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GRAFT COPOLYMERIZATION OF 2-HYDROXYETHYL METHACRYLATE ONTO BOVINE SERUM ALBUMIN: PREPARATION AND CHARACTERIZATION

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Key Words: Graft Copolymerization, Bovine Serum Albumin, 2-Hydroxyethyl Methacrylate

ABSTRACT

Bovine Serum Albumin (BSA) was separated from the whole blood of freshly slaughtered animals. This BSA was graft copolymerized with poly (2-hydroxyethyl methacrylate) and characterized. The infrared spectroscopy, thermogravimetric analysis, scanning electron microscopy, and percentage of grafting of the composites were studied. The graft copolymers have better physicochemical properties and, therefore, could be more useful in their field of application.

INTRODUCTION

Albumins are used in medicine for various purposes. Among other uses, it is used as a hydrogel for the controlled release of drugs. Albumin crosslinked fibrogels were prepared by free radical polymerization using 1-vinyl-2-pyrrolidinone as a monomer and functionalized albumin as a crosslinked agent for long term oral drug delivery [1, 2] . Polymerized albumin was used as a surface active compound

in drug delivery compositions containing coacervate systems which can deliver the physiologically active compounds through oral, parenteral, transdermal and trans-mucosal routes [3]. Macroparticles and nanospheres of bovine serum albumin were studied for their drug delivery properties [4-8]. Sinn *et al* [9] prepared conjugates by coupling drugs to serum albumin through linkers, which are useful for inhibition of tumor cells or pathogenic microbial cells. Hydrogels were prepared by crosslinking bovine serum albumin with polyethylene glycol, which are suitable for controlled drug delivery systems [10-12]. In the present study, bovine serum albumin was prepared from the serum available from the slaughter house, and graft copolymerized with 2-hydroxyethyl methacrylate. The graft copolymer was characterized for its percentage grafting, infrared spectroscopy, scanning electron microscopy and thermogravimetric analysis.

EXPERIMENTAL

Materials

1) Bovine Serum Albumin (BSA) (isolated from the serum available at the municipal slaughter house, Madras, India).

2) 2-Hydroxyethyl methacrylate (Fluka, Switzerland)

All other reagents used were of analytical grade.

Methods

Isolation of Bovine Serum Albumin (BSA)

Cattle blood, after defibrination and removal of cells, was obtained from the local municipal slaughter house. The liquid (serum) was heated over a water bath at about 65°C for 30 minutes with continuous stirring to precipate proteins other than albumin. The supernatent obtained was cooled and residual non-albumin proteins were precipitated out using polyethylene glycol (25% w/v) according to the method of Jimenez *et al.* [13]. Albumin was separated from the supernatent by ethyl alcohol (40% v/v) precipitation method followed by centrifugation. Thus, the obtained precip itate was dissolved in water and the pH of the solution was adjusted to 4.7 to get the albumin precipitated in pure form. Albumin was lyophilized and then made into a powder form.

Preparation of Bovine Serum Albumin-2-Hydroxyethyl Methacrylate Graft Copolymer (BSA-PHEMA)

0.5 gm of BSA was soaked in 25 ml of water overnight. To this, 0.025 g of potassium persulfate and 0.025 g of sodium metabisulfite were added followed by 2

ml of 2-hydroxyethyl methacrylate. This experiment was carried out at 50°C for 1 hour. The crude graft copolymer was extracted with acetone to remove poly hydroxyethyl methacrylate homopolymer and then dried at room temperature.

Percentage Grafting

Percentage grafting was determined by the following equation [14].

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

Scanning Electron Microscopy

Scanning electron micrographs were taken both for BSA (Figure 1) and BSA-PHEMA (Figure 2) using JSM-5300 Scanning Microscope.

Infrared Spectroscopy

To provide proof of grafting, the infrared spectra of BSA and BSA-PHEMA graft copolymer were studied by using Nicolet Impact 400 Fourier Transform Infrared Spectroscopy (FTIR) using KBr pellet 500 mg containing 2-6 mg of the sample.

Thermogravimetric Analysis (TGA)

The TGA of the samples were carried out using a Seiko SSC 5200 H in nitrogen atmosphere (80 ml/min) at a heating rate of 10°C/min. Primary weight loss of these materials as a function of temperature was recorded using this study.

