Studies on Biodegradable Polymeric Nanocomposites Based on Sheet Molding Compound for Orthopedic Applications

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Abstract- **Biodegradable polymeric bone plates suffer from warping, hollowing or substantial erosion inherent with the process of degradation. In order to solve the problem of warping and insufficient mechanical strength, forging of curable sheet molding polyester compound has been attempted. Polymeric nanocomposite was prepared by forging using compression molding with a sheet molding compound (SMC) consisting inorganically modified hydroxy terminated poly (propylene fumarate) (HT-PPF). Hydroxyapatite nanopowder (100-250nm sintered) and polyol modified-polymeric diphenyl methane diisocyanate (Empeyonate PM-35, NCO Content 27%) were used for modification of HT-PPF to get the SMC. The DTA studies on SMC mixed with methyl methacrylate reveal exothermic peak at 94.9^{o}C for the curing of SMC though crosslinking of HT-PPF with methyl methacrylate. SEM microphotographs (fractography) of as-prepared compression molded polymeric nanocomposite reveal serrated structure reflecting higher mechanical strength and good dispersion of hydroxyapatite in the polymer matrix and good interfacial bonding between the polymer and hydroxyapatite nanocrystals.The surface studies revealed the hydrophilicity of the composite. The *in-vitro* degradation of the nanocomposite was found to be more in Ringer solution than PBS which is due to the slow degradation by the buffering effect of the PBS and hydroxyapatite. The nanocomposite is nonhemolytic, noncytotoxic and blood compatible.**

Keywords- Sheet Molding Compound; Inorganically Modified Hydroxy Terminated Poly (Propylene Fumarate); Polymeric Nanocomposite; Interfacial Bonding; Blood Compatibility

I. INTRODUCTION

Metal plates used in fracture fixation are more rigid than bone. They transmit the majority of the stress received by the bone-plate system, resulting in localized bone atrophy and osteoporosis during latter stages of fracture healing. Stress shielding also interferes with blood circulation. The weaker bone may fracture again after implant removal [1]. Therefore metal plates and screws have to be surgically removed after they have served their stabilization function. Thus the demand for non-metallic bone fixation devices, which can exert less stress protection on bone and allow flexible fixation of fracture is increasing with time. Biodegradable polymeric devices have more advantages over the non-biodegradable.

For the success of the bone fixation devices, it is essential to match modulus and strength of the biomaterial to that of bone to prevent the stress protection with dimensional gradual biodegradation so that the regenerated natural tissue can grow over the biodegraded composite implant without any tissue necrosis. An ideal fracture fixation device should have optimum mechanical properties during the two stages of fracture healing [2].Initially, the implant must have sufficient stiffness to allow bony union without angulations [3].Some degree of mechanical stimulation at the fracture site is desired to allow the formation of cortical callus, the most rapid way to achieve bony union and restore the strength of the broken bone to its original level [4].During the second stage of fracture healing the implant must deform sufficiently under a given load to increase the strain within the bone, permitting remodeling to occur and avoid the effects of stress protection.

Bone plates of reinforced polymer composites have shown some success in the prevention of stress protection [5,6]. Fiber reinforced polymer composites having perfect alignment possess a wide range of properties such as specific strength and stiffness. The positive aspects of heterogeneity and anisotropy of synthetic composite biomaterials can be easily exploited. The elastic properties of composites can be accurately controlled and be tailor-made to match close to the elastic property of host bone tissue. Anisotropy can be exploited in biomedical structures which require specific stress and stiffness like longitudinal and transverse rigidities for bone replacement application. Heterogeneity can be utilized in a partially biodegradable composite implant, where the matrix will be gradually resorbed to make room for regenerated natural tissue. The latter will grow on to the reinforcement, which serves as a permanent scaffold [7].

Biodegradable plates also suffer from warping, hollowing or substantial erosion inherent with the process of degradation. To solve the problem of insufficient mechanical strength, polymers with high crystallinity have been explored [1], [8,9]. Reinforcing elements such as fibers of crystalline polymers, fibers of carbon in polymeric resins at particulate fillers have also

been studied [10,11]. Recently interpenetrating network in an implant was reported for improving the mechanical strength [12,13].

Though degradable linear poly (lactide) (PLA) and poly (lactide-*co*-glycolide) (PLGA) are used in experimental studies as fracture fixation devices, they are unsuitable for bone fixation because of insufficient mechanical properties and uncontrolled biodegradation. The excessive biodegradation and liberation of acidic components generated by PLA and PLGA-based devices at the vicinity of degradation site have resulted in tissue necrosis. Therefore PLA and PLGA polymers are not ideal candidate materials for bone fixation devices.

Crosslinkable and biodegradable polyester resin is alternate polymer for the development of most promising bone fixation devices. In order to solve the problem of warping and insufficient mechanical strength, forging of curable sheet molding polyester compound has been attempted with general purpose polyester resins. Generally polyester resins are widely used in molding applications in liquid form; such liquid resins comprise a solution of liquid or solid polyester dissolved in a liquid cross-linking agent. Solid forms of polyester resins can be made from the liquid polyester resin solutions by the addition of a chemical thickening agent such as an oxide or hydroxide of magnesium or calcium. Or the liquid polyester resin solution can be converted into solid form by adding there to solid filler, such as calcium carbonate, which absorbs the liquid resin. A serious disadvantage in relying on the use of filler to absorb sufficient amounts of a liquid resin is that the properties of the articles made from the cured composition can be affected adversely. In general, the higher the proportion of filler in the composition, the poorer the strength of articles made from them.

As it is essential to match modulus and strength of the biomaterial to that of bone to prevent the stress protection and allow dimensional biodegradation for the growth of regenerated bone, it is more relevant to prepare alternate crosslinked biodegradable poly (propylene fumarate) polyester composites and study the factors which influence the performance of the composite for orthopedic applications. The present paper deals with the studies on reinforced biodegradable polymer composite based on sheet molding compound of poly (propylene fumarate) for use as bone fracture fixation devices.

II. MATERIALS AND METHODS

A. Preparation and Characterization of Inorganically Modified HT-PPF Resin

Initially oligomeric hydroxyl terminated-poly (propylene fumarate) (HT-PPF) resin was synthesized as reported elsewhere [14]. Briefly, 2,5-furandione and 1,2-propanediol were refluxed, followed by vacuum-condensation at 140-200^{o}C and 1 mbar for 4h. The reaction was catalysed by sodium acetate and tetrahydro-1,4-oxazine. The reaction product was dissolved in acetone and then washed with 25% aqueous methanol to remove unreacted reactants. The polymer was reprecipitated in petroleum ether, filtered and dried under vacuum. A highly viscous, yellowish brown and transparent resin was obtained. HT-PPF was characterized. The molecular weight and polydispersity of HT-PPF resin were determined by gel permeation chromatography. Styragel-H-5E/4E/2/0.5 columns with mobile phase tetrahydrofuran having flow rate – 1.0 ml/min were used. Water GPC system with 600 series pump, 2414 refractive index detector were used. Infrared spectral analysis of HT-PPF resin was carried out using a Nicolet (5700 FT-IR) spectrophotometer by scanning a film of the viscous resin on NaCl window as per the test method ASTM E 1252-98.

The inorganically modified HT-PPF resin (Component ‘A’) was prepared by blending HT-PPF resin (1.338g) with polyol modified-polymeric diphenyl methane diisocyanate (0.5g), (Empeyonate PM-35, NCO Content 27%) (M/S Mitsu Takeda Chemicals, Inc., Japan) and hydroxyapatite nanopowder (100-250nm sintered) (0.67g) and 1,4 diazobicyclo (2,2,2) octane (DABCO) (0.006g) under ambient conditions for 15 min. The weight ratio of HT-PPF resin, Empeyonate PM-35 and hydroxyapatite is 53:20:27pbw. This product was analyzedusing attenuated total reflection (ATR) spectroscopy. The IR Spectrum was recorded using a Nicolet (5700 FT-IR) spectrophotometer. Differential thermal analysis (DTA) and thermo-gravimetric analysis (TGA) was carried out as per ASTME-1131-08 and ASTME-537-98. The sample was heated to a maximum temperature of 500 ℃ at the heating rate of 10 ℃ /mm in inert nitrogen (99.99% purity, moisture 1ppm and oxygen 1ppm) atmosphere. A SDT-Q600 simultaneous DTA-TGA instrument (M/S TA instrument) was used. ASTM D 3418-08 method was used for DSC analysis. The sample was heated from -50 to 200 ℃ at the heating rate of 10 ℃ /mm in inert nitrogen atmosphere.

