MOLECULAR ORGANIZATION, TOPOGRAPHY AND PHASE PROPERTIES OF ZEIN FILMS

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Biodegradable films help reduce the use of synthetic polymer films which take a long time to completely degrade. Recent research efforts to develop biopolymeric alternatives to polymeric packaging materials have focused on utilization of proteins. Among many protein sources, zein (the major storage protein in corn endosperm) has been actively investigated for its film forming properties and its potential to produce novel hydrophobic films with good water vapor and oxygen barrier properties.

The molecular organization of biopolymer films have a significant impact on their functional properties. The relationship between the functionality of the zein film and its molecular organization requires consideration of molecular organization during film formation. Understanding the properties of such films at the nano-levels can help understand their properties at macro levels better. A clear understanding of conformational features and forces (e.g. adhesion forces) characteristic to food matrices helps in gaining an insight into food structure and functionality. Atomic force microscopy (AFM) is a relatively new and useful technique that has been used by various research groups to analyze molecular structure and conformation of various food biopolymers such as titin, wheat glutelins, xanthan etc. (Panchapakesan, 2005). Studies prove that differences in mechanical properties of zein films can be related to their structural organizations (Lai and Padua, 1997). A more "uniform" network like structure would imply that the films have greater barrier properties (moisture and oxygen). Understanding the molecular organization of biopolymer films can help us understand their functional properties and thus help us improve them.

Phase behavior of biopolymers is very important in determining the physicochemical properties of several food components, primarily proteins and carbohydrates. The physical properties of many amorphous materials can be related to their glass transition temperature (Tg). For amorphous polymers at their Tg, the mechanical properties undergo a drastic change with temperature due to increasing mobility of the polymeric materials as glassy material converts into a rubbery structure. Predicting the changes in mechanical properties that occur as a result of plasticization with water or of temperature is critical to control the physical properties and the resulting quality and stability of the biofilms. Glass transition and phase behavior of several cereal proteins have been studied extensively in our laboratory. We have investigated the phase transitions of glutenin (Cocero and Kokini, 1991), gliadin (Madeka and Kokini, 1994), zein (Madeka and Kokini, 1996), 7S and 11S soy globulins (Morales and Kokini, 1997 and 1999), and gluten (Toufeili *et al.*, 2002) as a function of temperature and moisture content.

Differential scanning calorimetry (DSC) and rheometry, in particular small amplitude oscillatory measurements, are the most common techniques used to study the glass transition of biopolymers. Phase behavior of polymers can also be studied by measuring the adhesive forces between AFM probe tip and the molecules at the surface. As the cantilever approaches to the surface it experiences an attractive force from the sample. A pull-off force is experienced when the cantilever is pulled back to detach the tip from the surface, which is indicated as negative force in an AFM force curve.

AFM force curves have been used by many researchers as an aid in investigating the surface properties of polymeric materials such as surface heterogeneity (Eaton *et al*., 2002; Mizes *et al*., 1991). Bliznyuk *et al* (2002) measured the surface glass transition temperature of several amorphous polystyrene samples of different molecular weights (Mn) ranging between 3,900 to 1,340,000 by force-distance measurements at different temperatures using a scanning force microscope. The results related to existing theories on glass transition temperature of the bulk describing the decrease in Tg at lower Mn as a result of increased free volume in the system. The values of the surface glass transition for the samples with Mn>30,000 have been found to be the same as corresponding bulk values. The effect of temperature on surface phase behavior was also observed to be similar to that of the bulk. Pull-off forces measured at temperatures above the Tg of the samples were significantly lower than those measured at temperatures below Tg. Marti *et al* (1999) used heterogeneous polymer samples to analyze the effect of temperature on adhesion. They reported an increase in surface adhesive force with an increase in temperature. The increase in adhesion was attributed to the increased flexibility in the polymer chains as the polymer reached its glass transition regime.

The mechanical and barrier properties of biofilms depend on many factors such as formulation, processing technique as well as the state of film forming polymer at a particular temperature and water activity level. Understanding the phase behavior and molecular organization of biofilms is very important to control their functionality at their end usage. In this study, we utilized Atomic Force microscopy to study phase transitions at nano-level and show that mobility at the nano-scale is well correlated with mobility at the macro-scale. We studied the self-assembly of zein to understand the molecular organization at the nano-level in order to devise strategies on how to manipulate the self-assembly process to obtain favorable film properties.

