

Wheat Gluten Blends with Maleic Anhydride-Functionalized Polyacrylate Cross-Linkers for Improved Properties

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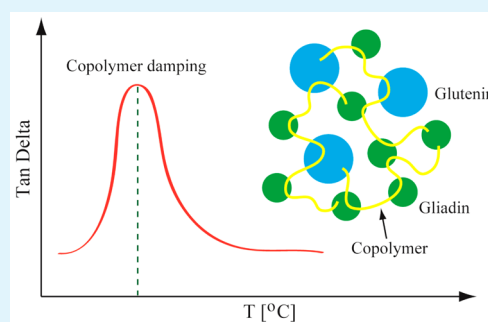
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S Supporting Information

ABSTRACT: A family of polyacrylate-based cross-linkers was synthesized to maximize the toughness of high T_g , high modulus wheat gluten blends in the glassy state. Mechanical testing and damping measurements were conducted to provide an example where the work of fracture and strength of the blend substantially exceeds polystyrene while maintaining flexure stiffness in excess of 3 GPa. The new rubbery cross-linkers, polymethyl acrylate-*co*-maleic anhydride and polyethyl acrylate-*co*-maleic anhydride, improve WG mechanical properties and reduce water absorption simultaneously. MDSC, FTIR, HPLC, and NMR data confirmed the cross-linking reaction with wheat gluten. Flexural, DMA, and water absorption testing were carried out to characterize the property improvements. DMA was conducted to investigate the relationship between energy damping and mechanical property improvement. If the cross-linker damping temperature is close to the testing temperature, the entire sample exhibits high damping, toughness, and strength.

KEYWORDS: wheat gluten, acrylate copolymer, cross-linking, damping, toughness, water absorption



INTRODUCTION

Wheat gluten (WG) is the protein extracted from wheat flour as the byproduct of starch and gluten-free food production.^{1,2} As demand for gluten-free food and starch has increased, more wheat gluten has been produced as byproduct. Currently, WG is often processed into low-value products or discarded as waste.^{1,3} On the other hand, as pollution caused by petroleum-based plastics soars, biodegradable plastics are highly expected to substitute some of the nondegradable plastics to slow the global pollution rate. WG has many advantages over other biomaterials, such as high molecular weight, high stiffness, low cost, and complete biodegradability; thus, converting WG into bioplastic is a goal pursued by several investigators.^{3–12} Molecular analysis shows that WG is mainly composed of two fractions, glutenin and gliadin, and both have large molecular weight. Gliadin's molecular weight ranges from 28 to 55 kDa, and glutenin's molecular weight ranges from 100 kDa to 10 MDa.¹³ After compression molding raw wheat gluten into a plastic, it has good mechanical properties with strength of 45 MPa and modulus of 4 GPa.^{10,14}

Nevertheless, WG's wide application is limited by two major problems, brittleness and high water absorption. Brittleness causes WG to fail at strains as low as 1%, giving very low toughness. Water absorption as high as 110 wt % can soften WG plastics and lead to premature biodegradation. Generally, two pathways have been followed to modify WG and address

the brittleness and water absorption problems to produce both glassy and rubbery materials, plasticizing and cross-linking by small molecules. Plasticizing produces rubbery WG with greatly increased ductility, but at the expense of modulus and strength, and may worsen water absorption.^{15,16} Cross-linking rubbery WG by small cross-linkers like aldehyde¹⁷ or isocyanate¹⁸ can increase modulus, but lowers failure strain that makes rubbery WG more brittle. Small molecular weight additives designed to either cross-link or reduce the thiols at the cysteine residues were also used with WG in the glassy state and simultaneously increased strength and strain to failure while maintaining high modulus.^{3,6,7,9} None of these cross-linkers provided a significant reduction of water absorption in a 48 h time period.

In an attempt to increase ductility while preserving modulus, macromolecular cross-linkers were developed to modify WG. Thiolated poly(vinyl alcohol) (TPVA) was used to first reduce WG disulfide bonds and then generate a cross-linked network structure by forming new disulfide linkages.¹¹ WG/TPVA blends displayed double the strength and ductility, with slightly greater modulus as compared to the base WG material. However, the water absorption most likely increased due to low cross-linking density, microphase separated morphology, and

Received: August 3, 2015

Accepted: September 22, 2015

Published: September 22, 2015

partial opening of the globular structure of WG. More recently, an alternating copolymer of ethylene and maleic anhydride, PEMA, was used in an effort to form an intermolecular interpenetrating network structure without opening the globular structure of WG protein and to increase the cross-linking density.¹⁴ The experimental results indicated that PEMA successfully increased mechanical properties just like TPVA and greatly reduced the water absorption simultaneously. Nevertheless, the best strain to failure was only 1.9%, which is still too low as compared to comparable plastics such as polystyrene and epoxies. The brittle failure mechanism of the WG/PEMA blends was attributed to the high glass transition temperature of PEMA (140 °C) and the formation of homogeneous cross-linked blends with very high T_g near 200 °C.

Toughening brittle polymers by adding rubber particles has been accomplished with several polymers such as epoxy,^{19,20} polystyrene,²¹ and others.²² Most recently, this idea was used to toughen brittle hydrogels.^{23–25} Rubber toughening natural protein polymers has been studied only recently.^{26,27} However, the addition of rubber particles appears most effective for the cases of highly cross-linked thermosets unless surface modification is used to increase surface adhesion. Therefore, in the current study, the rubber toughening and reactive blending ideas are combined in a hybrid approach by using rubbery macromolecular cross-linkers to toughen WG. The closest work on rubber toughened protein polymer obtained an increase in strain to failure but with a large reduction of strength and modulus.²⁶ The most probable reason for loss of strength was blending under dry conditions with subsequent large-scale phase separation. In this study, toughening is accomplished using new rubbery cross-linkers, polymethyl acrylate-*co*-maleic anhydride (PMA-MA) and polyethyl acrylate-*co*-maleic anhydride (PEA-MA), by cross-linking WG in solution without opening the globular structure of WG. The new WG/PMA-MA and WG/PEA-MA materials can also be regarded as reactive polymer blends. Much greater toughness is illustrated below. Thermal analysis by MDSC indicates a homogeneous blend is formed with high T_g , but DMA illustrates excellent damping properties due to the rubbery cross-linker.