B. Optimization of Formulation of Sheet Molding Compound

The sheet molding compound was formulated as three components consisting inorganically modified HT-PPF resin (Component ‘A’), Component ‘B’ and ‘C’. Component ‘B’ consists of monomer methyl methacrylate (MMA) and crosslinker diethylene glycol dimethacrylate. Component ‘C’ consists of initiator benzoyl peroxide, lubricant zinc stearate and filler calcium carbonate.

1) Setting and Hardening Characteristics of the Sheet Molding Compound:

a) Effect of Variation in Comonomer Concentration:

The effect of variation in comonomer methyl methacrylate concentration on the setting and hardening of the sheet molding

compound to a semisolid form was studied. Component 'A' was prepared as above. Component 'B' was prepared by mixing methyl methacrylate and diethylene glycol dimethacrylate (0.668g). Component 'C' was prepared by mixingbenzoyl peroxide (0.2g), zinc stearate (0.1g) and calcium carbonate (0.4g). The components 'A', 'B' and 'C' were mixed well and the setting was observed. The setting time and exothermic temperature during setting to a semisolid were determined as per ISO 5833/1-1999 E Standard. Methyl methacrylate was varied in the following concentrations 0.7, 1.0, 1.3 and 1.5g while keeping other components constant.

b) Effect of Variation in Cross-Linker Concentrations:

The effect of variation in PM35 concentration on the setting and hardening characteristics of the sheet molding compound was studied. The component 'A' was prepared by blending HT-PPF resin (1.338g) with modified diphenyl methane diisocyante [Cosmonate PM-35] and hydroxyapatite (0.67g) and 1,4 diazobicyclo (2,2,2) octane (DABCO)(0.006g). The component 'B' was prepared by mixing methyl methacrylate (0.668g) and diethylene glycol dimethacrylate (0.668g). Component 'C' was prepared as described earlier. The crosslinker PM35 was varied in the following concentrations 0.5, 1.0, 1.35 and 2.0g while keeping other components constant. The setting characteristics were determined as above.

c) Determination of Hardness of Sheet Molding Compound:

The variation of hardness with different concentrations of MMA 0.7, 1.0, 1.3 and 1.5g and of PM35 0.5, 1.0, 1.35 and 2.0g was studied. The sheet molding compound was prepared by mixing Component 'A', Component 'B' and "C', filled in the cavity of stainless steel cylindrical mould (12-mm diameter) and allowed to set. The molded compound was recovered from the mould. Hardness was measured using a Shore A-durometer as per ASTM standard D 2240-8 1. The hardness attained with concentrations of MMA 0.7, 1.0, 1.3 and 1.5g and of PM35 0.5, 1.0, 1.35 and 2.0g after hardening was recorded. The maximum hardness attained and the time taken to attain the maximum hardness was recorded.

C. Preparation and Characterization of Sheet Molding Compound

The sheet molding compound was prepared based on the optimized chemical components which offered favorable setting and hardening characteristics. The formulation consists Component 'A' withHT-PPF resin (4g), Cosmonate PM-35 (1.5g), hydroxyapatite (2.0g) and 1,4 diazobicyclo (2,2,2) octane (DABCO) (0.017g). Component 'B' consists of methyl methacrylate (3.9g), and diethylene glycol dimethacrylate (2.0g). Component 'C' consists of benzoyl peroxide (0.6 g), zinc stearate (0.3 g) and calcium carbonate (1.2 g). The components 'A', 'B' and 'C' were mixed well and kneaded to get a semi-solid. The sheet molding compound was stored in airtight container. The thermal behavior of the sheet molding compound was studied by thermo-gravimetric analysis (TGA) and differential thermal analysis (DTA) as mentioned in previous section.

D. Compression Molding of Sheet Molding Compound and Characterization of Compression Molded Composite Sheet

The sheet molding compound was compression mounded to get composite sheet. The molding process was carried out at a temperature of 130ºC and a pressure of 130 kg/cm^2 for a period of 15 min. The surface and interfacial morphology of the compression molded composite was investigated using scanning electron microscope (SEM). The samples were shock-frozen in a liquid nitrogen bath for 1 min. The frozen sample underwent brittle fracture without any external force. The surface and cross section of the fractured sample was analysed by scanning electron microscopy after gold sputtering. The thermal behavior of the compression molded composite was studied by thermo-gravimetric analysis (TGA) and differential thermal analysis (DTA) as mentioned in previous section. The hardness of the compression molded composite was determined. Hardness was measured using a Shore D-durometer as per ASTM standard D 2240-81. Dynamic mechanical analysis was carried out to identify the glass transition regions. The test conditions were: temperature sweep tensile mode, frequency 1Hz, temperature range between -150ºC to 150ºC and scan rate 1ºC/min.

E. Studies on the in-vitro Biodegradation of Compression Molded Composite

The degradation of the compression molded composite was evaluated by *in vitro* aging in two media, phosphate buffered saline (pH 7.4) and Ringer solution. Uniform samples of composite were accurately weighed and immersed in both the media. The aging of samples was carried out at 70^{0}C for 22 days. At regular intervals, the samples were taken out, dried and weight loss was determined. The weight loss (%) was plotted against duration of exposure. The aging stability was determined. A digital tablet disintegration apparatus (from Veego scientific) was used for the immersion experiments.

F. Evaluation of Blood Compatibility of Compression Molded Composite

The blood compatibility was investigated as per the procedure ISO 10993-4. The blood compatibility was evaluated by determining the percentage of hemolysis and RBC aggregation. Initially the composite was kept in sterile 1xPBS for 48h and the extract of the composite was collected. Hemolysis assay was carried out as per the reported procedure [15]. In brief 100µl of the plasma separated blood was diluted with 800µl of saline. To this diluted blood 100µl of the PBS extract of test sample was added along with the controls. Here normal saline was used as negative control and distilled water as positive control. The samples were incubated for half an h at 37^{0}C and centrifuged at 700 rpm for 5 min. The absorbance (OD) was measured at 541 nm by UV–Vis spectrophotometer (Varian). From the OD values the % hemolysis was calculated. *In vitro* RBC aggregation

assay was carried out as per the reported procedure [16].Anticoagulant (3.8% sodium citrate) human blood sample was collected. To obtain the RBC layer, the blood sample was centrifuged at 700 rpm for 10 min; the separated RBC layer was washed twice with saline and then diluted with saline (1:4). 100µl of this diluted RBC was incubated with 100µl of PBS extract of test sample, incubated for 30 min at 37^0C. PEI and saline were used as positive and negative controls.

G. In Vitro evaluation of Cytocompatibility of Compression Molded Composite

1) Direct Contact Method:

The cytotoxicity of composite was evaluated by directly exposing the composite material to L-929 mouse fibroblast cells as per ISO 10993-5. The test materials were initially incubated in culture medium overnight at 37˚C. Then the composite samples were placed on a subconfluent monolayer of L-929 mouse fibroblast cells and incubated at 37°C in a CO_2 incubator for 24h. The cell responses around the test samples were microscopically evaluated.

2) Quantitative Estimation of Surviving Fibroblast Cells to Assess Cytotoxicity:

Initially the extracts of the composite was prepared. The composite of 1cm×1cm was immersed in 2mL serum-supplemented DMEM cell culture media. The extracts were prepared by incubating composite at 37°C in a CO_2 incubator for 24 hs. The amount of surviving cells to assess cytotoxicity was evaluated by MTT Assay as per the reported procedure [17]. MTT assay is based on the capability of metabolically active cells to reduce the yellow water-soluble tetrazolium salt (MTT) to purple formazan crystals using the mitochondrial enzyme succinate dehydrogenase (SDH). The intensity of purple color so formed is proportional to the number of viable cells. Monolayer culture of L 929 was initiated at a density of $5x10^3$ cells per 24 well and incubated for 24h. Following incubation, the cell culture media were aspirated from the mono layers. All cultures were incubated at 37˚C for 72h in a CO_2 incubator. Then 50µl MTT solution (MTT 5 mg/vol dissolved in PBS and filtered through a 0.2µm filter before use) was added to the culture. The whole content was again incubated at 37^oC for 3h and added 300µl DMSO and incubated at room temperature for 30 min for all cells to get lysed. The homogenous color so obtained was centrifuged at 1000rpm for 2min to sediment cell debris. Then the optical density (OD) was measured spectrophotometrically at 540 nm using DMSO as blank.