MATERIALS AND METHODS

Atomic Force Microscopy (AFM) was used for adhesive force measurements and film topography. A multimode Scanning Probe Microscope with a NanoScope IIIa SPM Controller (Digital Instruments/ Veeco Instruments, CA) was used with a J/E scanner attached. Silicon nitride cantilevers with a nominal spring constant of 0.58 N/m were used. The dimensions of the cantilever as specified by the manufacturer were nominal thickness= 0.6 µm, thickness= 0.4-0.7 µm, tip height= 2.5-3.5 µm, maximum tip radius= 60 nm and tip angle= 35°. Typical scan rates ranged between 0.5-1.5 Hz.

Commercial food grade zein (Freeman Industries, NY) were used to form zein films with three different methods: Solvent cast films were prepared by dissolving zein at 16% concentration in 75% ethanol and heating to 75-80°C. Solution was cooled, cast onto petri

plates and dried at 30°C. Films by drop deposition were prepared by dissolving 0.1, 1, 2 and 4% zein in 75% ethanol and heating to 75-80°C. A drop of solution was placed on silicone wafers and allowed to dry. For spin-casting, zein was dissolved in 75% ethanol and spin-cast on silicon wafers at 3000 rpm for 10 seconds.

Zein fractions were extracted from commercial zein using based on differential solubility of zein in 90% ethanol and 60% ethanol. Topographic features of both purified and unpurified zein fractions were evaluated at concentrations in the range of 1-40 mg/ml in aqueous ethanol solutions using tapping mode of AFM to study the macromolecular organization of zein films.

Force measurements were made on 16% w/v solvent cast films to study the effect of plasticizer content on the molecular mobility of the film. Adhesive force measurements were conducted in the "contact mode" using the force analysis software. Equilibrated (Aw= 0.12-0.93) solvent cast zein samples were adhered to the sample holders and ~90-100 force curves were generated on each sample in order to obtain an average adhesive force measurement over the sample surface. Three different samples at the same Aw were measured and the average of 270-300 adhesive force readings was reported. Samples at high Aw were contained in an "atmospheric hood" made of polycarbonate to avoid any moisture loss from the sample. The phase properties of zein films at the nano-scale were correlated to those at macroscopic levels as measured using traditional methods such as DSC. Solvent cast films were cut into small pieces and equilibrated to water activities of 0.12, 0.43, 0.64, 0.84 and 0.93 at room temperature, by placing them in desiccators over supersaturated salt solutions. Thermal analysis was performed using a TA 4000 Thermal Analysis System with a DSC 30-S Cell/TC11 TA Processor (Mettler Instrument Inc., Highstown, NJ) at a heating rate of 5°C/min, and the scan range of -20 and 120°C.

RESULTS AND DISCUSSION

Characterization of nanoscale phase behavior of zein films:

Glass transition temperature of solvent cast (16%) zein films determined by DSC as a function of water activity are given in Figure 1. Phase behavior of zein films was compared to that of zein powders (Madeka, 1996) in order to investigate whether the film making process affects the phase behavior. The change in glass transition temperature of zein films as a function of water activity was found to be in close agreement with that of zein powder. Slight differences in the measured Tg values of zein powders and zein films are attributed to the differences in the method of sample preparation.

The phase behavior of polymers depends on various factors such as molecular weight, presence of plasticizers, cross-linking. The molecular structure at the surface may lead to notably different surface phases compared to the bulk. In the current study we investigated the phase behavior of zein films as bulk by measuring their Tg using DSC. Then we compared the results with the adhesive force measurements to investigate the mobility of zein films at the nano-scale. Glass transition temperature of solvent cast zein films decreased from 95°C to around 24°C with increasing Aw from 0.12 to 0.93 indicating the plasticization effect of water (Figure 1). The average adhesive force (Fad) increased from 67.2 nN at Aw= 0.28 to 138.8 nN at Aw= 0.93. The most significant increase in Fad was observed between Aw= 0.82 to 0.93 (Figure 2). This sudden increase in adhesive force was attributed to the zein changing from a

