

Studies on Chemically Modified Hen Egg White and Gelatin Composites

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ABSTRACT: Hen egg white (A) and gelatin (G) composites at different proportions were prepared and optimized by their stress–strain behavior. The optimized composite (AG) was graft copolymerised with methyl methacrylate (MMA). The AG and its graft copolymer AGMMA were characterized for their mechanical strength, water absorption capacity, Fourier transform infrared spectra, and circular dichroism (CD) spectroscopy. It was evident from the results that AGMMA possesses higher mechanical strength and lower water absorption capacity than does AG. The FTIR spectra of AG and AGMMA confirm the grafting phenomena of MMA onto AG, and the CD spectrum of AG

shows two negative bands at around 207 and 222 nm and a positive band at around 191.76 nm, which confirms the α -helix in the composite. The α -helical structure in AG was broken and converted into unordered random coil in AGMMA because of the reaction conditions while grafting MMA onto AG, which was confirmed by the increase in mechanical strength of AGMMA. © 2006 Wiley Periodicals, Inc. *J Appl Polym Sci* 100: 318–322, 2006

Key words: hen egg white; gelatin; methyl methacrylate; composites; biopolymers

INTRODUCTION

Hen egg white (A) consists of water (85%), protein (11.5%), carbohydrates (0.6%), and ash (0.5%). The protein of (A) constitutes mainly albumin, with 6% globulin and appreciable amount of sulfur.¹ The egg albumin contains 20 amino acids, including essential amino acids. Eventhough A is mainly used for edible purposes, we have explored it as a binder in combination with gelatin in this study. Gelatin is a degraded form of collagen, which is a connective tissue protein present in most of the vertebrates. The gelatin has an unordered random coil structure, because of the temperature conditions employed in its preparation from collagenase materials.^{1–3}

Gelatin was also used as a protein binder for leather finish, by grafting it with phosphorylated gelatin–acrylate,² acrylonitrile,³ poly(ethyl acrylate),⁴ ethyl acrylate and methyl methacrylate (MMA),⁵ acrylonitrile in zinc chloride,⁶ MMA,⁷ butyl acrylate, acrylic acid, and wax.⁸ Huang et al.⁹ studied the influence of gelatin graft copolymers on photographic properties of the film. Gelatin¹⁰ was also chemically modified by crosslinking with trimethylphenol, which represents the smallest molecule of phenol formaldehyde resins. Sastry et al.,¹¹ developed a leather filler by grafting

vinyl polymers onto hair and feather keratin, and Padma bai et al.,¹² studied the interaction of 7-dehydrocholesterol, with serum albumin as the leather finish.

As the film made of A exhibits very poor mechanical strength, it has to be reinforced with a compatible protein to get the required properties to the binder. In the present investigation, we have chosen gelatin for this purpose. Neither natural polymers nor synthetic polymers can offer all the essential properties for a binder. A judicious combination for the natural and synthetic polymers offers better physico chemical properties to the end products. Hence in this study, poly(methylmethacrylate) (PMMA) was grafted onto albumin and gelatin (AG) to get the required mechanical properties with protein binders.

EXPERIMENTAL

Materials

Fresh hen egg white was separated from chicken egg and used as such. Gelatin from MBD Gelatins (Mumbai, India) was used. MMA was obtained from Fluka, Switzerland. All other reagents used were of analytical grade.

Methods

Preparation of albumin film

Twenty five milliliters of isolated hen egg white was poured into a polythene tray of size 100 × 50 mm² and

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spread uniformly and dried at 40°C in a hot air oven. The film obtained was denoted as A and stored in a polythene cover.

Preparation of gelatin film

Ten grams of gelatin was dissolved in 100 mL of distilled water at 55°C, in a water bath. The solution was poured into a polythene tray of the dimension denoted earlier, and spread uniformly and dried at 40°C in a hot air oven. The gelatin film obtained was denoted as G and stored in a polythene cover.

Preparation of AG film

Known amount of hen egg white and gelatin solutions were mixed (Table I) thoroughly and poured into a polythene tray and dried as mentioned earlier. The film so formed is denoted as AG.

Graft co polymerization of hen egg white—gelatin with MMA

Fifty milliliters of gelatin solution (10%) was added to 25 mL of egg white and made up to 100 mL with water. To this solution, 0.25 g of $K_2S_2O_8$, 0.25 g of $Na_2S_2O_8$, and 4 mL MMA were added. The mixture was stirred for 45 min at 50°C. Then, 1 mL of ethylene glycol was added and stirred for another 5 min. Finally, the contents were poured into a polythene tray and dried at 40°C to form a film. This film was extracted with acetone for 72 h, to remove PMMA homopolymer. The obtained film is denoted as AGMMA.

