Physico-mechanical properties of hen egg-gelatin-glutaraldehyde composite

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Abstract - *Hen egg white (A) and gelatin (B) composition was prepared and the product is cross-linked with Glutaraldehyde (C). The composition thus formed with A, B and C was standardized with tensile strength and characterized with FTIR, TGA, CD spectra and morphological properties. From the results it was found that the composition ABC possesses better strength than uncross linked AB composition. The CD spectra shows helical structure for AB and unordered random coil for ABC composites and this result confirms the concept of cross linking of AB composite with Glutaraldehyde.*

Key words: hen egg white, gelatin, Glutaraldehyde, FTIR, TGA, CD

1. Introduction

Hen egg white is a viscous fluid that contains albumen, poggle, glair, proteins and water. Hen egg white in combination with gelatin was used as a binder [1]. The glutaraldehyde was cross linked with Bovine serum albumin and used as bio-glue [2] for tissue adhesive and as a sealing agent for kidney during partial nephrectomy. Glue was prepared using albumin and glutaraldehyde as a tissue adhesive for sealing pulmonary parenchyma and bronchial anastomoses [3]. The immobilization of Lysozyme in hen egg white was cross linked with glutaraldehyde that can be used for continuous lysis of bacterial cells. Enhancement of Lysozyme action by cross linking hen egg white in the presence of N-acetyl glucosamine was observed by Kamalroohk [4]. The unique property of hen egg white to form stable foam has been reported by Marolia [5] in developing a novel method for immobilization of naturally present Lysozyme in egg with glutaraldehyde. Kulkarni [6] has developed a new inter penetrating polymer network of sodium alginate and gelatin with egg albumin cross linked with glutaraldehyde for in-vitro release of cefadroxil. Gelatin is common substance used extensively in pharmaceuticals, leather, food ingredient and packing. Bigi [7] has investigated gelatin- glutaraldehyde films for its mechanical, thermal and swelling properties and verified the influence of glutaraldehyde concentration on the stability of the films. Tabata [8] had prepared gelatin- glutaraldehyde hydrogels and implanted the same after incorporating bFGF in the hydrogel in the rats to find out the revascularization of bFGF release from the hydrogels. Chen [9] had prepared anticancer biodegradable and hydrophilic glutaraldehyde cross-linked microspheres and quantified the in-vivo release of anticancer drugs from microspheres. Sharma [10] studied the LASER light scattering of gelatin-glutaraldehyde solutions using static and dynamic Laser light scattering measurements. Similar method has been studied by Kennedy [11] for the surface mobilization and entrapping of enzymes on glutaraldehyde cross linked gelatin particles. Martucci [12] has studied creep behavior of glutaraldehyde-gelatin films by short time flexural tests at 30^{o}C. Chemical cross linking yields an increase in strength and decrease in viscous creep. In the present study, I have developed hen egg white-gelatin-glutaraldehyde composite and characterized it for FTIR, TGA, CD and Tensile strength.

2. Experimentation:

2.1 Materials:

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

2.2 Methods:

2.2.1 Preparation of Albumin solution (A):

Egg white was separated from broken egg and kept in a conical flask.

2.2.2 Preparation of Gelatin solution (B):

20 grams of gelatin powder was dissolved in 200 ml of water at 55°C in a water bath and stored in a flask.

2.2.3 Preparation of AB film:

5 ml of solution (A) was added to 20 ml of (B) at ration of 1:4 and stirred for about 10 minutes to get uniform solution. It was then poured in a polythene tray and dried at room temperature (20-25°C) for about 24 hours. Then so formed film was named as (AB).

2.2.4 Preparation of ABC film:

30 ml of solution (A) was added to 120 ml of (B) and different composition films were prepared by adding solution (C) as per concentrations shown in below table.

Table-1: Preparation of ABC composites

Sample ID	AB (ml)	C (ml)
1	25	0.25
2	25	0.50
3	25	0.75
4	25	1.00
5	25	1.25
6	25	1.50

These six samples were dried and made into films as per procedure mentioned in section 2.2.3.