RESULTS AND DISCUSSION

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 bovine serum albumin offer an attractive technique of improving its surface characteristics. In this study, grafting of PHEMA on BSA was established using potassium per sulfate-sodium bisulfite initiation technique.

Percentage Grafting

The grafting percentage result was found to be as high as 200%. This indicated that the PHEMA was readily grafted onto BSA in the high order in the

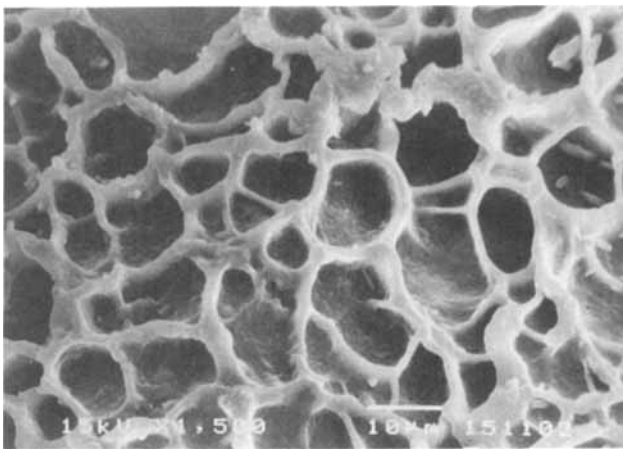

Figure 1. Scanning electron mictrograph of BSA (1000 X)

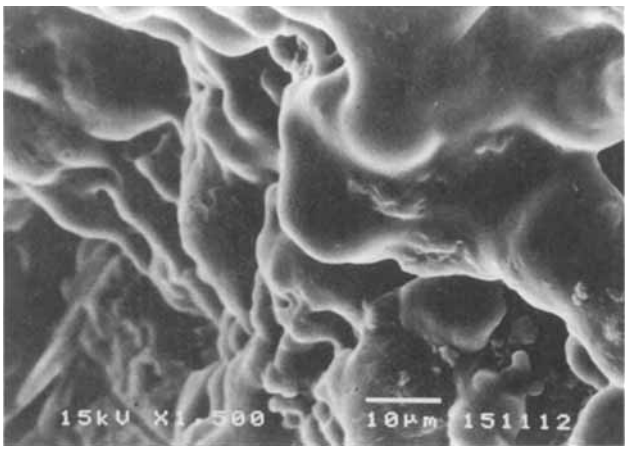

Figure 2. Scanning electron micrograph of BSA-PHEMA graft copolymer (1000 X)

present experimental conditions wherein, potassium persulfate and sodium meta-bisulfite were used as initiators.

Scanning Electron Microscopy

Figures 1 and 2 show the Scanning Electron Micrography of BSA and BSA-PHEMA, respectively. It could be seen from Figure 1 that the morphological structure of BSA is expressed in the network form. On the other hand, after