Characterization

The products prepared, i.e., A, G, AG, AMMA, and AGMMA, were characterized for their Fourier transform infrared (FTIR), tensile strength, water absorption, and circular dichroism (CD) spectroscopy.

Water absorption capacity

Water absorption capacities of different compositions of A, G, AG, and AGMMA were determined, according to the method followed by Sastry and Rao¹³ A small piece of each sample of known weight, dried to a constant weight at 100°C, was allowed to swell in distilled water at room temperature (22°C). The swollen weight of the samples was determined by first blotting the samples with filter paper, and then by accurately weighing the sample. The weight of the swollen samples was recorded at every 1, 2, 3, and 24 h. The percentage of swelling of the samples at a given time was calculated using the formula:

$$E_s = ((W_s - W_0) / W_0) \times 100$$

where W_s is the weight of the sample (moist) at a given time, W_0 is the initial weight of the sample, and E_s is the percentage of swelling at a given time.

Tensile strength

Two dumbbell-shaped specimens of 4 mm wide and 10 mm length were punched out of the prepared films using a die. Mechanical properties, such as tensile strength (MPa) and percentage of strain at break (%), were measured by using a Universal Testing Machine (INSTRON-Model 1405) according to Vogel,¹⁴ at an extension rate of 5 mm min⁻¹.

Infrared spectroscopy

To provide proof of grafting of MMA onto AG composite, IR spectra of AG composite and graft copolymer of AGMMA composite were taken using a Nicolet Impact 400 FTIR spectroscopy, using a 500 mg KBr pellet, containing 2–6 mg of the sample.

CD spectroscopy

The CD measurements were recorded on a Jasco J-715 spectropolarimeter (Japan). The instrument was calibrated using ammonium-*d*₁₀-camphor sulfonic acid, as described by the instrument's 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 recordings. The resulting spectra were baseline-corrected and smoothed. The samples A, G, AG, and AGMMA were obtained by dissolving 0.5 mg of the sample in 1 mL of distilled water. All the spectra were collected from 190 to 250 nm, the range that is suitable to study the secondary structure of most of the proteins and peptides.

RESULTS AND DISCUSSION

Tensile strength

The mechanical properties of the composites with varying amounts of individual constituents are given in Table I. Albumin exhibits very poor tensile strength whereas gelatin shows better strength. With increase in the amount of gelatin in the AG composite, the tensile strength also increases. Similarly, with the increase in the concentration of albumin, the tensile strength of the composite decreases. Similar phenomena were observed in the case of elongation of the composite. Egg albumin has α -helical structure with simple polypeptide, and hence exhibits very poor tensile strength. Gelatin is extracted from collagen, which

TABLE I
Mechanical Properties of AG Composites Prepared Using Different Amounts of A and G

S. no.	A (mL)	G (mL)	Tensile strength (N/mm ²)	Elongation at break (%)
1.	10	0	0.014	23.88
2.	0	10	80.22	40.10
3.	10	10	1.010	79.14
4.	10	20	9.936	163.19
5.	10	30	12.510	204.82
6.	10	40	28.448	203.17
7.	20	10	1.080	79.0
8.	30	10	0.626	79.06
9.	40	10	0.496	79.03

is triple helix in nature. The degraded form of collagen (gelatin) exhibits better tensile strength, as it may contain peptides of double helix in nature. This may be the reason for the increase in the mechanical strength of AG, with increase in the amount of gelatin in the composite. We have selected the composition of A and G in the ratio 1:4, which results in better tensile strength and elongation for AG. The graft copolymerization of MMA was carried out on AG with the aforementioned ratio.

The tensile strength of the graft copolymers at varying pH was studied (Table II). The graft copolymer exhibits maximum tensile strength at neutral pH, whereas decreased strengths were observed in both acidic and alkaline media. This is due to acidic and alkaline hydrolysis of proteins at the respective pH ranges. Because of this protein hydrolysis, the peptide bonds (backbone) are broken, and hence the decrease in their strength. The mechanical strength of AG composite, using different monomer concentrations, were also studied (Table III). The AG composite, grafted with 2 mL monomer (MMA), exhibited better tensile strength. In fact, the tensile strength increases initially up to 2 mL monomer concentration, and later decreases. The initial increase is due to the increased graft copolymerization onto the available sites on the backbone. The decrease in tensile strength above 2 mL concentration may be explained as due to the functional groups of the backbone that could have been exhausted, and hence homo polymerization of MMA might have taken place. Hence, a decrease in the ten-

TABLE II
Tensile Strength of AGMMA at Different pH

pH	Tensile strength (N/mm ²)	Elongation at break (%)
2	23.92	182.99
4	26.32	198.40
7	29.23	224.65
9	21.56	204.24
10	19.29	199.28

TABLE III
Mechanical Properties of AGMMA Prepared by Varying Amounts of Monomer (MMA)

S. no.	Monomer (mL)	Tensile strength (MPa)	Elongation at break (%)
1	1.0	0.5	147.4
2	1.5	0.97	85.7
3	2.0	2.68	79.9
4	2.5	1.31	58.6
5	3.0	0.88	57.8

sile strength is observed. The comparative tensile strengths of A, G, AG, and AGMMA are shown in Figure 1.

