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Preparation of Feather Keratin Hydrolyzate-Gelatin Composites and Their Graft Copolymers

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With the aim of utilizing the Keratinous waste material, poultry feather, which is up till now discarded as a waste, was hydrolyzed to form keratin hydrolyzate (FH). As FH does not form a film, it was mixed with gelatin (G) and FH-G composite was prepared in film form. FH-G was further graft co-polymerized with 2-hydroxyethyl methacrylate (HEMA) to achieve better physico-chemical properties for the resultant hydrogels. Percentage grafting studies and IR studies confirmed the grafting of PHEMA onto FH-G. FH-G-PHEMA exhibited better mechanical properties compared to FH-G and FH-PHEMA. TG studies clearly indicated the grafting of HEMA onto FH and FH-G. SEM (Scanning Electron Microscopy) pictures of FH-G and FH-PHEMA films exhibited brittle nature on their surface, whereas continuity and A smooth surface was observed on for the FH-G-PHEMA films.

Keywords feather keratin hydrolyzate (FH), gelatin, 2-hydroxyethyl methacrylate (HEMA), hydrogel, feather keratin-gelatin composite (FHG-PHEMA)

Introduction

Gelatin is obtained by controlled alkaline hydrolysis from fibrous insoluble protein collagen, which is widely found in nature as a major constituent of skin, bone, and connective tissues of the animal body (1). Gelatin is well known for its wound healing properties (2–4). Sinha et al. (5) treated burns with gelatin sheets and deducted that these biological dressings prevented secondary infection and fluid loss due to exudation. Yannas et al. (6) mentioned a process for preparing gelatin from animal products for the purpose of wound healing. Eckmayer (7) reported the favorable properties of gelatin-based biomaterials for use as haemostatic and wound healing agents. Gelatin is a unique and valuable protein and does not occur in nature as gelatin. Gelatin is well known for its biological properties and is used in various cosmetic and surgical applications. Polymeric hydrogels based on gelatin are of considerable importance due to their potential application as biomaterials. Thus, it is of interest to synthesize hydrogels

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containing protein composites and study their utility in various applications, especially in biomedical areas. Xiao et al. (8) prepared blend films using Gelatin and polyacrylamide solutions and characterized their physical properties. They reported improved water absorption properties for the blend films. Anne George et al. grafted gelatin with Ethyl acrylate in a water-acetic acid medium (1 : 1 v/v). The effects of experimental variables, such as reaction time and concentration of monomer, initiator and gelatin, were studied.The results are discussed in terms of rate of grafting and percentage of grafting. These quantities were found to be lower than for gelatin-g-PEA copolymers in aqueous and water-isopropanol media. Dhara et al. synthesized a series of IPNs of poly(N isopropyl acrylamide) with anionic poly acrylic acid and cationic poly (3-(methacrylolamino)propyl)trimethyl ammonium chloride) [PMAPTAC]).The swelling behavior of the interpenetrating polymers is monitored as a function of both PH and temperature. The swelling ratios of IPNs are significantly larger than those reported for pure PNIPA gels [PNIPA-PAA]. IPNs show a continous volume phase transition (VPT) at PHs below the Pka of polyacrylic acid and at PH $>$ Pka the IPNs show a temperature independent swelling. Noorjahan et al. prepared fibrin gelatin composite films crosslinked with glutaraldehyde and graft copolymerized with poly(2-hydroxyethyl methacrylate) and poly(2-hydroxypropyl methacrylate) and characterized the hydrogels. Fibrin used in the systems acted as a reinforcement material. Krishna et al. (12) synthesized full and semi-interpenetrating polymer network based on polyacrylic acid and gelatin, using N,N′-methylene bis-acrylamide (BAM) and gluteraldehyde as cross-linking agents.

