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GRAFT COPOLYMERIZATION OF GLYCIDYLMETHACRYLATE ONTO FIBRIN PREPARED FROM SLAUGHTER-HOUSE WASTE

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Key Words : Fibrin, poly glycidylmethacrylate, graft-copolymerisation, percentage grafting, thermal decomposition.

ABSTRACT

Fibrin collected from the slaughter house in crude form was purified. The purified fibrin was graft copolymerised with poly-glycidylmethacrylate (PGMA) and characterised for the

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percentage of grafting. The infrared spectroscopy and thermogravimetric anlysis, of the composite were studied. This biomaterial could be used as a drug delivery system after coupling the suitable antibiotic through the epoxy functional groups introduced by PGMA.

INTRODUCTION:

In the blood clotting process fibrinogen is converted to fibrin in the presence of the proteolytic enzyme thrombin. Fibrin monomers has the subunit surface structure of $(\alpha\beta\gamma)_2$. Fibrin monomers spontaneously assemble into ordered fibrous arrays called fibrin (1).

Fibrin is having good haemostatic and wound healing properties and it can be made in the form of sponge, film, powder, fibrin glue etc (2-5). Fibrinogen has been isolated from whole blood using centrifugation in combination with cryoprepcipitation, ethanol, ammomium sulfate or polyethylene glycol precipitation. Concentrated solutions of fibrinogen, when resolubilised and mixed with thrombin are used as fibrin glue or a fibrin adhesive to adhere tissues together or as a fibrin sealant to close tissue defects in many phases of surgery. Fibrin glue has been used to eliminate dead space beneath skin grafts and to promote healing (6-8). It is also useful in decreasing the number of natures required in cosmetic and reconstructing surgery. Fibrin sealant is used to seal anastomoses in vascular grafts, leaks and anastomoses in common bile duct, superficial and deep hepatic injury and haemorrhage, splenic injury, enterocutaneous fistulae, renal injuries and uteral anastomoses. (9-12)

In the local municipal slaughter house, daily, fibrin is isolated from 1000 litres of blood by churning the blood with

a stirrer and the defibrinated blood is collected by a few pharmaceutical companies to isolate biochemicals like haemoglobin. The crude fibrin is wasted or sold at a cheaper rate to be used as a fertiliser for plants. This fibrin is used in the present graft copolymerisation studies.

In the present study fibrin is graft copolymerised with polyglycidyl methacrylate (PGMA). Grafting of selected polymers onto fibrin will improve the function of the product. Antibiotics like gentamicin and tetracylin could be coupled to the grafted composite through the epoxy functional groups introduced by PGMA. These graft copolymers coupled with antibiotics can be used in the case of above said infected tissue repair.

EXPERIMENTAL:

Materials: 1) Fibrin (purified from the crude fibrin available at local slaughter house, Madras).

2) Glycidyl methacrylate (Fluka, Switzerland)

All other reagents used were of analytical grade.

Methods:

1. Purification of fibrin (F)

The fibrin collected from the slaughter house was in crude form containing blood clots. It was washed thoroughly under running water to remove the blood clots, and further treated with 0.5 M sodium acetate solution to remove remaining blood stains. The resultant material was bleached with 20 ml hydrogen peroxide solution (30% v/v) per litre at pH 8.0 (pH was adjusted by using 0.1.N sodium hydroxide solution). Thus bleached fibrin, was removed from the bleaching bath, washed thoroughly with cold running water and ground to pulp by using a mixer. The ground mass was casted to form film. It may be stored as a film or in the form of a powder.

2. **Preparation of Fibrin - Glycidylmethacrylate graft copolymer (F-PGMA).**

A 5 gm of fibrin (powder form) was soaked in 50 ml of water overnight. To this, added 25 ml of initiator solution [containing potassium persulfate (1.8×10^{-3}M) and sodium bisulfite (1.2×10^{-3}M)], followed by 10 ml of glycidylmethacrylate. The experiment was carried out at room temperature for 2 hrs. The crude graft copolymer was extracted with acetone to remove poly glycidyl methacrylate homopolymer and then dried at room temperature.

CHARACTERISATION:

The characterisation of the F-PGMA was carried out for percentage of grafting, nitrogen content, infrared spectroscopy and thermogravimetric analysis.

1.**Percentage grafting**

Percentage of grafting was determined by the following equation(13):

$$\text{Percentage grafting} = \frac{\text{(Total weight of graft copolymer - weight of the fibrin)}}{\text{Weight of fibrin}} \times 100$$

2. **Determination of Nitrogen Content**

Total nitrogen contents were determined by the micro Kjeldahl method after separately digesting the known weights of fibrin and F-PGMA samples.