Water absorption capacity

The water absorption capacities of A, G, AG, and AGMMA are shown in Table IV. Gelatin shows high percentage of water absorption, when compared to other composites. This indicates that gelatin is having more hydrophilic functional groups, when compared to albumin. The reduced percentage of water absorption in AG composite is due to the presence of A. The PMMA chains on the backbone of AG have further reduced the % of WA of AGMMA. This is due to the hydrophobic nature of PMMA.

Infrared spectroscopy

The FTIR spectrum of AG composite (Fig. 2) shows protein characteristic peaks at 1641 cm⁻¹ (Amide-I), 1566 cm⁻¹ (Amide-II), and 1241 cm⁻¹ (Amide-III). In AGMMA, IR spectrum apart from the amide absorption bands, the carbonyl (C=O) peak at 1740 cm⁻¹ was also observed. This carbonyl peak represents the ester group in MMA and confirms the graft polymer-

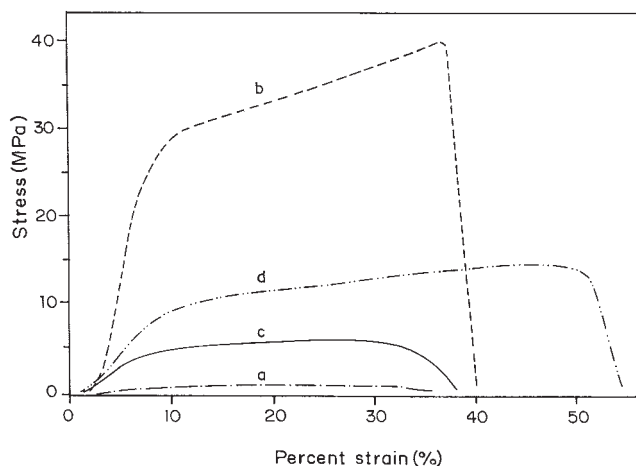


Figure 1 Tensile strength of (a) A, (b) G, (c) AG, and (d) AGMMA.

TABLE IV
Water sorption Capacities of A, G, AG, and AGMMA

Sample	Percentage swelling time			
	1 h	2 h	3 h	24 h
A	8	13	54	62
G	252	310	460	594
AG	110	190	350	359
AGMMA	60	80	96	129

ization of MMA onto AG. The peak at 3300 cm^{-1} (Amide-I) confirms the hydrogen bonded —NH stretching, and the peak at 620 cm^{-1} (Amide-V), which arises from CO—NH group, supports the peptide backbone in AG. The peak at 1650 cm^{-1} [$\nu(0)$] and 1653 cm^{-1} [$\nu(\theta)$], corresponds to Amide-I, which confirms the α -helix structure in AG. Also, the peak at 1548 cm^{-1} , which corresponds to Amide-II [$\nu(0)$], supports the existence of the α -helix structure in AG. In AGMMA (Fig. 3), the peak at 1657 cm^{-1} (Amide-I) confirms the unordered random coil in the composite.

Circular dichroism

The far-UV spectral studies [185–250 nm] were conducted on the composites formed by A, G, AG, and AGMMA, for their conformational analysis. Linear dichroic effects of the composite samples were obtained by rotating the film-coated slide to 90° . The CD spectrum of composites A, G, AG, and AGMMA are shown in Figure 4. The CD spectra of A shows features characteristic of α -helical turn conformation, with a negative maxima near 207.4 nm (Amide $\pi\text{—}\pi^*$ transition) [molar ellipticity (θ) = $-13,272\text{ deg cm}^2\text{ dmol}^{-1}$]

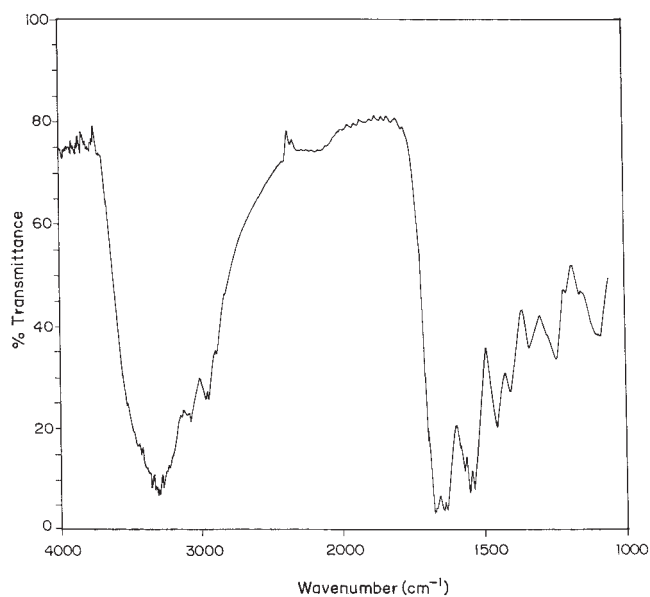


Figure 2 FTIR spectrum of AG.