2.3 Characterization:

The AB and ABC films were characterized by using FTIR spectra, Thermo Gravimetric Analysis, Circular Dichroism, Tensile strength and water absorption.

2.3.1 Water Absorption (%):

The samples were tested for water absorption capacity according to the following method.

Each sample was cut in to 6 pieces of 10 mm x 10 mm in size. These six samples were placed in a chamber which maintains at 20°C and 65% RH for about 24 hours in order to get uniform condition for all samples. Then take weight for each sample individually and denote it as (W_i). Then place all samples in a beaker of 100 ml size and pour 50 ml of double distilled water in to the beaker. Then take weight of each sample (W_f) after 1 hr, 2 hr, 3 hr and 24 hrs and recorded. Before taking weight, the sample should be blotting with filter paper to remove unabsorbed water particles on the surface of the sample. The water absorption was calculated in terms of percentage per unit area as follows.

Water Absorption: $\mathbf{E_s = \{(W_f - W_i) / W_i\}}$ %/mm^2

2.3.2 Tensile Strength:

Three samples of dumbbell shape of sized 4 mm wide and 10 mm length were used to find tensile property using Universal Testing Machine (INSTRON Model 1405) according to method described by Vogel [11]at elongation rate of 5 mm/min.

2.3.3 Infrared Spectroscopy

The concept of cross linking of AB composite with glutaraldehyde is tested with FTIR spectra using Nicolet Impact 400using 500 mg KBr pellet containing 2-6 mg of the sample.

2.3.4 CD Spectroscopy

The CD measurements were recorded using JASCO J-715 spectropolarimeter (Japan). The instrument was calibrated using ammonium-d10-camphor sulfonic acid. The path length used was 1 mm. The spectra were recorded with 1 nm bandwidth and 0.2 nm step resolution. This spectrum will record average value of 5 readings. The resulting spectra were baseline-corrected and smoothened. The samples AB and ABC 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 which is the band in analyzing the secondary structure of protein and peptides.

2.3.5 Thermal Gravimetric Analysis

The thermal stability of the substance was determined using Perkin-Elmer over temperature range of 37^{o}C to 585^{o}C at heating rate of 20^{o}C / min under nitrogen atmosphere.

3. Results & Discussion

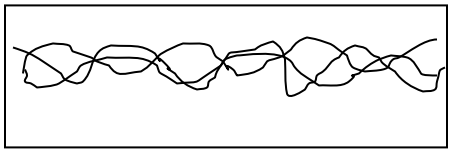
3.1 Tensile Strength

In this study AB is cross linked with varying amounts of glutaraldehyde and the tensile strength of these components were given in Table-1.

Based on studies [1], AB sample was prepared at a ratio of 1:4 (A: B) which gave highest tensile strength (20.9 Mpa) and hence it was used as a standard composition in the present study. With increasing the amount of glutaraldehyde, the tensile strength increases first and then decreases. The composition ABC with 2% glutaraldehyde gives highest tensile strength when compared to other concentration. At this concentration GTA would have been reacted completely with NH_2 groups present in AB backbone as shown in fig-1. With increase in glutaraldehyde concentration, it homo polymerizes and hence decrease in tensile strength and elongation at break was observed.

Table-1: Tensile Strength of hen egg white-gelatin-glutaraldehyde composite

Sl. No	AB (ml)	C (ml)	Tensile Strength (Mpa)	Elongation at break (%)
1	25	0.00	20.9	112
2	25	0.25	33.3	129
3	25	0.50	40.0	170
4	25	0.75	36.2	143
5	25	1.00	32.1	68
6	25	1.25	28.4	45
7	25	1.50	25.2	39



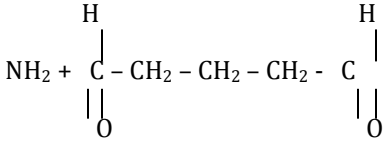
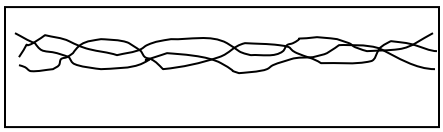