3. **Infra-red spectrum**

To provide proof of grafting the infrared spectra of fibrin, and Fibrin -PGMA graft copolymer was measured with NICOLET IMPACT 400 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) using KBr pellet 500 mg containing 2-6 mg of the sample.

4) **Thermogravimetric analysis (TGA)**

The TGA was carried out using a Gener V4 1C DU PONT 2000 in nitrogen atmosphere at a heating rate of $10^{o}C$/min. Primary weight change of these materials as a function of temperature was recorded using this study.

RESULTS AND DISCUSSIONS

During the past few years, significant progress has been made in the chemical modification of natural macromolecules with the aim of improving physico-chemical properties in the resulting products. The grafting of organic monomers onto the fibrin offer an attractive technique of improving its characteristics that may be useful in its field of application. In this study, grafting of PGMA on fibrin was established using potassium per sulfate - sodium bisulfite initiation technique.

Percentage grafting:

According to the percent grafting determination, it is understood that the formation of F-PGMA graft copolymer requires almost equal parts of its constituents. The percentage of grafting determined by weight difference method and by nitrogen estimation were 102% and 101% respectively. These results clearly indicate that PGMA was readily grafted onto fibrin.

Infrared Spectroscopy

The infrared spectra of fibrin (fig.1a) and fibrin - PGMA graft copolymer (fig.1b) were compared. The fibrin being protein showed the characteristic amide absorption bands at 1660 cm^{-1}, 1550 cm^{-1} and 1250 cm^{-1}. The IR spectrum of F-PGMA showed absorption bands of ester carbonyl group of PGMA at 1740 cm^{-1} and C-O stretching at 1270 cm^{-1} in addition to the absorption bands of fibrin. It is understood from these results that the PGMA was grafted onto fibrin.

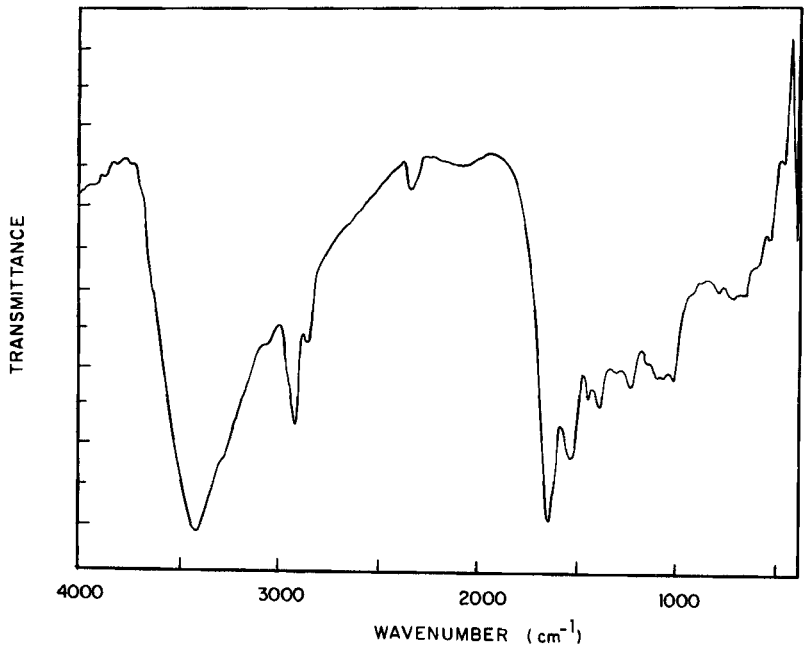

Figure 1a: The infra-red spectrum of fibrin sample, run from 4000 - 400 cm^{-1}.

