

## Zein–Iodine Complex Studied by FTIR Spectroscopy and Dielectric and Dynamic Rheometry in Films and Precipitates

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In the present study the effect of iodine on properties of zein films and zein precipitates obtained after hydrophobic aggregation was evaluated. Zein films were cast with and without glycerol (as plasticizer) after incorporation of iodine at different levels (2–8%, zein wt basis). Zein films were characterized by secondary structure (determined by infrared spectroscopy) and dielectric and mechanical properties. The rheological properties of zein precipitates as a function of frequency and temperature were evaluated using a dynamic rheometer. Inclusion of iodine changed the secondary structure of zein films and decreased their tensile strength as well as strain at failure. In aggregates, changes in  $G'$  (elastic modulus) and  $G''$  (viscous modulus) during heating were affected by the presence of iodine due to the inhibition of aggregation. The water-holding capacity of precipitates precipitated in the presence of iodine was higher than that of those without iodine.

**KEYWORDS:** Zein; film; glycerol; iodine; biodegradable; FTIR; rheology; mechanical strength

### INTRODUCTION

Edible films from plant materials have already been reported to have potential uses in food protection and preservation. These films are used in food packaging and coating of fruits and vegetables and are of great interest due to their environmental benefits (1).

Among the proteins from various sources such as corn, rice bran, wheat, and soyabean, zein has received more attention because of its low price and readily availability. Zein films function well as selective barriers to the transport of gases, water vapor, or solutes (1–4).

Biodegradable films from plant proteins have possible potential uses in the pharmaceutical industry. Indeed, zein proteins forming microspheres or films showed promising drug delivery properties. O'Donnell et al. (5) reported that zein film coated tablets have controlled release characteristics. This was further supported by Wang et al. (6), who demonstrated that zein microspheres could successfully be used to deliver heparin for vascular devices.

The mechanical, water absorption, and barrier properties of zein-based films appear to be dependent on their three-dimensional network structure and on the interaction between proteins, plasticizers, and other functional agents (7). The structural characterization of edible films is carried out by chemical analysis, X-ray, FT-IR, and other techniques (8, 9). It has been reported that the secondary structure of zein in solution was 50–60%  $\alpha$ -helix and 15%  $\beta$ -sheet with the remainder of the molecule being aperiodic (7, 10). Tatham

et al. (11) reported that  $\alpha$ -helix predominates in the central domains of zein. Recently, the work of Momany et al. (12) reported that zein comprises nine helical segments based on three sets of three interacting coiled-coil helices with segments positioned end to end.

Iodine is also known for its antimicrobial properties. Ointments and salves containing iodine are well-known and are often applied as wound dressings. However, these are not satisfactory because the ointment does not form a film and upon drying changes to a cake and subsequently to a crumbly mass. The release of iodine from such ointment is generally slow, and higher concentrations of additional antimicrobial agent are required for efficient protection against pathogenic microorganisms and/or infections. These ointments contain substantial amounts of substances with oily characteristics, which tend to smear and spread over areas surrounding the site of application, leading to reduction in efficiency as well as spoiling and staining of clothing. Systems such as polyvinylpyrrolidone mixed with iodine used as a coating antibacterial polymeric complex was reported by Jones et al. (13). Iodine gives blue color with starch, which has been linked to the formation of an inclusion complex with the amylose coil (14); it seems that similar a complex formation may occur between iodine and  $\alpha$ -zein. In addition, iodine tends to partition from polar to nonpolar environments; thus, the hydrophobic nature of zein may also favor the absorption of iodine. The objective of the present work was to examine the effect of iodine on the properties of zein films and precipitates obtained after hydrophobic aggregation with a view to evaluating the system as a possible antimicrobial wound dressing.

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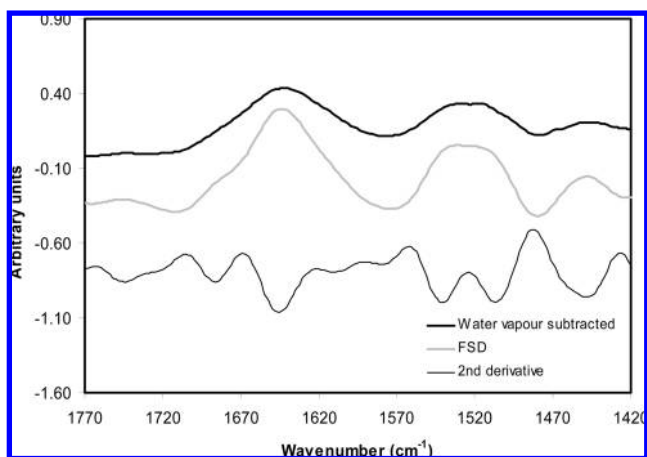
## MATERIALS AND METHODS

**Zein.** Zein (purified) was obtained from Acros Organic (Geel, Belgium). Ash and lipids contents of zein were 1.0 and 1.3%, respectively. Glycerol and iodine were procured from BDH (Pool, U.K.) and Lancaster (U.K.), respectively.