Keratin hydrolyzate is prepared by hydrolyzing keratinous protein like feathers, horns, hooves, hair, etc. Seghal et al. (13) solubilized poultry feather under controlled alkaline conditions and characterized the resultant keratin hydrolysate for its pH stability range, gels strength, viscosity and molecular weight. Sastry et al. (14) employed the keratin hydrolyzate as a filler in the re-tanning operation of leather processing with excellent results. From the electron microscopic data, it was found that keratin hydrolyzate penetrated through pores of chrometanned leathers and filled their available gaps in the network of collagen matrix. Feather and hair keratins were also graft copolymerized using vinyl polymers and these graft co-polymers were used in leather impregnation and finishing of leathers (15). Hydrolyzed feather meal was prepared using different techniques such as digesting the feathers at various pressures, employing enzymatic hydrolysis and sulphate treatment followed by enzymatic hydrolysis and protein digestibility of all these products were analyzed (16). Chemical modification of proteins with selected polymers improves the functionality, thermal stability and other physicochemical properties of the products (17–19). Acrylic polymers based on HEMA have been considered as materials for many biomedical applications because of their biological inertness, nontoxicity, biocompatibility, and chemical stability (20). Hydrogels based on these polymers are biodegradable and are being used in various fields of medicine, such as ophthalmology, plastic surgery, urology and so forth (21, 22). The presence of hydroxyl groups ensures the hydrophilicity and biocompatibility of polymers. Polymers can be degraded by the cells of monocytes-macrophages and degraded compounds can be excreted by a renal filtration process. Hydrogels possess good compatibility, and their soft and rubbery nature minimizes irritation to the surrounding tissue (23–24).

In the present investigation feather keratin hydrolyzate and gelatin composite were prepared and these composites were graft copolymerized with hydroxyethyl methacrylate. The grafted copolymers were analyzed for their physico-chemical parameters.

Experimental

Materials

Poultry feathers were collected from local poultry dressing units. Pharmaceutical grade gelatin was obtained from MBD gelatin, Mumbai, India. 2-Hydroxyethyl methacrylate (HEMA) was obtained from FLUKA, Switzerland. All other agents used were of analytical grade.

Preparation of Feather Keratin Hydrolyzate (FH)

FH was prepared from the poultry feathers by a method described earlier (13). Briefly, Poultry feathers were washed in running water and were transferred to a steam jacketed steel kettle, Ca $(OH)_2$ 10% was added, based on the wet weight of feathers, and the lot allowed to soak for 24 h. Later, water sufficient to cover the feathers was added and steam was fed into the jacket and the contents were boiled for 10 h. The solubilized material was filtered and the residue was discarded. The filtrate was neutralized to pH 7, dried at 100°C and the resultant dry matter was feather keratin hydrolyzate (FH).

Preparation of FH-G

Gelatin (5 g) was dissolved in 50 mL of water at 55°C in a water bath and to this solution a known amount of 10% aqueous FH solution was added (Table 4), stirred to a homogeneous solution and then 1 mL of ethylene glycol was combined with the solution. Later, the solution was poured into a polythene tray (11 × 7 cm), spread uniformly, and dried at 45°C in a hot air oven. The film obtained was denoted as FH-G.

Graft Copolymerization of PHEMA on to FH (FH-PHEMA)

FH (5 g) was dissolved in 50 mL water. To this 0.25 g of potassium persulfate, 0.25 g of sodium metabisulfate and 2 mL of HEMA were added and stirred for 45 min. Later, 0.35 mL gluteraldehyde was added and the stirring was continued for 10 min. Then, 2 mL of ethyleneglycol was added and stirred for another 5 min. The reagents gluteraldehyde and ethylene glycol acts as crosslinking and plasticizing agents, respectively. Throughout the reaction, a 55°C temperature was maintained. Finally, the contents were poured into a polythene tray (11 × 7 cm) and dried at 45°C to form a film. The Keratin polymer composite film (crosslinked) washed with acetone to remove excess poly (HEMA) homopolymers. This is denoted as FH-PHEMA.

Graft Copolymerization of PHEMA on to FH-G (FH-G-PHEMA)

Gelatin (5 g) was dissolved in 100 mL water at 55°C and to this solution 2 g of FH was added and stirred to obtain a homogeneous solution. The latter stoichiometric ratio was taken, since it exhibited better tensile strength. Then, 0.50 g of potassium per sulfate, 0.50 g of sodium metabisulfate and 4 mL of HEMA were added and stirred for 45 min. Later, 0.70 mL glutaraldehyde was added and the stirring was continued for 10 min. Then, 2 mL of ethylene glycol was added and stirred for another 5 min. The rest of the procedure was the same as explained in the preparation of FH-PHEMA. The final film was denoted as FH-G-PHEMA.

