

Synthesis and Characterization of hen egg albumin and Gelatin composite cross linked with Glutaraldehyde

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Abstract- Hen egg (A), Gelatin (G) and their composite (AB) in film form was prepared in different proportions and cross linked with glutaraldehyde (GTA) and the composite was named as (AGG). The characterization of the composite (AGG) was evaluated with GTA interms of tensile strength. The structure and properties of AGG composite was studied for its FTIR, TGA, CD and mechanical strength. From the results obtained from above studies, it was evident that the cross linked AGG film exhibits higher Tensile strength than uncrosslinked AG films. Thermal decomposition studies show AG was stable up to 375°C. The CD spectrum shows an unordered random coil to AGG and helical structure for A, G, AG composites which shows confirmation of crosslinking of AG with GTA.

Keywords: hen egg albumin, Gelatin, Glutaraldehyde, Tensile strength, FTIR, CD, TGA

1. INTRODUCTION

Hen egg white a cytoplasm of egg is a single cell until fertilization. Gelatin cross linked with glutaraldehyde finds applications in determination of α -amylase activity and β -galactosidase. In our previous study [1] we synthesized a binder using egg white with gelatin. In this study we cross linked AG composite with glutaraldehyde and characterized for its physical & chemical properties. Bovine serum albumin cross linked with glutaraldehyde was used by Hidas [2] as bio glue in tissue as an adhesive and also as a sealing agent for kidney during partial nephrectomy. Similarly albumin-glutaraldehyde glue was used as a tissue adhesive for sealing pulmonary parenchyma and bronchial anatomizes by Georg [3]. The composite developed by Kamalrookh [4] uses Egg white which is cross linked with glutaraldehyde has been used for continuous analysis of bacterial cell. The property of hen egg white cross linked with Lysozyme was reported by Marolia [5] that used in immobilization of Lysozyme. Anandrao [6] has developed a new interpenetrating polymeric network of sodium alginate, gelatin, egg white cross linked with glutaraldehyde used for in-vitro release of cefadroxil. Bigi [7] studied mechanical, thermal, swelling properties of glutaraldehyde cross-linked gelatin films. Tabata [8] has prepared biodegradable gelatin cross linked glutaraldehyde hydrogels and implanted the same in rats to find neovascularization of bFGF release. Yan Changhong [9] has prepared anticancer composite using glutaraldehyde cross linked microspheres and quantified the in-vitro release of drug.

Jitendra Sharma [10] has reported on Laser light scattering of gelatin – glutaraldehyde film for static & dynamic studies. Kennedy [11] has studied surface immobilization and entrapping of enzymes on glutaraldehyde cross linked gelatin films. Creep behavior of glutaraldehyde cross linked gelatin was evaluated by Martucci [12].

2. MATERIALS & METHODS

2.1. Materials

Hen egg white was separated from chicken egg and used as such. Gelatin from MBD, Mumbai, India was used as such. Glutaraldehyde was obtained from Merck, Germany and used as such. All other reagents used were of analytical grade.

2.2. Methods

2.2.1. Preparation of Albumin solution (A)

Isolated hen egg white was kept in a conical flask and denoted as (A).

2.2.2. Preparation of Gelatin solution (G)

20 gm of gelatin powder was dissolved in 200 ml distilled water at 55°C in a water bath for about 3 hr, cooled and stored in a conical flask and is denoted as (G).

2.2.3. Preparation of AG film

5 ml of Albumin solution (A) was added to 20 ml gelatin solution at the ratio of 1:4 and the mixture is stirred thoroughly and poured in to a polythene tray and dried as mentioned in our earlier study [1]. This ratio has given better results in our earlier study and the film so formed is designated as (AG).

2.2.4. Cross linking of AG with Glutaraldehyde (AGG)
30 ml of solution (A) was added to 120 ml of solution (G) and the mixture is divided into six equal parts of 20 ml each and marked as I, II, III, IV, V and VI. Known amount of glutaraldehyde namely 0.25 ml, 0.5 ml, 0.75 ml, 1.0 ml, 1.25 ml and 1.5 ml respectively) was added to the above six solutions (AG) and mixed thoroughly and poured in to six polyethylene trays and dried as mentioned earlier. These films are denoted as AGG.

