

Improved Zein Films Using Polyethylenemaleic Anhydride

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ABSTRACT: The effect on the physical properties and solubility of corn protein (zein) films was studied after reaction of zein with polyethylenemaleic anhydride (PEMA). Reactions were carried out in dimethylformamide (DMF) solution where the concentration of PEMA was varied between 0 and 6%. After reaction at room temperature, cast films were prepared, and the physical and solubility properties were determined. Incorporating more than 2% PEMA provided films with increased tensile strength and elongation. Incorporating 6% PEMA provided films with higher tensile strength (32–42 MPa) and improved solvent resistance (100–21% solubility) relative to control. If the films were heated in an oven, the film's solubility decreased further. Solution rheology experiments demonstrated that the zein and PEMA formed a cross-linked gel with time in DMF. Dynamic mechanical analysis experiments have shown that the PEMA-modified zein films undergo a dramatic loss in modulus on reaching 116°C, whereas the control experiences this loss at 98°C. © 2013 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2014**, *131*, 40122.

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INTRODUCTION

Zein is the predominant protein found in the endosperm of corn. It is defined as a prolamine based on its solubility characteristics.¹ Because zein is one of the main components found in the co-products of the bioethanol industry, developing higher value products using it would improve the economics of this industry. Historically, zein was used in the textile and coatings industries.¹ In the textile industry, fabrics containing zein fibers would be exposed to dyeing and finishing processes that required the zein fiber to not undergo morphological changes during these treatments. For example, the fabric may be treated with organic solvents or aqueous detergent systems to remove fabric finishes. Dyeing operations are typically performed at elevated temperatures in aqueous systems in the presence of various surfactants or other agents. In the coatings industries, the zein could be exposed to various solvents and abrasion that could lead to failure. Historically, to be used in these markets, the zein was treated with formaldehyde.^{2–6} This formaldehyde treatment delivered improved tensile strength, improved solvent resistance, and improved resistance to fabric treatments (such as dyeing). Formaldehyde has been defined as a probable human carcinogen by the U.S. Environmental Protection Agency, so

commercial use of formaldehyde requires significant investment to be performed safely. Because of this expense, other chemistries are being explored as an alternative to formaldehyde to improve the properties of zein-based materials.^{5–22} Glutaraldehyde has been used to modify zein providing films and fibers with improved tensile strength (29 to 49 MPa).^{16–18} However, in order for the zein glutaraldehyde samples to be resistant to solvents known to dissolve zein, the samples needed to be heated at elevated temperatures. Glyoxal has been investigated and found to provide films, fibers, and injection molded samples with improved tensile strength (22 to 55 MPa).¹⁹ However, these samples also required heating to become resistant to dissolution by known zein solvents.^{13,14,19–21} Cinnamaldehyde¹⁴ and citric acid²³ were shown to provide samples with improved physical properties (4 to 5 MPa for cinnamaldehyde; and 2 to 4 MPa for citric acid); however, there was no evaluation of solvent resistance. Anhydrides have been demonstrated to react with proteins giving products with improved properties.^{24–35} In some of these studies, the protein of interest was zein.^{30–35} When zein was the protein used, the anhydride was of relatively low molecular weight (octenyl succinic anhydride or smaller) which will have some amount of vapor pressure at atmospheric

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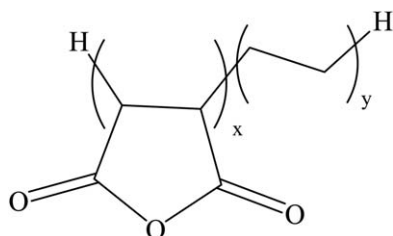


Figure 1. Structure of PEMA.

conditions leading to potential inhalation exposures. In addition, in these studies the solvent resistance of the products produced was not discussed. By selecting a polymeric anhydride, similar to that used by other researchers with other proteins,^{24,27,29} the overall safety of the reaction can be improved.