**Film Casting.** Film casting was carried out by dissolving zein (15% w/v) in aqueous ethanol (90% v/v) with and without glycerol (30% on zein wt basis) as plasticizer. Aliquots (3 g) of this filmogenic solution were weighed into 5 cm diameter plastic Petri dishes and gently swirled to coat the bottom of the dish. The Petri dishes were placed without lids on a level surface (checked with a spirit level) in an oven at 40 °C for 24 h. Three replicate films were cast for each film mix. For casting zein-iodine films, iodine (3% w/w) was dissolved in aqueous ethanol (90% v/v). The aqueous ethanol solution was replaced with iodine solution to produce zein films containing different concentrations of iodine (2, 4, 6, and 8% on zein wt basis).

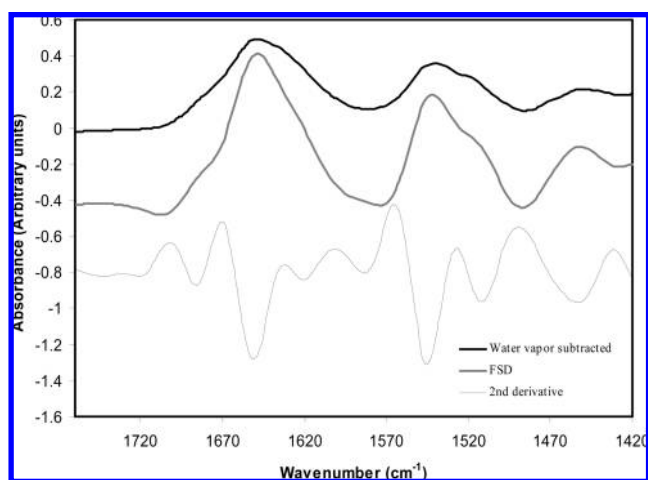
**FTIR Spectroscopy.** Spectra were recorded on a Bio-Rad FTS 165 FTIR (Varian Limited, Yarnton, Oxford, U.K.) spectrometer with a mercury/cadmium/telluride detector as described earlier (15). All films were stored in a desiccator over P<sub>2</sub>O<sub>5</sub> under vacuum for at least 2 weeks until constant weights were obtained before spectra were taken; subsequent storage was always over P<sub>2</sub>O<sub>5</sub>. No evidence of any water signal was found in the IR spectra. Films were taken from the desiccator, placed on a single-reflection diamond attenuated total reflectance (ATR) accessory (SPECAC, Orpington, U.K.), and pressed down to ensure good contact with the ATR crystal. The spectra (200 spectra at 4 cm<sup>-1</sup> resolution for each sample) were averaged. Three replicates of each sample were taken using the empty ATR crystal as a reference. The spectrum for water vapor was measured and subtracted from the sample spectra using Omnic v6.1A software (Thermo Nicolet Corp., Madison, WI) as described by Wellner et al. (16). Each spectrum was baseline corrected following the method of Wellner et al. (16) by ensuring that the spectrum was zeroed at 1800 cm<sup>-1</sup>, where the baseline was relatively flat. Fourier self-deconvolution (FSD) was also carried out with an enhancement factor of 1.3 and a bandwidth of 30. The positions of the absorbance peaks located in the amide I region were determined using the second derivative (17). In the amide I region, ratios between the absorbance peaks at 1650 and 1610–1620 cm<sup>-1</sup> and in the amide II region ratios between the absorbance peaks at 1545 and 1510 cm<sup>-1</sup> were determined.

**Dielectric Properties.** Dielectric measurements were carried out on a BDC-N broadband dielectric converter (Novo-control GmbH, Germany) linked to a frequency response analyzer (Solartron-Schlumberger, Germany). A parallel plate stainless steel electrode system had electrodes of area 254.5 mm<sup>2</sup> and a frequency range 10<sup>6</sup>–10<sup>-2</sup> Hz and an applied voltage of 0.5 Vrms. All of the measurements were carried out in quadruplicate.

