

Engineering Zein Films with Controlled Surface Morphology and Hydrophilicity

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A new method to engineer zein films with controlled surface morphology and hydrophilicity has been developed. The resulting surface morphology and surface hydrophilicity have been studied by tapping mode atomic force microscopy (TP-AFM) and a combination of water contact angle measurements and X-ray photoelectron spectroscopy (XPS), respectively. Our AFM results revealed that zein films cast from acetic acid showed much smoother surfaces as compared to those cast from ethanol aqueous solutions. Furthermore, zein films of controlled hydrophilicity have been engineered through the use of UV/ozone treatment, which can efficiently decrease the water contact angles of zein films from $\sim 80^\circ$ to less than 10° within 130 s. XPS results suggest that the difference in surface hydrophilicity of zein films is due to the difference in surface elemental composition, and UV/ozone treatment converted some of the surface methyl groups mainly to carbonyl groups, therefore decreasing the water contact angles and increasing the surface hydrophilicity of zein films. This research opens up new opportunities of using zein as barrier materials and delivery vehicles for functional food ingredients, drugs, and dietary supplements.

KEYWORDS: Zein; surface morphology; hydrophilicity; tapping mode atomic force microscopy; X-ray photoelectron spectroscopy

INTRODUCTION

Biodegradable polymers from renewable resources have attracted a lot of interest in recent years because they have positive impacts on the economy and environment. As the world energy demand soars while petroleum resources shrink, the energy sector focuses more on the conversion of biomass into useful products, and ethanol (EtOH) is often mentioned as an energy alternative. However, the advantage of green biomass will be fulfilled only if the “waste” products can be efficiently utilized. Zein, a major protein found in the endosperm of corn, is a main byproduct of the bioethanol industry.

Several proteins have been developed as barrier materials or delivery carriers for food ingredients, drugs, and dietary supplements, mainly because of their superior biodegradability, edibility, low toxicity, and convenient absorbability (1, 2). Among these proteins, zein is an excellent film former upon chemical modification, or physical plasticization, or through certain processing (3–6). Zein films are unique because they are tough, glossy, hydrophobic, greaseproof, and resistant to microbial attack, simply by evaporation of the solvent from zein solution (7). These physical properties ensure zein films'

suitability as coating and packaging materials in food and pharmaceutical industries. In the area of controlled release applications, zein has been utilized to coat medical tablets to delay the release of drugs and to protect the drugs from the extremely acidic environment in the stomach (7). In addition, zein has also been used to produce protein microspheres to encapsulate drugs, pesticides, and selected dietary fats (8). Recently, several attempts have been made to further investigate the potential of using zein for delivery applications through the in situ formation of zein beads and zein hydrogels, because their unusual hydrophobic property helps the delivery vehicles to resist water uptake and maintain the network structure (9–16). Furthermore, zein films gain additional interest because they can be used as biomaterials for cells and tissue engineering applications (17–21).

The applications of zein films as protective coatings or packaging materials are largely dependent upon their surface properties, such as surface morphology, which is defined as the form and structure of a surface, and hydrophilicity. Previous research suggested that surface properties of zein-containing microspheres could significantly affect the loading or encapsulation efficiency of active compounds (16, 22). Gao et al. revealed that the surface morphology of zein in situ gels affected the release profile of pingyangmycin, where a smoother surface formed a better barrier on the surface of the gel to eliminate an initial burst of release (11). However, until now, the majority of the research on zein films is focused on improving their

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mechanical properties, and little work has been done on direct modification of zein film surfaces to achieve controlled surface morphology and hydrophilicity.

Zein is insoluble in water but can be readily dissolved in various solvents, among which EtOH/water mixtures with EtOH contents ranging from 60 to 95% are commonly used. Studies showed that certain solvents, such as either 60–90% aqueous EtOH or acetic acid (AcOH), could alter the secondary and tertiary structures of zein in solutions (23). It has also been reported that zein in alcohol solution acidified by AcOH can be electrospun to produce fibers with an appearance distinct from those without AcOH (24). However, a clear understanding of the effects of solvent types (i.e., AcOH versus EtOH) on the surface properties as well as the underlying mechanism, which is essential in designing effective methods to control the physical properties of zein films, is still lacking.

To use zein as barrier materials or delivery vehicles, it is desirable to accurately control the surface properties of this naturally inert protein. The combination of ultraviolet light ($\lambda = 184\text{--}257\text{ nm}$) and ozone gas (UVO) treatment has been recognized as an effective method for the chemical modification of synthetic polymer surfaces (25, 26). During the UVO treatment, UV light, in combination with the ozone gas generated from the UV-induced gas-phase reactions, leads to extensive and rapid oxidation of the very top surfaces of a polymer. However, the bulk properties, such as thermal and mechanical properties as well as moisture permeability of the polymer, may not be altered. This convenient and promising surface modification technique is worthy trying on the edible biomaterials.