Polyethylenemaleic anhydride (PEMA, Figure 1) has a molecular weight as received of $\sim 100,000$ kDa and is ~ 50 mol % maleic anhydride (Mfg. specifications). With such a high molecular weight, the primary route of exposure would be because of dust inhalation. This greatly improves the safety of this protein derivatization technique relative to others that use relatively low molecular weight reagents with higher volatility. Given that PEMA and zein are both soluble in dimethylformamide (DMF), a solution study of the reaction of zein with PEMA in DMF was undertaken to determine if the resulting cast films would have improved physical properties and reduced solvent solubility. It was found that by modifying zein with PEMA, both physical properties and solvent resistance were improved.

EXPERIMENTAL

Materials and Equipment

Zein was obtained from Flo Chemical Corp. (Ashburnham, MA Lot #F40006124C, 14.39% Nitrogen, 4.8% moisture). Tris (hydroxymethyl)aminomethane, sodium dodecyl sulfate (SDS), ethylenediaminetetraacetic acid, acetic acid (AcOH), DMF, 4-morpholineethanesulfonic acid, were obtained from Sigma-Aldrich (St. Louis, MO), and PEMA was obtained from Poly-Sciences, Inc. (Warrington, PA). NuPAGE Novex 10% Bis-Tris gels and lithium dodecyl sulfate sample buffer were obtained from Life Technologies (Carlsbad, CA). Protein molecular weight standard was obtained from Lonza (Allendale, NJ). Physical property testing was done using an Instron Universal Testing Machine (Model 4021, Canton, MA). Solution rheology was performed on a controlled strain fluids rheometer (ARES LS1 TA Instruments, New Castle, DE). Dynamic mechanical analyses were carried out on an ARES LS2 controlled strain rheometer (TA Instruments, New Castle, DE).

Preparation of Zein Solutions and Films

Separate solutions were prepared for both the zein and the PEMA. The zein solution was prepared by slowly adding 5.0 g zein to 18.0 g DMF followed by stirring for 30 min at room temperature. The PEMA solution was prepared by adding various amounts of PEMA, to deliver the desired PEMA concentration, to 4.0 g of DMF followed by stirring for 30 min at room

temperature. For example, for 4.0% PEMA, 0.208 g of PEMA was added to 4.0 g DMF followed by stirring. After stirring, the two solutions were combined, and then allowed to stir for an additional 15 min. Solutions were then placed in a vacuum oven to degas at room temperature and cast onto a glass plate covered with Bytac® having a suitable sized rubber gasket in place so that the total area of the film was on the order of 200 cm². After the films had fully dried, they were placed in a 70% relative humidity (RH) chamber set at 23°C for 2 days to facilitate cutting.

Solution Rheology

The control solution was prepared by adding 2.5 g of zein to 10 g of DMF. For the 6% PEMA item, 2.5 g of zein was dissolved in 8.5 g of DMF and a separate solution was prepared by adding 0.156 g of PEMA (6%) to 1.5 g of DMF. Upon dissolution, the two solutions were combined and allowed to mix for 1 min at room temperature before rheology testing. Both the control and 6% PEMA solutions were tested on a controlled strain fluids rheometer fitted with a humidity cover to reduce solution evaporation. Tests were performed with a 50 mm diameter cone-and-plate geometry at a strain of 10% and a frequency of 1 radians/sec. A Peltier plate was used to maintain the temperature at $25 \pm 0.1^\circ\text{C}$. All measurements were run three times, and the median data is reported.

Mechanical Property Determination

Films were cut with a die conforming to the ASTM D-638-V Standard for testing.³⁶ Sample thickness was measured with a micrometer (Model No. 49-63, Testing Machines Inc., Amityville, NY) at five places within the testing region and averaged. Five bars from each film were then stored at 50% RH (23°C) for 3 days to determine the tensile strength (MPa), percent elongation at break (%), and Young's modulus (MPa) with a crosshead speed of 10 mm/min and a gauge length of 7.62 mm.