3. CHARACTERIZATION

3.1 Tensile strength

The samples AG and AGG were characterized for their Tensile strength using INSTRON 1405 according to Vogel [11] at speed of 5 mm/min. Two dumbbell shaped specimens of 4 mm wide and 10 mm length were punched out using a die. Mechanical properties such as Tensile strength (MPa) and elongation (%) were measured.

3.2 FTIR Spectroscopy

To provide proof of cross linking of glutaraldehyde with AG we have taken FTIR spectra of AG and AGG using Nicolet Impact 400 using a 500 mg KBr pellet containing 2-6 mg of the sample.

3.3 Circular Dichroism Spectroscopy

The CD measurements were recorded on a JASCO J-715 spectropolarimeter, Japan. The instrument was calibrated using Ammonium-d10-camphor sulfonic acid as described by the manufacturer. The path length used was 1 mm. The spectra were recorded with 1 nm bandwidth and 0.2 nm step resolution. The CD spectra represent an average value from five readings. The resulting spectra were baseline-corrected and smoothed. The samples AG and AGG were obtained by dissolving 0.5 ml of the sample in 10 ml of distilled water. All the spectra were collected from 190-250 nm range which is a suitable bandwidth to study the secondary structure of most of the proteins and peptides.

3.4 Thermal gravimetric Analysis

Thermal stability of the films was determined with a thermo gravimetric (TG) Analyzer (Perkin-Elmer TGA) over a temperature range of 37°C–585°C at a heating rate of 20°C/min under Nitrogen atmosphere.

4. RESULTS & DISCUSSION

4.1 Tensile Strength

Tensile strength of samples of different proportions AG and GTA was given in below Table.1.

Table.1. Tensile strength of AGG

S. No.	AG (ml)	GTA (ml)	Tensile strength (MPa)	Elongation at brake (%)
I	25	0	20.9	126
II	25	0.25	33.3	112
III	25	0.5	40.0	170
IV	25	0.75	36.2	143
V	25	1.0	32.1	68
VI	25	1.25	28.4	45
VII	25	1.5	25.2	40

Based on our previous study [1] we have prepared a mixture of A and G at a ratio of 1:4 which is an optimum AG composite interms of its tensile strength. From above table, it is clear that the Tensile strength of AGG increases initially with glutaraldehyde concentration and then decreased. AG containing 0.5 ml GTA gave better result of tensile strength due to complete reaction of GTA with NH₂

groups in AG composite as shown in Fig.2 where NH₂ in Fig.1 is vanished. With increase in GTA, it homo polymerizes and hence shows decrease in tensile strength which is shown in fig.3. Due to the homo polymerization, elongation at break was also decreased. The elongation at break for the sample-3 was also observed as 170% which is highest among all samples