III. RESULTS AND DISCUSSION

A. Preparation and Characterization of Inorganically Modified HT-PPF Resin

Slow degradable and bioassimilable polyester, poly(propylene fumarate) [PPF] is required for orthopedic applications. Synthesis of PPF resin has been attempted by various researchers. Sanderson has prepared PPF by transesterification reaction [18]. Gerhart and Hayes [19] and Domb *et al* [20] have also synthesized low molecular weight PPF (M_n = 500-1200) by a condensation reaction involving propylene glycol and fumaric acid. Yazemski *et al* [21] and Peter *et al* [22] also have produced PPF by a two-step process involving the synthesis of bis (2-hydroxy propyl fumarate). The synthesis of PPF resin (condensation reaction involving the acid/anhydride and alcohol) is generally interfered with reverse reactions as well as side reactions at the double bonds. Therefore the molecular weight of PPF is limited by the reversibility of polyesterification and transesterification reactions proceeding in temperatures above 180 °-200 ℃ for several hours. Moreover, the condensation reaction at 190 ℃ involves cyclization side reactions involving maleic anhydride and glycols which reduces the unsaturation (about 10-20%). In order to get low molecular weight PPF with less strained, highly reactive and more planar trans fumarate configuration, hydroxy terminated-poly(propylene fumarate) (HT-PPF) oligomeric resin was prepared by esterification and isomerisation of maleate during the refluxing and vacuum condensation [14]. The reaction scheme and chemical structure of HT-PPF is shown in Figure1.The present HT-PPF is a structopendant unsaturated polyester having double bonds within the polymer chain. The molecular weight and polydispersity of hydroxy terminated-poly (proplyene fumarate) (HT-PPF) resin were determinedusing gel permeation chomatography. The molecular weight of HT-PPF was 1406 g mol^{-1} (Mn), 2456 g mol^{-1} (Mw) and polydispersity 1.75 which suggest oligomeric nature of HT-PPF.

The inorganically modified HT-PPF resin prepared by end capping the HT-PPF resin and hydroxyapatite with Cosmonate PM35 diisocyanate has resulted in the formation of urethane linkages (Figure1) through the reaction of hydroxyl groups in HT-PPF and hydroxyapatite with isocynate. The inorganically modified HT-PPF resin also contains reactive fumarate functional groups in PPF units for cross linking with vinyl monomer.

FT-IR analyses of HT-PPF resin inorganically modified HT-PPF polymer reveal inorganic modification in the latter. The IR spectrum of HT-PPF resin (Figure2 A) shows a strong peak for ester(C=O) group at 1720 cm^{-1} and an intense broad band for hydroxyl group (-OH) at 3446-3511 cm^{-1}. The absence of peak at 3400 cm^{-1} reveals that the end groups are hydroxyl groups instead of carboxyl groups. The peak around 2985 cm^{-1} is due to the aliphatic C-H group in the chain. The peak for unsaturated double bonds(C=C) between carbon atoms of fumarate linkage was observed at 1645 cm^{-1} and 982 cm^{-1}. Other pertinent peaks observed were methylene scissoring and methyl asymmetric bend in the 1455cm^{-1} region and C-O stretch at 1262-1297 and 1159cm^{-1}. The AT-IR spectrum of inorganically modified HT-PPF polymer (Figure2 B) shows a strong peak for ester(C=O) group at 1715 cm^{-1} and weak band for hydroxyl group (-OH) at 3335cm^{-1} for HT-PPF unit.

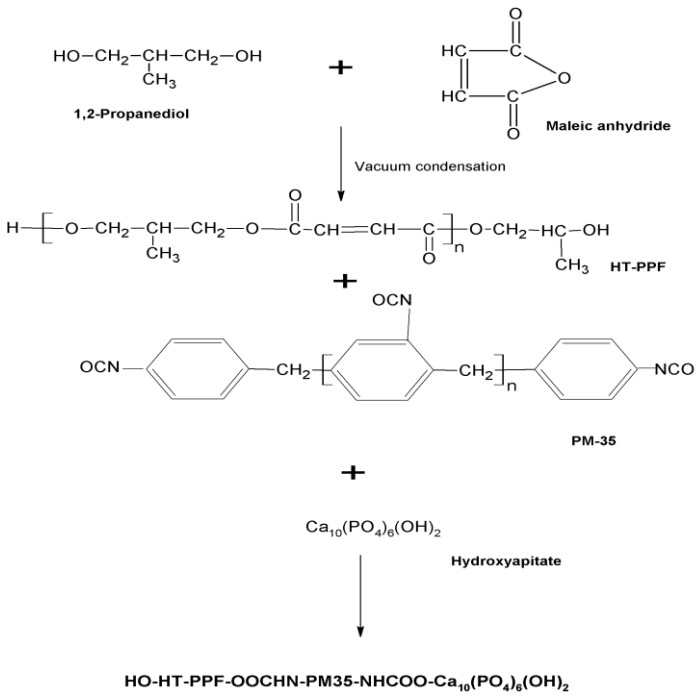

Fig.1 Formation of inorganically modified HT-PPF polymer

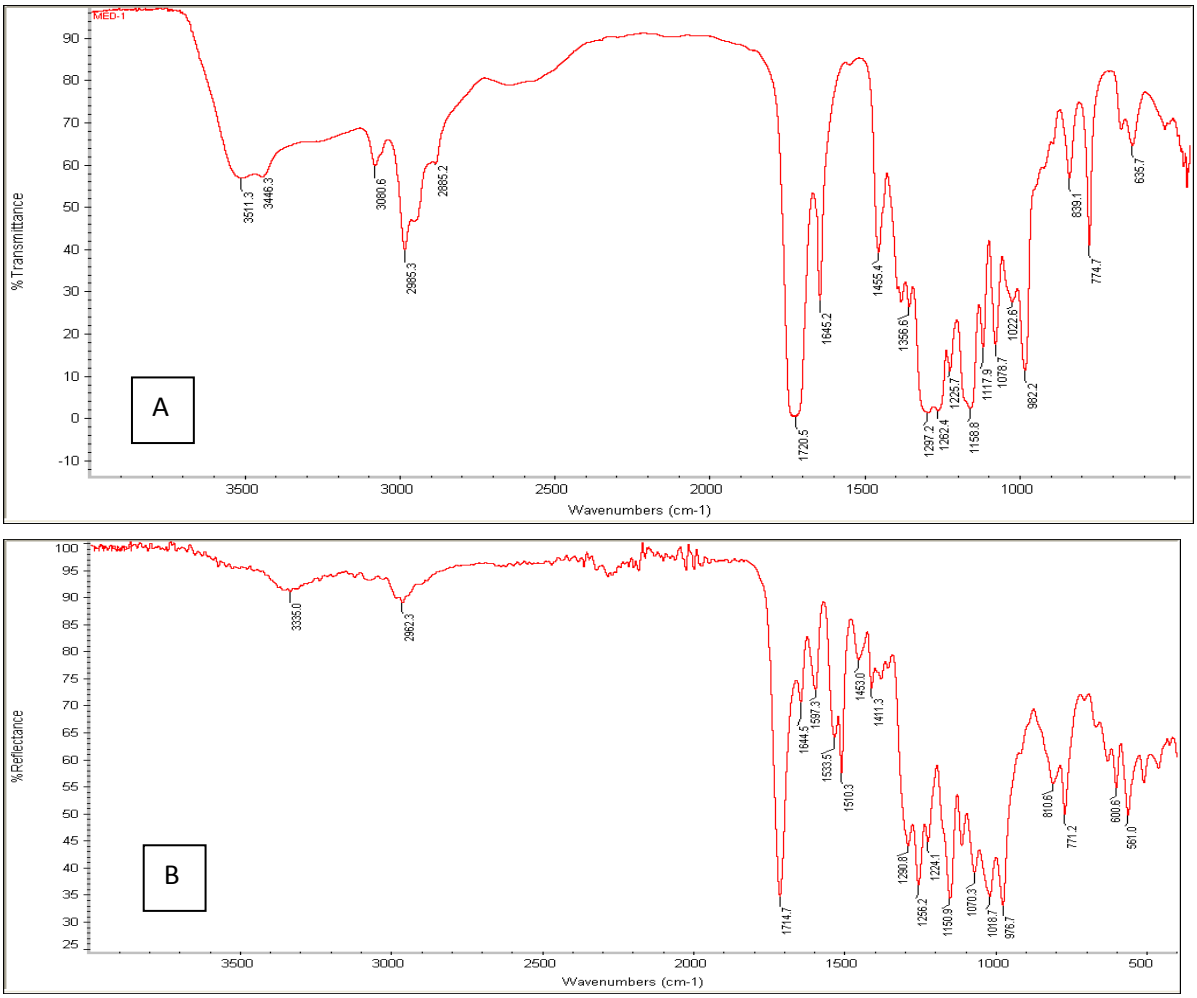

Fig. 2 FTIR Spectrum of hydroxy terminated- poly (proplyene fumarate) (HT-PPF) (A) and AT-IR spectrum of inorganically modified HT-PPF polymer (B)