Dynamic Mechanical Rheology

Dynamic mechanical rheology (DMA) of zein films was carried out on films with dimensions of 0.3–0.4 mm thickness, 5–10 mm width, and 10–15 mm length. Dynamic temperature ramps were run on these films from 25 to 200°C at 1°C/min with a strain of 0.5% and a frequency of 1 rad/sec. A constant normal force of 0 ± 10 g was placed on the sample so that sample expansion or contraction could be monitored.

Gel Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), was performed to monitor changes in the molecular weight of the zein protein.³⁷ The presence of the SDS denatures the protein so that the depth that the protein penetrates the gel is proportional to its molecular volume. By using protein standards, the relative molecular weight can be determined. The zein proteins were dissolved in DMF to insure that the most material possible would go into solution and still provide a quality analysis.³⁸ Films having higher amounts of PEMA, had limited solubility in DMF, and the gel traces are not representative of the entire sample but only the soluble portions. The zein solutions were then diluted to an appropriate concentration with water and loading buffer, loaded onto a NuPAGE Novex

10% Bis-Tris gel, and run at 200 V for 40 min. Gels were stained with Coomassie Blue.

Dissolution of Films

Aluminum pans were heated at 125°C for 2 h before testing to remove adsorbed moisture. As-is film samples (~30 mg, recorded to the nearest 0.001 mg) were placed in weighed aluminum pans and then allowed to soak in 10 mL of AcOH for 2 h. After carefully removing the AcOH with a pipette, the films were rinsed once with fresh AcOH, once with acetone and then dried by placing them in a 125°C oven for 5 h. The amount of film that did not dissolve was determined gravimetrically. Samples were not dried before testing, so that moisture and other AcOH soluble materials (e.g., residual corn oil) present in the sample would be counted as being protein sample that had dissolved. All samples were done in triplicate.

RESULTS AND DISCUSSION

PEMA has essentially zero vapor pressure, making it an attractive reagent for derivatizing zein. Zein has many serines and threonines,³⁹ which may react with the anhydride moieties on PEMA to create esters. The PEMA may also react with the terminal amine end of the protein to form an amide. Given the molecular weight of the PEMA and the mole fraction of the maleic anhydride groups, there are 100s of anhydride moieties per PEMA chain that may react with the nucleophilic sites on zein. These reactions would lead to branching and possibly a cross-linked network. To determine if the PEMA was crosslinking the zein or acting as a chain extending or branching reagent, solution rheology was used. Solution rheology has been used by numerous researchers to study the reaction of polymeric systems which may undergo crosslinking reactions.^{40,41} This is the first rheological study of the reaction of zein with a reagent that may induce cross-linking. To determine if PEMA is acting as a cross-linking reagent, a representative zein-PEMA reaction, using 6% PEMA, was carried out in a controlled strain rheometer. Using this instrument, the elastic (G') and viscous (G'') modulus of the solution was monitored with time at the desired temperature (Figure 2). The G' and G'' of the control sample were both on the order of 0.10 MPa, near the lower limit of sensitivity for the instrument, and remained at this value for the duration of the experiment. With the addition of 6% PEMA, both G' and G'' increased with reaction time. Initially G' was lower than G'' . However, G' increased at a much higher rate than G'' . One definition for the formation of a cross-linked network is when G' and G'' are equal.^{40,41} After