"glass" to a "rubber" at this particular water activity. Increased mobility in the rubbery state molecules resulted in stronger adhesion to the AFM tip when the tip contacts the surface of the film. Increase in adhesive forces with increasing molecular mobility has also been reported in several other studies where temperature was used as the driving force for phase transition to occur. Marti *et al*. (1999) and Tsui *et al*. (2000) found that the measured adhesive force increased when the temperature of the samples were increased. There is a good agreement between the properties that were observed on zein film surfaces at the nano-scale levels and the properties exhibited by the zein films as bulk, when measured by traditional methods.
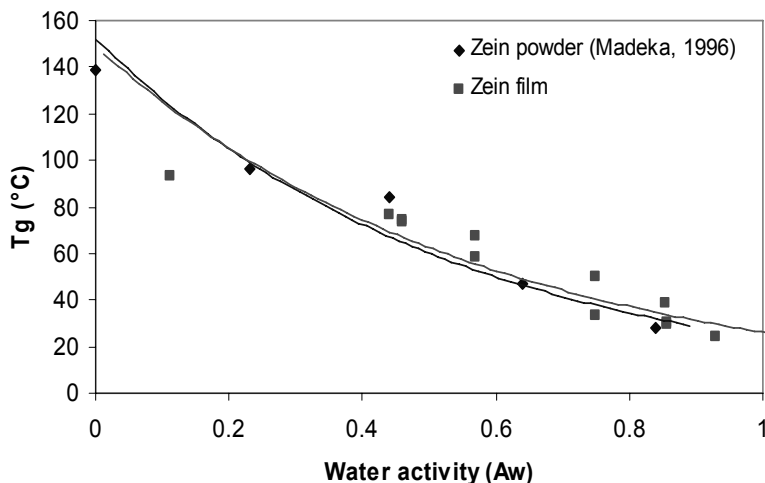
**Figure 1.** Glass transition temperature (Tg) as a function of water activity
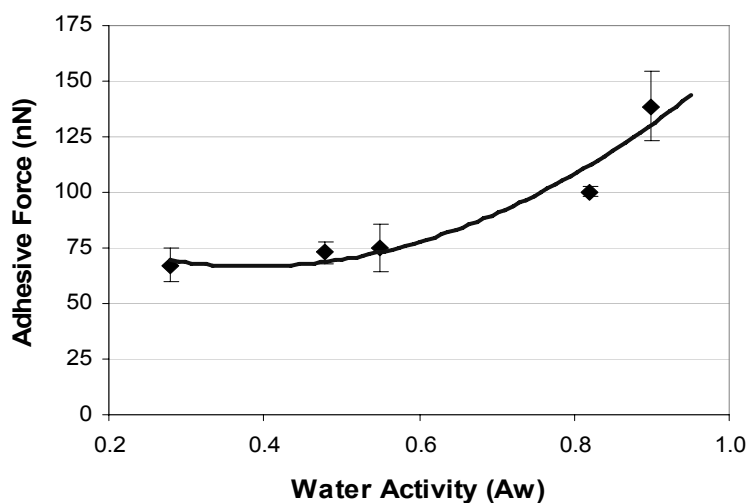
Figure 2. Adhesive force as a function of Aw (average of total 230-300 measurements for each Aw level).

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In addition to magnitude of measured adhesive forces, AFM force curve itself gives a variety of information about the sample being analyzed such as sample stiffness. During force measurements as the cantilever approach to the surface of the sample it experiences an attractive force from the sample, which is indicated, by a negative force on force-displacement curve. As the cantilever is slowly retracted from the sample an adhesive force or pull-off force is needed to detach the cantilever from the surface of the film. The deflection on the Y-axis as shown in Figure 3, is directly related to the force between the cantilever tip and the sample. Force and displacement are related by

$$F_{ad} = k * d$$

where F_{ad} is the adhesive force, k is the cantilever spring constant and d is the deflection/displacement of the cantilever.