Fig-1: Partial Cross linking of hen egg white-gelatin composite with Glutaraldehyde



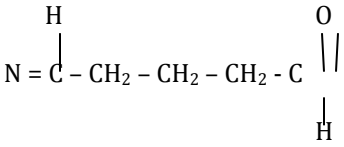
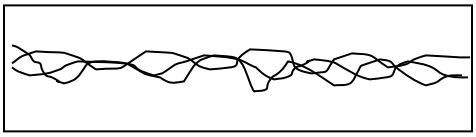


Fig-2: Fully cross linking of hen egg white-gelatin composite with Glutaraldehyde



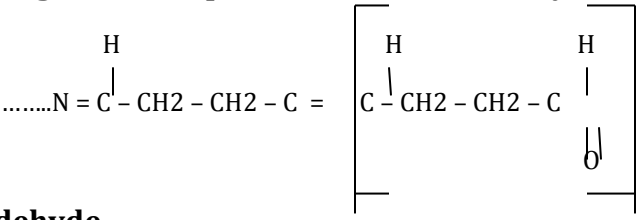


Fig-3: Homo polymerization of Glutaraldehyde

3.2 Water Absorption

Water absorption property of above samples is shown in below table-2.

Table-2: Water absorption of hen egg -gelatin and hen egg-gelatin-glutaraldehyde composites

Sl. No.	Time (hr)	Sample-1 AB alone	Sample-2 %	Sample-3 %	Sample-4 %	Sample-5 %	Sample-6 %	Sample-7 %
1	1	Disintegrated after 1 hour	187	173	170	167	157	125
2	2		256	233	224	219	198	148
3	3		368	245	234	228	205	156
4	24		300	153	125	129	119	103

The hen egg white-gelatin composite as such was disintegrated within a hour whereas samples cross linked with glutaraldehyde were intact even after 24 hours. With increase in glutaraldehyde concentration, the water absorption property increases first and then decreases, this is due to hydrophilic groups (NH_2, COOH, OH) on the AB backbone would have contributed to dissolving membrane in water. With the addition of glutaraldehyde, the NH_2 groups on the AB would have reacted with the –CHO group on glutaraldehyde and increased the hydrophobicity. With further increase in concentration of glutaraldehyde, lower values of percentage water absorption were observed due to the homo polymerization of glutaraldehyde.

3.3 FTIR Spectroscopy

The FTIR spectra of hen egg – gelatin composite shows protein peaks at 1654 cm^{-1} (Amide-I), 1561 cm^{-1} (Amide-II), 1244 cm^{-1} (Amide-III) as shown in fig-4.

