

**CHAPTER - III**

**EXPERIMENTAL**

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### **3.1 Materials**

Styrene (LOBA CHEMIE) and methylmethacrylate(MMA)(M/S ROBERT JOHNSON) were freed from inhibitors by washing with aqueous 5% sodium hydroxide, followed by washing with distilled water, drying and then they were distilled over cuprous chloride under reduced pressure.

Azobisisobutyronitrile (AIBN)(ALFA BIOCHEM) was crystallized from methanol and dried under vacuum.

Cellulose triacetate (ALDRICH), Ethyl Cellulose, (s.d.Fine-Chem Ltd, 48.2% ethoxy content) starch (BDH) were obtained from commercial sources.

Thioglycollic acid (TGA) was mixed with equal volume of benzene, then benzene was distilled out to dehydrate the acid. After heating to 100°C to remove most of the benzene the residue was distilled under vacuum.

Pyridine, tetrahydrofuran, n-hexane, benzene, N,N-dimethylacetamide, acetic acid, chloroform, mercaptoethanol obtained commercially were purified using documented procedures ( 74 ).

### **3.2 Carboxyl-Terminated Polystyrene (CPS)**

In a 200 ml three necked round bottomed flask equipped with a reflux condenser, a nitrogen inlet tube were placed 36 g styrene (345.65 mmol), 50 ml tetrahydrofuran, 0.5725 g

AIBN (3.49 mmol), 0.6 ml thioglycolic acid( 8.63 mmol). The reaction mixture was flushed with nitrogen for 15 min and then heated to 60°C for 2 h under nitrogen atmosphere using an oil bath. The prepolymer was precipitated into dry hexane and purified by reprecipitation and freeze drying with benzene.

Yield = 12 g

acid value = 23.65

$\overline{M}_n$  = 4700

### 3.3 Hydroxyl Terminated Polystyrene (HPS)

In a 200 ml three necked round bottomed flask equipped with a reflux condenser, a nitrogen inlet tube were placed 36 g styrene (345.65 mmol), 50 ml tetrahydrofuran, 0.5725 g AIBN (3.49 mmol), 0.01 ml mercaptoethanol (0.1424 mmol). The reaction mixture was flushed with nitrogen for 15 min and then heated to 60°C for 2 h under nitrogen atmosphere using an oil bath. The prepolymer was precipitated into dry hexane and purified by reprecipitation and freeze drying with benzene.

Yield = 8 g

Hydroxyl Value = 8.3

$\overline{M}_n$  = 13400,

### 3.4 Carboxyl-Terminated Poly(methylmethacrylate) CPMA

In a 200 ml three necked round bottomed flask equipped with a reflux condenser, a nitrogen inlet tube were placed 24 g MMA (239.71 mmol), 50 ml tetrahydrofuran, 0.450 g AIBN (2.743 mmol), and 0.5 ml thioglycolic acid (7.191 mmol). The reaction mixture was flushed with nitrogen for 15min and then heated to 60°C for 2 h under nitrogen atmosphere using an oil bath. The prepolymer was precipitated into dry hexane and purified by reprecipitation and freeze drying with benzene.

Yield = 15 g

Acid value = 16.93

$\overline{M}_n$  = 3300

### 3.5 Hydroxyl-Terminated Poly(methylmethacrylate) HPMMA

In a 200 ml three necked round bottomed flask equipped with a reflux condenser, a nitrogen inlet tube were placed 26 g MMA (259.68 mmol), 50 ml tetrahydrofuran, 0.450 g AIBN (2.743 mmol) and 0.4 ml mercaptoethanol (5.70 mmol). The reaction mixture was flushed with nitrogen for 15 min and then heated to 60°C for 2 h under nitrogen atmosphere using an oil bath. The prepolymer was precipitated into dry hexane and purified by reprecipitation and freeze drying with benzene.