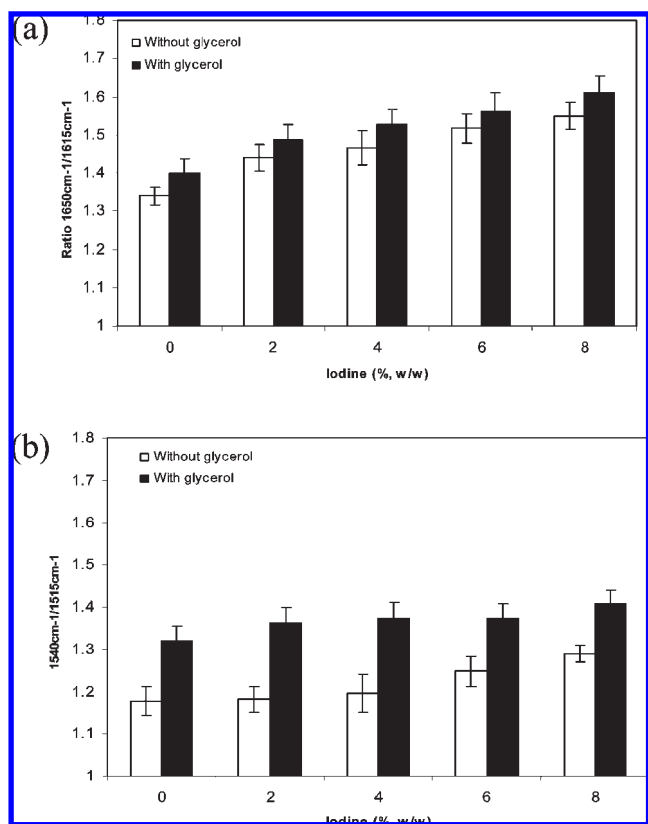


**Figure 1.** Typical Fourier self-deconvoluted (FSD) spectrum with a second-derivative of zein protein powder.

**Mechanical Properties.** The mechanical properties of zein-glycerol films containing different levels of iodine were measured using a Texture Analyzer (TAXT2, Stable Microsystem, Godalming, U.K.) with a 5 kg load cell as described earlier (18). The speed was kept constant at 1 mm/min. To measure the separate tensile properties, films of a well-defined geometry were used. A film 5 mm wide and 50 mm long was cut. The average thickness of films was 0.095 ± 0.005 mm, which was measured using a micrometer (outside, range 0.01–0.25 mm). Ends were glued to two metal plates using cyanoacrylate glue. Film strips were single edge notched to a depth of 2.5 mm midway along their length. Although this method results in stress concentration at the notch tip, it has proved to be reproducible (18) and is suitable for a comparative method. The



**Figure 2.** Typical Fourier self-deconvoluted spectrum (FSD) with a second derivative of zein film.



**Figure 3.** Ratio of the 1650 to 1630 cm<sup>-1</sup> (a) and 1540 to 1500 cm<sup>-1</sup> (b) intensities in the amide I and amide II band, respectively, of the infrared spectra as a function of iodine concentration.

mechanical tests were performed to obtain force/displacement data, and tensile strength (MPa), strain at failure, and estimated modulus (MPa) were calculated. Tensile strength (MPa) was measured using

$$\sigma = \frac{F_{\max}}{(w-a)t}$$

where  $F_{\max}$  is the maximum force associated with failure,  $w$  is the width of the strip,  $a$  is the notch length, and  $t$  is the thickness of the strip.

Strain at failure was calculated as

$$\varepsilon_{\text{fail}} = \frac{\Delta l_{\max}}{l}$$

where  $\Delta l_{\max}$  is the change in length at  $F_{\max}$  and  $l$  is the initial length.

Estimated modulus (MPa) was calculated as

$$E_{\text{est}} = \frac{dF}{(w-a)t} \frac{l}{\Delta l}$$

where  $\Delta l/l$  is the strain obtained from a fit of the initial linear portion of the force/displacement curve and  $dF$  is the force at this particular strain.

Ten replicates were tested, and values of the mean and standard deviation of mean were reported.