Characterization

The physico-chemical properties of the prepared biomaterials were analyzed by the following methods. The percentage grafting was determined by the following equation.

$$\textit{Percentage of grafting} = \frac{\text{Total weight of the graft co-polymer} - \text{Weight of the FHG}}{\text{Weight of the FHG}} \times 100$$

The water absorption capacities of different compositions of FH-G, FH-PHEMA and FH-G-PHEMA were determined according to the method followed earlier (25). A small piece of each sample of known weight, dried to the constant weight at 100°C, was allowed to swell in distilled water at room temperature (22°C). The swollen weight of the samples were determined by first blotting the samples with filter paper followed by accurately weighing the sample. The weights of the swollen pieces were recorded every 1, 2, and 3 h and after 24 h. Percentage swelling of the samples at a given time was calculated from the formula.

$$\text{Es} = \frac{W_s - W_0}{W_0} \times 100$$

where, W_s is the weight of the sample (moist) at a given time, W_0 is the initial weight of the sample, Es is the percent of swelling at a given time.

IR spectra were taken for FH-G, FH-PHEMA and FH-G-PHEMA using Nicolet impact 400 Fourier transform infrared Spectrophotometer, and a 500 mg, KBr pellet containing 2.6 mg of the sample. The tensile tests of the films were obtained by using an Instron 4501 tensile testing system. Two dumb-bell shaped specimens, 4 mm width and 10 mm length were punched out from the films prepared. Mechanical properties such as tensile-strength and percentage of strain at break were measured. According to Vogel (26) at an extension rate of 10 cm/min.

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

The surface morphology was examined by SEM using a (SEM model LEICA Stereo Scan 440 with a 15 V accelerating voltage. For the study, dried film samples were coated with gold ions using an ion coater (Fisons sputter coater) under 0.1 Torr pressure, 20 mA current, and 70 s coating time conditions.

Results and Discussion

Today, combination of synthetic and natural polymers are finding increased applications in various industries such as leather, paper, printing press, and bio-medical industries, such as pharmaceuticals, hard capsules and cosmetics. However, the biodegradability of the product is of utmost importance in the present day world to reduce the environmental pollution. Therefore, an attempt has been made to combine the two biodegradable compounds, keratin and gelatin. Gluteraldehyde is used as a crosslinker in the study to impart stability to the stored biological materials.

Percentage Grafting (PG)

The grafting of FH-G was carried out using potassium persulfate and sodium metabisulfate as initiators. The results of the present investigation indicate that the PHEMA are readily grafted onto gelatin. The percentage grafting of FH-PHEMA and FH-G-PHEMA were found to be 25 and 30%. As gelatin and keratin are fibrillar proteins and the functional groups (–OH) on the amino acids in the peptide chains of both the proteins are more or less similar, little difference in the PG was observed between the graft copolymers.

Moisture Content

The moisture content of the films FH-G, FH-PHEMA and FH-G-PHEMA are given in Table 1. The moisture content varied between 7.5–12. This is the ideal moisture content for the biomaterial to store at room temperature. At this moisture level, the possibility of bacterial attack is reduced.

Water Absorption Capacity

Previously, chemically modified gelatin sheets were used on wounds (27). Sometimes the sheets did not adhere to the wound properly and adsorption was very poor. To circumvent this problem the FH and FH-G were graft-copolymerized by 2-hydroxyethyl methacrylate. The equilibrium water absorption values of FH-G, FH-PHEMA and FH-G-PHEMA are given in Table 2. The FH-G and FH-PHEMA films disintegrated in 1 h. Whereas FH-G-PHEMA has shown 257% water absorption capacity after 1 h, after 24 h, it exhibited 268%. The results indicate the importance of gelatin in the composite as it gives stability and acts as a reinforcement material to the composite.

Infrared Spectroscopy

The IR spectra of FH-G, FH-PHEMA and FH-G-PHEMA were shown in Figure 1. The characteristic amide absorption peaks of both FH and G are observed around 1632 and 1538 cm^{-1} in the FH-G film. These two peaks represent amide 1 and amide 2 for proteins. Whereas, the IR spectra of FH-PHEMA and FH-G-PHEMA show the bands around 1730 and 1737 cm^{-1}, respectively. Representing the ester carbonyl bond (C═O) vibration, in addition to the amide absorption bands of FH and G. The result in the amide bands FH- PHEMA (1647 and 1543 cm^{-1}) and FH-G-PHEMA (1632 and 1537 cm^{-1}) may be due to the chemical modification of the backbone by the monomer. These results clearly indicate the grafting of polymer P-HEMA on to FH and G.