Yield = 16g

Hydroxyl Value = 13.10

$\overline{M}_n$  = 4200

### 3.6 Hydroxyl Terminated Cellulose Triacetate (HCTA)

Cellulose triacetate was obtained from Aldrich chemicals and dissolved in chloroform and reprecipitated by methanol and dried under vacuum at 60°C

$[\eta]_{\text{CHCl}_3} = 1.85 \text{ dL/g at } 30^\circ\text{C}$

In a 250 ml three necked round bottomed flask fitted with a stirrer, a dropping funnel and a nitrogen inlet tube, 40 g cellulose triacetate and 400 ml glacial acetic acid were placed and heated to 80°C using an oil bath. When the dissolution was complete, 6.6 ml acetic anhydride and 2 ml conc. sulphuric acid were added. Two minutes later 2.4 ml water was added. The clear solution was stirred at 80°C for 5 h, cooled to 35 °C and 30 g of 21% aqueous solution of manganese acetate was added, and stirred for 30 min. The solution was poured into petroleum ether with stirring. The stirring was continued overnight and the petroleum ether was decanted off. The solvent was removed under reduced pressure to near dryness using a Rotavapour. The solid was washed with ethanol, stirred overnight in ethanol, filtered and dried under vacuum at 60°C.

Yield = 17 g

Acetyl content = 44.98%

Degree of substitution = 3.02

$[\eta]_{\text{CHCl}_3} = 0.392 \text{ dL/g at } 30^\circ\text{C}$

### 3.7 Acetylated Starch

Soluble starch (BDH) (20 g) was dissolved in minimum amount of hot water. It was kept for one day and then filtered through buckner funnel. Water was removed from the above paste of starch by successive washings with acetic acid. To the above material mixture of acetic acid (100 ml) and acetic anhydride (30 ml) was added and kept at room temperature for 15-20 h. Then precipitated into methanol, washed several times with methanol, filtered and dried under vacuum at 60°C.

Yield = 10 g

Acetyl content = 41.55

Degree of substitution = 2.63

$[\eta]_{\text{CHCl}_3} = 0.3958 \text{ dL/g at } 30^\circ\text{C}$

### 3.8 Ethyl Cellulose-Methylmethacrylate Graft Copolymers

#### 3.8.1 Coupling of HPMMA with Ethyl Cellulose in Dichloromethane using TDI Coupling Agent (GCP1)

In a 100 ml three necked R.B.flask equipped with a reflux condenser and a nitrogen inlet tube were placed 75 ml dichloromethane, stirred well with magnetic needle and added slowly 2 g dried Ethyl Cellulose (8.66 mmol). After dissolution 8 drops of dibutyltin dilaurate (DBTDL) and 3.3

ml (23.21 mmol) tolylene-2,4-diisocyanate<sup>^</sup> were added in succession at room temperature. The reaction mixture was stirred at 40°C bath temperature under dry nitrogen atmosphere for 3 h. Then, the contents were cooled to room temperature. Excess of solvent was removed under vacuum. Contents of the flask were washed with hexane for 3 to 4 times under nitrogen atmosphere using a stainless steel cannula. Then solution of 5 g of HPMMA (1.001 mmol) in minimum quantity of dichloromethane was added to the reaction flask under nitrogen atmosphere followed by 8 drops of DBTDL. The reaction mixture was heated to and maintained at 40°C for 15 h. The contents were then cooled and precipitated into excess of hexane and filtered, the residue was washed with hexane and acetone-methanol mixture (30:70 V/V), and dried under vacuum at 60°C.

Yield = 4.14 g

Solubility = Insoluble in most common solvents.

### 3.8.2 Coupling of HPMMA with Ethyl Cellulose in Dichloromethane using TDI Coupling Agent (GCP1a).

General procedure adopted was same as described in section 3.8.1. except that the EC-TDI reaction time was 1 1/2 h instead of 3 h

Yield = 2.7 g

Solubility = Insoluble in most of common solvents.

### 3.8.3 Coupling of CPMMA with Ethyl Cellulose in N,N-Dimethylacetamide (GCP2)

In a 100 ml three necked round bottomed flask equipped with a reflux condenser, a nitrogen inlet tube and a pressure equalizing dropping funnel were placed 5 g dried CPMMA (1.385 mmol) and 20ml benzene when CPMMA dissolved completely the reaction flask was cooled to 0°C using ice salt bath and 10 ml thionyl chloride was added dropwise through pressure equalizing funnel. Two/three drops of dry pyridine were added. The ice-salt bath was removed and the contents of the flask were stirred at room temperature for 1 h. Then the temperature of reaction mixture was raised to 80°C using an oil bath. The reaction was allowed to proceed at 80-90°C under nitrogen atmosphere for 72 h. Benzene and excess of thionyl chloride were removed under reduced pressure, and the solid acid chloride was dried under vacuum at room temperature.