In this paper, the surface morphology and surface hydrophilicity of zein films prepared from using either EtOH/water mixtures or AcOH as solvents have been studied by tapping mode atomic force microscopy (TP-AFM) and static water contact angle analyses, respectively. UVO treatment has been applied to modify zein film surfaces with controlled surface hydrophilicity. The possible mechanism of UVO surface modification has been investigated by a combination of contact angle measurements and X-ray photoelectron spectroscopy (XPS) analysis.

MATERIALS AND METHODS

Materials. Zein (lot #F40006031C1, regular grade) was obtained from Freeman Industries, LLC (Tuckahoe, NY). Zein was stored in a desiccator under room temperature before experiment to prevent moisture uptake. AcOH (ACS reagent grade, >99.7%) was purchased from Sigma-Aldrich, Inc. (St. Louis, MO). EtOH (95%) was purchased from Fisher Scientific, Inc. (Pittsburgh, PA). Mixed solvents were diluted from above AcOH and anhydrous EtOH on a volume to volume basis. Milli-Q water was used throughout the experiment.

Zein Films Preparation. Zein was dissolved in 60–95% (v/v) anhydrous EtOH/water mixtures or 60–100% (v/v) AcOH/water mixtures by magnetic stirring at ambient temperature. Either 0.5 (w/v) or 4% (w/v) zein solutions were prepared to study the effects of solvents or the effects of UV/ozone (UVO) treatment on surface properties of zein films, respectively. All of the zein solutions were shaken vigorously overnight, filtered through 0.45 μm filters (Whatman Inc., Florham Park, NJ) to remove insoluble impurities, and then spin coated onto silicon (Si) wafers, which were precleaned with piranha solutions using a Laurell model WS-400A-6NPP/LITE spin coater (Laurell Technologies Corp., North Wales, PA). Zein in EtOH/water solutions was spun at 4000 rpm for 80 s, while zein in AcOH/water solutions was spun at 800 rpm for 20 s, followed by 3000 rpm for 20 s. Clear and homogeneous zein films were obtained and dried in a 50 °C vacuum oven overnight to remove the residual solvent. Subsequently, these zein films were stored in desiccators at room temperature before further

analysis. The zein films were denoted according to sample preparation methods. For example, the first letter A or E denotes that either AcOH or EtOH was used as the solvent, while the number that follows corresponds to the initial zein concentration.

UVO Treatment. Si wafers bearing zein films were prepared by spin coating 4% zein in glacial AcOH solution. These zein films were exposed to ultraviolet emission from a low-pressure quartz-mercury vapor lamp at the distance of 1 in. in the cabinet of a UV/Ozone Cleaning System (model 42, Jelight Co. Inc., Irvine, CA) with an exposure time ranging from 0 to 180 s.

Contact Angle Measurement. The static water contact angle measurements were carried out using a VCA Optima XE Dynamic Contact Angle Analyzer (AST Products Inc., Billerica, MA) at ambient condition. The image was recorded by a CCD camera immediately after the water drop was deposited onto the zein film surface. At least six measurements were averaged for each sample.

AFM. TP-AFM images were collected by NanoScope IIIA Multi-mode AFM (Veeco Instruments Inc., Santa Barbara, CA) with a Si-etched RTESP7 cantilever (Veeco Nanoprobe, Camarillo, CA) under ambient conditions. Images were analyzed using the software provided by the manufacturer.

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR). The ATR-FTIR spectra were collected using a Thermal Nicolet Nexus 670 FT-IR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) under ambient conditions. Each spectrum was averaged by 512 scans with 4 cm^{-1} resolution.

XPS. Elemental compositions of different zein films were characterized using a PHI5400 spectrometer (Physical Electronics, Inc., Chanhassen, MN) with a dual anode X-ray source (Mg and Al) for excitation. The electron takeoff angle was 45°, and the analyzer was operated in the constant energy mode for all measurements. XPS survey spectra over a binding energy range of 0–1000 eV were acquired using analyzer pass energy of 160 eV and 40 eV for high-resolution elemental scans. The vacuum pressure was around 7.5×10^{-8} mbar during spectral acquisition.

Statistical Analysis. All data were expressed as means \pm standard deviations (SDs) with n equal to 6. A single-factor analysis of variance (ANOVA) technique was used to assess the statistical significance of results between groups. The statistical analyses were performed at a confidence level of 95%.

RESULTS AND DISCUSSION

Effects of Solvent on the Surface Properties of Zein Films.