Table 1
Moisture content

S. no	Sample	Percentage of moisture (%)
1	FH-G	7.5
2	FH-PHEMA	11
3	FH-G-PHEMA	12

Table 2
Water absorption capacity

S. no	Sample	Water absorption (h)			
		1	2	3	24
1	FH-G		Film dissolved		
2	FH-PHEMA		Film dissolved		
3	FH-G-PHEMA	257%	260%	264%	268%

Tensile Strength

Gluteraldehyde is widely used in protein chemistry as a cross-linking agent, since it reacts primarily with the protein amino groups. It has been used in manufacture of medical devices, tissue prostheses, and as a sterilizing agent. Gluteraldehyde is also being used for improving the mechanical strength of collagenous and gelatinous materials (28). With this view, the cross-linking agent was used here to obtain a biomaterial with enhanced mechanical properties and relatively increased resistance to biodegradation. The tensile strength data of the FH-G, FH-PHEMA and FH-G-PHEMA are shown in Table 4.

The FH-G tensile strength data, prepared with various stiochiometric ratios of FH and G, FH-PHEMA and FH-G-P-HEMA are shown in Tables 3 and 4. Among the various

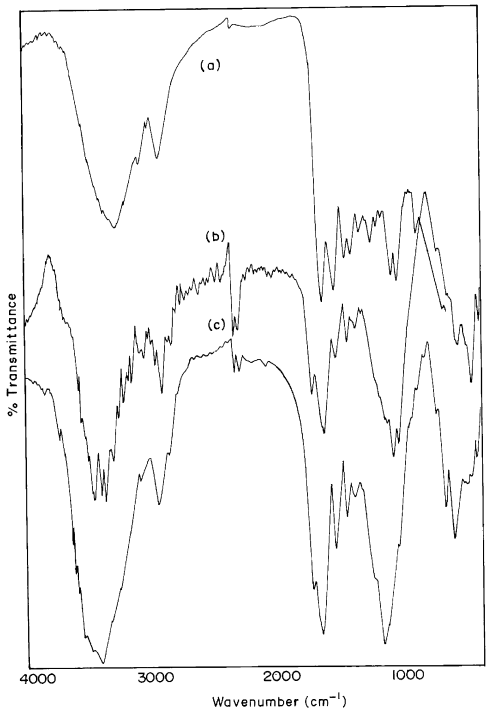

Figure 1. IR spectrum of (a) FH-G, (b) FH-PHEMA and (c) FH-G-PHEMA.

Table 3
Tensile strength of FH-G prepared using various stoichiometric ratios of FH and G

S. no	FH : G	Tensile strength (MPa)	Elongation (%)
1	0 : 5	3.659	171.554
2	1 : 5	4.868	237.330
3	2 : 5	5.736	432.484
4	3 : 5	4.076	749.323
5	4 : 5	2.220	524.326
6	5 : 5	3.228	366.458

stiochiometric ratios, FH and G (2 : 5) gave the best tensile strength (5.73 MPa) and 3 : 5 (FH:G) gave the best percentage elongation at break (749.32%) for the FH-G composite. Whereas, FH-G-PHEMA exhibited far better mechanical properties than FH-PHEMA. The FH-G composite prepared with various stiochiometric ratios of FH and G have also shown better mechanical properties compared to FH-PHEMA. From these results, it is inferred that keratin alone could not form better graft copolymer in terms of mechanical properties, whereas gelatin and keratin mixture has given better mechanical properties. It is evident that in the film of FH-G composite, the gelatin has served as a reinforcement material to give strength to the end product, such as keratin and gelatin. Gluteraldehyde is used as a cross-linker in the study to impart storage stability to the biological materials.