The weak band reveals that the hydroxyl end groups are consumed in the reaction with isocyanate groups of PM35. The peak for unsaturated double bonds for fumarate linkage was observed at 1645 cm^{-1} and 977 cm^{-1}. Other pertinent peaks

observed were methylene scissoring and methyl asymmetric bend in the 1455 cm^{-1} region and C-O stretch at 1262-1297 and 1159 cm^{-1}. With these features additional peaks for urethane linkages -NHCOO- also appeared at 1533 $cm^{-1}$for N-H bending of urethane linkage. The double peak at 600 cm^{-1} and 561cm^{-1} are due to bending of P-O bonds in phosphate groups of hydroxyapaptite.

The DSC thermal analysis of inorganically modified HT-PPF polymer reveals glass transition at temperature 40.74^{0}C.The DSC trace is given in Figure3A.

DTA analysis reveals softening around 50-75^{0}C which is due to the disruption of physical crosslinks. The DTA trace is shown in Figure3B. The DTA curve also indicates a mild endotherm above 225^{o}C for chain scission and degradation of inorganically modified HT-PPF resin. The DTA curves also indicate one exotherm at 310^{0}C for cross linking of the degraded fragments. The TGA analysis also confirms the degradation by two step degradation starting from 225 to 385^{0}C and from 385 to 495^{0}C. The TGA trace is shown in Figure3C. The thermal analyses confirm the thermoplastic character with a characteristic glass transition and softening temperature.

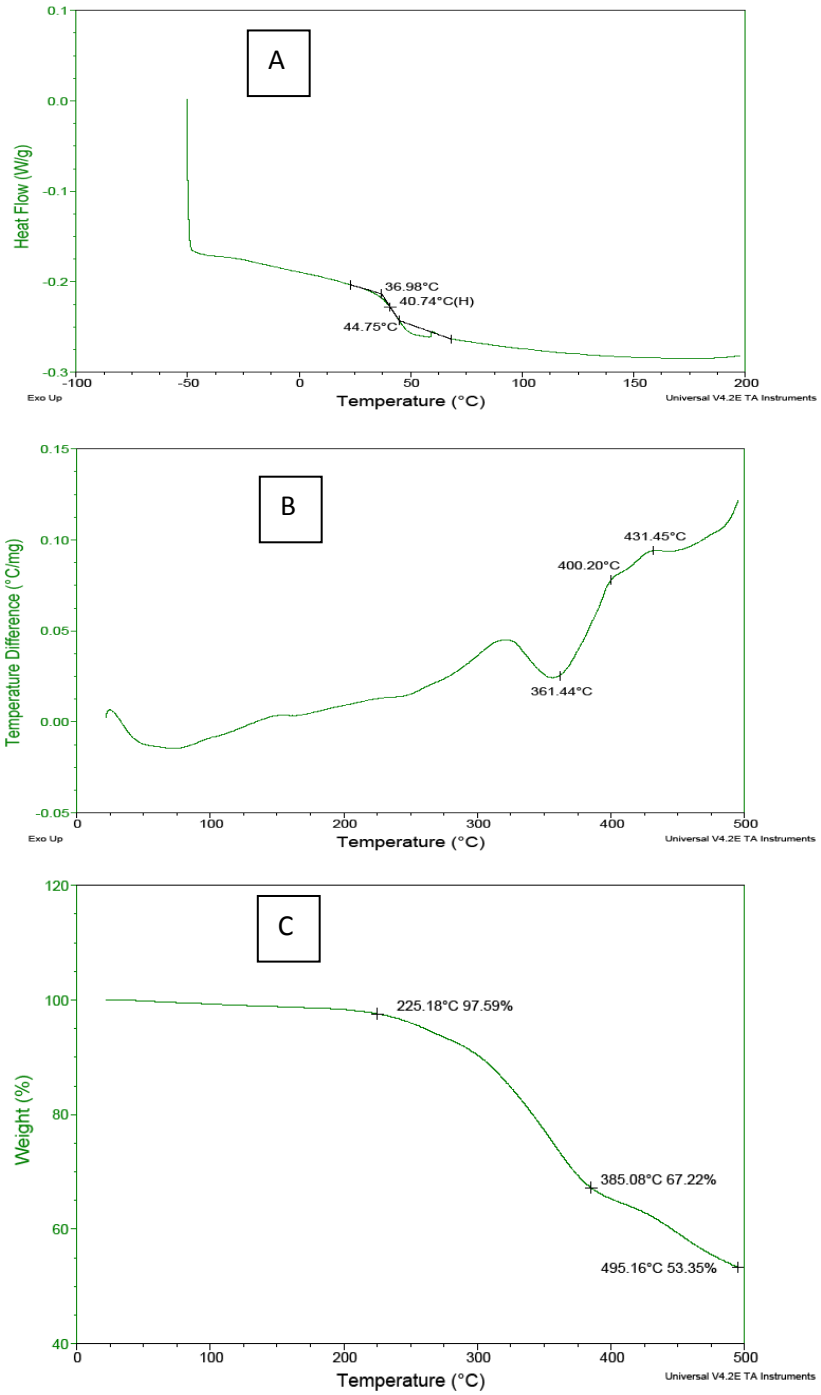

Fig. 3 Thermal analyses of inorganically modified HT-PPF DSC trace (A), DTA trace (B), TGA trace (C)

B. Optimization of Formulation of Sheet Molding Compound

The sheet molding compound of polyester resin is generally formulated using chemical thickening agent such as an oxide or hydroxide of magnesium or calcium or by absorption of liquid resin by the solid filler, such as calcium carbonate. In the present investigation the thickening of liquid polyester resin to a semi solid and doughy mass was achieved by inorganically modifying the HT-PPF resin and also by absorption by the solid hydroxyapatite and calcium carbonate fillers. In the present studies, the sheet molding compound consists of three components comprising inorganically modified HT-PPF resin (Component 'A'), Components 'B' and "C'. Component 'B' consists of methyl methacrylate and diethylene glycol dimethacrylate. Component 'C' consists of benzoyl peroxide, zinc stearate and calcium carbonate.

The effect of variation in comonomer MMA and PM35 in inorganically modified HT-PPF resin (Component 'A') on the setting and hardening characteristics of the sheet molding compound reveal interesting results. The setting time ($t_{setting}$) and setting temperature ($T_{setting}$) are more relevant parameters in the formation of the sheet molding compound. The setting time ($t_{setting}$) of the present polymer systems is largely influenced by the concentration of comonomer, methyl methacrylate which interferes with the absorption by the solid hydroxyapatite and calcium carbonate fillers. Similarly, the setting is interfered by the extent of inorganic modification of HT-PPF resin by and PM35. The setting and hardening characteristics of the sheet molding compound are given in Tables 1 and 2.

TABLE I SETTING AND HARDENING CHARACTERISTICS OF THE SHEET MOLDING COMPOUND WITH MMA

Concentration of MMA (g)	Setting time (min)	Setting temperature (℃)	Hardening time (min)	Hardness attained (Shore A)
0.7	60	32	1250	40
1.0	60	32	1270	25
1.3	70	33	1290	17.5
1.5	90	32	1330	12.5

TABLE II SETTING AND HARDENING CHARACTERISTICS OF THE SHEET MOLDING COMPOUND WITH PM35

Concentration of PM35 (g)	Setting time (min)	Setting temperature (℃)	Hardening time (min)	Hardness attained (Shore A)
0.5	35	33	1440	45
1.0	50	34	1410	48
1.35	60	36	1400	50
2.0	90	37	1380	60

With the variation of MMA, the setting time ($t_{setting}$) of the present the sheet molding compoundis found to vary from 60 to 90 min with the exothermic setting temperature remains at around 32^0C (Table 1). The time required for hardening increases with the increase of concentration of MMA. The hardness (Shore A) is reduced with the increase of concentration of MMA as given in Table1. It is clear that the setting and hardening time is comparatively lower with 0.7g MMA which may be due to the presence of both inorganically modified HT-PPF polymeric component and comonomer with sufficient concentration to complete the crosslinking reaction. This has resulted to higher hardness. Among the five combinations, longer setting time was observed with 1.5g MMA which is attributed to the slow and prolonged curing due to lack of sufficient concentration of inorganically modified HT-PPF polymeric component. This has resulted to low hardness which is attributed to the plasticization by the excess MMA.