■ EXPERIMENTAL SECTION

Materials. American Vital Wheat gluten was obtained from Arrowhead Mills and vacuum-dried for 12 h before use with a resulting moisture content of approximately 8%. Methyl acrylate, ethyl acrylate, maleic anhydride, AIBN, methanol, and ethanol were purchased from Sigma-Aldrich. Diethyl ether, DMSO, and petroleum ether were purchased from Fisher Scientific.

Polymethyl Acrylate-*co*-maleic Anhydride and Polyethyl Acrylate-*co*-maleic Anhydride Synthesis. PMA-MA and PEA-MA were synthesized through free radical polymerization at monomer ratios of 100:0, 95:5, 90:10, and 80:20. The reaction was performed at 65 °C for 8 h. The synthesized polymer was precipitated in petroleum ether, and the collected polymer was then redissolved in acetone and precipitated in petroleum ether three times. The purified polymers were vacuum-dried at 50 °C overnight and then kept in a vacuum before experiments. Each copolymer was synthesized at least three times.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis. ATR-FTIR spectra were taken using a Nicolet Magna-IR 560 with 32 scans at 4 cm⁻¹. The spectra were analyzed with Omnic software from Thermo Electron Corp.

Nuclear Magnetic Resonance (NMR) Analysis. ¹H NMR analysis was conducted on purified PMA, PMA-MA, PEA, and PEA-

MA samples in CDCl₃ to quantitatively calculate anhydride content by using a Bruker DMX 500 MHz. Experimental data were analyzed with Mnova 8.1.

Wheat Gluten Modification. WG and acrylate copolymers were mixed at an 80:20 mass ratio for all experiments. 1.6 g of WG was dispersed in 45 mL of DMSO at 65 °C and stirred for 1 h. 0.4 g of PMA-MA or PEA-MA was dissolved in 20 mL of DMSO. The PMA-MA, or PEA-MA, solution was added dropwise into the WG dispersion under vigorous stirring. An increasing viscosity upon mixing indicates association and perhaps reactions between PMA-MA or PEA-MA with WG. The mixture was stirred at 65 °C for 4 h to blend homogeneously. 60 mL of methanol was added to the mixture right before precipitation to increase compatibility of DMSO and diethyl ether. Subsequently, the mixture was precipitated in diethyl ether at the ratio of 1:10 (v/v). The white precipitate was collected carefully and washed three times with ethanol to remove residual DMSO to prevent residual DMSO from plasticizing the blends. The powder then was washed three times with petroleum ether to remove ethanol and subsequently dried under vacuum overnight before thermo-molding. Control experiments were performed by preparing samples of WG with the same procedures used to prepare the WG blends. Experimental results verified that the DMSO was removed to a level low enough so no discernible plasticizing effects were detected in any of the measured properties. Because of the slight solubility of pure PEA in diethyl ether, the WG/PEA blend, without MA, was dialyzed against water for 2 days and freeze-dried after blending in DMSO. All of the recipes were repeated at least four times to generate statistically independent samples.

Differential Scanning Calorimetry (DSC). PMA, PMA-MA5%, PMA-MA10%, PMA-MA20%, PEA, PEA-MA5%, PEA-MA10%, and PEA-MA20% were analyzed using DSC (DSC-Q100 from TA Instruments). Ten milligram samples were loaded into nonhermetically sealed aluminum pans. Samples were heated at 10 °C/min from -50 to 220 °C, held at 220 °C for 0.5 min, cooled at 5 °C/min back to -50 °C, held at -50 °C for 5 min, and heated to 220 °C at 5 °C/min. The moisture in the WG was driven off in the first heating. The T_g from the second heating is therefore for well-dried WG and is reported below. WG/PMA, WG/PMA-MA5%, WG/PMA-MA10%, WG/PMA-MA20%, WG/PEA, WG/PEA-MA5%, WG/PEA-MA10%, and WG/PEA-MA20% samples were analyzed using modulated DSC (DSC-Q100 from TA Instruments). Modulation amplitude was 0.5 °C every 60 s. Ten milligram samples were loaded and sealed in aluminum pans. Samples were heated at 10 °C/min from -50 to 220 °C, held at 220 °C for 0.5 min, cooled at 5 °C/min back to -50 °C, held at -50 °C for 5 min, and heated to 220 °C at 5 °C/min. Data from the second heating are reported below. At least three DSC spectra were obtained for each recipe using independent samples. Glass transition temperature (T_g) values were obtained by analyzing the data using the Universal Analysis software from TA Instruments.

Size Exclusion-High Performance Liquid Chromatography (SE-HPLC). Samples of dry powdered WG and WG blends were dispersed in extraction solutions at 1.0 mg/mL and extracted for 1 h at room temperature with 0.05 M sodium phosphate buffer (pH 6.8) containing 2.0% (w/v) sodium dodecyl sulfate (SDS)¹¹ and centrifuged (10 min, 10 000g). Supernatants were filtered (0.45 μm) and loaded (50 μL) on a Phenomenex BioSep-SEC-S4000 (300*7.8 mm) column (Phenomenex, Torrance, CA). The proteins were eluted at room temperature with 50.0% (v/v) acetonitrile containing 0.05% (v/v) trifluoroacetic acid (flow rate, 0.5 mL/min). The detection was performed with a Shimadzu SPD-10A VP UV-vis detector at 210 nm. WG proteins were classified into three groups: (1) unextractable polymeric proteins, (2) glutenin, and (3) gliadins ($\alpha/\beta/\gamma/\omega$ gliadins).⁸ At least three SDS extractions and HPLC runs were conducted for each recipe using independent samples.