**Rheological Measurements.** Zein (30%, w/v) was dissolved in aqueous ethanol (90% v/v) by stirring on magnetic stirrer

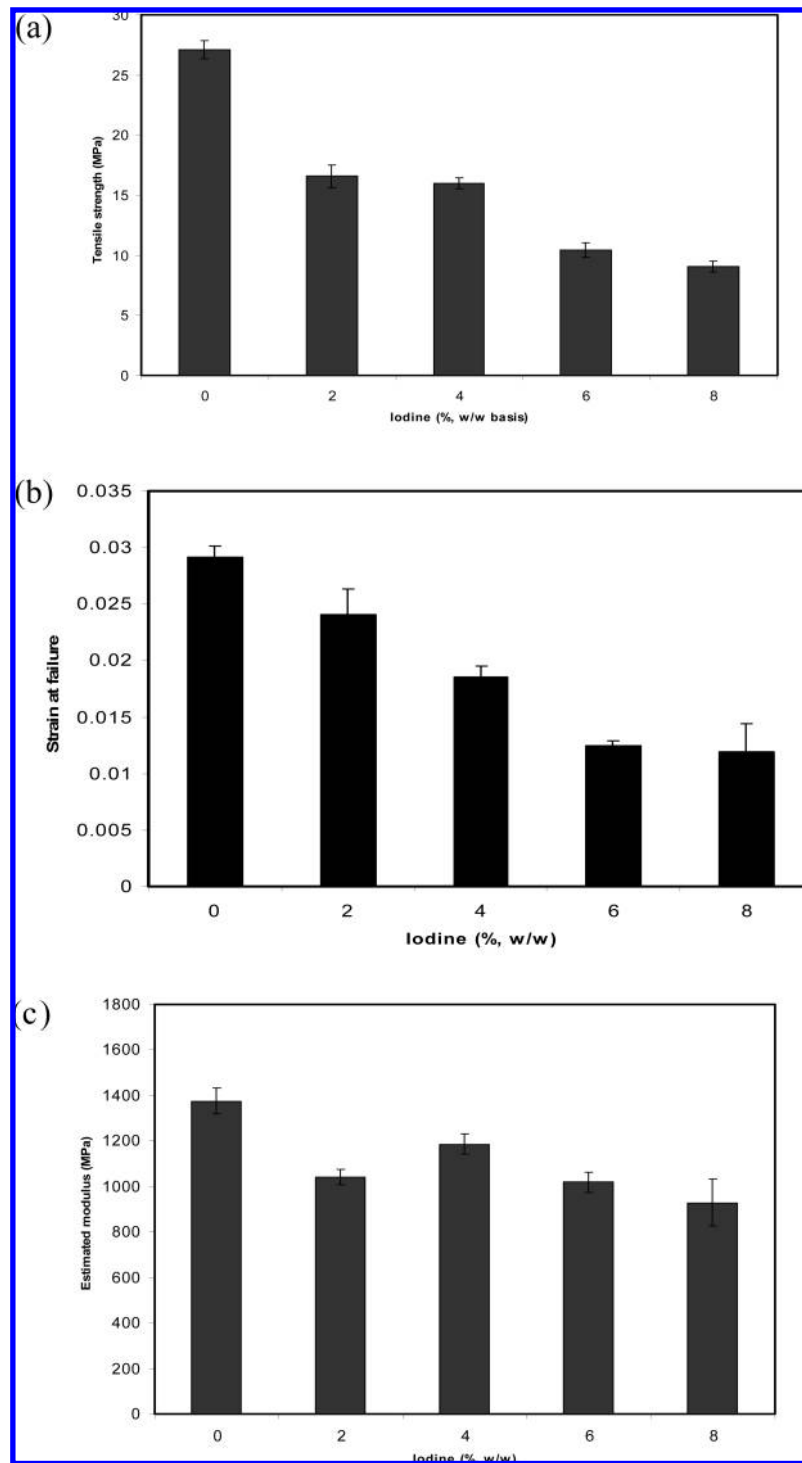


Figure 4. Tensile strength (a), strain at failure (b), and estimated modulus (c) of zein-glycerol films as a function of iodine concentration.

for 15 min. Zein dispersions were precipitated by the addition of distilled water (9-fold). Zein precipitates (termed resin) were separated by centrifugation at 3000 rpm for 30 min. For evaluating iodine effects, iodine (3%) was dissolved in aqueous ethanol (90% v/v). The aqueous ethanol solution was replaced with iodine solution to produce aggregated zein in the presence of different concentrations of iodine (1, 2, 3, 4, 5, and 10% on zein wt basis). Zein dispersions containing iodine were precipitated as described above. Small-amplitude oscillatory shear measurements were performed on the resin with TA (AR 1000, TA Instruments, New Castle, DE) a controlled-stress rheometer. The plate–plate geometry was used with a diameter of 60 mm and a gap between two plates of 1 mm. For each test, approximately 2.2 g of aggregated zein was placed on the rheometer plate. Frequency sweeps were carried out at a constant temperature of 25 °C. Frequency sweep tests were made between 0.1 and 20 Hz at linear viscoelastic ranges (LVR). Initially, oscillation stress sweeps (at 1 Hz) were carried out. On the basis of the results, an oscillation stress value of 10  $\mu\text{N}\cdot\text{m}$  was selected. Temperature ramp tests were performed by increasing the temperature from 25 to 90 °C at a heating rate of 2 °C/min while

using an oscillatory frequency of 1 Hz. Solvent trap was used to avoid drying during experiment. The dynamic rheological data, elastic ( $G'$ ), and viscous modulus ( $G''$ ) was obtained from the TA Rheology Advantage Data Analysis software V 5.1.42 (AR 1000, TA Instruments). The average of the three runs was reported as the measured value.

**Water-Holding Capacity.** Resins were produced as described under Rheological Measurements, and the water-holding capacity of the resin was determined by weight difference between the dry zein and the centrifuged resin (3000 rpm/15 min).

**Statistical Analysis.** Each experiment was performed in triplicate unless otherwise stated, and data were expressed as means  $\pm$  SEM.

## RESULTS AND DISCUSSION

**FTIR Spectra of films.** A typical Fourier self-deconvoluted spectrum with a second-derivative spectrum of zein is illustrated in Figure 1. Zein protein showed three major bands in the amide I region at 1685  $\text{cm}^{-1}$  and around 1650  $\text{cm}^{-1}$  and a band that had its maximum between 1610 and 1620  $\text{cm}^{-1}$ . Two bands in the amide II region of similar intensity were