With the increase of PM35, the setting time also increases which may be due to the presence of excess PM35 and solvation of the cured compound, as shown in Table 2. However the hardening time decreases with increase of PM35 which is attributed to the crosslinking with hydroxyl groups of hydroxyapatite by excess diisocyantae of PM35; this has resulted to higher hardness. Based on the setting and hardening characteristics, the formulation consisting 0.5g of PM35 and 1.35g of MMA exhibiting optimum 60 min setting time, 36°Csetting temperature and 50 Shore Ahardness was selected for the preparation of sheet molding compound.

C. Preparation and Characterisation of Optimized Sheet Molding Compound

The sheet molding compound having optimized setting and hardening characteristics consists Component 'A' with HT-PPF resin (4g), modified diphenyl methane diisocyante [Cosmonate PM-35] (1.5g), hydroxyapatite (2.0g) and 1,4 diazobicyclo (2,2,2) octane (DABCO) (0.017g). Component 'B' consists of methyl methacrylate (3.9g), and diethylene glycol dimethacrylate (2.0g). Component 'C' consists of benzoyl peroxide (0.6 g), zinc stearate (0.3 g) and calcium carbonate (1.2 g). The Components 'A', 'B' and 'C' were mixed well and kneaded to get a semi solid.

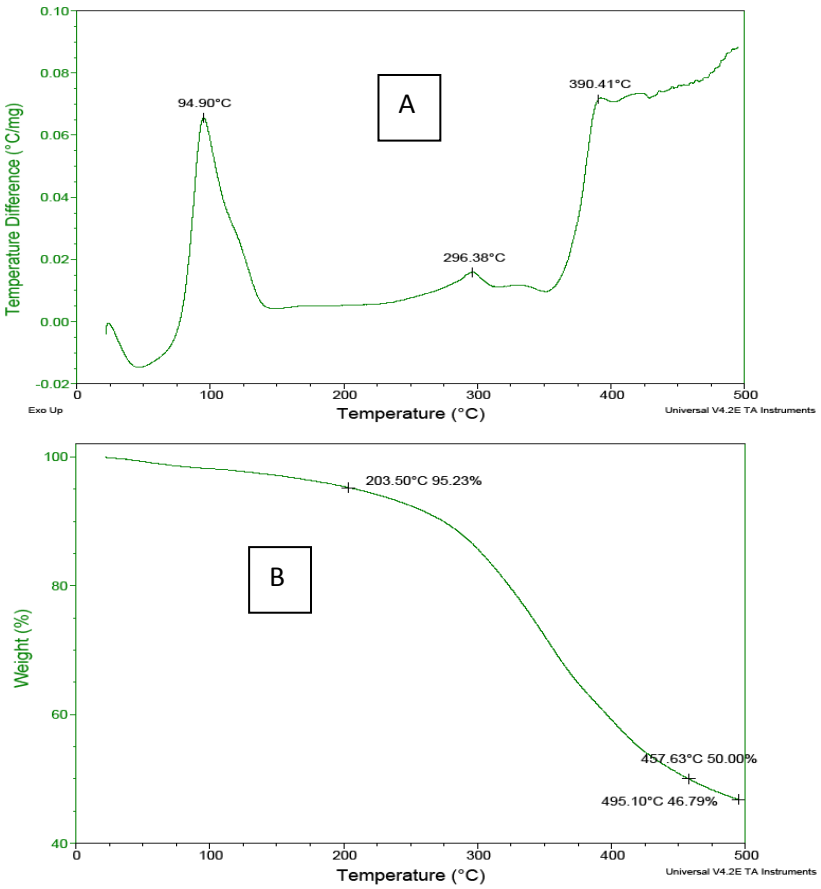

Fig. 4 Thermal analyses of sheet molding compound DTA trace (A), TGA trace (B)

The DTA analysis of optimized sheet molding compound reveals an exothermic peak at 94.9^{0}C which is due to the crosslinking reaction (Figure4 A). The crosslinking of the present inorganically modified HT-PPF can be accomplished by crosslinking the fumarate linkage in the polyester with methyl methacrylate initiated by dibenzoyl peroxide under heat. The plateau arrived after the sharp exothermic curve indicates the completion of all the reaction within the material. With thermo gravimetric analysis of optimized sheet molding compound a gradual weight loss of material is observed with about 95% remaining at 203^{0}C and about 45% remaining at 500^{0}C (Figure4B). The analyses reveal crosslinking sheet molding compound and appreciable thermal stability of the crosslinked compound.

D. Compression Molding of Sheet Molding Compound

In order to solve the problem of warping and insufficient mechanical strength in cured polyester materials, forging of curable sheet molding polyester compound is more promising tool. The crosslinking of unsaturated polyesters can be accomplished by crosslinking of the alkene linkage in the polyester with a monomer such as styrene, methyl methacrylate, etc. initiated by free radical catalyst, dibenzoyl peroxide by free radical mechanism under high temperature and pressure during compression molding as shown in Figure5.

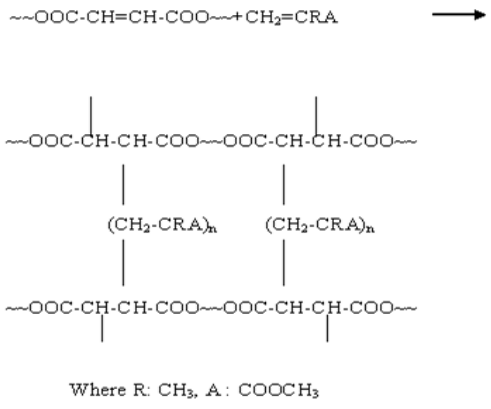

Fig. 5 Crosslinking of inorganically modified HT-PPF polymeric component of the sheet molding compound with comonomer, methyl methacrylate (MMA)

Accordingly the present inorganically modified HT-PPF polymeric component of the sheet molding compound undergoes crosslinking of the fumarate linkage in the HT-PPF units with methyl methacrylate and networking by the crosslinker diethylene glycol dimethacrylate. The crosslinking is accelerated under high temperature and pressure during compression molding. Since the molecular weight of HT-PPF is very low, the propagation of crosslinking chain and growth lead to a highly crosslinked the dimensional structure.

E. Evaluation of Compression Molded Composite

FT-IR (ATR) analysis of compression molded composite spectroscopy reveals spectral features of HT-PPF. The IR spectrum (Figure6) shows a strong peak for ester(C=O) group at 1718 cm^{-1} and weak band for hydroxyl group (-OH) at 3280cm^{-1} for HT-PPF unit.