The acid chloride was then dissolved in 15 ml DMAc and to it was added a solution of 2 g dried ethyl cellulose in 20 ml DMAc at room temperature. The temperature was raised to and maintained at 80-90°C and the reaction was allowed to proceed for 24 h under nitrogen atmosphere. After the stipulated reaction time the contents were cooled to room temperature and precipitated into excess of water. The

precipitate was washed several times with water and it was further purified by reprecipitation from DMAc solution by addition of deionized water. The precipitate was filtered and dried under vacuum at 60°C.

Yield = 6.0 g

$[\eta]_{\text{CHCl}_3} = 0.5205 \text{ dL/g at } 30^\circ\text{C}$

### 3.9 Acetylated Starch-Methylmethacrylate Graft Copolymers.

#### 3.9.1 Coupling of HPMMA with Acetylated Starch in Dichloromethane using TDI Coupling Agent (GCP3)

In a 100 ml three necked RB flask equipped with a reflux condenser and a nitrogen inlet tube were placed 75 ml dichloromethane, stirred well with magnetic needle and added slowly 1 g dried acetylated starch. After dissolution, 8 drops of DBTDL and 1 ml (7.033 mmol) tolylene-2,4-diisocyanate were added in succession at room temperature. The reaction mixture was stirred at 40°C bath temperature, under dry nitrogen atmosphere for 1 1/2 h. Then, the contents were cooled to room temperature. Excess of solvent was removed under vacuum. Contents of the flask were washed with hexane for 3 to 4 times under nitrogen atmosphere using a stainless steel cannula. Then, solution of 2.5 g of HPMMA (0.5005 mmol) in minimum quantity of dichloromethane was added to the reaction flask under nitrogen atmosphere followed by 8 drops of DBTDL. The reaction mixture was

heated to and maintained at 40°C for 15 h. The contents were then cooled and precipitated into excess of hexane and filtered, the residue was washed with hexane and acetone-methanol mixture (30:70 V/V), and dried under vacuum at 60°C.

Yield = 3.1 g

$[\eta]_{\text{CHCl}_3} = 0.21 \text{ dL/g at } 30^\circ\text{C}$

### 3.9.2 Coupling of CPMMA with Acetylated Starch in N,N-Dimethylacetamide (GCP4)

In a 100 ml three necked RB flask equipped with a reflux condenser, a nitrogen inlet tube and a pressure equalizing dropping funnel were placed 10 ml thionyl chloride. The flask was cooled to 0°C using ice-salt bath and a drop of dry pyridine was added. Then, 5 g of dried CPMMA (1.385 mmol) was slowly added. Ice-salt bath was removed and contents of flask were stirred at room temperature for 1 h. Then the temperature of reaction mixture was raised to 80°C using an oil bath. The reaction was allowed to proceed at 80-90°C under nitrogen atmosphere for 24 h. Excess of thionyl chloride was removed under reduced pressure. Solid acid chloride was dried under vacuum at room temperature.

The acid chloride was then dissolved in 15 ml DMAc and to it was added a solution of 2 g acetylated starch in 20 ml DMAc, at room temperature. The temperature was raised to and maintained at 80-90°C and the reaction was allowed to

proceed for 24 h under nitrogen atmosphere. After the stipulated reaction time the contents were cooled to room temperature and precipitated into water. The precipitate was washed several times with water, then with acetone-methanol mixture (30:70 V/V), and dried under vacuum at 60°C.

