CHAPTER – 2

MATERIALS AND METHODS

2.1 Preview :

In this chapter, different materials used for the studies, the experimental procedures used for polymer synthesis and different methodologies adapted for the characterization of polymers are given. This chapter also describes the methods used for studying the solution properties and biodegradability of various polymers.

2.2 Materials :

Acrylamide (Mitsubishi Chemical Ltd.), acrylic acid (National Chemicals, Baroda), acrylonitrile (BDH, Poole, England), N-vinylpyrrolidone (Fluka, Switzerland), ethyl acrylate (BDH, Poole, England), *n*-hexyl acrylate, cyclohexyl acrylate and benzyl acrylate (gifted by Indian Petrochemical Corporation Limited, Baroda, India) were used for polymerization without any prior purification. Vinyl acetate (SD's Lab-Chem. Industry, Bombay, India), styrene (Fluka, Switzerland), methyl acrylate, ethyl acrylate and methyl methacrylate (National Chemicals, Baroda, India) were distilled at reduced pressure. The middle fraction of distillate was collected and used.

Hydrogen peroxide (Glaxo, India, 30% w/w), potassium persulfate (Merck, India) and sodium dodecyl sulphate (Qualigens, India) were used as received. Technical grade azobisisobutyronitrile (AIBN) was recrystallized from warm methanol and benzoyl peroxide (BPO, Fluka) was purified by dissolving it in chloroform at room temperature and reprecipitating it by adding methanol before it was used.

2.3 Experimental Methods :

(a) Polymer Synthesis :

Polymerization of monomer(s) was carried out by the free radical solution / microemulsion polymerization techniques[1,2]. The monomer-to-solvent ratio was 1:4 (w/v). The initiator concentration was 1.0% (w/w) to total monomer weight. The reaction set up consisted of a three necked round-bottom flask equipped with a water condenser at one side neck and the nitrogen inlet at the other. The reaction mixture was mechanically stirred through the center neck. The whole assembly was placed in a thermostated water bath at required temperature. After polymerization, the reaction mass was poured into an excess of nonsolvent. The precipitated products were purified, dried '*in vacuo*' and then characterized.

(b) Characterization of Polymers :

Polymer characterization is an essential step in working with the polymers[3]. Characterization of the polymers was done by Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (NMR), elemental analysis, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and gel permeation chromatography (GPC).

(i) FTIR analysis :

The FTIR spectra of homo-, co- and tercopolymers were done either by preparing films or by making pellets. The films were prepared by dissolving the polymers in water and pouring them over a pool of mercury. The films were obtained by vacuum evaporation of the solvent. The pellets were prepared by thoroughly mixing solid polymers and KBr (spectroscopic grade) and then this mixture was pressed under vacuum. IR-spectra of the films / pellets were recorded on a Bomem-MB140, Canada, FTIR spectrometer at Physics Department, M.S.University, Baroda, India in the range 4000-600 cm^{-1}.

(ii) NMR analysis :

The ^{1}H- and ^{13}C-NMR measurements were carried out in deuterium oxide (D_2O, 99.9 atom %D, Aldrich), chloroform-*d* ($CDCl_3$) and dimethyl-d_6-sulphoxide (DMSO) solvent at about 30°C. The NMR spectra of various polymer solutions were recorded at concentration of 1-2% (w/v) for ^{1}H-NMR and 20-25% (w/v) for ^{13}C-NMR, respectively. The spectra were recorded on JEOL JNM FX-100 FTNMR spectrometer at Indian Petrochemicals Corporation Ltd., Baroda, India in ^{13}C mode at a frequency of 25 MHz and ^{1}H mode at a frequency of 100 MHz. Tetramethyl silane (TMS, Aldrich) was used as an internal reference and deuterated solvents (i.e. D_2O, DMSO and $CDCl_3$) as an internal lock.

(iii) Elemental analysis :

The elemental analysis (viz. C and H) was carried out with a Coleman C, H analyzer and nitrogen was estimated by the Dumas method[4].

(iv) TGA studies :

TGA thermogram ranging from room temperature to 600°C of the synthesized polymers were recorded on Shimadzu thermal analyzer DT-30B. Approximately 10 mg polymer samples were taken into platinum pan and then heated in presence of air / nitrogen atmosphere. The measurement of weight loss as the reaction temperature increases by means of programmed rates of heating (i.e. 10, 15 and 20°C min^{-1}) were recorded. The residue remaining at high temperature represents the percentage of ash content of the sample.

(v) DSC measurements :

DSC of the polymer samples were recorded on a modulated differential scanning calorimetry (MDSC) (MDSC^{TM}, TA Instruments Inc., USA) DSC 2910 system at IUC-DAEF, Indore, India. DSC measurements were carried out from –75 to 200°C in nitrogen atmosphere. The heating rate was 10°C min^{-1}.

(vi) GPC analysis :

GPC of the polymer samples were run in toluene at ambient condition (~30°C) on "Waters" 510 HPLC, Millipore Corporation, with a differential refractive index detector, at the Electrical Research and Development Association (ERDA), Baroda, India. The 500 μL polymer solution (1.0%, w/v) was injected in styragel column packed with nominal pore sizes of 500, 1000 and 10,000 Å connected in series. The instrument was calibrated using standard polystyrene samples of molecular weight in the range 15,000-4,70,000[5]. The refractive index difference between the polymer solution and the pure solvent eluting out the sample and reference column, respectively were measured by using differential refractometer.

(c) Properties of Polymers :

(i) Swelling studies :

The swelling behaviour of the copolymers with various composition was studied in different hydrocarbon solvents (i.e. *n*-hexane to *n*-nonane, Qualigens, India) at room temperature (~27°C). 1.0 g of approximately uniform particle-size polymer was taken into 25.0 mL standard joint conical flask. 10.0 mL of the solvent was added onto it and the flask was kept for 24 h before the solvent was completely decanted and the polymer was dried using tissue paper. The swollen polymer was then weighed (*W*). The swelling in different solvents was calculated using the following relation[6] :

$$S = \% \text{ swelling} = \frac{W - Wo}{Wo} \times 100$$

where *W* and *Wo* are weights of the swollen and dry polymers, respectively.

(ii) Viscosity measurements :

The viscosity measurements for the polymer solution (2-3%, w/v) were done using a four armed Ubbelohde viscometer at different temperatures of 30, 35, 40 and 45°C. The viscometer was placed in a thermostated water bath maintained at constant temperature (± 0.1°C). The flow times of a polymer solution and of the pure solvent at specific conditions of temperature and concentrations were determined with the help of a hand held stop watch. The resulting ratio is customarily termed as relative viscosity (η_r). The intrinsic viscosity $[\eta]$ value is obtained as the zero-concentration intercept of the extrapolation of η_{sp}/C [$= (\eta_r-1)/C$] and $\ln\eta_r/C$ Vs C (concentration) plots[7].

The empirical equation $[\eta] = KM^{\alpha}$ relates molecular weight to intrinsic viscosity through the constants K and α. The reported K and α values of homopolymer were used to determine the molecular weight of copolymers. This is not a good method though it was done as K and α of copolymers were not available and by this way a comparative idea about the molecular weights were possible to obtain.

(iii) **Biodegradation studies** :

For biodegradation studies, various polymer solution of different concentrations (0.25 – 4.0%, w/v) were prepared in minimal salt medium. Isolated cultures (i.e. BA-1 or TED-1) were then cultivated in 5.0 mL of the above polymer solutions in 50 mL culture tubes at 30°C on a rotary shaker at 180 rpm. The growth was followed turbidimetrically at 600 nm for different time period (upto 72 hours).

2.4 References :

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