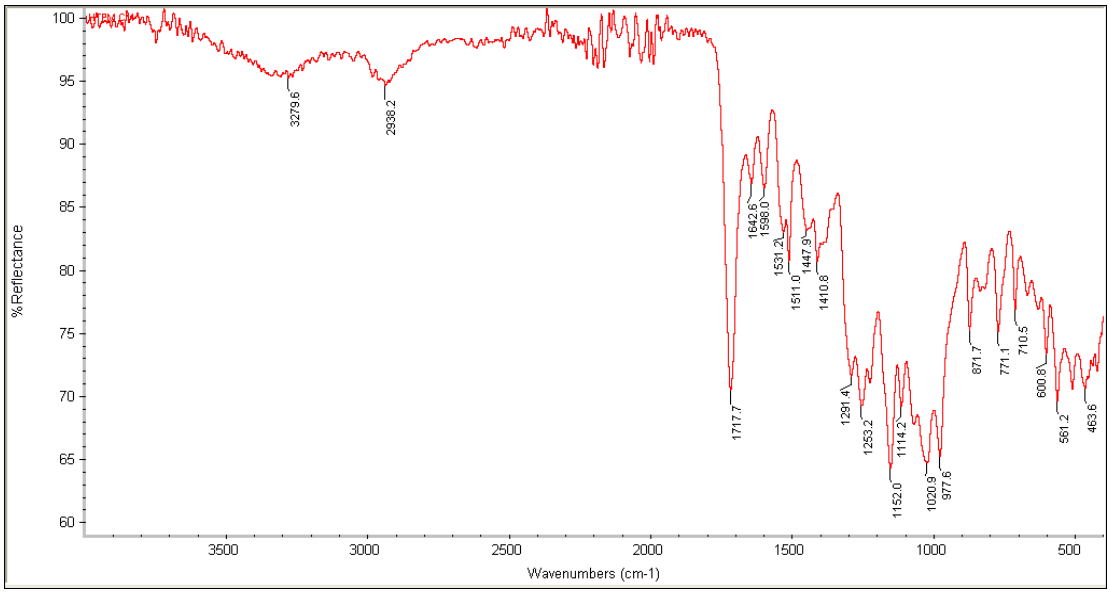

Fig. 6 AT-IR spectrum of compression molded composite sheet

The intensity of the peak for unsaturated double bonds(C=C) for fumarate linkage was observed at 1645 cm^{-1} is significantly reduced with the peak at 1643 cm^{-1}. This reveals the crosslinking of fumarate linkages with MMA units during the compression molding. The peak for urethane linkages –NHCOO- is also appeared at 1533 $cm^{-1}$for N-H bending of urethane linkage. The double peak at 601 cm^{-1} and 561cm^{-1} also appeared for the bending of P-O bonds in phosphate groups of hydroxyapatite.

The surface studies of thecompression molded composite reveal hydrophilic character. The advancing and receding contact angle of composites are found to be 45 and 56 respectively. The low contact angle under dynamic conditions suggests hydrophilicity. Hardness of the compression molded composite increases from that of sheet molding compound to 48 Shore D. The sheet molding compound has the hardness (Shore A) value of 50. The hardness is influenced by the degree of crosslink density. Maximum crosslinking was achieved in the present composite sheet by compression molding with heat and pressure. The SEM micro photograph of as-prepared compression molded composite is given in Figure7.

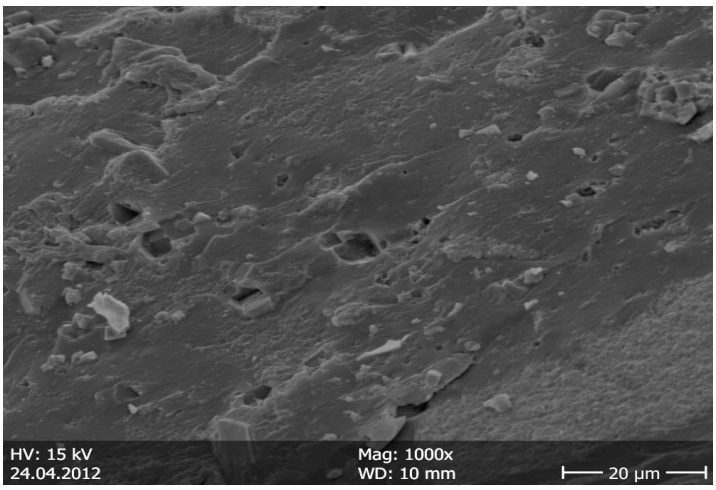

Fig. 7 SEM image of compression molded composite

The fracture morphology showed a serrated structure. The serrated structure reflects the high mechanical strength of the composite. The hydroxyapatite nanoparticle dispersed throughout the resin matrix. The agglomerated structure of hydroxyapatite normally observed has disappeared. This is due to the chemical modification of HT-PPF resin by covalently crosslinking of hydroxyapatite and HT-PPF resin with diisocyanate. The chemical modification enabled good dispersion of the hydroxyapatite in the polymer matrix and good interfacial bonding between the polymer and the hydroxyapatite nanocrystals.

Thermal analysis facilitates to judge whether the compression molded composite undergo any adverse thermal transition, softening and stability around the autoclaving temperature of 121^{o}C. Medical devices and Implants are generally sterilized by autoclaving at 121^{o}C. DTA analysis generally shows thermal responses for glass transition, softening, crosslinking reactions and decomposition and degradation. Endotherms observed in low temperature region of DTA traces are generally obtained due to softening by cleavage of feeble physical cross links such as hydrogen-bonds which has resulted in a virtually cross linked structure in some domains. Endotherms observed in high temperature region of DTA traces are generally obtained due to chain scission. Exotherms are due to cross linking reaction through newly formed chain ends.

The DTA curve of compression molded composite is given in Figure8A. The present compression molded composite does not exhibit glass transition and softening distinctly. However, a mild glass transition at 50^{o}C and mild endotherm appeared at 125-150^{o}C for slight softening which may be exhibited by uncrosslinked PPF segment. A broad endotherm centering at 191^{o}C appeared which is attributed to the chain scission of the crosslinked polymer in the composite. The area of the endothermic peak corresponds to the degree of chain scission. The exothermic peaks observed above 300°C are mild suggesting lesser degree of residual crosslinking during the thermal analysis. The area of the exothermic peak corresponds to the degree of crosslinking. Comparing the area of endothermic and exothermic peaks, the area of the former is more which reveals only chain scission and degradation as a major event during the thermal treatment. DTA analyses reveal that the compression molded composite can withstand the autoclaving temperature. The TGA analyses exhibit single stage decomposition (Figure8B). The compression molded composite underwent degradation from around 200^{o}C and exhibited 60-70% weight loss around 350^{o}C. The TGA analysis also reflects trend of thermal stability observed in the DTA analysis.

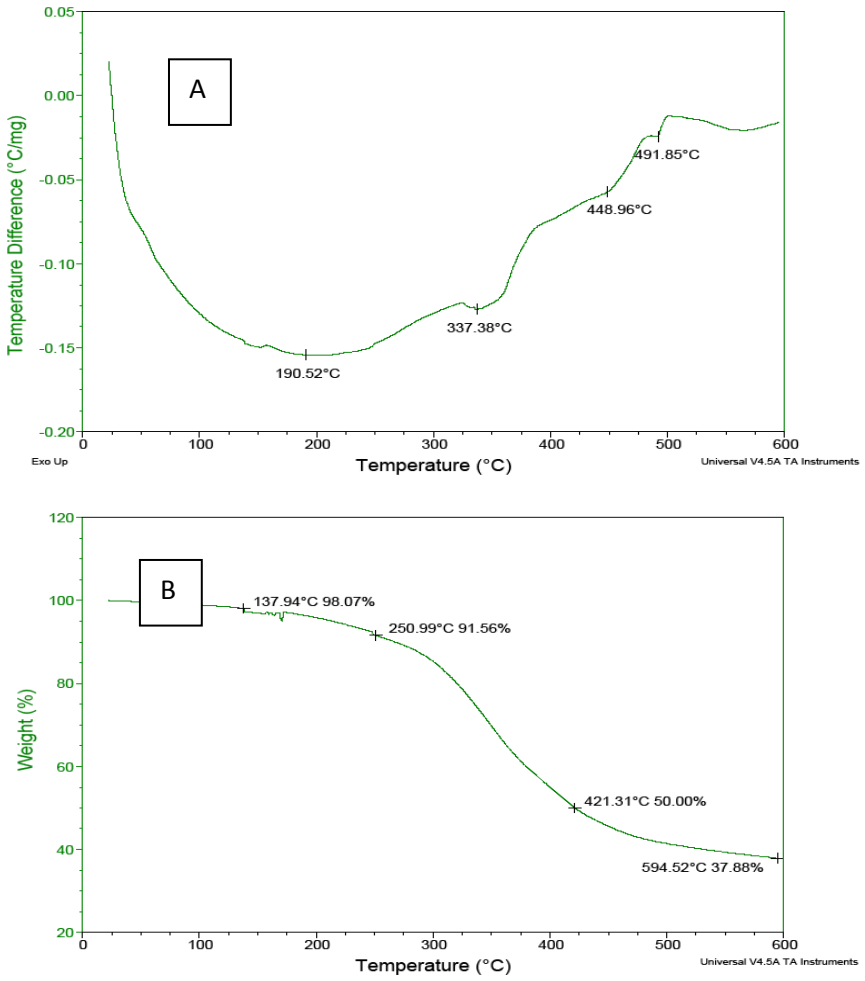

Fig. 8Thermal analyses of compression molded composite DTA trace (A), TGA trace (B)

Polymers having macromolecular chains have unique viscoelastic properties, which combine the characteristics of elastic solids and Newtonian fluids. The viscoelastic property of a polymer is studied by dynamic mechanical analysis where a

sinusoidal force (stress σ) is applied to a material and the resulting displacement (strain) is measured. For a perfectly elastic solid, the resulting strain and the stress will be perfectly in phase. For a purely viscous fluid, there will be a 90 degree phase lag of strain with respect to stress. Viscoelastic polymers have the characteristics in between where some phase lag will occur during DMA tests. The storage modulus measures the stored energy, representing the elastic portion, and the loss modulus measures the energy dissipated as heat, representing the viscous portion. The tensile storage and loss moduli, phase angle are defined in the following Equations (1), (2) and (3).

$$E' = \frac{\sigma_0}{\varepsilon_0}\cos\delta \tag{1}$$

$$E'' = \frac{\sigma_0}{\varepsilon_0}\sin\delta \tag{2}$$

$$\tan\delta = \frac{E''}{E'} \tag{3}$$

The variation of modulus and mechanical loss factor (tanδ) with temperature are given in Figure9. The Tan (delta) peaks at 65 and 130°C represent mild glass transition and slight softening which may be exhibited by uncrosslinked PPF segment.