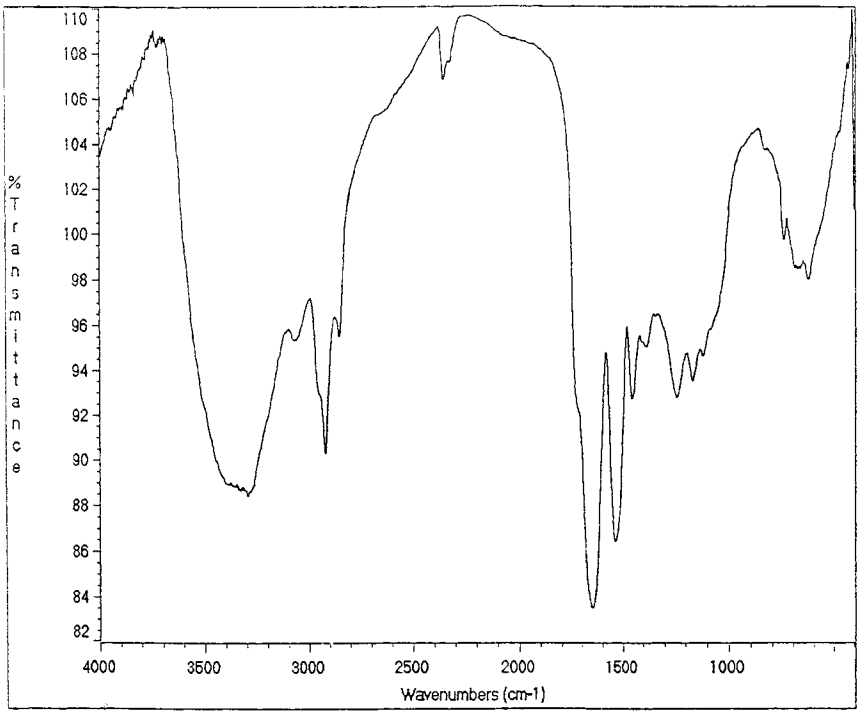

Figure 3. FTIR Spectrum of BSA

grafting, the network structure of protein is completely covered by the PHEMA molecules (Figure 2). The difference in the surface morphology of protein before and after coating with the polymer confirms the grafting of PHEMA onto BSA. It should be noted that there is no free PHEMA in the system, which may give just physical coating to the protein particles, as the homopolymer has been removed completely by washing with acetone prior to the SEM Study.

Infrared Spectroscopy

The IR spectra of BSA (Figure 3), PHEMA (Figure 4) and BSA-PHEMA (Figure 5) were compared. The BSA, being a protein, showed the characteristic amide absorption bands at 1660 cm^{-1}, 1550 cm^{-1} and 1250 cm^{-1}. In the IR spectrum of PHEMA (Figure 4) the absorption at 1720 cm^{-1} characteristic of ester carbonyl was seen. The IR spectrum of BSA-PHEMA showed characteristic absorption bands of the ester carbonyl group of PHEMA at 1720 cm^{-1}, besides the absorption bands of BSA. As mentioned in the Experimental section, the presence of free homopolymer, if any, in BSA-PHEMA was washed with acetone. It is understood from these results that the PHEMA was grafted onto BSA.