Flexural Property Characterization. WG, WG/PMA-MA blends, and WG/PEA-MA blends (650 mg) were compression molded at 150 °C for 10 min at pressure of 1.1×10^8 N/m², corresponding to an applied force of 8.9×10^4 N (20 000 lbf) in a stainless steel mold to form $4 \times 0.5 \times 0.2$ cm³ bars. All samples were subjected to three-point bending tests performed according to the

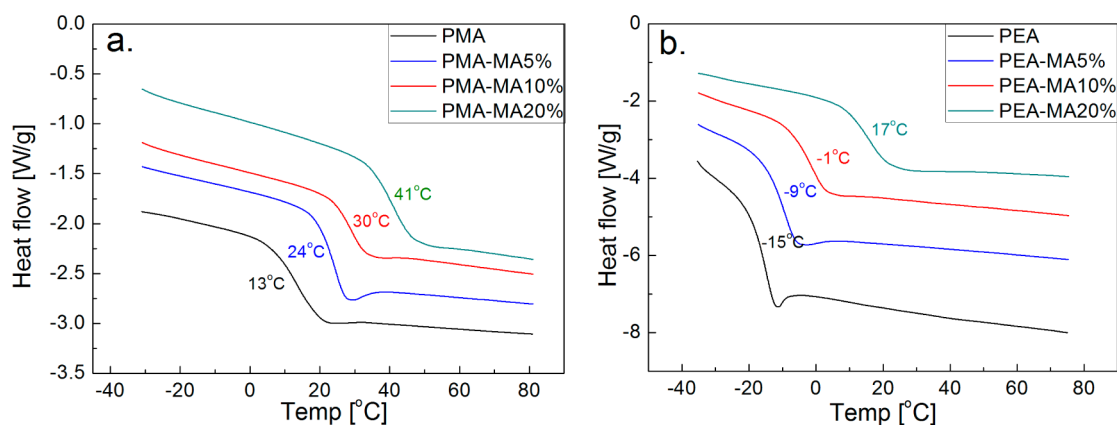


Figure 1. DSC data showing glass transition temperatures: (a) PMA-MA; (b) PEA-MA.

Table 1. PMA-MA and PEA-MA Characterization Data Summary

	M_n	PDI	MA [%]	T_g [°C]		M_n	PDI	MA [%]	T_g [°C]
PMA	73k	1.88	0	13	PEA	90k	1.83	0	-15
PMA-MA5%	31k	2.45	4.1	24	PEA-MA5%	54k	2.31	5.3	-9
PMA-MA10%	25k	2.46	8.4	30	PEA-MA10%	43k	2.32	9.3	-1
PMA-MA20%	28k	2.15	15.2	41	PEA-MA20%	41k	2.29	17.1	17

ASTM D790-02 standard on a computer interfaced Instron-1011 with a 500 N load cell. The rate of crosshead motion was 1 mm/min with the data acquisition rate of 10 points per second. At least four measurements were performed for each recipe.

Dynamic Mechanical Analysis (DMA). WG, WG/PMA-MA, and WG/PEA-MA powder were compression molded into solid bars with dimension of 32 mm × 5 mm × 2 mm. Three independent samples of each recipe were tested with the TA DMA 2980 instrument to investigate damping caused by the copolymer component in the blends. All samples were subjected to three-point bending with a span of 20 mm and temperature sweep from -50 to 110 °C at a heating speed of 2 °C/min, at frequencies of 1 and 50 Hz, respectively. Higher temperatures could not be investigated straightforwardly due to sample instability at high temperature and evaporation of moisture, which led to dimensional changes and resulted in noisy data.

Water Absorption. The original weights of molded WG, WG/PMA-MA, and WG/PEA-MA samples were recorded. Specimens were subsequently immersed in DI water. At certain times, specimens were taken out, surface dried, and weighed. The sample mass reached a steady value within 48 h. Three measurements were performed for each recipe using independent samples.

RESULTS

PMA-MA and PEA-MA Characterization. PMA-MA and PEA-MA were synthesized at molar monomer ratios of 100:0, 95:5, 90:10, and 80:20, respectively. NMR, FTIR, DSC, and GPC were performed to characterize PMA-MA and PEA-MA structures, with experimental results summarized in Figure 1 and Table 1. NMR and FTIR spectra are provided in the Supporting Information. The FTIR provided qualitative evidence of the copolymerization by tracking the anhydride doublet peaks and the acrylate carbonyl peak, and the NMR provided quantitative measurement of the anhydride fraction in the copolymer. DSC results given in Figure 1 and Table 1 show that the glass transition temperature of PMA-MA and PEA-MA increases as maleic anhydride content increases, which is due to chain backbone stiffness induced by maleic anhydride ring structure. The increasing glass transition temperature permits investigation of glass transition temperature effects on toughening.

FTIR Characterization of WG/PMA-MA and WG/PEA-MA Blends. Upon blending, the amine (1.4 mol % lysine), hydroxyl (8.5 mol % serine and threonine), and the very low fraction of free thiol functional groups in WG can react with the anhydride in PMA-MA and PEA-MA.^{14,28} WG has a very heterogeneous and complex structure with many functional groups, making it difficult to characterize the cross-linking reaction. However, the anhydride group in the copolymers shows a unique doublet at 1850 and 1780 cm^{-1} (see Figure S1), which is absent in both WG and the acrylate base polymers (PMA and PEA). This doublet peak can be used to characterize the reaction between WG and PMA-MA or PEA-MA. As shown in Figure 2, before reaction, the anhydride doublets are easily seen, while after reaction the anhydride doublets are greatly reduced in the blend spectra.

MDSC Analysis of WG/PMA-MA and WG/PEA-MA Blends. In Figure 3, without maleic anhydride, WG/PMA and WG/PEA blends display two distinct glass transition

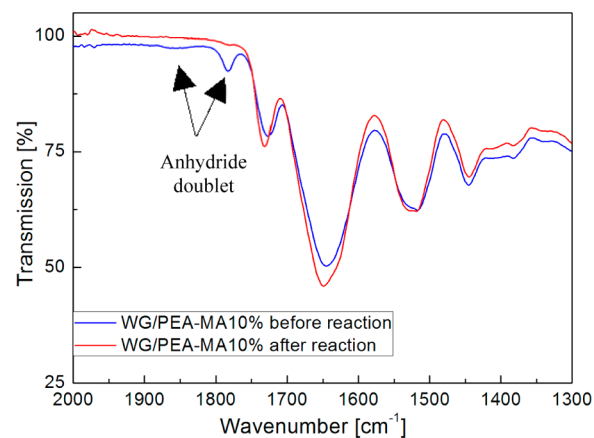


Figure 2. Representative FTIR spectra of WG/PEA-MA10% before and after reaction; other spectra are included in the Supporting Information.