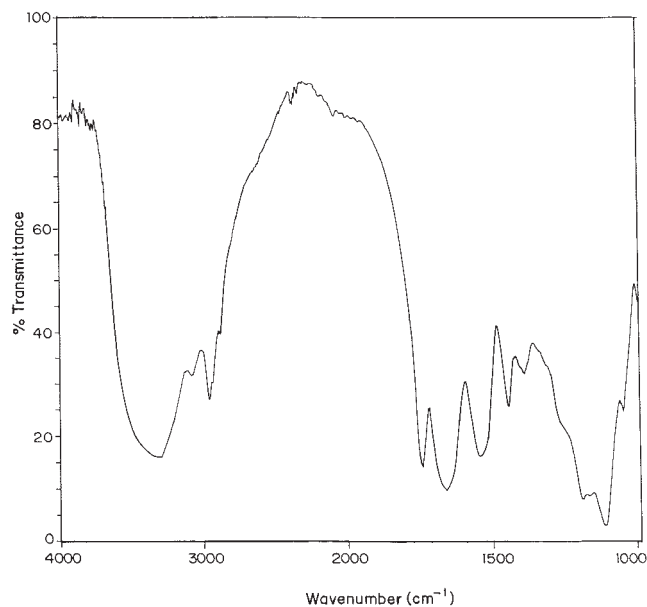


Figure 3 FTIR spectrum of AGMMA.

and 222 nm (amide $n\text{—}\pi^*$ transition) [molar ellipticity (θ) = $-16,123\text{ deg cm}^2\text{ dmol}^{-1}$] respectively, and with positive peak at 191 nm .¹⁵ The CD spectrum of G shows features characteristic of denatured protein, having unordered random coil conformation, with a negative CD band near 200 nm [$(\theta) = -29,939\text{ deg cm}^2\text{ dmol}^{-1}$].¹⁶ But, the CD spectra of the AG confirms the α -helical turn, with reduced intensity in the positive band, near 191 nm compared to A. It has two negative bands at around 207.8 nm [$(\theta) = -7719\text{ deg cm}^2\text{ dmol}^{-1}$] and 222.4 nm [$(\theta) = -7256\text{ deg cm}^2\text{ dmol}^{-1}$].

But the CD spectra of the copolymer composite AGMMA shows an unordered random coil structure, with negative peak at around 199 nm [$(\theta) = -29,939$

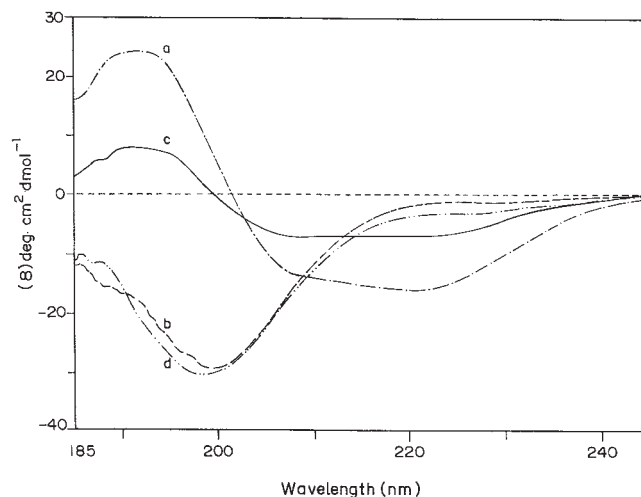


Figure 4 CD spectra of (a) A, (b) AG, (c) G, and (d) AGMMA.

deg cm² dmol⁻¹]. This is due to the broken α -helical structure in AG, which is converted into random coil because of polymerization of MMA onto AG.

CONCLUSIONS

The AG composite prepared and graft copolymerized with MMA (AGMMA) has shown better tensile strength compared to AG alone. The FTIR spectrum of AGMMA confirms the grafting of MMA onto AG. The CD spectrum of AG shows α -helix structure conformation, while AGMMA shows unordered random coil due to grafting of MMA onto AG. The grafting phenomenon was also confirmed by an increase in tensile strength of the AG composite.

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