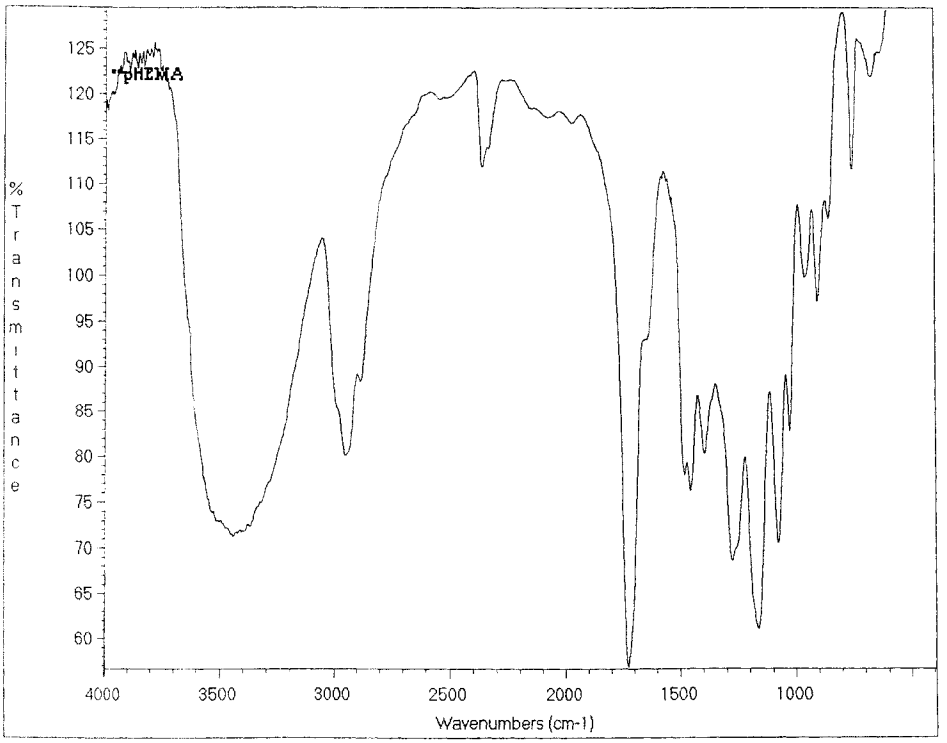

Figure 4. FTIR Spectrum of PHEMA

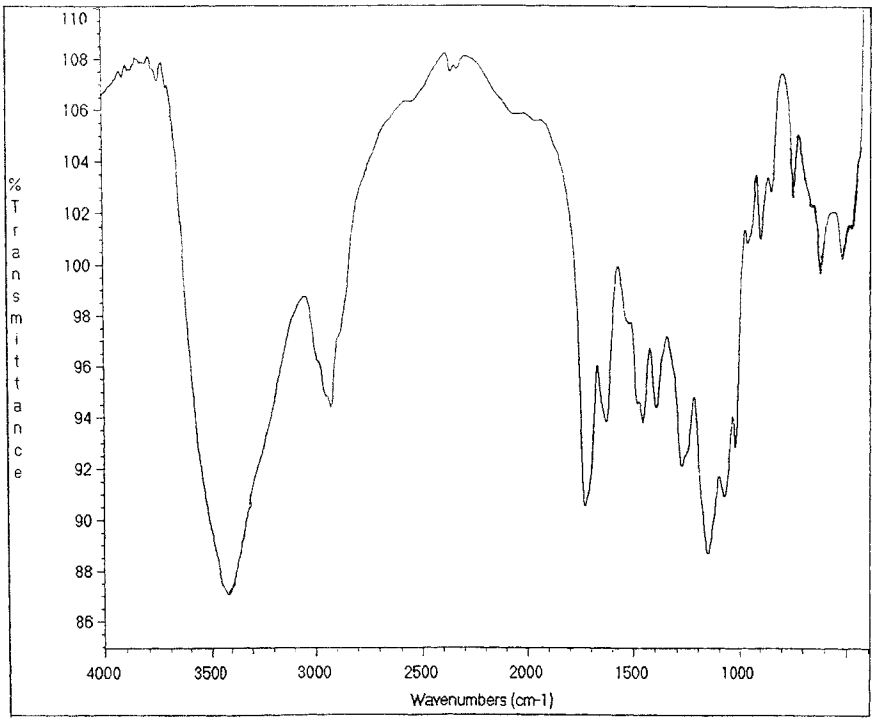

Figure 5. FTIR Spectrum of BSA-PHEMA graft copolymer

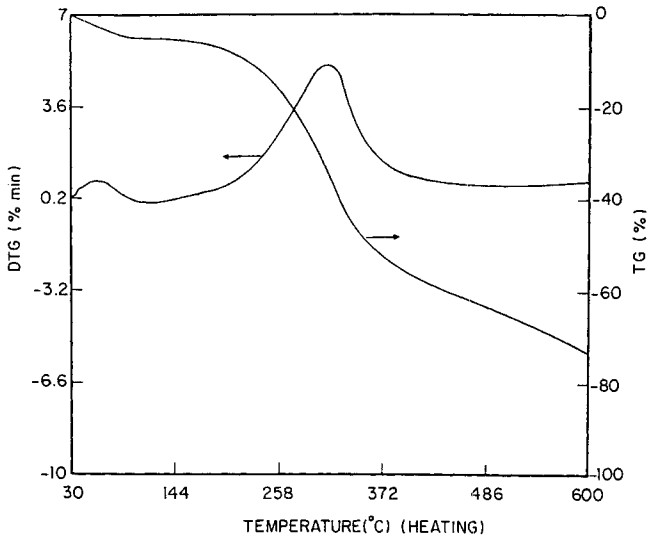

Figure 6. Thermogram of BSA

Thermogravimetric Analysis

Figures 6, 7 and 8 illustrate the thermal decomposition profile of BSA, PHEMA and BSA-PHEMA respectively. According to the thermogram obtained for the bovine serum albumin (Figure 6), it is understood that the decompostion takes place at a single stage which starts after 102°C and completes at around 360°C. During this phase, a net weight loss of about 45% was seen with a maximum loss at about 310°C. On the other hand, the PHEMA (Figure 7), another constituent of the graft copolymer undergoes decomposition at a temperature between 210 and 310°C with a net weight loss of about 90%, whereas the thermal decomposition profile of BSA-PHEMA (Figure 8) showed a three stage decompositon. Evidently, this did not exhibit the pattern of its constituents and, therefore, proves the chemical combination of BSA and PHEMA. As per this thermogram, a present experimental conditions wherein, potassium persulfate and sodium metabisulfite were used as initiators.