Thermogravimetric Analysis

Figure 2 shows the thermal decomposition profile of the FH-G-PHEMA graft copolymer. In thermogravimetric analysis, the loss of weight due to evolution of water, carbon monoxide and evaporation of other pyrolysis products are collectively measured as percentage of the original weight. In this investigation, FH-G-PHEMA was heated at a steadily increasing temperature from 30–600°C in an atmosphere of nitrogen (80 mL/min). The initial weight loss of 9.5% observed below 145°C was due to the water evaporation. The second weight loss, occuring between 259 and 317°C, was due to the decomposition of protein and about 40.7% of protein (FH-G) loss was observed. The third weight loss between 390–435°C was attributed to the graft copolymer moiety. The weight loss of

Table 4
Tensile strength of graft copolymers

S. no.	Sample	Tensile strength (MPa)	Elongation
1	FH-PHEMA	1.384	12.709
2	FH-G-PHEMA	6.273	190.833

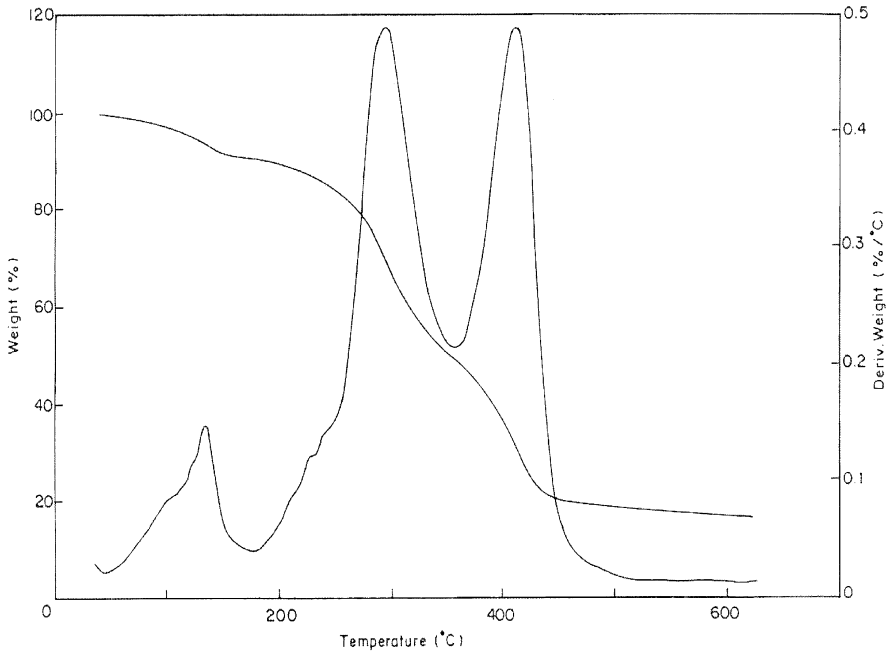

Figure 2. TGA curve for FH-G-PHEMA.

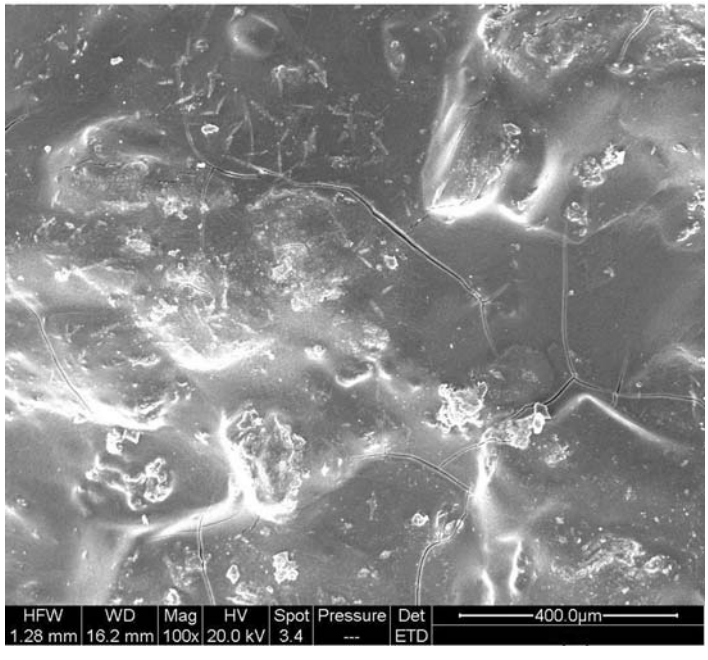

Figure 3. SEM of FH-G.