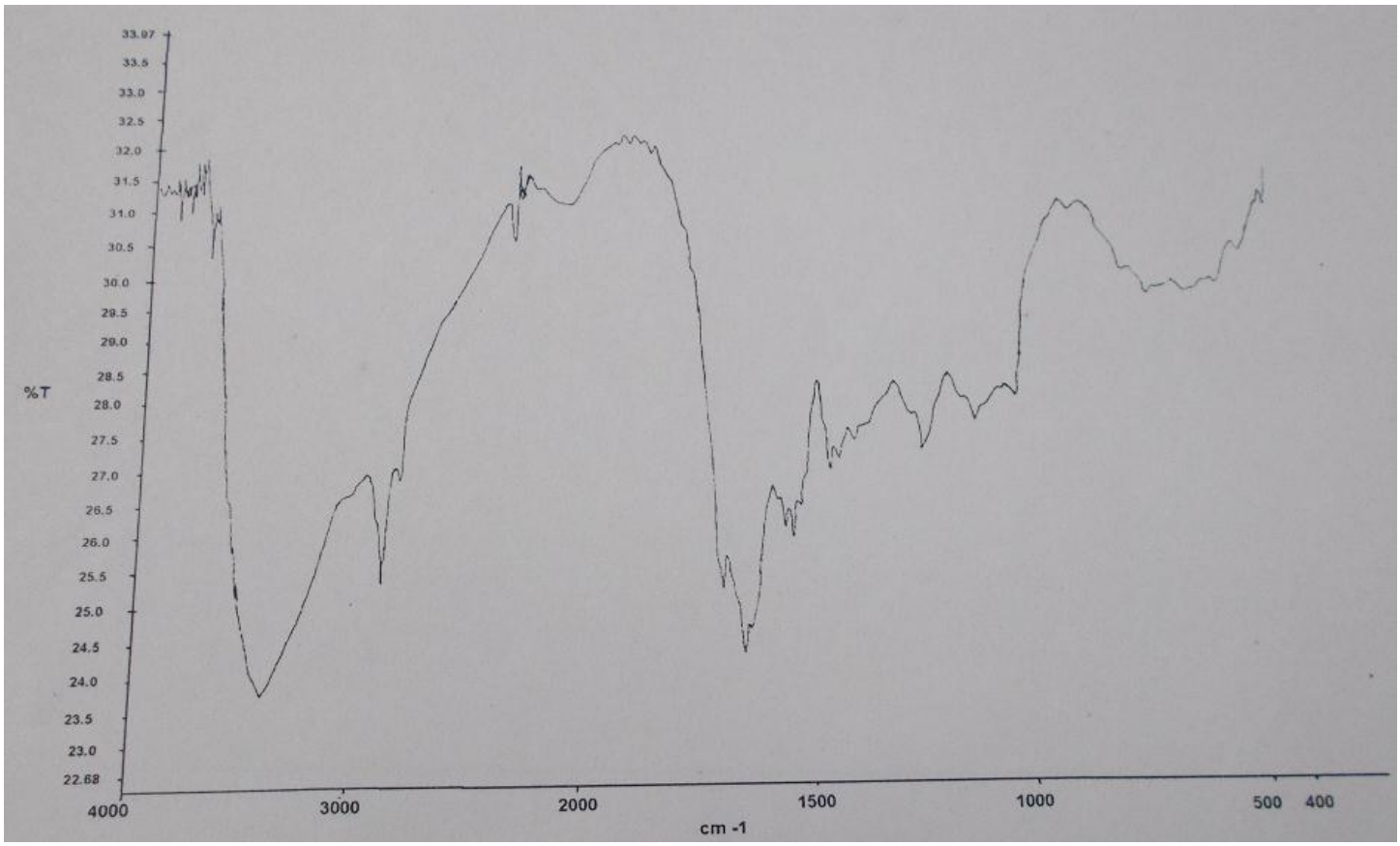


Fig-4: FTIR Spectra of Hen egg white-Gelatin composite

In the FTIR spectra of hen egg-gelatin cross linked glutaraldehyde, the amide-III band is absent; this is due to linking of glutaraldehyde with amide groups as shown in fig-5.