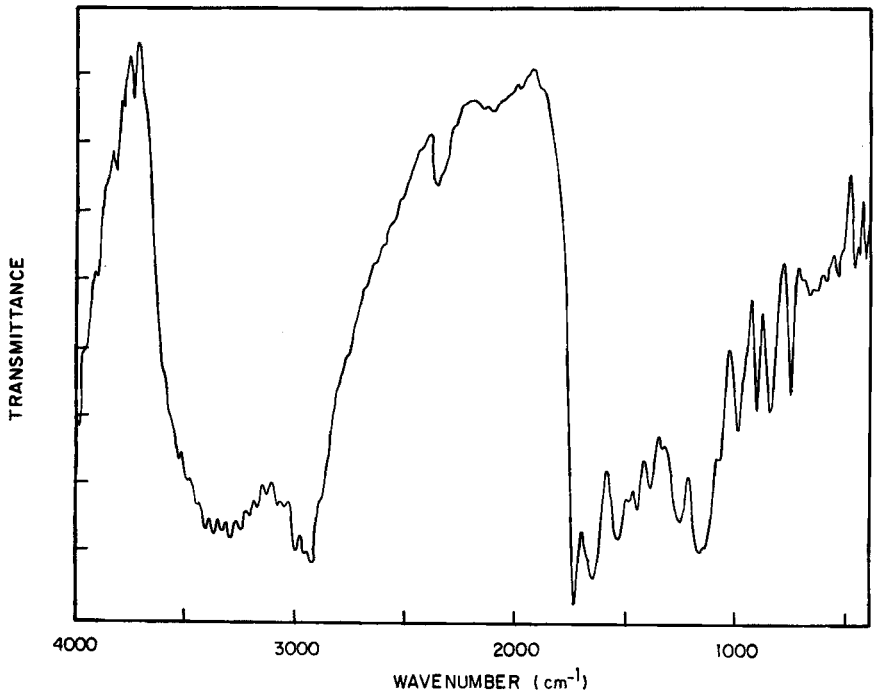

Figure 1b: The infra-red spectrum of fibrin-poly glycidylmethacrylate graft copolymer.

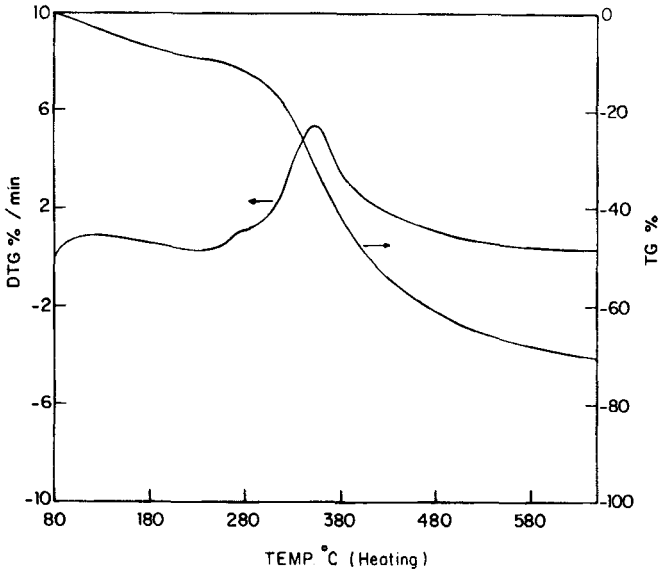

Figure 2: Thermogram showing the decomposition pattern of fibrin heated from 30°C to 600°C in the presence of nitrogen (Control 1)

Thermogravimetric Analysis

Figures 2, 3 and 4 illustrates the thermal decomposition profile of fibrin (protein), the poly-GMA and the polymer grafted protein respectively. In thermogravimetry, the losses of weight due to evolution of water, carbon monoxide, carbondioxide and evaporation of other pyrolysis products are collectively measured as percentage of original weight. In this investigation, the PGMA grafted fibrin and its individual constituents were heated at steadily increasing temperature from 30°C (room temperature) to 600°C in an atmosphere of nitrogen (100ml/min). It was observed from the results that the fibrin decomposition - was maximum at about 290°C (Fig.2) with single stage decomposition. Whereas, the thermogram obtained for the poly GMA (Fig.3) showed a two-stage decomposition at about 330°C