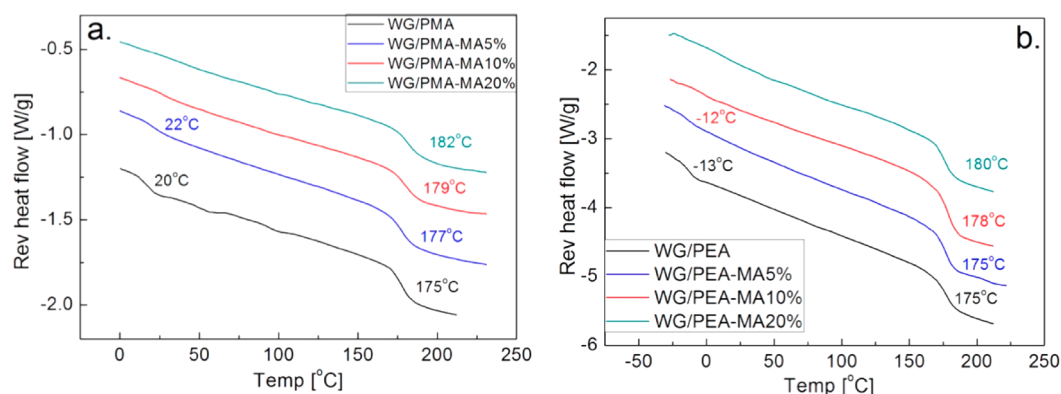


Figure 3. MDSC characterization of blends: (a) WG/PMA-MA; and (b) WG/PEA-MA.

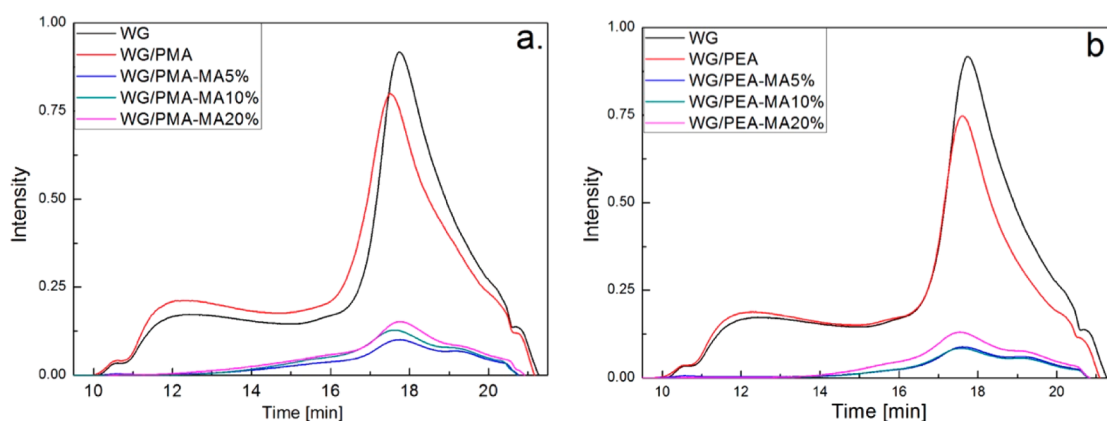


Figure 4. HPLC study of (a) WG/PMA-MA and (b) WG/PEA-MA blends.

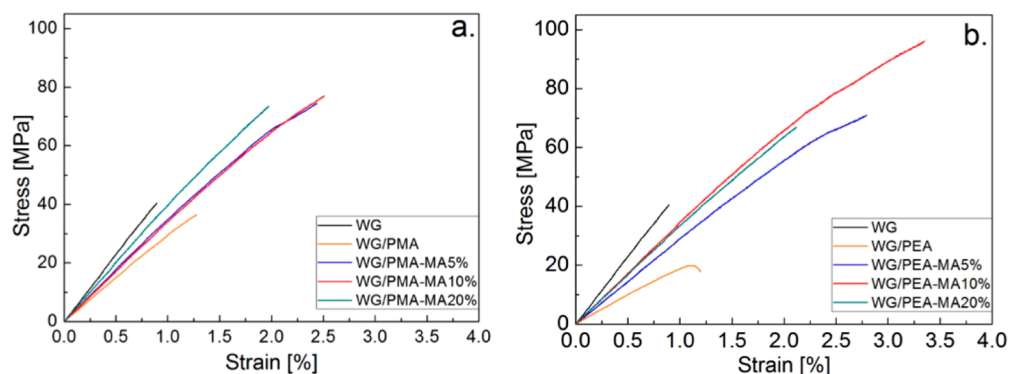


Figure 5. Stress–strain curve of (a) WG/PMA-MA and (b) WG/PEA-MA blends.

temperatures due to phase separation because there is no reaction between WG and the copolymers. In contrast, with addition of maleic anhydride in copolymers, T_g of the copolymer phase starts weakening. As the maleic anhydride content increases to 10%, the copolymer phase T_g disappears for both blends. Simultaneously, T_g of the WG matrix phase increases slightly, which is attributed to increasing cross-linking induced by more maleic anhydride content in copolymers.

SE-HPLC Study. Besides FTIR and MDSC, SE-HPLC was also performed to characterize the cross-linking reaction. As shown in Figure 4, WG extraction greatly decreased with the addition of MA in the copolymers. However, a slight increase in WG extraction is observed as the MA content increases from 5% to 20%, which is opposite to the trend expected if increasing MA content led to increased cross-linking as indicated by

MDSC. This is thought to be due to the copolymer's increasing polarity. As compared to methyl acrylate and ethyl acrylate monomers, maleic anhydride is much more polar. Therefore, PMA-MA and PEA-MA polarity increases as maleic anhydride content increases, which would increase the blend hydrophilicity and thus increase its extraction in SDS buffer. In our previous work,¹⁴ WG and polyethylene-*alt*-maleic anhydride (PEMA) blends were prepared, in which the PEMA loading varied, but PEMA polarity remained constant because PEMA is always 50% MA. Therefore, the decreasing extractability of WG/PEMA blends as PEMA loading increased clearly indicated increasing cross-linking density. In contrast, the increasing polarity of the PMA-MA and PEA-MA with increasing MA content complicated the extraction results and