Force curves for zein films at the lowest and the highest water activities are shown in Figure 3. The force curve generated at Aw= 0.28 shows straight and sharp lines, indicating that the sample is "hard" or "glassy". At the highest water activity level (Aw= 0.93), the lines that constitute the graph are very smooth and curved. The adhesive part of the force curve is very large indicating a large adhesive force between the cantilever and the sample at this water activity. The adhesiveness causes the sample and the cantilever tip to stick to each other, causing them to be in contact for a longer period of time, until the cantilever is able to overcome this force and leave the surface of the zein film. This adherence of the tip and the cantilever cause the extended adhesive portion at high water activity levels as shown in Figure 3b. The force curve at 0.28, which exhibits a very sharp retraction curve, indicates minimal tip-sample interaction during the retraction process (Figure 3a).


(a)   (b)
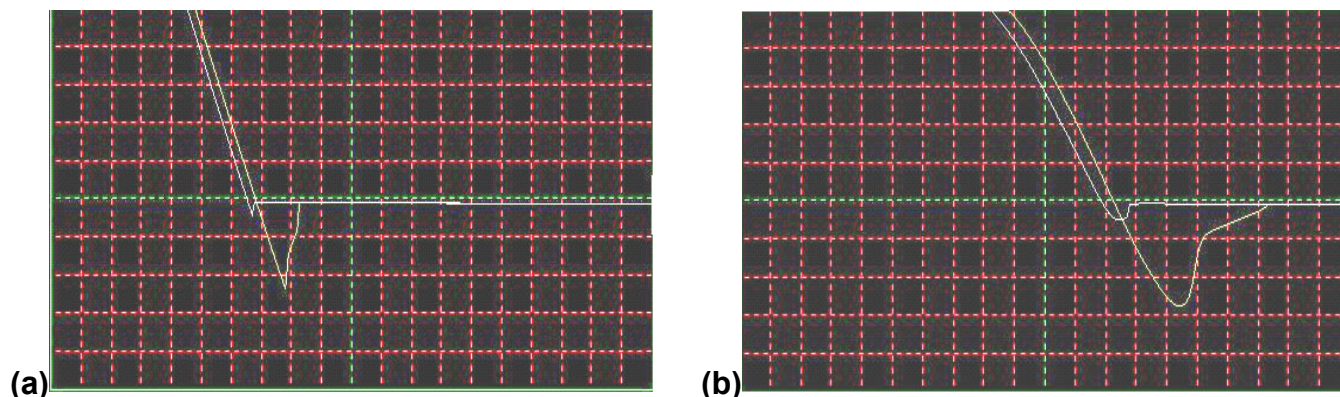
Figure 3. Force curves at the (a) Aw= 0.28 and (b) Aw= 0.90 as measured by the AFM. Scale: (a) Y-axis: 44 nm/div, X-axis: 85 nm/div, (b) Y-axis: 98 nm/div, X-axis: 130 nm/div.

Film topography:

Significant differences were observed in the topography of commercial zein films prepared by different techniques. 10 µm scan of solvent cast films at a data scale of 760 nm (Figure 4a) were featureless, except the presence of several pores on the surface of the film.