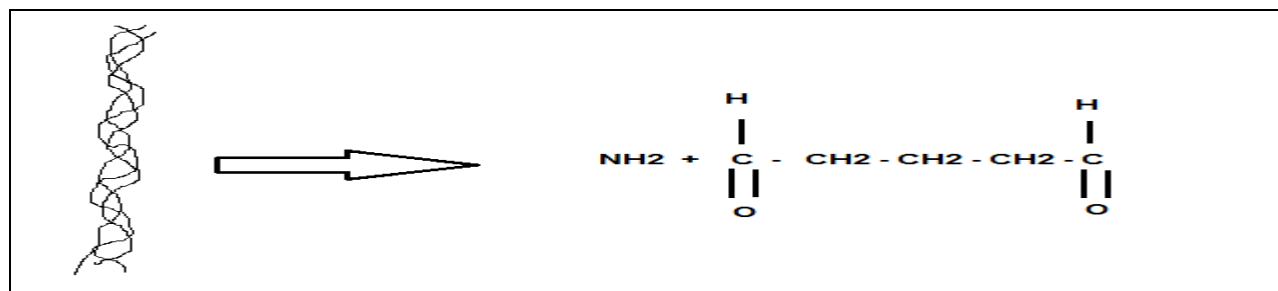


Fig.1. AG composite

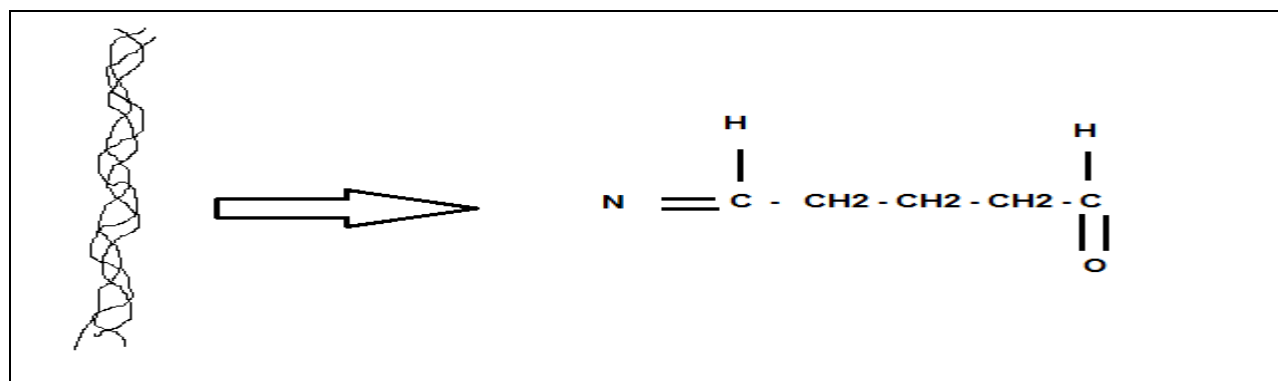


Fig.2. Cross linking of glutaraldehyde

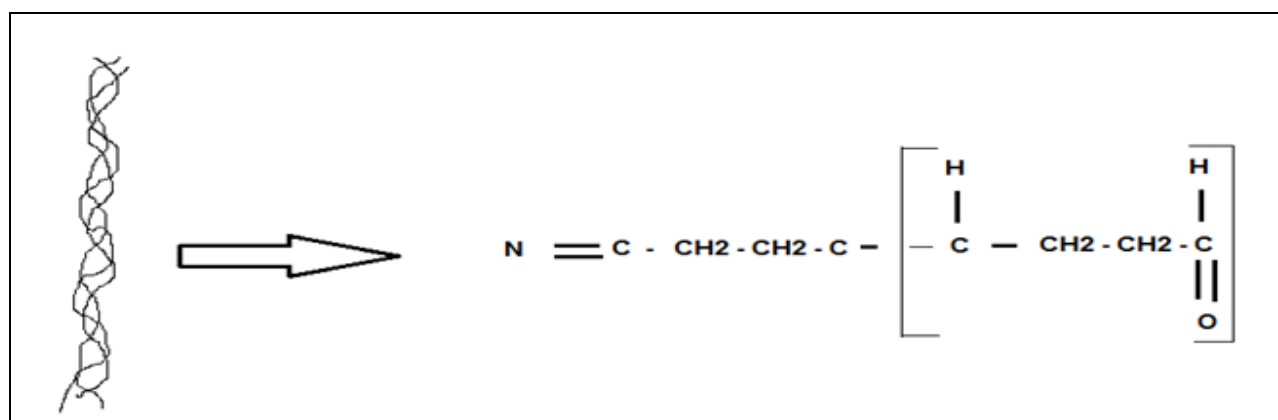


Fig.3. Homo polymerization of glutaraldehyde

4.2 Water Absorption Test

Water absorption capacity for all the above prepared samples is shown in below Table.2.