Table 2. Mechanical Properties Summary of WG/PMA-MA and WG/PEA-MA Blends

	stress [MPa]	strain [%]	modulus [GPa]	toughness [MJ/m ³]
WG	42.7 ± 2.2	0.97 ± 0.09	4.40 ± 0.1	0.21 ± 0.03
PS ²⁹	76	3.3	2.7	1.25
WG/20%PEMA ¹⁴	92.9 ± 3.3	1.89 ± 0.12	5.24 ± 0.16	0.93 ± 0.10
WG/PMA ^a	36, 35	1.28, 1.26	3, 2.9	0.24, 0.23
WG/PMA-MA5%	76.33 ± 2.08	2.49 ± 0.09	3.47 ± 0.05	1.03 ± 0.06
WG/PMA-MA10%	77.4 ± 2.33	2.55 ± 0.12	3.43 ± 0.17	1.07 ± 0.04
WG/PMA-MA20%	70.38 ± 3.9	1.92 ± 0.07	3.90 ± 0.14	0.71 ± 0.07
WG/PEA ^a	19, 23	1.2, 1.4	1.9, 2	0.14, 0.19
WG/PEA-MA5%	71 ± 2.45	2.75 ± 0.14	2.95 ± 0.06	1.06 ± 0.09
WG/PEA-MA10%	89.38 ± 5.8	3.44 ± 0.5	3.20 ± 0.14	1.76 ± 0.41
WG/PEA-MA20%	68.97 ± 5.3	2.28 ± 0.22	3.30 ± 0.1	0.84 ± 0.15

^aNote: Only two samples were measured for WG/PMA and WG/PEA, so both results are shown.

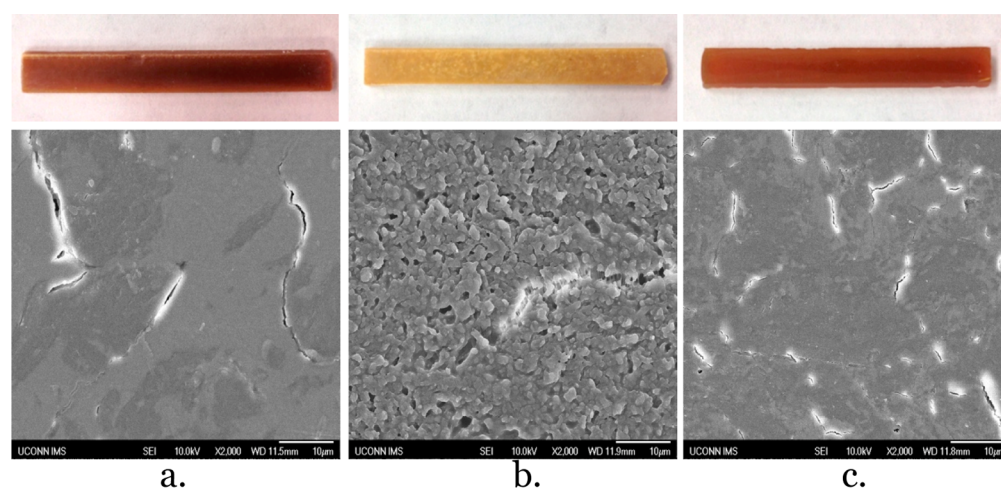


Figure 6. Optical and FE-SEM micrographs (2000 \times) of (a) WG, (b) WG/PMA or WG/PEA, and (c) WG/PMA-MA10% or WG/PEA-MA10%. The blends with 5% MA and 20% MA content appeared as shown in (c). (Note: The small cracks resulted from flexural tests performed on the samples before imaging.)

may be responsible for the slightly reversed trend observed in the extraction results.

Flexural Properties Characterization. The strength and modulus of WG/PMA and WG/PEA are somewhat reduced as compared to WG (see Figure 5 and Table 2), most likely due to the observed large-scale phase separation because there was no cross-linking reaction between WG and PMA or PEA. Figure 6 shows optical and SEM micrographs of WG, WG/PMA (or WG/PEA), and WG/PMA-MA (or WG/PEA-MA). The WG and WG/PMA-MA (or WG/PEA-MA) samples all have a homogeneous appearance. The WG/PMA (or WG/PEA) samples have a yellowish, opaque appearance and granular structure, which indicates incompatibility and large-scale phase separation. For blends with anhydride in copolymers, as shown in Figure 5a and b, the flexural properties are improved significantly, with the highest mechanical properties achieved with blend of WG/PEA-MA10%. Its strength and strain-to-failure are increased by 110% and 255%, respectively, with some decrease in modulus, as compared to WG. The WG/PMA-MA blends show much less sensitivity to the MA content than the WG/PEA-MA blends. This difference is discussed below in terms of the T_g of the copolymers and the viscoelastic damping behavior.

One measure of toughness is the work required to fail a sample, obtained by integration of the flexural stress–strain curve. As shown in Table 2 and Figure 7, most of the PMA-MA

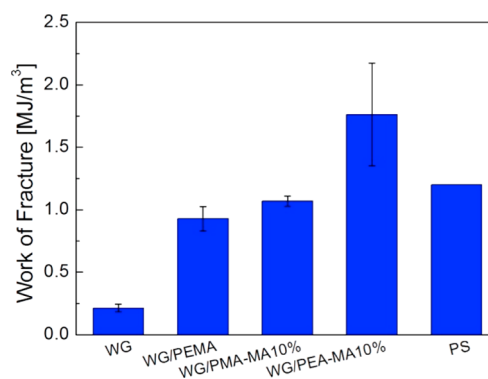


Figure 7. Work of fracture of WG, modified WG, and polystyrene (PS).

and PEA-MA blends with WG have toughness 4–5 times higher than that of pure WG, which is thought to be due to the newly formed network structure. WG/PEA-MA10% has a toughness that is 8.5 times higher than that of pure WG, and this case is discussed further below. Polystyrene data are shown in Table 2 and Figure 7 for comparison.²⁹