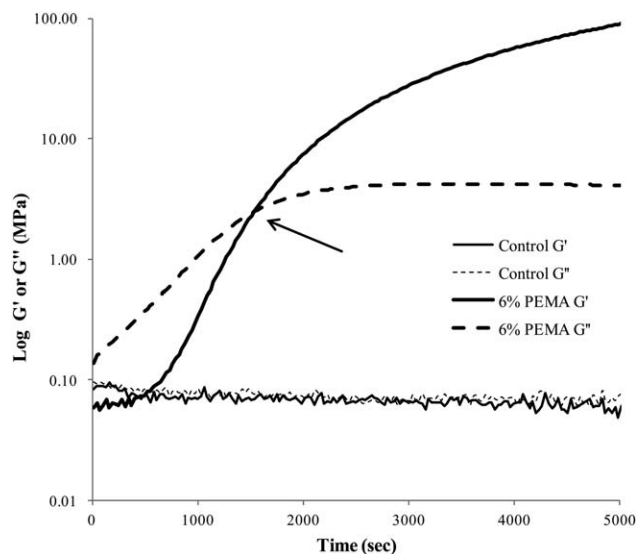


Figure 2. Elastic (G' , solid lines) and viscous (G'' , dashed lines) modulus for control (thin lines) and 6% PEMA (thick lines) DMF solutions at 25°C with time. Arrow signifies gelation point.

carrying out the reaction of zein with 6% PEMA for 1540 sec, G' and G'' equal each other, signifying that a cross-linked network has formed. After longer reaction time, G' is much larger than G'' . The rheology of zein-PEMA solutions is under further study to evaluate the impact of PEMA concentration and temperature.

Solvent sensitivity is one of the main shortcomings of materials made using biobased proteins. With so many anhydride groups present on PEMA leading to cross-linking, the resulting film should have reduced solubility in solvents known to dissolve zein. It has been shown that AcOH is a good solvent for zein^{12,42–44} and reduced solubility in this solvent would be a good indication of increased value. Shown in Table I is the formulations prepared and the amount of each cast and dried film that is soluble in AcOH. In this test, the amount of oil (~6%) and water (~4%) was counted as soluble material. Zein readily absorbs moisture, so attempts to remove moisture before or during film formation were not practical as the films take many days to dry. Heating in an oven has been shown to alter the properties of zein,^{13,18} so removing water by heating was not an option before testing. Therefore, the impact of water contained in the film could not practically be avoided. Commercially, oil is removed from corn before the isolation of zein.¹ Using an

Table I. Amount of Film Soluble in AcOH (Dissolution) and Physical Properties (Tensile Strength, TS MPa, Elongation, Elo % and Young's Modulus YM, MPa as well as Corresponding Standard Deviations) of Zein-PEMA Films

% PEMA	Dissolution	Dissolution st. dev.	TS	TS st. dev.	Elo	Elo st. dev.	YM	YM st. dev.
0%	100	0	32	3	6	1	642	16
1%	88	4	31	2	8	2	506	64
2%	49	1	36	1	14	4	618	28
4%	29	1	42	3	18	1	671	110
6%	21	2	41	3	12	4	590	23

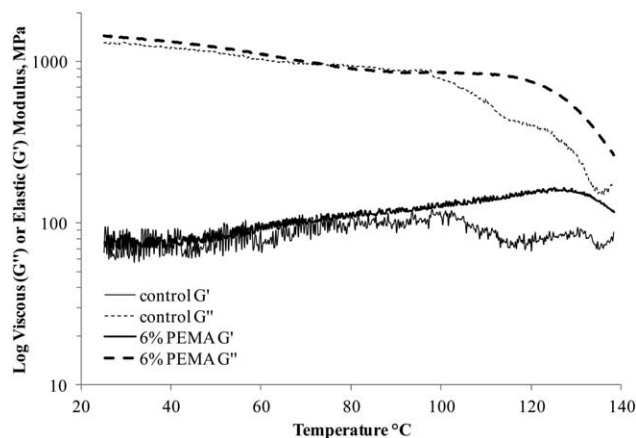


Figure 3. DMA for control (thin lines) and 6% PEMA (thick lines)—elastic (G' , solid lines) and viscous (G'' , dashed lines) modulus of films with increasing temperature.