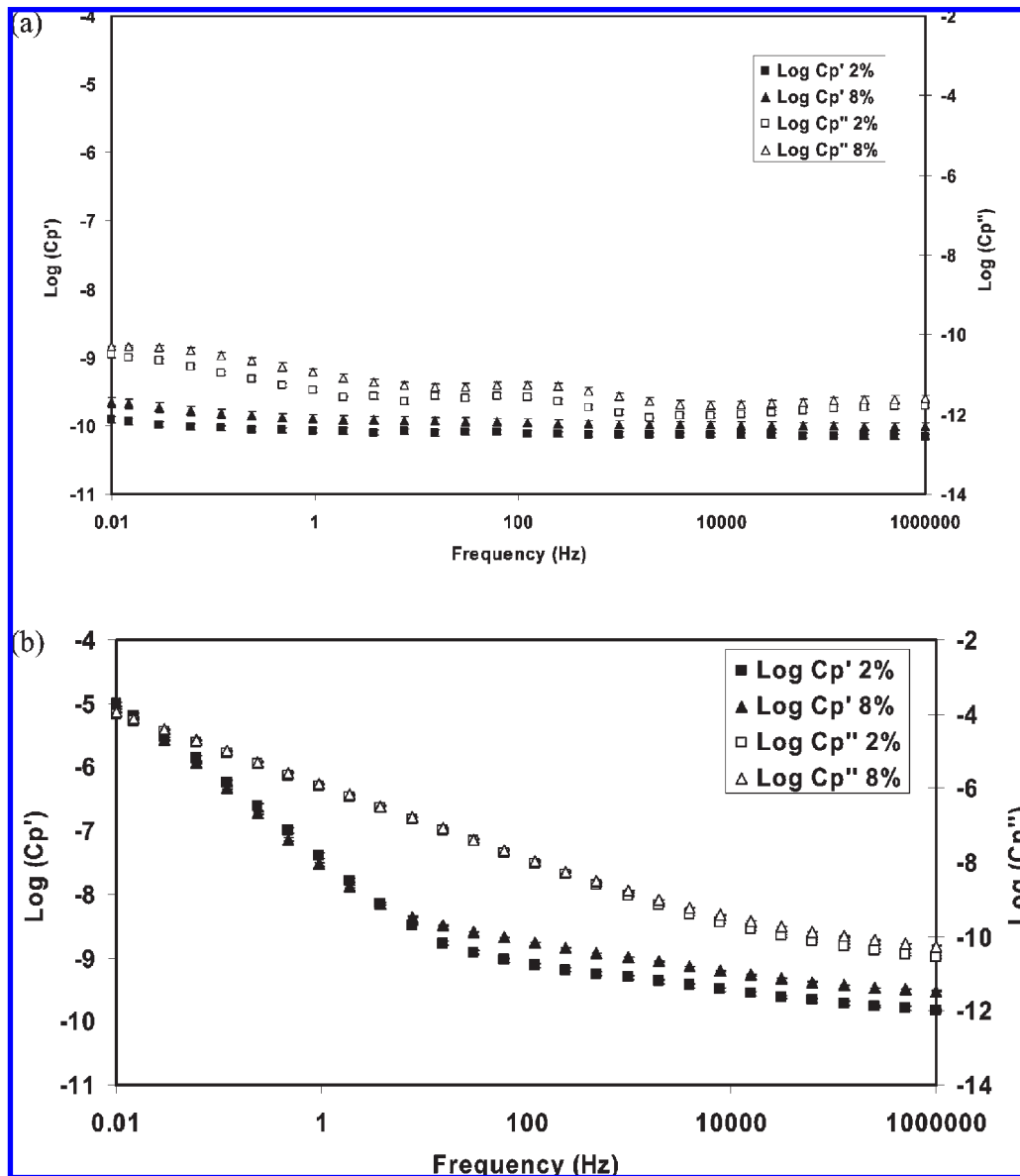
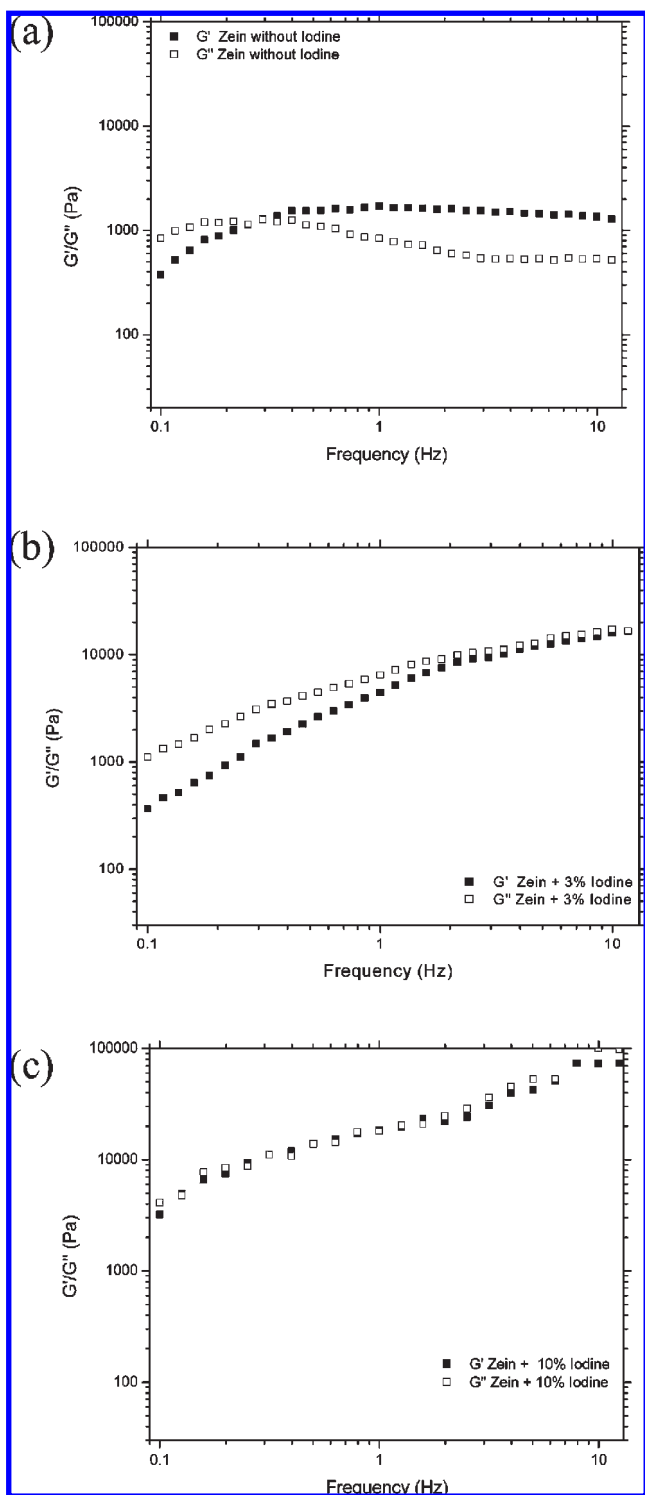