Yield = 6.0 g

$[\eta]_{\text{CHCl}_3} = 0.2515 \text{ dL/g at } 30^\circ\text{C}$

### 3.10 Cellulose Triacetate-Styrene Block Copolymers

#### 3.10.1 Coupling of CPS with HCTA in N,N-Dimethylacetamide (BCP1)

Dried CPS (0.083 mmol) was converted into acid chloride as described in section 3.8.3 and dissolved in 15 ml dry DMAc. To it was added a solution of 2 g of (0.0838 mmol) hydroxy terminated cellulose triacetate (HCTA) in 15 ml DMAc at room temperature. The temperature was then raised to 70°C. The reaction was allowed to proceed at 70-80°C under nitrogen atmosphere for 24 h. The contents were cooled to room temperature and precipitated into excess of methanol. The precipitate was filtered, washed with methanol and dried under vacuum. The dried product was immersed overnight in acetone methanol (30:70 V/V) mixture and filtered. The residue was washed with acetone-methanol mixture and dried under vacuum at 60°C.

Yield = 2.7 g

$$[\eta]_{\text{CHCl}_3} = 0.3790 \text{ dL/g at } 30^\circ\text{C}$$

### 3.10.2 Coupling of HPS with HCTA using TDI Coupling Agent (BCP2)

In a 100 ml three necked RB flask equipped with a reflux condenser, a nitrogen inlet tube were placed 3 g (0.2246 mmol) HPS and 15 ml dry pyridine. When HPS was completely dissolved, 3.91 ml of 1% solution of TDI in pyridine corresponding to 0.2246 mmol of tolylene 2,4-diisocyanate was added through a syringe at room temperature. The reaction mixture was stirred at 115-120°C using an oil bath under nitrogen atmosphere for 24 h. Then the contents were cooled to room temperature and solution of 5.36 g (0.2247 mmol) hydroxy terminated cellulose triacetate in 15 ml dry pyridine was added under nitrogen atmosphere. The reaction mixture was heated to and maintained at 115-120°C for 48 h. The contents were then cooled and precipitated into excess of methanol, filtered, washed with methanol and dried under vacuum. The dried product was placed overnight in acetone and filtered. The residue was washed with acetone-methanol mixture (30:70 V/V) and dried under vacuum at 60°C.

Yield = 2.7 g

$$[\eta]_{\text{CHCl}_3} = 0.2900 \text{ dL/g at } 30^\circ\text{C}$$

### **3.11 Biodegradability of Starch Based Copolymers.**

#### **3.11.1 Deacetylation of Starch Based Copolymers**

A modification of Vink's (75) procedure was used. Sodium (1.0 g) was added to anhydrous methanol(300 ml) contained in a three necked flask equipped with an agitator and nitrogen inlet and outlet tube. After dissolution was complete, the starch based copolymer (10 g) was introduced. The heterogenous mixture was stirred continuously at room temperature for 3 h. The solid was collected, washed with methanol and dried at 60°C under vacuum.

#### **3.11.2 Enzymatic Hydrolysis**

1 ml of saliva was diluted 25 times with 0.85% NaCl and the diluted enzyme solution was used for hydrolysis of starch based copolymers.

The copolymer(0.2 g) was added to enzyme solution (10 ml) contained in a 50 ml stoppered conical flask. It was kept for 15 h and filtered. The filtrate was used for estimation of glucose by titration using  $K_4 [Fe(CN)_6]$  as an external indicator.

### **3.12 Chemical Analysis**

Acetyl content and degree of substitution of acetylated starch and cellulose triacetate was determined by saponification with standard sodium hydroxide followed by titrating the excess of alkali with standard hydrochloric

acid as described in literature (72).

The carboxyl group content of CPMMA and CPS was determined by titration in THF with 0.02 N aqueous potassium hydroxide using phenolphthalein as an indicator ( 71 ).

The Hydroxyl group content of HPMMA and HPS was determined by acetylation with standard acetylating mixture followed by titration with standard alkali.

### 3.13 Instrumental Methods

The viscosities of various polymer solutions were determined by using Ubbelohde viscometer. A constant temperature bath was maintained at 30°C.

IR spectra were taken on Perkin Elmer 883 IR spectrophotometer. About 1 to 1.5 mg of the sample was dispersed in 80 mg of dry KBr and KBr pellets were prepared using the standard procedure. The spectra were scanned in 4000 to 600  $\text{cm}^{-1}$  region.

Proton NMR spectra were obtained on a F X 90 Q F T NMR spectrophotometer in deuteriated chloroform using TMS as an internal standard.