additional step to remove residual oil would add expense to the process and so this was not used in this testing. The amount of PEMA was varied between 1 and 6%. With higher levels of PEMA, gelation quickly became an issue during sample preparation so these formulations were not studied. As seen in Table I, the amount of material soluble in AcOH was inversely proportional to the amount of PEMA used. Control zein films were completely soluble in AcOH. The amount of AcOH soluble material drops to 21% when 6% PEMA was used. Taking into account that the oil and water were removed by the AcOH, the amount of zein that has been rendered insoluble approaches 90% when 6% PEMA was used. Unlike previous zein derivatization techniques which provide solvent resistance,^{4,12,13} the use of PEMA does not require an additional heating step to provide solvent resistance. Although the heating step was not required for solvent resistance, the addition of a heating step did show improved solvent resistance. After heating the 1% PEMA film for 1 h at 135°C, the amount of material that dissolved on exposure to AcOH was reduced from 88 to 55%. The effect on dissolution by heating the generated film diminished with increasing % PEMA. The film formulation having 2% PEMA had its dissolution decrease from 49 to 40% after heating 1 h at 135°C.

The physical properties of the films also improve with the incorporation of PEMA. As the amount of PEMA was increased above 2%, TS was seen to increase (Table I). With increasing amounts of PEMA, Elo was not negatively affected and when 4% PEMA was used Elo was higher than control. The improved solvent resistance and physical properties observed in these films demonstrate that PEMA modification gave an article with a better balance of properties.

DMA was used to study the impact temperature had on films made with and without PEMA. Shown in Figure 3 is the DMA plot for the film with 6% PEMA and control. As can be seen, the control film displays a T_g at 98°C, whereas the film with 6% PEMA has a T_g at 116°C. An increase in T_g with cross-linking is commonly observed.^{17,45} The zein film produced after chemical modification with PEMA has improved thermal resistance. Further DMA experiments are in progress.



Figure 4. SDS-PAGE gel of zein-PEMA film formulations.

The use of SDS-PAGE has been shown to accurately monitor the molecular weight of zein after derivatization.^{11–13,15,46} Shown in Figure 4 is the SDS-PAGE gel of the zein-PEMA formulations detailed in Table I. As the amount of PEMA was increased, the number of bands visible and the overall amount of protein present (determined by the overall darkness of the bands) decreases. This was a result of the lower solubility of the zein-PEMA film in the DMF used to prepare samples for this test; only the soluble portion of the film was observed in this test. Interestingly, the pattern observed in Figure 4 was different than that observed in other zein modification techniques. When formaldehyde⁴, glyoxal¹³, glutaraldehyde,¹² or 4,4'-diisocyanodiphenylmethane¹⁵ reagents were allowed to react with zein, higher molecular weight protein dimers, trimers, and streaking (indicative of proteins with molecular weights across this region) were observed. This overall shift to higher molecular weight was observed as each molecule of these reagents will bind only two proteins. When PEMA was used as the reactive reagent, dimers, trimers, and other higher molecular weight materials (evidenced by streaking) were not observed. With PEMA, many zein proteins may be bound to a single PEMA molecule. When this occurs dimers and higher homologs are not readily apparent as the molecular weight growth, and resulting insolubility, occurs too rapidly once started. Only unreacted zein can be observed via SDS-PAGE after derivatization with PEMA.

CONCLUSIONS

Zein can be modified using polyethylene maleic anhydride (PEMA) in DMF solution to provide films with improved properties. The reaction of zein with PEMA was shown to produce a cross-linked network based on solution rheology experiments. The films had improved resistance to dissolution by acetic acid with the amount of modified zein film that dissolved on exposure to AcOH going from 100% for control to 21% after treatment with 6% PEMA. Unlike treatments using aldehydic reagents, additional heat treatments were not required. The films also had increased tensile strength (32 to 42 MPa) and

appeared to provide some degree of increased elongation (6 to 18%). Utilizing SDS-PAGE techniques to monitor molecular weight did not show the formation of protein dimers and trimers. With so many reactive sites on PEMA, the branching reactions that occurred immediately rendered the protein insoluble in the solvent used to run the SDS-PAGE test. Therefore, only the unreacted proteins are observed in this test. The improved balance of properties, reduced solubility coupled with the improved safety will make this approach for modifying zein useful in more traditional markets served by products based on petroleum-based feedstocks.

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