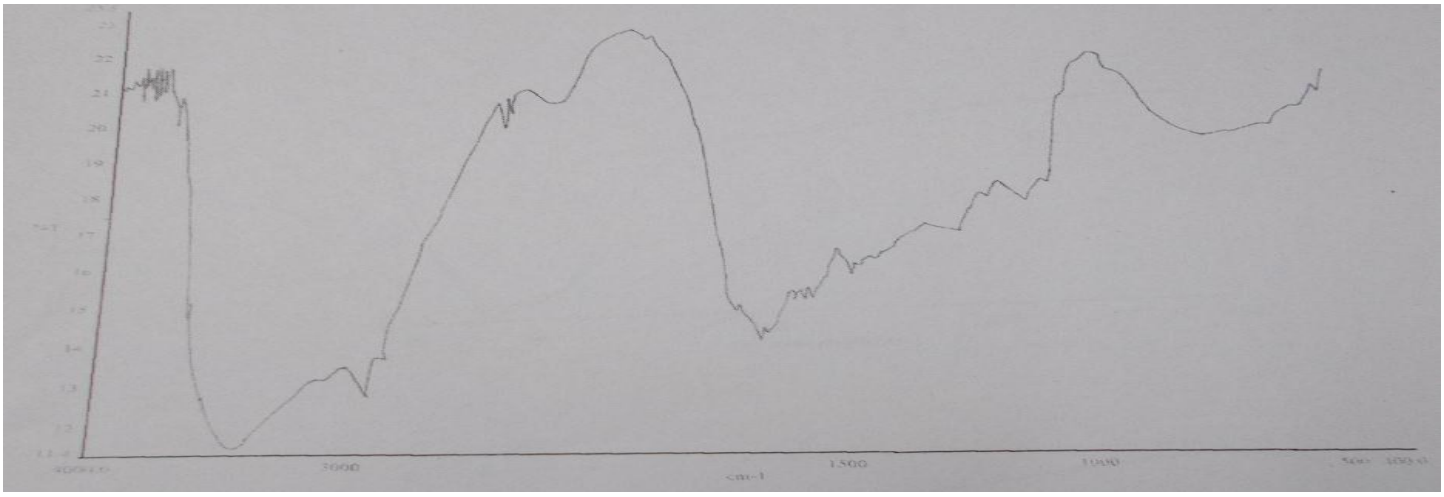


Fig-5: FTIR spectra of Hen egg white-Gelatin-Glutaraldehyde composite

This is an indication of cross linking of glutaraldehyde with hen egg-gelatin composite.

3.4 Thermal Gravimetric Analysis (TGA)

In thermo gravimetric analysis, the loss of weight due to evolution of H_2O and CO and evaporation of other pyrolysis products are collectively measured as percentage of original weight. In this investigation, AB and ABC were heated steadily from 37°C to 585°C. The initial weight loss of 25.38% and 13.08% were observed at 235°C for AB composite and ABC composite respectively. About 60% of weight loss was observed between 235°C to 400°C for AB as shown in fig-6 whereas for ABC about 8% of weight loss was observed between 235°C – 375°C as shown in fig-7.