Table.2. Absorption coefficient of AGG

S.No.	Time (hr)	Sample-1 (%)	Sample-2 (%)	Sample-3 (%)	Sample-4 (%)	Sample-5 (%)	Sample-6 (%)	Sample-7 (%)
I	1	Disintegrated after 1 hr	187	213	170	230	157	125
II	2		256	213	170	230	157	125
III	3		368	230	170	230	157	125
IV	24		300	273	188	240	171	133

From the above table, it is clear that AG sample as such was disintegrated within a hour whereas samples cross linked with GTA were intact even after 24 hr. With increase in GTA concentration, the water absorption capacities were decreased. The hydrophilic groups like NH₂, COOH and OH on AG backbone would have contributed to the dissolving of the membrane in the water. With the addition of GTA, the NH₂ groups on AG would have reacted with -CHO and increases hydrophobicity. With further increase of GTA, lower values of %

absorption were observed due to homo polymerization of GTA.

4.3 FTIR Spectroscopy

FTIR spectra of AG shows protein characteristic peak at 1654 cm⁻¹ (Amide-I) 1561 cm⁻¹ (Amide-II) and 1244 cm⁻¹ (Amide-III) as shown in fig-4. In AGG sample, the Amide band is absent due to cross linking of GTA with AG as shown in fig.5. This confirms the cross linking of GTA with AG.