Scanning Electron Microscopy

Figures 1 and 2 show the Scanning Electron Micrography of BSA and BSA-PHEMA, respectively. It could be seen from Figure 1 that the morphological structure of BSA is expressed in the network form. On the other hand, after grafting, the network structure of protein is completely covered by the PHEMA

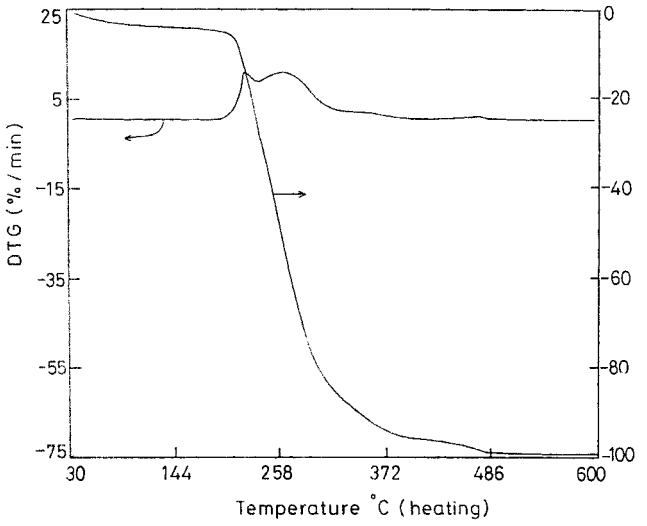

Figure 7. Thermogram of PHEMA

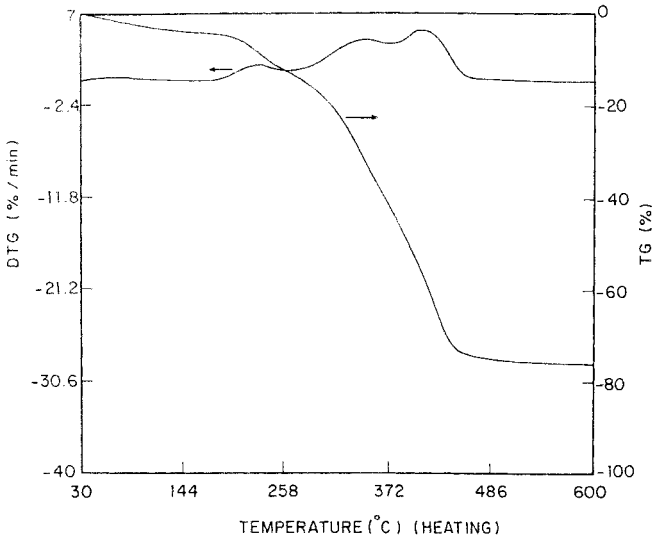

Figure 8. Thermogram of BSA-PHEMA graft copolymer

molecules (Figure 2). The difference in the surface morphology of protein before and after coating with the polymer confirms the grafting of PHEMA onto BSA. It should be noted that there is no free PHEMA in the system, which may give just physical coating to the protein particles, as the homopolymer has been removed completely by washing with acetone prior to the SEM Study.

Infrared Spectroscopy

The IR spectra of BSA (Figure 3), PHEMA (Figure 4) and BSA-PHEMA (Figure 5) were compared. The BSA, being a protein, showed the characteristic amide absorption bands at 1660 cm^{-1}, 1550 cm^{-1} and 1250 cm^{-1}. In the IR spectrum of PHEMA (Figure 4) the absorption at 1720 cm^{-1} characteristic of ester carbonyl was seen. The IR spectrum of BSA-PHEMA showed characteristic absorption bands of the ester carbonyl group of PHEMA at 1720 cm^{-1}, besides the absorption bands of BSA. As mentioned in the Experimental section, the presence of free homopolymer, if any, in BSA-PHEMA was washed with acetone. It is understood from these results that the PHEMA was grafted onto BSA.

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CONCLUSIONS

The potassium persulfate and sodium metabisulfite seem to be a good redox initiator system in the grafting of PHEMA onto BSA, though other initiators were not tried. A high yield of graft copolymer was evidenced by a high percentage

of grafting (>200%). Uniform surface morphology of BSA was seen in the scanning electron micrograph. There was a delay in the decomposition of graft copolymer, when compared with that of BSA or PHEMA. It (Figure 8) seems to follow the thermolytic pattern of the PHEMA, as the graft copolymer contains a high level of PHEMA chemically bonded with BSA.

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