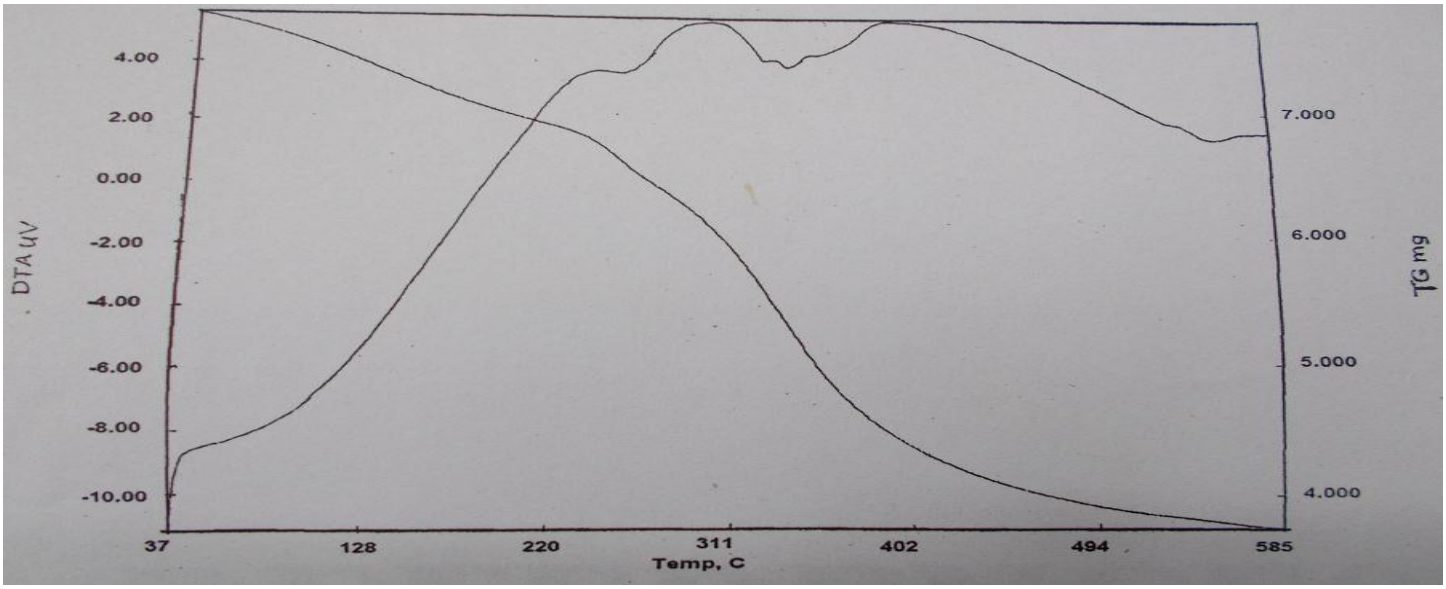


Fig-6: TGA analysis of Hen egg white-Gelatin composite

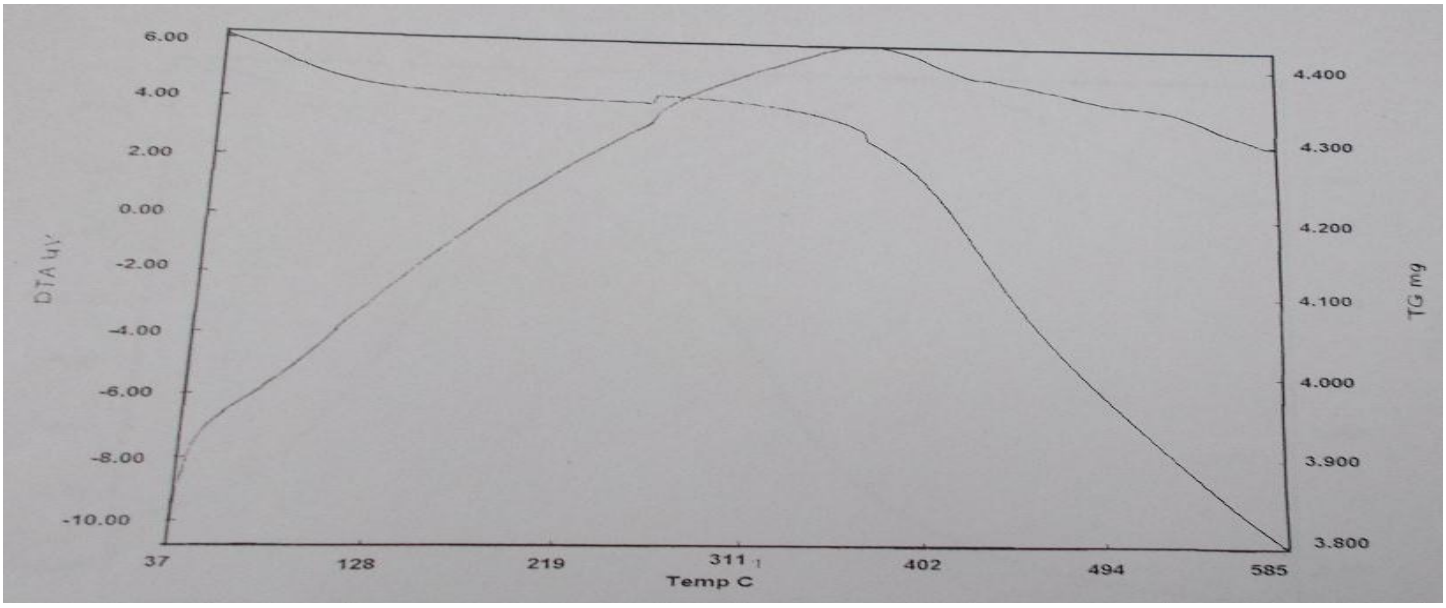


Fig-7: TGA analysis of Hen egg white-Gelatin-Glutaraldehyde composite

A sudden decomposition was observed between 375°C – 585°C for ABC. The ABC sample was thermally stable up to 375°C whereas gradual thermal decomposition was observed between 235°C – 585°C in the case of AB. A 100% thermal

decomposition was observed in both the cases at 585°C. Higher value of thermal stability in ABC composite is due to cross linking of glutaraldehyde with the functional groups in the backbone of AB composite.