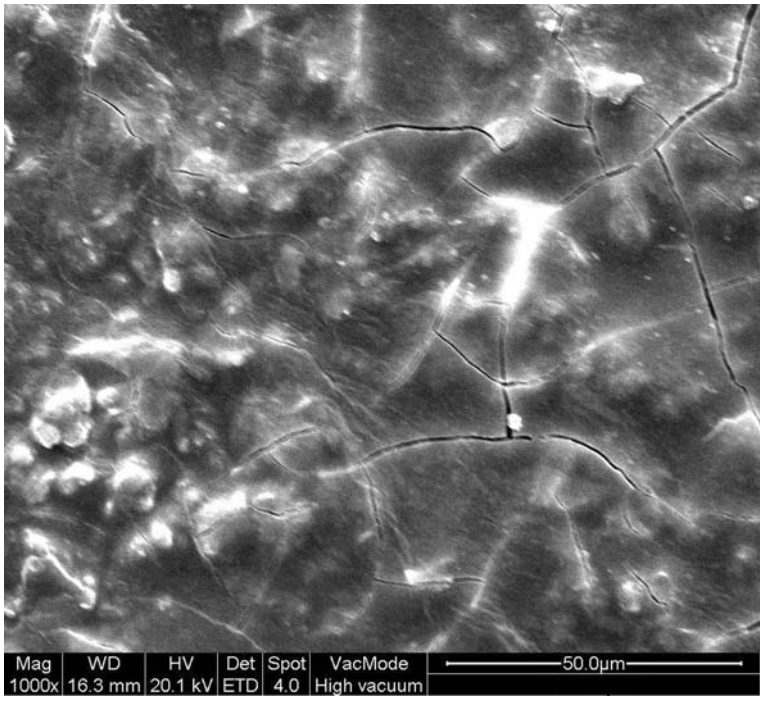

Figure 4. EM of FH-PHEMA.

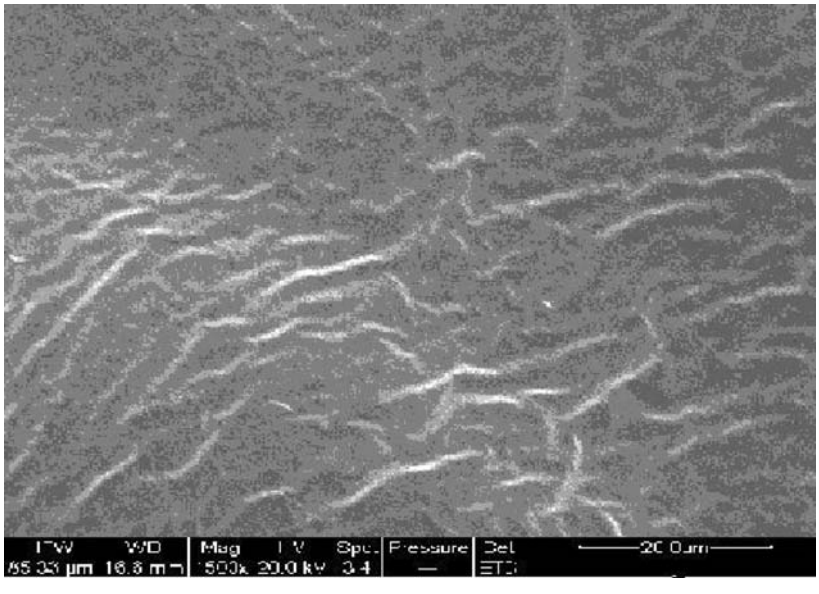

Figure 5. SEM of FH-G-PFHEMA.

polymer observed was 32.85%. The TGA data clearly indicates the presence of PHEMA in the graft copolymer.

Scanning Electron Microscope

The SEM pictures of FH-G, FH-PHEMA and FH-G-PHEMA are shown in Figures 3–5. The SEM of the FH-G and FH-PHEMA shows the brittleness of the film, as cracks are also clearly visible in the surface of the films, whereas no cracks were observed in FH-G-PHEMA. Continuous coating of P-HEMA on FH-G was seen and the surface was smooth without exhibiting any cracks. This confirmed the grafting of P-HEMA onto FH-G.

Conclusions

This study highlighted the grafting of P-HEMA onto FH and FH-G. The optimum stoichiometric ratios of FH and G that gave maximum mechanical properties were standardized. The results showed FH-G-PHEMA exhibited better mechanical properties and water absorption properties, when compared to FH-G and FH-PHEMA. The SEM and IR studies revealed the grafting of P-HEMA onto FH and FH-G. FH-G-PHEMA is being evaluated as a wound dressing materials with rats as animal models.

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