mixed and left standing for 5 days to complete binding (Jarvis and Wagener 1995, Kobayashi et al 1987).

The PEI and metal ions containing solutions were filled in the feed reservoir. For each run, the first few ml of the permeate was discarded. The permeate flux was measured by collecting the permeate at a pressure of 345 kPa. The concentration of each metal ion in feed and permeate was measured by using Atomic Absorption Spectrophotometer (Perkin Elmer-2380). The pH of feed and permeate were measured with Elico pH meter. In the absence of metal ions, the concentration of PEI was also confirmed by UV-Visible Spectrophotometer (Hitachi, model U-2000) at λ max = 269 nm.