3.5 Circular Dichroism

It was known from the previous study [1] that the composite AB shows α-helical structure which confirms protein present in the composite. The far UV spectral studies (185 nm - 250 nm) were conducted on ABC composite and it was observed that an unordered random coil structure with negative peak at around 197 nm as shown in fig-8.

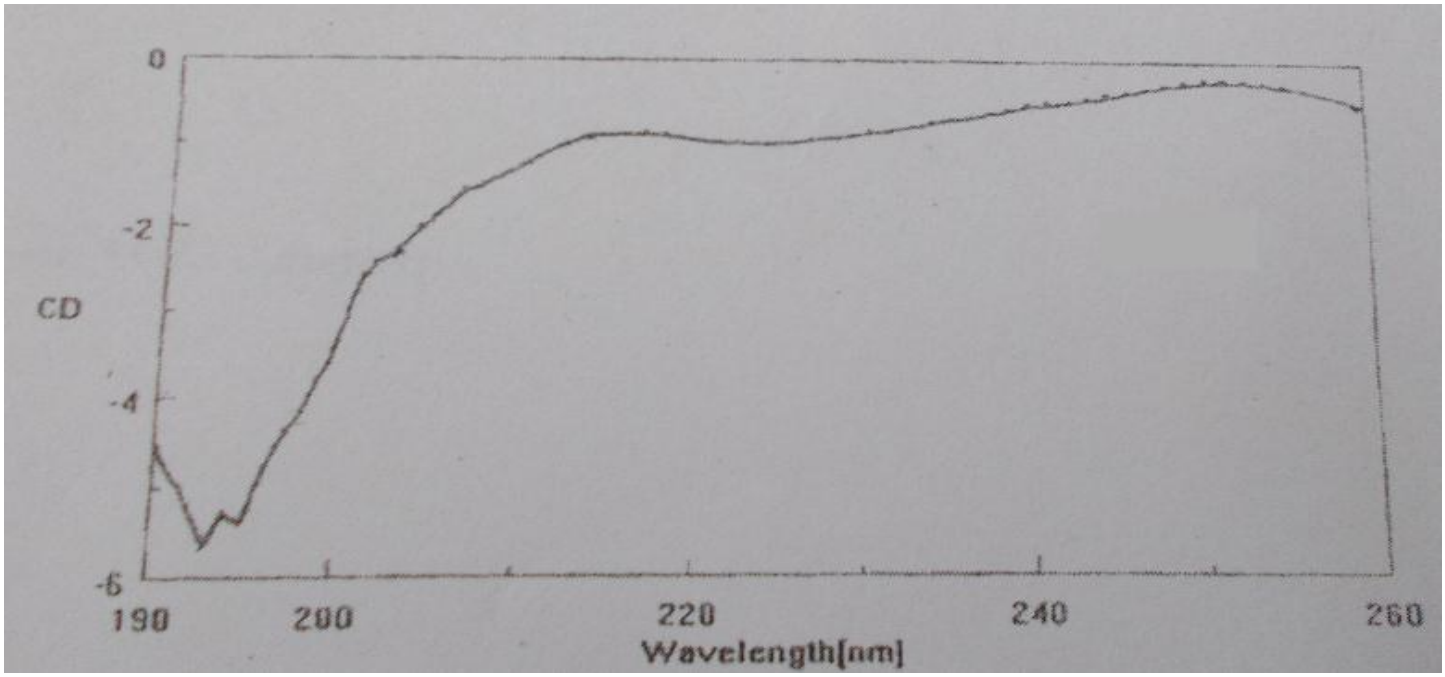


Fig-8: CD spectra of Hen egg white-Gelatin-Glutaraldehyde composite

The breakage of helical structure in AB and converted in to random coil is due to cross linking of glutaraldehyde with AB composite.

4. Conclusion

Hen egg white which is natural food nutrient is used in making a bio-polymer material by cross linking with gelatin and glutaraldehyde. This bio-polymeric film possesses excellent tensile strength and high temperature withstands and hence the product can be used as a coating agent on materials to prevent rust and thereby improving the life of the material.

5. REFERENCES

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