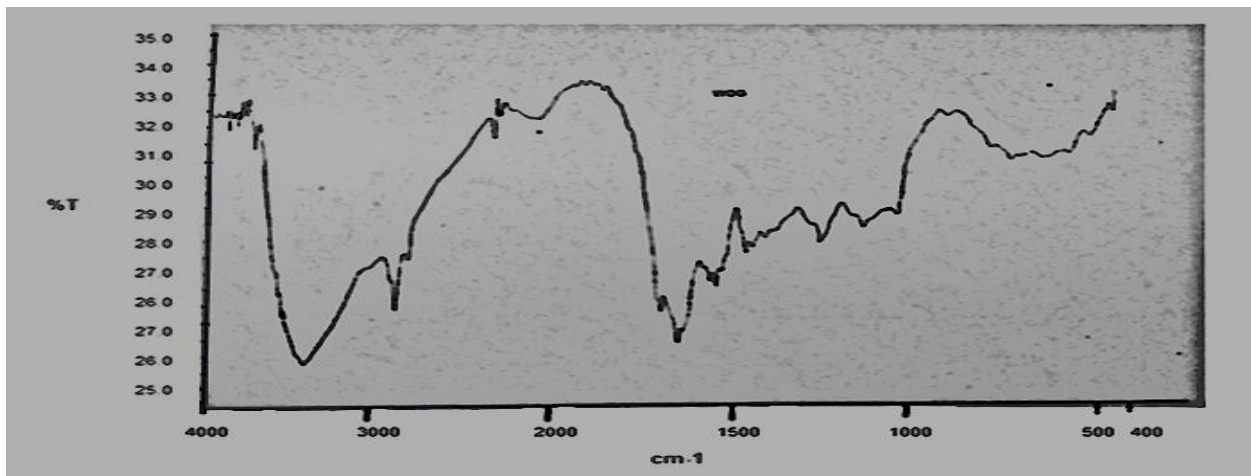


Fig.4. FTIR spectra of AG composite

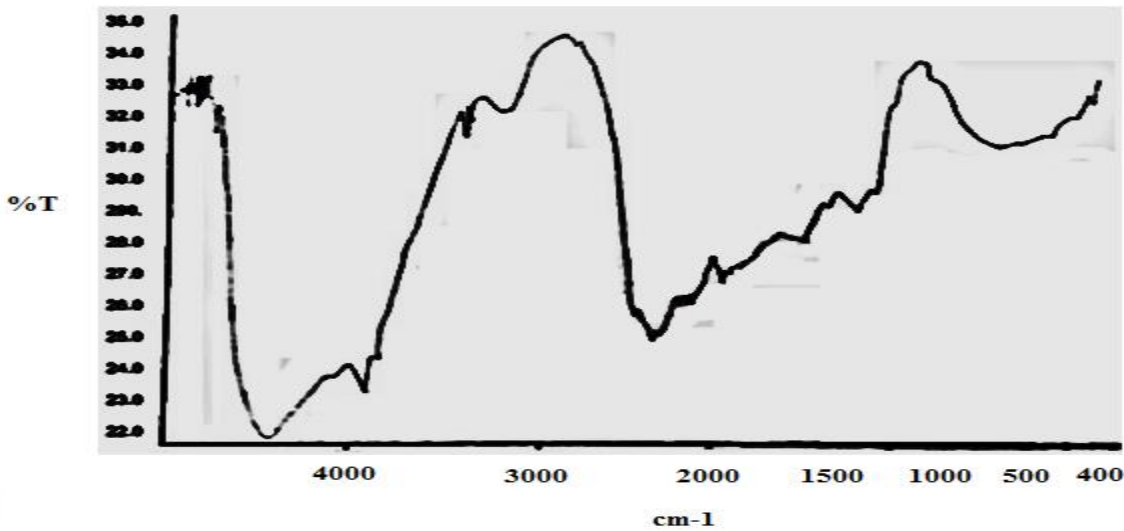


Fig.5. FTIR spectra of AGG composite

4.4 Circular Dichroism Spectroscopy

The far UV spectral studies (185-250 nm) were conducted on AG and AGG. In our earlier study, we reported that AG composite consists of α -helical

structure whereas AGG shows random coil structure with negative peak at around 197 nm. This is due to the broken of α -helical to random coil structure due to cross linking of GA on to AG. This is shown in fig-6a and fig-6b respectively.