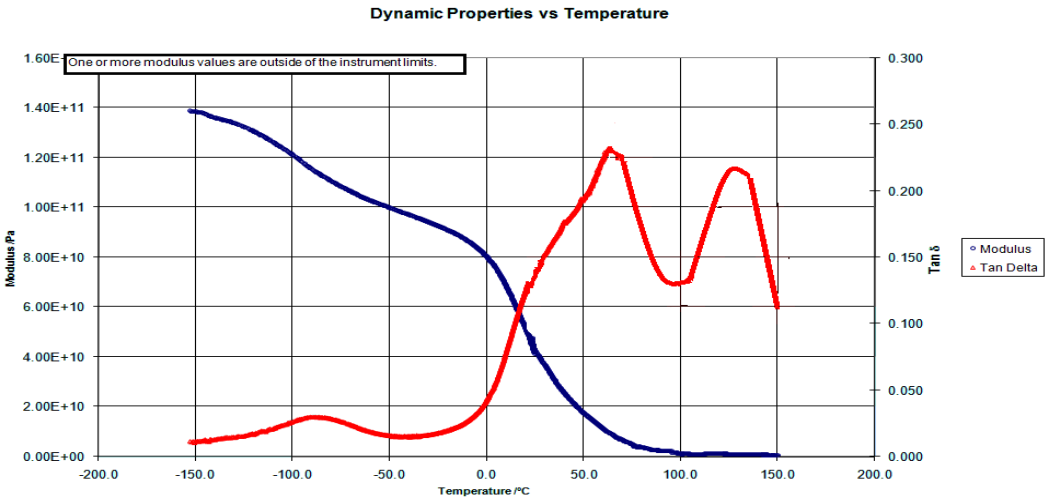

Fig. 9 DMA trace of compression molded composite

F. Studies on the In-Vitro Biodegradation of Compression Molded Composite

Degradation in the present compression molded composite occurs by ester hydrolysis in crosslinked HT-PPF units with the generation of fragments with hydroxyl and carboxyl end groups as shown in Figure10.

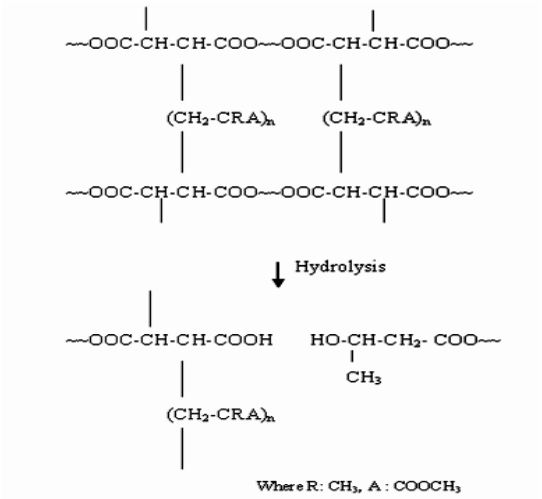

Fig. 10 Hydrolysis of crosslinked HT-PPF units in compression molded composite

The degradation is preceded by absorption of medium by the crosslinked composite material. Comparing the absorption of medium, absorption of PBS is relatively higher than in Ringer's solution. Though the spreading and wettability of PBS is relatively lesser than that of Ringer's solution, higher absorption of PBS has been observed. This is attributed to the diffusion of ionic components of PBS solution. The diffusion is slightly hindered in the Ringer's solution.

It was observed that with aging, pH of the media is found to be reduced significantly especially in Ringer's solution. The appreciable reduction of pH in Ringer's solution leading to a level of highly acidic condition is due to the hydrolysis at ester linkages and generation of soluble low molecular weight fragments with -COOH groups. *In-vitro* degradation in PBS mimics the physiological conditions, i.e. constant osmolarity and neutral pH (7.32) at 37°C. The use of phosphate-buffered PBS medium for the *in-vitro* degradation enables stabilization of pH around the physiological range. Comparing the pH of the Ringer solution and PBS, the reduction was not higher in PBS. The stabilization of pH in PBS around 7 is due to the combined buffering effect of the inorganic ions of PBS and hydroxyl apatite.

The weight loss (%) in Ringer's solution increases more with time in comparison with that in PBS medium, shown in Figure11.It is inferred that the buffering effect of the PBS and hydroxyl apatite slowed down the degradation as reported elsewhere [23]. Peter et al [23] have reported that *β*-tri calcium phosphate appears to act as a buffering agent *in vivo*, maintain local pH and prevent accelerated degradation of poly propylene fumarate.

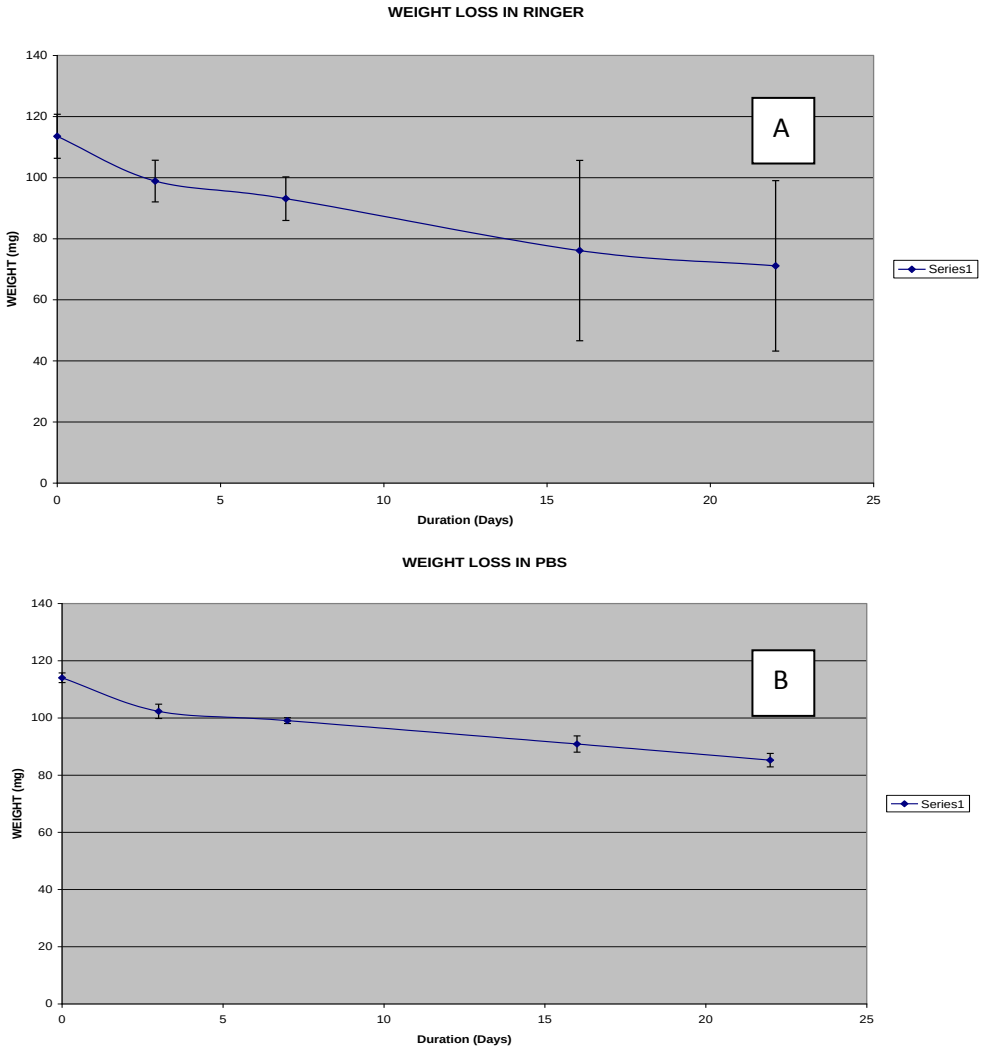

Fig. 11 Degradation and weight loss of compression molded composite Ringer's solution (A), PBS medium (B)