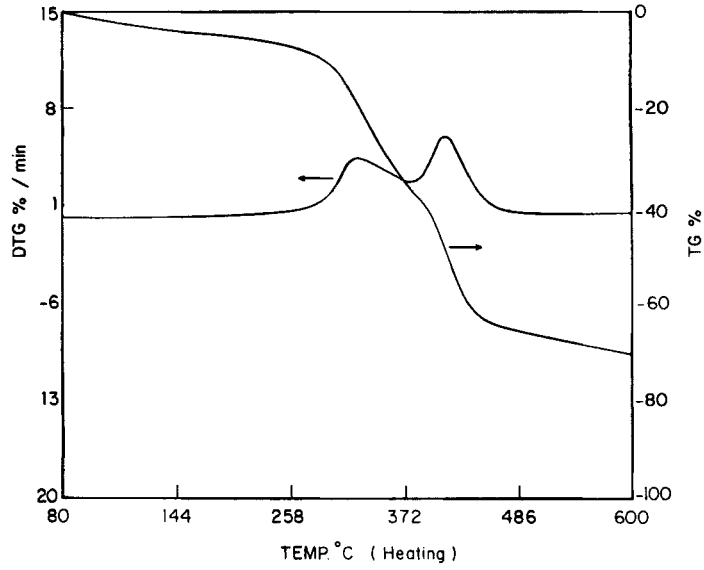

Figure 3: Thermogram showing the decomposition profile of poly-GMA heated from 30°C to 600° in the presence of nitrogen (Control 2)

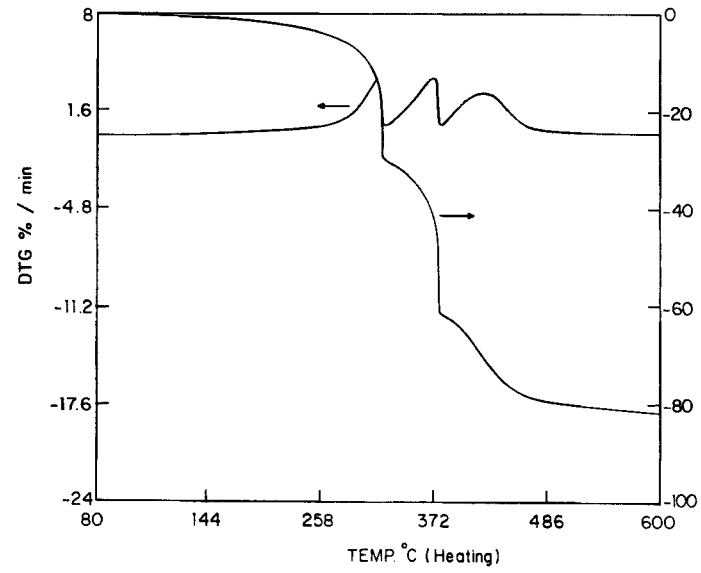

Figure 4: Thermogram showing the decomposition profile of Fibrin - poly GMA heated from 30° - 600°C in the presence of nitrogen.

and 410°C respectively. On the other hand, the graft copolymer showed combination of these decomposition patterns but with minor shifts for the respective constituents (Fig.4). The thermogravimetric curve, as seen in figure 4, indicates that weight loss begins at 240-250°C and proceeds very rapidly until approximately 320°C where the first stage of decomposition appear to be complete with a weight loss of nearly 30%. The maximum weight loss peak (DTG curve) was obtained at 310°C (Fig.4) which might attribute to the pyrolysis of fibrin fraction. This indicates that the shift in thermal decomposition, temperature of fibrin is due to its interaction with the PGMA in the graft copolymer. The second stage of pyrolysis starts immediately (at 321°C), as evidenced by both TGA and DTG curves, and completes almost at 380°C with a weight loss of 60% with maximum weight loss at 370°C. As has happened to the protein part, the polymer part (PGMA) also had a pyrolysis shift when it is grafted onto the fibrin back bone.

The 30% and 60% weight loss in the subsequent stages decomposition of graft copolymer indicate that the fibrin and PGMA are copolymerised in 1:1 ratio. This observation was also well demonstrated by the nitrogen determination results by micro Kjeldahl experiment.

In our earlier report (Sastry 1989), the DTG curve showed a weight loss peak for demineralised bone protein (DMBP) at about 280-290°C; and the DMBP - PGMA copolymer showed a three stage decomposition with weight loss peaks at 310°C, 370°C and 460°C. These results seemingly support the observation of the present investigation.

In this study, (Fig.4) the second stage decomposition which takes place in between 321-380°C may be due to the depolymerization of the PGMA part of graft copolymer. And the

third stage decomposition which begins at about 380°C and proceeds until upto 480°C may be due to further degradation of the decomposed polymer substances which was evidenced by both TGA as well as DTG of Fig.4.

CONCLUSIONS

Fibrin could be salvaged from slaughter house waste by simple chemical treatment. This protein could be easily graft copolymerised with polyglycidylmethacrylate. The composition of the fibrin - poly GMA graft copolymer appears to be in the ratio of 1:1. The thermal stability of the graft copolymer, in an inert atmosphere, appears to be slightly more when compared with that of its individual constituents. However, it was observed that the decomposition took place within short period of time.

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