The zein film formed by spin casting was observed to form a more "uniform" distribution across the surface of the film. This can be attributed to the high speeds employed during the film formation technique (3000 rpm) which helps to spread the molecules across the surface of the film and cause lesser amounts of clumping than observed in drop deposited films (Figure 4b and 4c). Figure 5 shows the self-organization of spin cast zein films prepared at 0.1 and 0.01% concentrations. Films prepared at high concentration showed significantly higher surface roughness than that the films at low concentration. Topography and surface roughness of spin cast films of 90% and 60% ethanol soluble zein factions showed slight differences (Figure 6). The difference in the hydrophobicity of the two fractions affected their self-assembly behavior of on silicon substrate surfaces.
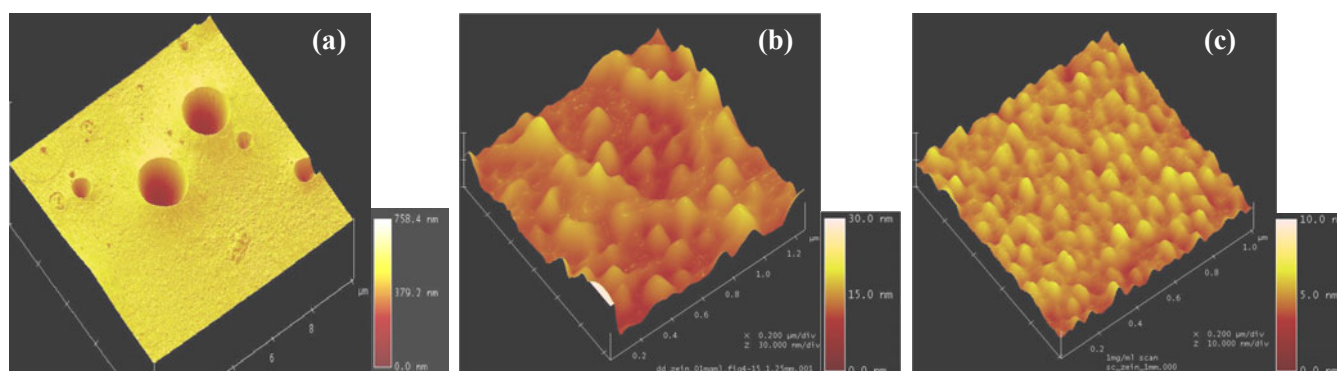
Figure 4. AFM images of unpurified zein films formed by (a) solvent casting (10 μm scan size, 760 nm data scale), (b) drop deposition at 1mg/ml (scan size 1 μm, 30 nm data scale), (c) spin-casting (1 μm scan size, 10 nm data scale).
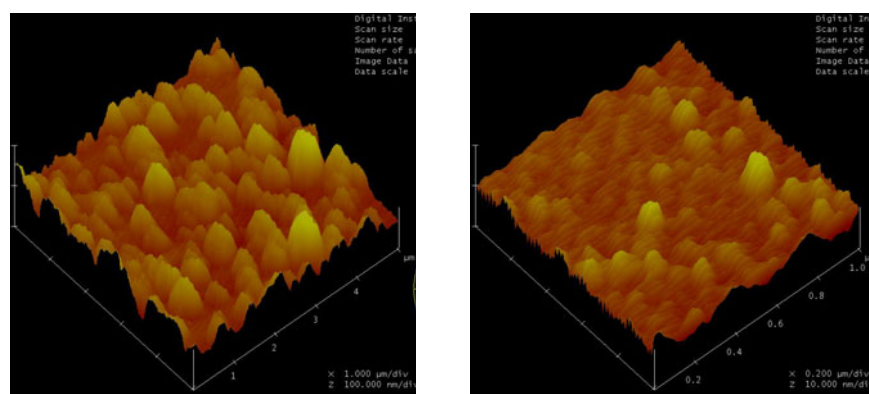
Figure 5. Spin cast zein films prepared at different concentrations (a) 0.1% (1x1 μm scan size, 100 nm data scale), and (b) 0.01% (1x1 μm scan size, 10 nm data scale)
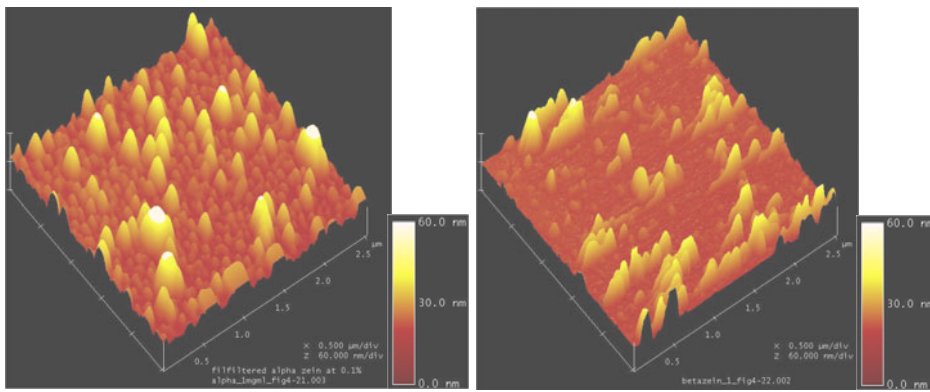
Figure 6. AFM images of spin cast films of zein fractions (a) 90% ethanol soluble fraction, (b) 60% ethanol soluble fraction (scan size: 2.5 μm, data scale: 60 nm).

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