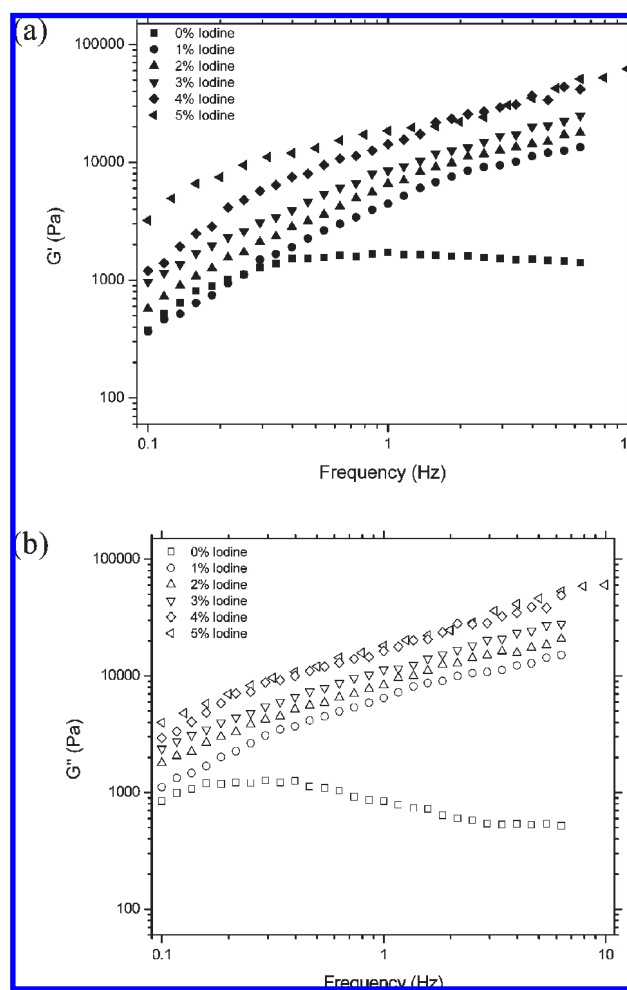
Figure 5. Dielectric properties of zein films without (a) and with (b) glycerol containing 2 and 8% iodine.

observed at 1545 and 1510  $\text{cm}^{-1}$ . A band at 1745  $\text{cm}^{-1}$  characteristic of C=O stretch, corresponding to lipids, was also observed. The most prominent band in the amide I region was at about 1650  $\text{cm}^{-1}$  followed by band intensities in the 1685 and 1610–1620  $\text{cm}^{-1}$  regions (**Figure 2**). The amide I band around 1650  $\text{cm}^{-1}$  originates from  $\alpha$ -helical structures, and the band at 1685  $\text{cm}^{-1}$  is due to  $\beta$ -sheets. The 1610–1620  $\text{cm}^{-1}$  band is also due to  $\beta$ -sheets but may contain contributions from  $\beta$ -edges (15). The low-frequency  $\beta$ -sheet band



**Figure 6.** Frequency dependence of  $G'$  and  $G''$  of zein aggregates prepared with and without iodine measured at 25 °C (a, without iodine; b, 3% iodine; c, 10% iodine).

occurred at 1620  $\text{cm}^{-1}$  in films without glycerol but shifted to 1610  $\text{cm}^{-1}$  when glycerol was added. This may be due to solvation of the  $\beta$ -edges. The relative intensities of the peaks present in both the amide I and amide II bands change with addition of glycerol and iodine. Iodine resulted in an increase in the ratio of the intensities of the  $\alpha$ -helical band to low-frequency  $\beta$ -sheet band; the increase was iodine concentration dependent. The relative intensity of the  $\alpha$ -helical band in the presence of glycerol was greater than in the material without glycerol, but the effect of glycerol was much less than that of iodine. Addition of 30% by weight of glycerol (0.32 mol/100 g of zein) results in an increase in the 1650–1630  $\text{cm}^{-1}$  band ratio from 1.35 to 1.4, whereas an addition of 8% by weight of iodine (0.031 mol/100 g of zein) results in an increase of the ratio from 1.35 to 1.5 (**Figure 3a**). The relative intensity of component  $\alpha$ -helical band of films with and without glycerol did not differ significantly in the presence of >2% iodine. In the amide II region a maximum band intensity was observed around 1545  $\text{cm}^{-1}$ . The ratio of 1545/1510  $\text{cm}^{-1}$  bands increased with the increase in the concentration of iodine. Comparison between the films with and without glycerol revealed that the relative intensity of the component 1545  $\text{cm}^{-1}$  also increased in the presence of glycerol and that, in contrast to the amide I bands, the effect of glycerol was greater than that of iodine (**Figure 3b**). This is consistent with an earlier paper (19) and has been attributed to two factors: (i) more extensive hydrogen bonding between the protein