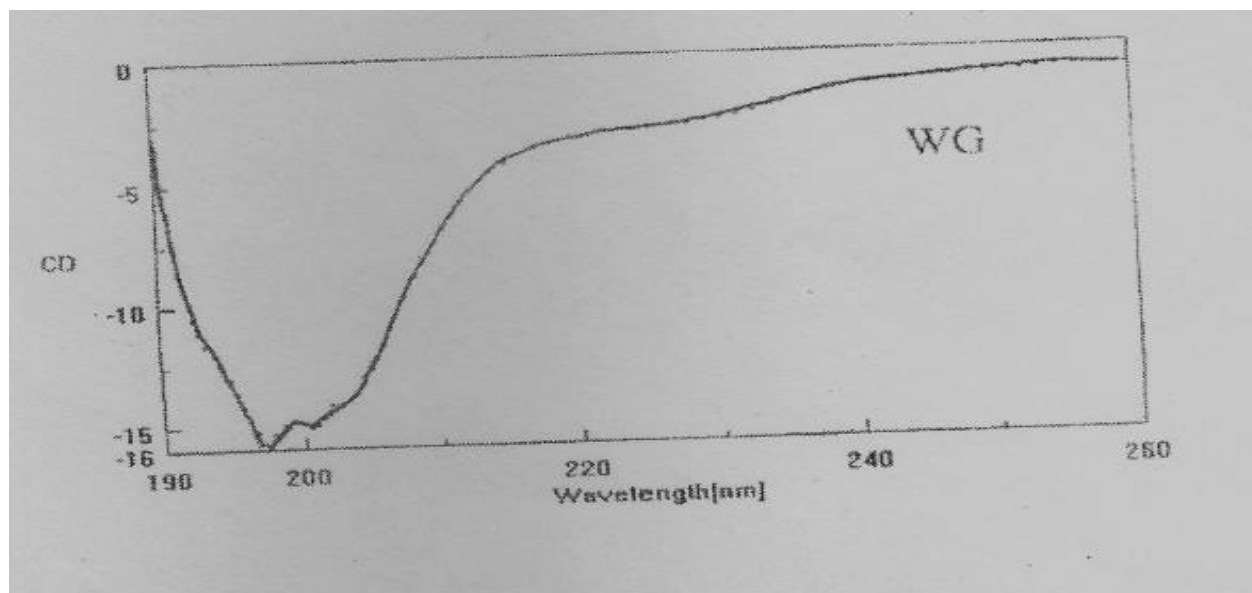


Fig.6a. CD spectra of AGG composite marked as WG

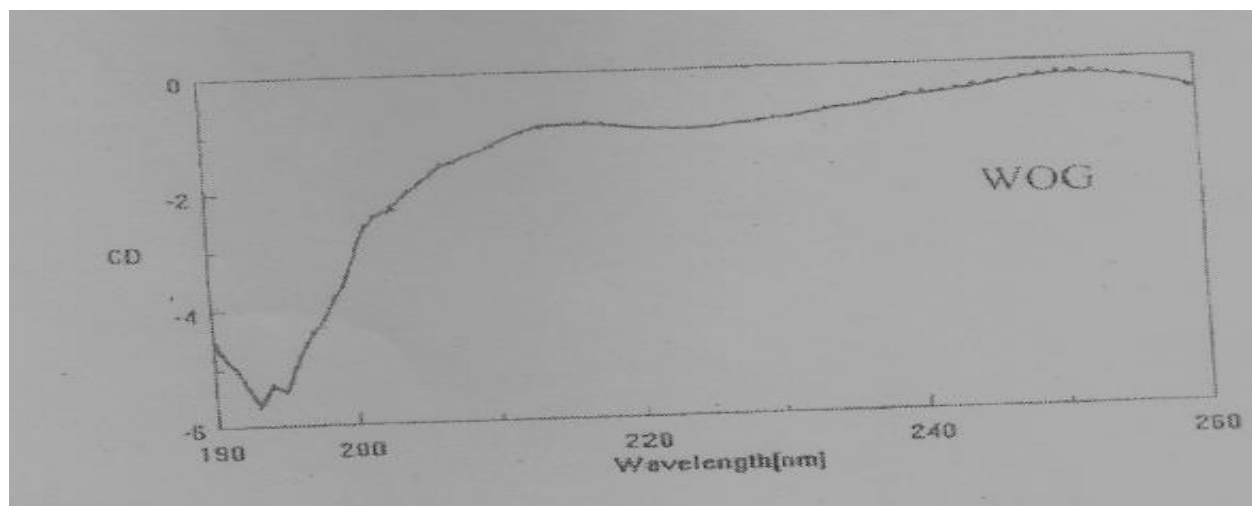


Fig.6b. CD spectra of AG composite marked as WOG

4.5 Thermal Gravimetric Analysis (TGA)

Thermal decomposition profile of AG and AGG shown in fig.7 and fig.8 respectively.