G. Blood Compatibility of Compression Molded Composite

The studies on RBC aggregation and hemolysis with thecompression molded composite reveal blood compatibility.The hemolytic potential of the biomedical materials is the measure of the extent of haemolysis that is induced by the materials when it comes in contact with blood. The hemolysis (%) of the compression molded compositeis 1.37**.** The compression molded compositeis found to be nonhemolytic. The extent of hemolysis falls under the acceptable limit of <5%. The response of RBCs to the compression molded compositewas monitored using an inverted phase contrast microscope using the DIC mode. No rouleaux formation was observed (Figure12). The absence of the rouleaux formation of RBCs in contact with blood

is a favorable as the material does not affect the fluidity and viscosity of blood [24].Moreover it is also obvious that there is no unfavorable adsorption of macromolecules or leaching of particles onto the membrane surfaces between erythocytes. From these assays, it is clear that all the compression molded compositeis blood compatible.

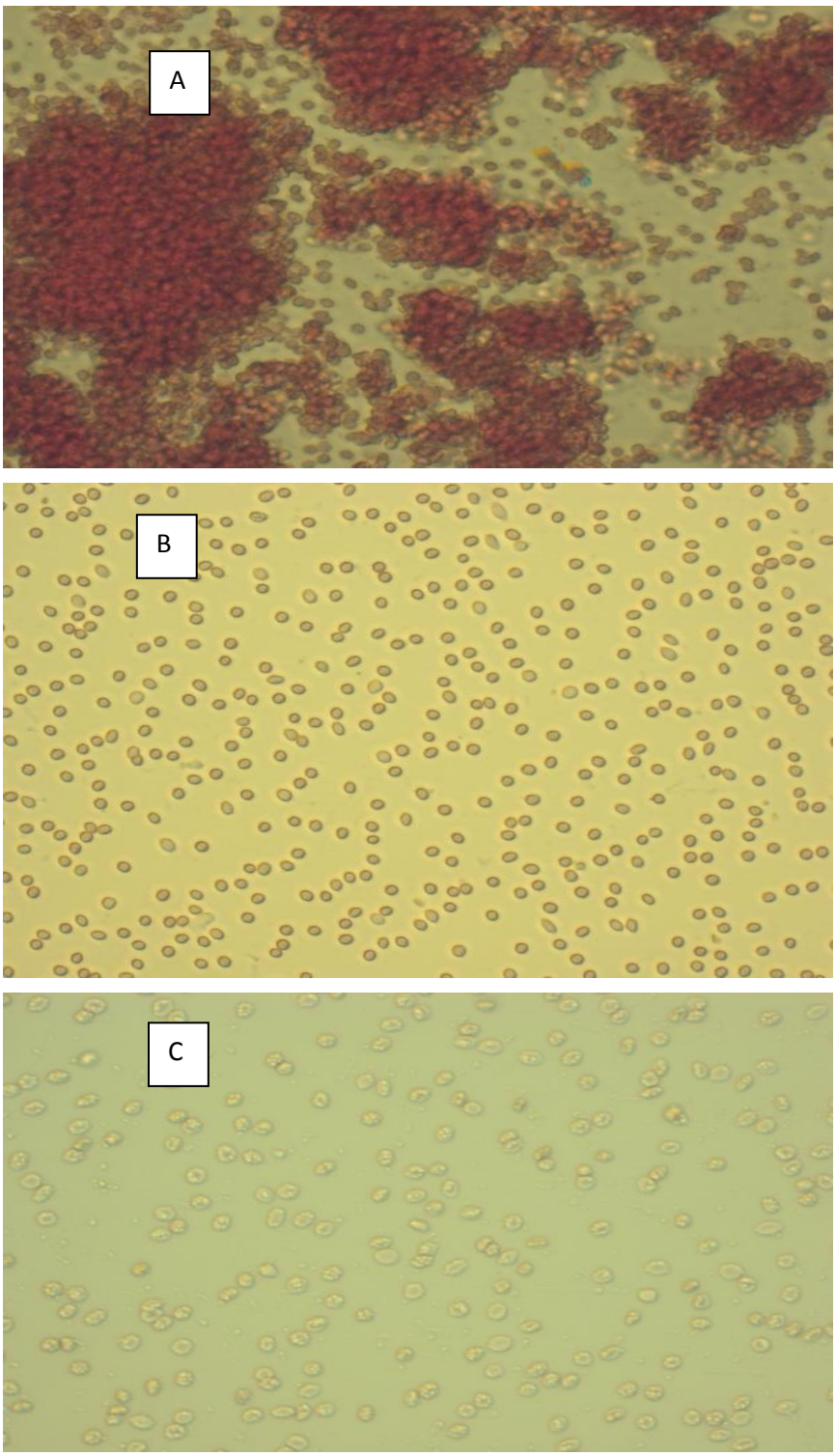

Figure 12 Aggregation of RBC on compression molded composite Positive control (A), Negative control (B), Compression molded composite (C)

H. Cytocompatibility of Compression Molded Composite

The studies on cytocompatibility of compression molded composite reveal non cytotoxic to the L-929 fibroblast cells (0 in cytotoxicity scale) (Figure13). The cells surrounding the material maintained their characteristic spindle shape. Negative control (ultrahigh molecular weight poly ethylene) elicited non cytotoxic response (0 in cytotoxicity scale) while the positive control (cytotoxic PVC) elicited severely cytotoxic (3 in cytotoxicity scale) response. Adhesion and spreading of fibroblast

cells are also observed on the present material surface. Generally, cell adhesion is more favored by the hydrophobicity of a material in comparison with the hydrophilic counterpart in serum-containing culture medium. This is due to the fact that proteins that act as ligands for cells have a greater tendency to bind to hydrophobic surfaces as compared to hydrophilic surfaces [25,26]. In order to test the cytotoxicity of the polymer quantitatively MTT assay was performed using L929 fibroblast cell lines. Quantitative assessment of the cytotoxicity to cells after contact with the material extract showed 90% metabolic activity when compared to cells without the material for 24h of contact. Therefore the present composite material is cytocompatible.

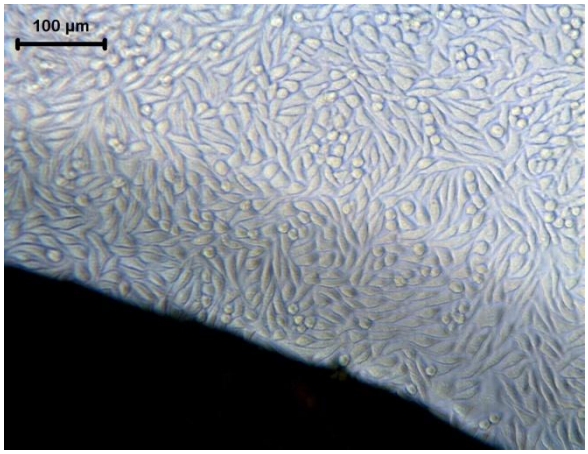

Figure 13 Optical photograph - viable L929 fibroblast cells surrounding the compression molded composite (20X)

IV. CONCLUSION

Biodegradable and crosslinked polymeric nanocomposite was prepared by compression molding usingsheet molding compound containing inorganically modified polypropylene fumarate (HT-PPF) unsaturated polyester.Hydroxyapatite nanopowder (100-250nm sintered)and polyol modified-polymeric diphenyl methane diisocyanate (Empeyonate PM-35, NCO Content 27%) were used for modification of HT-PPF. The fractographic studiesreveal higher mechanical strength and good dispersion of hydroxyapatite in the polymer matrix and good interfacial bonding between the polymer and hydroxyapatite.Though the present composite is hydrophilic, the in-vitro degradation in PBS is slowed down which is due to the buffering effect of the PBS and hydroxyapatite. The nanocomposite is nonhemolytic, noncytotoxic and blood compatible. Thus, it can be concluded that the present methodology and material offer great scope in developing biodegradable bone fixation devices with appreciable properties.

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