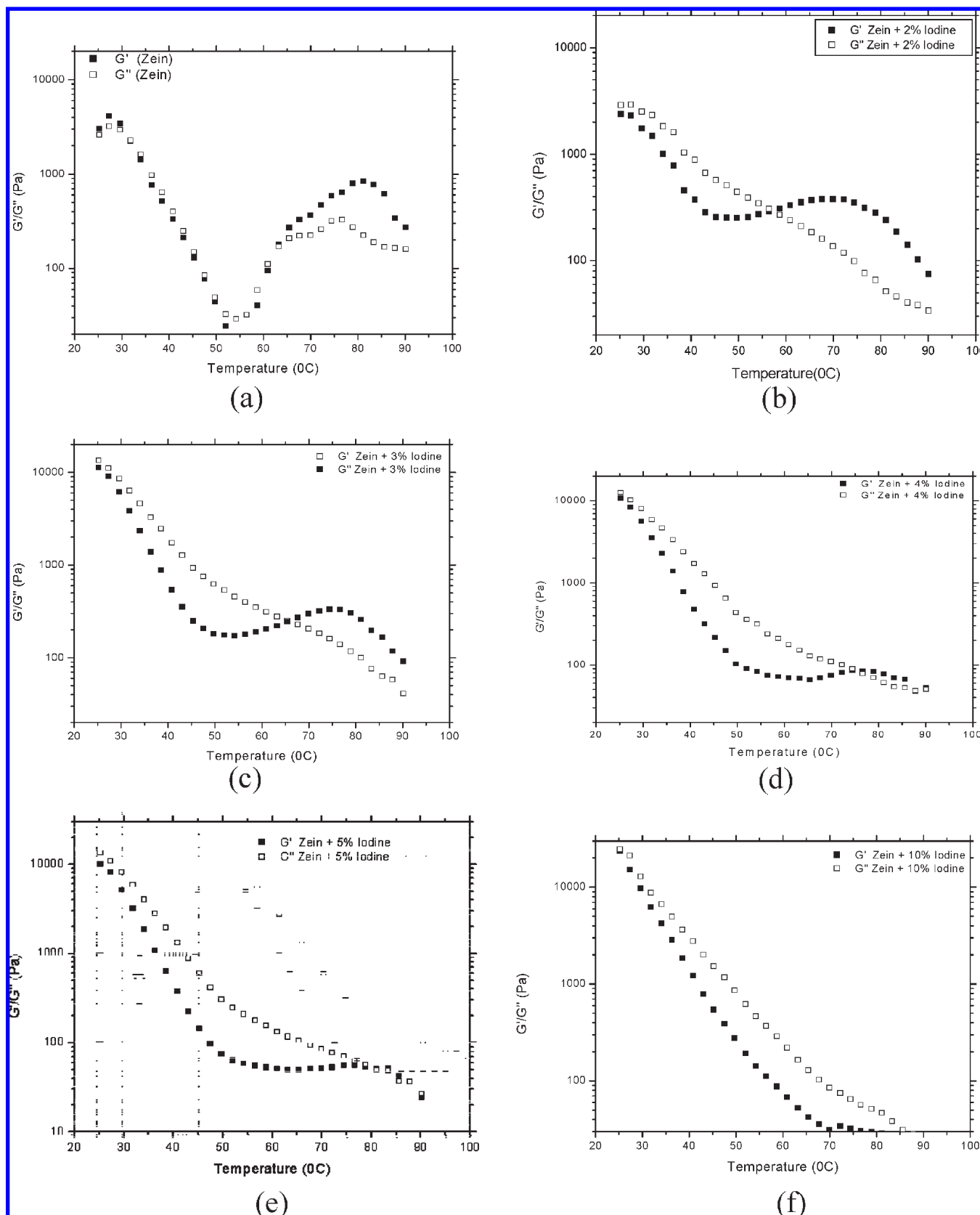


**Figure 7.** Frequency dependence of  $G'$  and  $G''$  of zein aggregates in the presence of different concentrations of iodine measured at 25 °C (a,  $G'$ ; b,  $G''$ ).

backbone and the glycerol, causing a reduction in the number of nonbonded peptide groups; and (ii) a shift from  $\beta$ -sheet structure, which absorbs around  $1525\text{ cm}^{-1}$ , to  $\alpha$ -helical structure, which has its amide II maximum at  $1545\text{ cm}^{-1}$ .

**Film Characteristics.** The films have mean thickness of  $0.095 \pm 0.005\text{ mm}$ . The tensile strength, strain at failure, and

modulus of zein–glycerol films containing different levels of iodine are illustrated in **Figure 4**. The tensile strength and strain at failure of zein–glycerol films progressively decreased with increase in the level of iodine. Zein–glycerol film without iodine showed tensile strength and strain at failure of  $27\text{ MPa}$  ( $\pm 0.77$ ) and  $0.029$  ( $\pm 0.0009$ ), which



**Figure 8.** Changes in  $G'$  and  $G''$  in zein aggregates during heating (frequency, 1 Hz; heating rate,  $2\text{ °C min}^{-1}$ ).