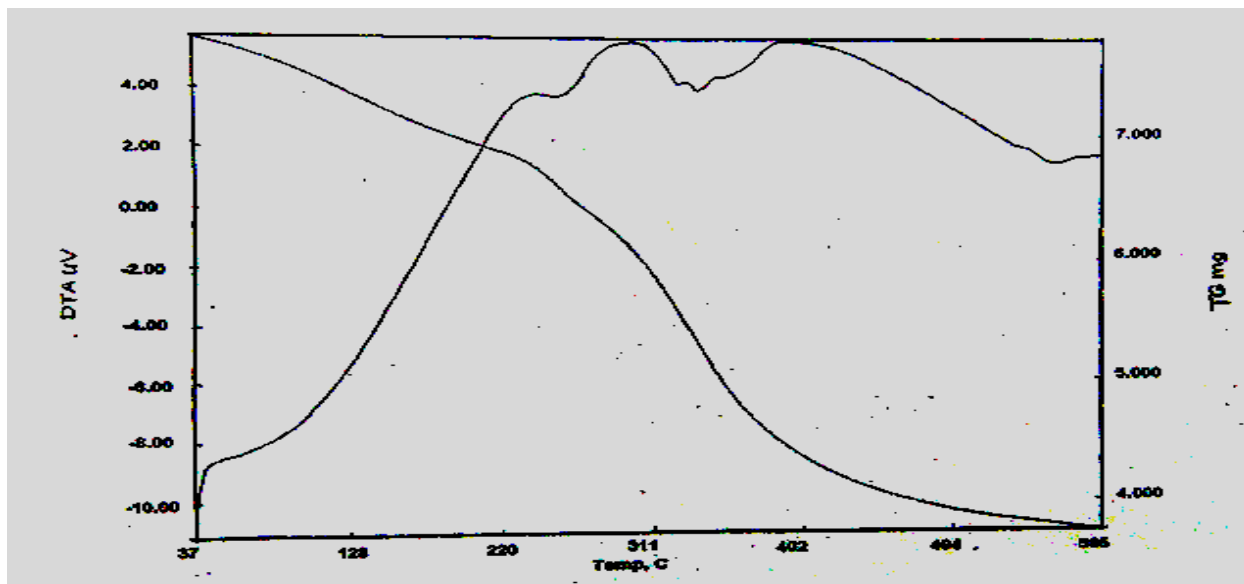


Fig.7. TGA Analysis of AG composite

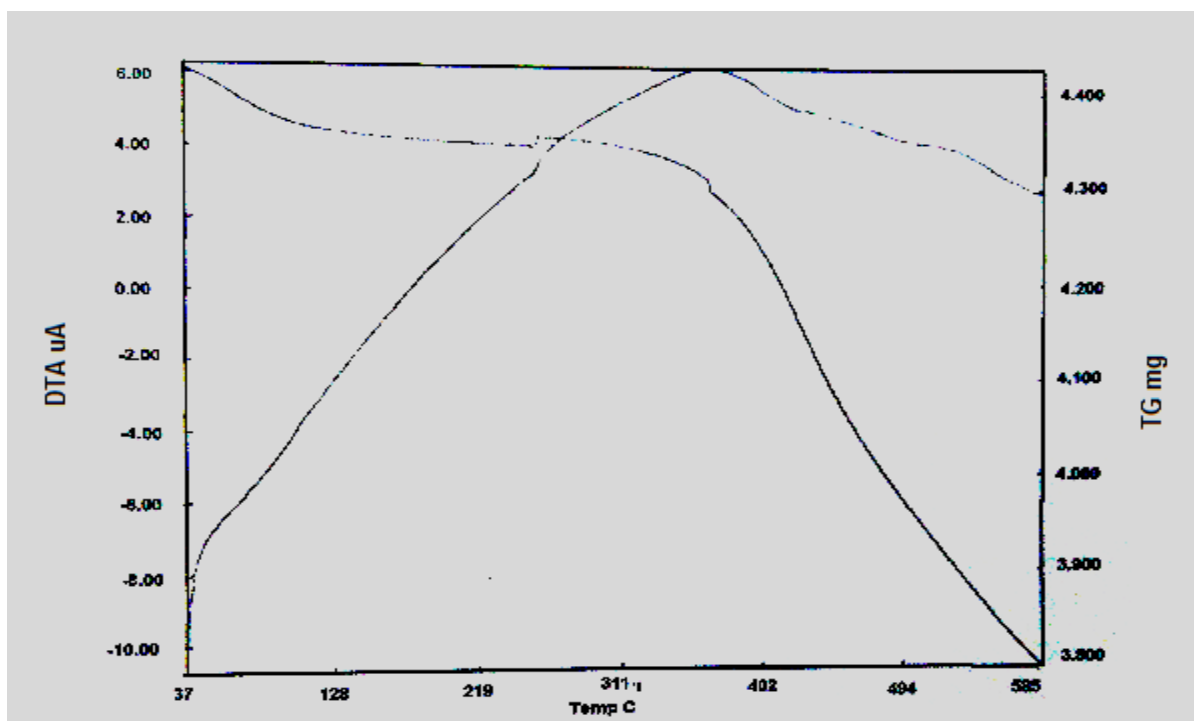


Fig.8. TGA Analysis of AGG composite

In TGA analysis, the loss of weight due to evaporation of water and CO and evaporation of other pyrolysis products are collectively measured as percentage of original weight. In our present study, AG and AGG were heated steadily from 37°C–585°C. The initial weight loss was observed f 25.38% and 13.08% were observed at 235°C for AG and AGG respectively. Above 60% of weigh loss was observed between 235°C to 400°C for AG whereas for AGG about 8% of weight loss was observed between 235°C–375°C. A sudden decomposition was observed between 375°C–585°C for AGG. The AGG was thermally stable up to 375°C and gradual thermal decomposition was observed from 235°C–585°C in case of AG. A 100% thermal decomposition was observed at 585°C for both the samples. The thermal stability of AGG may be due to cross linking of GTA with the functional groups present in backbone of AG.

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