Dynamic Mechanical Analysis (DMA) of Blends. The rubbery cross-linkers used above have different glass transition temperatures, leading to different damping temperatures in their blends with WG. DMA testing was performed to detect

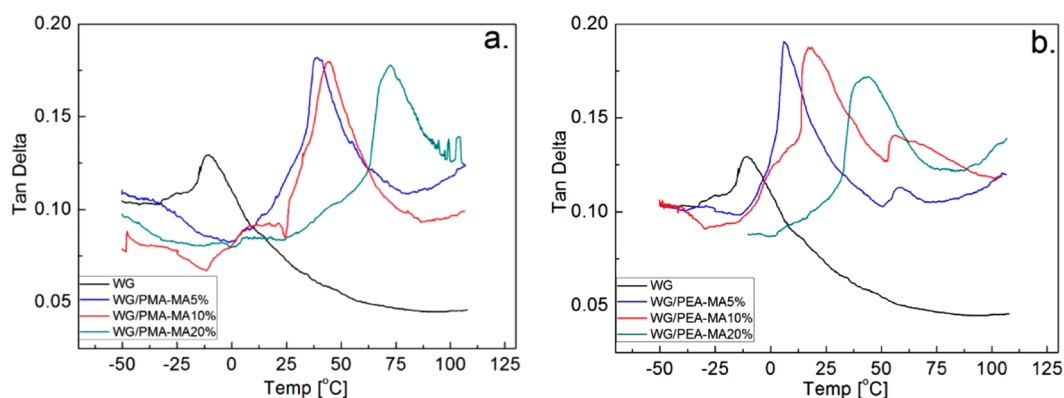


Figure 8. DMA characterization of damping peak at 50 Hz: (a) damping of WG/PMA-MA; and (b) damping of WG/PEA-MA.

Table 3. Damping Temperature of WG/PMA-MA and WG/PEA-MA Blends at 50 Hz

	copolymer T_g [°C]	damping temp [°C]		copolymer T_g [°C]	damping temp [°C]
WG/PMA-MA5%	24	34 ± 4	WG/PEA-MA5%	-9	5.5 ± 0.7
WG/PMA-MA10%	30	43 ± 1.5	WG/PEA-MA10%	-1	25 ± 2.5
WG/PMA-MA20%	41	66 ± 5	WG/PEA-MA20%	17	38 ± 4.8

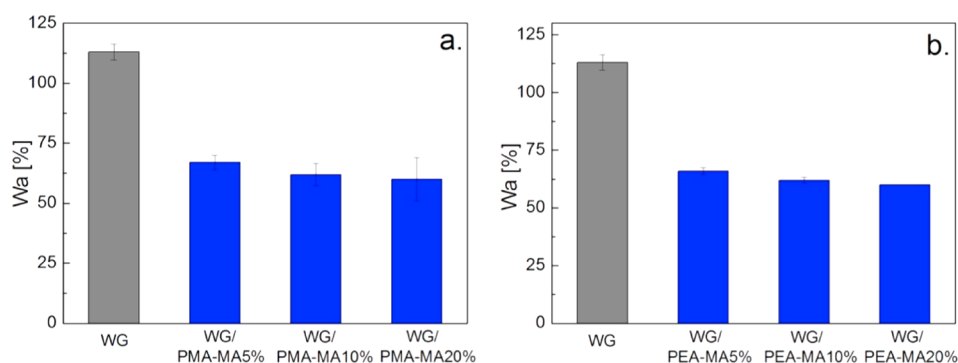


Figure 9. Water absorption of (a) WG/PMA-MA and (b) WG/PEA-MA blends.

the damping peak of each cross-linked blend. The damping peak is taken as the peak in $\tan \delta$, which is the ratio of the loss modulus to the storage modulus as measured by the DMA instrument. As stated in the [Experimental Section](#), the highest DMA testing temperature reached was 110 °C; therefore, the main damping peak of the WG backbone cannot be seen. In [Figure 8](#), below 110 °C, only one weak damping peak is observed at -15 °C for pure WG, which is thought to originate from the amino acid side group vibration.³⁰ For WG/copolymer blends, large damping peaks are observed, and they increase to higher temperatures as the copolymer T_g increases. Comparing [Figure 8a](#) to [b](#), WG/PEA-MA damping temperatures are lower than those of WG/PMA-MA due to the lower glass transition temperatures of the PEA-based materials as compared to the PMA-based materials.

The damping peak is observed at temperatures unrelated to the high T_g values observed for each blend in the MDSC. However, the damping peak is observed to track the T_g values of the rubbery cross-linkers, although at somewhat higher temperatures ([Table 3](#)).

Water Absorption. As shown in [Figure 9a](#) and [b](#), the water absorption decreased for both WG/PMA-MA and WG/PEA-MA blends as compared to WG. The water absorption also decreased slightly with increasing MA content, and increasing cross-linking most likely caused the decreased water absorption.

DISCUSSION

GPC results in [Table 1](#) indicate that the M_n of PEA-MA copolymers are roughly 15k higher than that of PMA-MA. However, no systematic difference in the properties was found between WG/PMA-MA and WG/PEA-MA. The lack of differences in the blend properties indicates that chemical bonding-based interactions are most likely more important than nonbonding interactions such as entanglements.

In the IR spectra shown in [Figure 2](#) and in the [Supporting Information](#), anhydride is completely consumed either by the reaction with WG or by hydrolysis.¹⁴ However, it is reasonable to expect that the majority of the anhydride was consumed by reactions with the protein because it is well-known that the reactions with protein functional groups are much faster than those with water.^{14,28}

Because FTIR cannot unambiguously confirm the cross-linking reaction with WG, MDSC measurements were carried out to characterize the blend glass transition temperatures to provide evidence of the cross-linking reaction with WG. The disappearance of the copolymer phase T_g in [Figure 3](#) illustrates the increasing compatibilization of the copolymer with WG as MA content increases. At 10% and 20% anhydride content, PMA-MA and PEA-MA do not appear in the MDSC as a separate phase because a low temperature transition is not detected. In polymer blends that do not cross-link, compatibi-

lization leads to a single T_g intermediate between the T_g values of the two blend components.^{30–32} Therefore, a blend of WG with the copolymers of much lower T_g would have produced a blend with T_g lower than WG in the absence of cross-linking. The increased T_g values illustrated in Figure 3 are therefore strong evidence that significant cross-linking occurred.