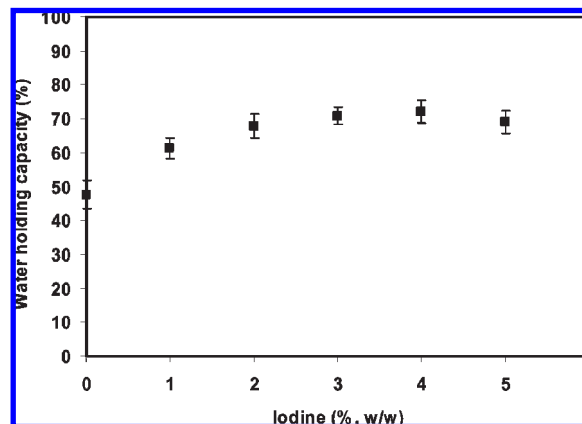


decreased to 9 MPa ( $\pm 0.47$ ) and 0.0118 ( $\pm 0.0025$ ), respectively, with the incorporation of 8% iodine. Estimated moduli of films containing iodine ranged between 1041 ( $\pm 57$ ) and 928 ( $\pm 102$ ) MPa and were significantly lower than that of 1376 ( $\pm 57$ ) MPa of films without iodine.

The effect of iodine is to weaken the films and make them more brittle. This may be rationalized in terms of the observation that iodine enhances the  $\alpha$ -helical content of the zein. Recent work on zein structure (12) has indicated that it has a triple superhelix structure that is capable of binding hydrophobic molecules such as lutein in the core region. The binding of iodine in a similar way would enhance the formation of  $\alpha$ -helix and diminish the interaction between proteins by reducing the number of residues available for protein–protein interactions. This in turn would result in weaker, more brittle film.

**Dielectric Properties of Films.** The variation of the real and imaginary permittivities for films containing 2 and 8% iodine with and without glycerol is shown in **Figure 5**. It is evident that when 30% glycerol is present, the dielectric behavior is dominated by it, such that the amount of iodine makes virtually no difference to the behavior. At low frequencies real and imaginary parts are close to parallel with slopes of 1.22 (real) and 0.99 (imaginary), which are characteristic of a low-frequency dispersion due to proton hopping (20, 21). The absence of Maxwell–Wagner dispersion, which implies a bulk and an electrode barrier region (22), may be taken to imply that the glycerol is closely associated with the protein surface and that no pools of glycerol exist. In the absence of glycerol an effect of iodine is seen, but it is mainly to increase the intensity of both the real and imaginary signals without changing their shapes. This suggests that the high polarizability of iodine is causing a frequency-independent addition to the permittivity. The imaginary permittivity also shows a weak maximum at around 100 Hz, which may be due to some slow motions in the protein film. There is no evidence that iodine causes any significant changes to the dynamics of the system.

**Rheology of Precipitates.** **Figures 6 and 7** illustrate changes in elastic ( $G'$ ) and viscous modulus ( $G''$ ) as a function of frequency for zein resins precipitated in the presence different concentrations of iodine measured at 25 °C. Both  $G'$  and  $G''$  showed frequency-dependence behaviors: both of the moduli increased with increase in frequency.  $G'$  of zein resins was greater than  $G''$ , indicating predominance of elastic character. However,  $G''$  became greater than  $G'$  in the zein aggregates with the inclusion of iodine, indicating predominance of viscous characteristics over elastic characteristics. Both moduli increased with increase in the concentration of iodine. As the temperature of zein resins increased, both  $G'$  and  $G''$  decreased gradually followed by a minimum value (**Figure 8a**). As the material was heated beyond 55 °C, both  $G'$  and  $G''$  increased sharply until a temperature of around 77 °C was reached and showed a crossover at 63.2 °C. The increase in  $G'$  was greater than that in  $G''$ . On the other hand, zein precipitated in the presence of 1, 2, 3, 4, and 5% of iodine showed crossover points of  $G'$  and  $G''$  at 56.5, 58.7, 65.4, 74.4, and 76.6 °C, respectively. The results clearly indicated that crossover temperature was iodine concentration dependent (**Figure 8b–e**). Zein resins containing iodine showed higher  $G''$  than  $G'$ .  $G'$  increased after reaching a minimum value at around 60 °C in zein precipitated in the presence of iodine up to a 5% level. In zein aggregated in the presence of 10% iodine, no crossover of  $G'$  and  $G''$  was observed; that is, both moduli progressively decreased (**Figure 8f**). The effects



**Figure 9.** Water-holding capacity of zein resins prepared in the presence of different levels of iodine.

of iodine on  $G'$  are consistent with a model in which iodine inhibits protein–protein interactions, thereby reducing the elastic response of the system.

**Water-Holding Capacity (WHC).** The WHC of zein resins precipitated in the presence of iodine was greater than that of those precipitated without iodine (**Figure 9**).

**Conclusions.** Iodine can profoundly alter the behavior of zein films and aggregates. The changes in the molecular structure with the addition of iodine are consistent with a model in which the iodine intercalates into the helical structure of zein, thereby stabilizing this conformation and reducing the flexibility of the protein and protein–protein interactions. This results in weaker and more brittle films. Similar effects are also seen in aggregates. The changes in water-holding capacity may be the result of a less compressible aggregate formed by the more  $\alpha$ -helical structures. Zein–iodine solution can be used as a film-forming medication (ointments) when high antimicrobial activity against pathogenic microorganisms or infections is required. Such ointments have the potential to replace substantially oily ointments that are inefficient and can spoil and stain cloths.

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