In Figure 3, relative to pure WG, the glass transition temperature of the WG phase increases progressively as anhydride content increases. However, the increase in T_g is not as great as seen previously with WG/PEMA blends.¹⁴ In WG/PEMA blends, PEMA has a maleic anhydride content of 50 mol %. In blends of WG/PMA-MA and WG/PEA-MA, PMA-MA and PEA-MA have maleic anhydride contents of only 5, 10, and 20 mol %. This lower maleic anhydride content might lead one to think the current blends have less cross-linking than the PEMA case and thus smaller increases in glass transition temperature. Moreover, the WG phase T_g region does not broaden as the maleic anhydride content increases in the current work, whereas a notable broadening in the T_g transition occurred in the WG/PEMA blends. This could also be construed as another indication of a lower degree of cross-linking in the current work as compared to the previous work. Before concluding that the current blends have less cross-linking than the previous WG/PEMA blends, it is important to note that there are several contributions to the T_g detected in MDSC, including the T_g of each individual component and the effect of cross-linking. Because of the low T_g of the rubbery cross-linkers used here, compatibilization is expected to lower the blend T_g below that of WG. However, cross-linking is expected to raise T_g , as modeled by, for example, DiBenedetto's equation.³⁰

While a quantitative model of these blends is beyond the scope of this work, the trade-off between cross-linking effects and mixing effects on the T_g can be briefly explored. In the earlier case of blending WG with PEMA, note that the T_g of PEMA was 141 °C and that of hydrolyzed PEMA was 158 °C, so that the T_g of the partially hydrolyzed PEMA in the blend with WG would lie between 141 and 158 °C if cross-linking did not occur. Therefore, in the absence of cross-linking between PEMA and WG, a 20% blend of PEMA and WG would have T_g near 170 °C, a small decrease from the 175 °C T_g of WG. However, in the cases of the PMA-MA and PEA-MA used above, with much lower T_g values, 20% blends with WG in the absence of cross-linking would lead to T_g values of the blends below 150 °C.

Cross-linking reactions are expected to raise the T_g of the blend, and we can use the calculated T_g of the un-cross-linked blend and the measured T_g of the cross-linked blend to estimate the cross-linking efficiencies of the macromolecular cross-linkers. In the case of the WG/20%PEMA blend, the increase in T_g due to cross-linking was roughly 30 °C because the calculated T_g of the un-cross-linked blend is 170 °C and the measured T_g of the actual cross-linked blend is 200 °C. Because the PEMA copolymer is 50 mol % MA, the entire blend was roughly 10 mol % MA, and therefore 10 mol % MA in the blend led to cross-linking that raised the T_g by 30 °C.

A comparison with the current blends illustrates the difference in the efficiency of MA on raising the T_g . The calculated T_g for the WG/PEA-MA10% would be 140 °C if no cross-linking occurred. The WG/PEA-MA10% blend had an observed T_g of 178 °C, which is 38 °C higher than the calculated T_g of the un-cross-linked blend of WG with PEA-MA10%. However, because the PEA-MA10% copolymer is only

10 mol % MA, the blend of WG/PEA-MA10% is only roughly 2 mol % MA. Therefore, 2 mol % MA in this blend led to cross-linking that raised the T_g by 38 °C.

These examples and the results for the other compositions indicate that the rubbery random copolymer blending agents synthesized in this work are much more efficient for raising the T_g than the more expensive PEMA alternating copolymer. These differences are most likely due to the length scale between cross-linking sites on the WG protein. Because only 10% of the WG amino acid residues are reactive with MA, the physical distance between reactive sites on the protein is expected to be much larger than the monomer length scale in the PEMA copolymer. Thus, if a MA group in PEMA reacts with a site on the WG, several nearby MA groups in the PEMA will not be able to react with WG due to steric hindrance. In contrast, the random copolymers synthesized in the current work have a much larger average spacing between MA groups, leading to a higher fraction of the MA groups reacting with the WG. Finally, because the current acrylate/MA copolymers raised the T_g of the WG blends more than the PEMA copolymers did, relative to the calculated T_g values of the un-cross-linked blends, the degree of cross-linking in the current blends may be higher than achieved in the PEMA blends.

In addition to the effects of cross-linking, energy damping at the testing temperature by PMA-MA and PEA-MA may also contribute to increased mechanical properties by toughening the material. Comparing the flexure property results, Table 2, with the damping temperature results, Table 3, provides insight into the high flexure property results found with the WG/PEA-MA10% material. The toughness of a material is related to the capacity of the material to dissipate energy during the crack propagation process leading to failure.³³ High rates of energy dissipation slow and may stop crack propagation. Energy dissipation in polymeric materials is typically a strong function of temperature, with maximum dissipation near the glass transition temperature.^{30,34} In polymer blends and cross-linked systems, the specific relationship between toughness and glass transition is more complex, and the rubbery cross-linkers used above were synthesized to increase the toughness of their blends with WG over and above the previously used glassy PEMA copolymer.¹⁴

As shown in Figure 5a, the WG/PMA-MA blends have very similar mechanical properties regardless of the maleic anhydride content, with WG/PMA-MA20% being slightly stiffer and more brittle. Table 3 indicates that the damping peaks for all of the WG/PMA-MA blends are at higher temperatures than the testing temperature of 23 °C. Therefore, damping did not play a significant role in the behavior of the WG/PMA-MA blends.

In comparison, the damping peaks of WG/PEA-MA blends appear at lower temperatures than the corresponding WG/PMA-MA blends as a result of the lower T_g of PEA-MA copolymers relative to PMA-MA copolymers. For WG/PEA-MA5%, the damping peak appears at a temperature of 5 °C, well below the testing temperature of 23 °C, and thus it would be expected to exhibit poor energy damping during testing. For WG/PEA-MA10%, the peak damping temperature is 25 °C, which is very close to the test temperature. Therefore, this blend is expected to exhibit high energy dissipation during testing. For WG/PEA-MA20%, the damping temperature is well above the test temperature, and therefore the damping efficiency is expected to decrease relative to WG/PEA-MA10%. These results may therefore indicate the possibility of tuning

the blend properties to provide maximum performance at specified application temperatures.

In the current blends, the copolymer phase gives a strong signal in DMA even though the copolymer phase cannot be detected well in MDSC. The disappearance of the copolymer phase in MDSC illustrates the increasing compatibility and distribution of PMA-MA and PEA-MA into WG matrix; thus, they become thermally homogeneous and give one single glass transition temperature. However, because the copolymers are not densely covalently bonded to WG, the flexible copolymer chain movement can still be detected by DMA. The copolymer chain mobility likely contributed to the efficient energy dissipation and toughness increase.

The damping peak is observed at temperatures unrelated to the high T_g values observed for each blend in the MDSC. However, the damping peak is observed to track the T_g values of the rubbery cross-linkers, although at somewhat higher temperatures (Table 3). While there may be several explanations for the differences between the damping temperatures and copolymer T_g values, two typical reasons for such discrepancies are noted. First, due to differences between the physics of MDSC and DMA measurements, damping temperatures observed in DMA are between 5 and 10 °C higher than glass transition temperatures observed in MDSC for homopolymers.³⁰ Second, DMA testing was done at 50 Hz to mimic the flexural testing time scale, which shifts damping temperatures to higher values.³⁰ This is also supported by the DMA testing at 1 Hz (see the Supporting Information) that gives a damping temperature slightly higher than T_g from DSC for the copolymer. For example, for the WG/PEA-MA10%, the damping temperature at 1 Hz was 5 °C, which is 6 °C higher than the T_g measured by DSC of -1 °C for the copolymer PEA-MA10%.

Crack propagation occurs on millisecond time scales, which is slightly faster than the time scale accessed by testing at a frequency of 50 Hz. However, DMA testing the current materials at higher frequencies, for example, 100 Hz, 200 Hz, did not give reliable results due to viscous heating and sample failure. According to WLF theory, higher testing frequency shifts damping temperature upward in an approximately logarithmic relationship.^{30,35} When frequency increased by 50 times from 1 to 50 Hz, damping temperature increased by 20 °C with the current materials. Testing frequencies between 100 and 1000 Hz, which would correspond to time scales in the millisecond range, would only further increase the damping temperatures by 2–7 °C. Therefore, testing at 50 Hz provides sufficient insight into the relationship between the copolymer structure and its effects on the blend mechanical properties.

Water absorption is another important property of the blend where the results are not easily interpreted. As compared to WG, the W_a values of WG/PMA-MA and WG/PEA-MA are significantly decreased. As the maleic anhydride content of the copolymer increased, WG/PMA-MA and WG/PEA-MA blends show very slight additional decreases in W_a . In previous WG/PEMA blends, increasing MA content by increasing PEMA loading continuously and significantly decreased W_a values, which was not observed in the current blends. As indicated by the SE-HPLC results, increasing the MA content in the acrylate copolymers enhances the copolymer hydrophilicity, which is expected to increase water absorption. Therefore, higher cross-linking induced by higher MA content in the acrylate copolymers may have been balanced by concurrent increases in their hydrophilicity, resulting in the very slight differences

observed in the W_a values in Figure 9 as MA increased from 5% to 20%.

The water absorption of the current WG/polyacrylate-MA blends was in general higher than the water absorption of the WG/PEMA blends. For example, the WG/20%PEMA blend absorbed roughly 37 wt % water and the WG/PEA-MA10% blend absorbed roughly 62 wt % water. This difference in W_a values appears opposite to the expected trend based on the discussion of the MDSC and T_g results, where it appears the WG/PEA-MA10% blend achieved a higher degree of cross-linking than the WG/20%PEMA blend. One possible explanation for these differences lies in the much lower T_g values of the polyacrylate-MA copolymers as compared to the PEMA copolymer. Most of the polyacrylate-MA copolymers have T_g values lower than the temperature of the water absorption tests, 23 °C, and so were rubbery while the PEMA T_g is far above 23 °C. This is consistent with the observed swelling, where the WG/PMA-MA and WG/PEA-MA blends swelled more than the WG/20%PEMA blend (see the Supporting Information). This explanation requires further investigation, however, because the PMA-MA10% and PMA-MA20% copolymers have T_g values of 30 and 41 °C, respectively. Perhaps the absorbed water plasticized the current polyacrylate-MA copolymers so they were all rubbery, permitting greater swelling and higher water absorption than the glassy PEMA copolymer.

CONCLUSION

Two macromolecular cross-linkers, PMA-MA and PEA-MA, were synthesized to modify WG. DSC, FTIR, GPC, and NMR were performed to characterize the PMA-MA and PEA-MA structure and properties. Upon blending, PMA-MA and PEA-MA effectively modified WG to improve mechanical properties and reduce water absorption. MDSC, FTIR, SE-HPLC, flexural, and DMA testing were conducted to characterize the blend properties. The WG blend with PEA-MA10% showed the best results, with strength, strain, and toughness increased by 110%, 255%, and 880%, respectively. These new macromolecular cross-linkers provided strong damping in the blends due to their low T_g and lightly cross-linked morphology. The peak damping temperature of the blends increased as the T_g of the cross-linker increased, and when the peak damping temperature was near the test temperature for the flexure tests, the blend displayed maximum toughness, strength, and strain to failure. This new family of cross-linkers can be tailored to provide damping over a range of temperatures ranging from about 5 to 70 °C.

The water absorption of the blend was reduced to about 55 mass % from 115 mass % for virgin WG. While significant, further decreases in water absorption are desirable. The reactions of the maleic anhydride with WG leave polar carboxylic acid residues in the blend, which may have contributed to the absorption of water. The rubbery nature of the cross-linkers and resultant swelling may have limited the effectiveness of the MA at reducing water absorption.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b07136.

NMR and FTIR characterization of PMA, PMA-MA, PEA, and PEA-MA copolymers; FTIR characterization of

WG/PMA-MA and WG/PEA-MA blends; WG/PEA-MA10% DMA testing at 1 Hz; and pictures of WG, WG/PEMA, and WG/PEA-MA blends' water absorption (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge the generous support of the Carpet America Recovery Effort (CARE) program.

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