Osborne reported that zein could be readily dispersed in 50–95% ethyl alcohol/water mixtures as well as in glacial AcOH aqueous solutions but not in either absolute alcohol or water (27). In our study, the zein sample used contains mainly α -zein and can be readily dissolved in 60–100% AcOH aqueous solutions and 60–95% EtOH water solutions at room temperature. To study the effect of solvent on the surface properties of zein films, the concentration of zein is fixed at 0.5% w/v. Even though all solutions are transparent, zein in AcOH solutions exhibited less yellowness than in EtOH solutions after overnight shaking, suggesting that the solvent can affect the color of the zein samples. By taking into account the viscosity of the zein solutions and the different evaporation rates for AcOH and EtOH, we used slightly different procedures to prepare clear and homogeneous zein films from either EtOH aqueous solutions or AcOH water solutions. At the same zein concentration, zein solutions using AcOH water solutions as the solvent showed a lower viscosity as compared to those using EtOH aqueous solutions as the solvent. Therefore, a lower spin speed was used for zein in AcOH solutions. However, we find negligible change in the surface properties of zein films within the range of spin methods used in current research. The reason for the lower viscosity of zein in AcOH solutions has been reported as due to the plasticization effects of the carboxylic acid (28). The thicknesses of the zein films obtained were

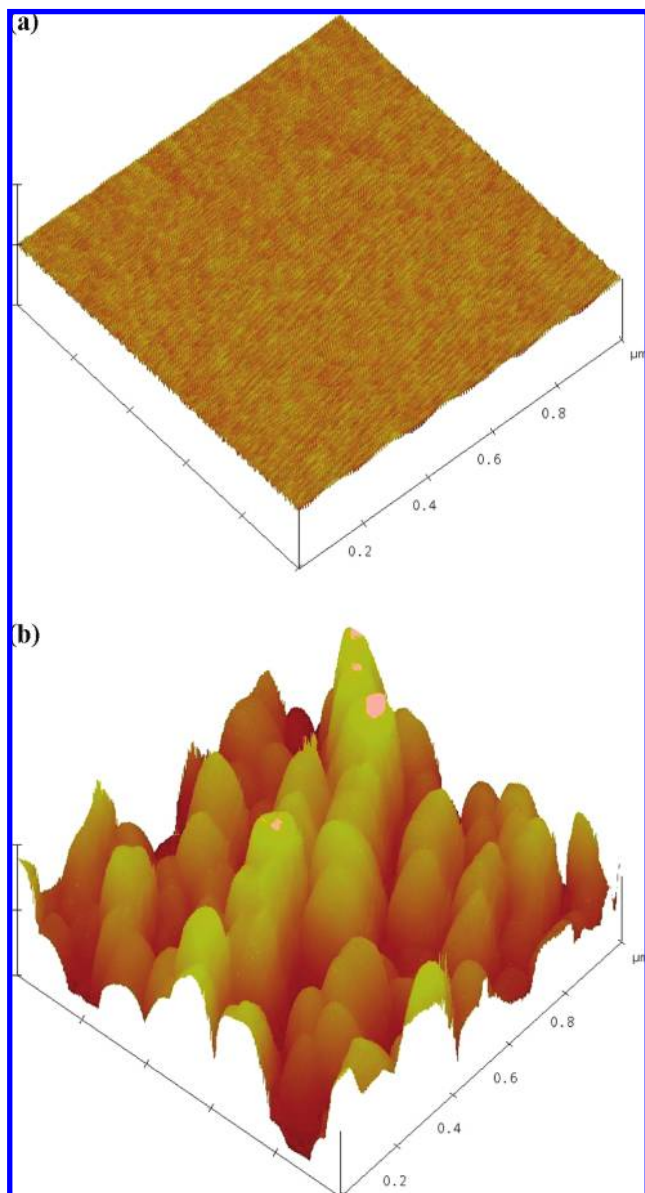


Figure 1. TP-AFM images of zein films prepared from either acidic acid (a) or 95% EtOH/water mixture (95:5 v/v) (b). The scale size is $1 \mu\text{m} \times 1 \mu\text{m}$, and the z scale is 60 nm.

usually thicker than $1 \mu\text{m}$, as estimated by ellipsometry. Within our thickness range studied, the surface confinement effects of the substrate on the surface properties of zein films, which usually occur at film thickness lower than 50 nm, can be neglected.

Figure 1 shows the typical TP-AFM images of zein films cast from either AcOH (**Figure 1A**) or 95% EtOH–water solution (**Figure 1B**). The surface of zein film prepared from AcOH appeared to be smooth and featureless, while the surface prepared from 95% EtOH solution was very rough and composed of granules with diameters ranging from 30 to 80 nm. Root mean square (rms) values calculated using NanoScope v5.30 (a software provided by the manufacturer) were used to describe the surface roughness of zein films, as shown in **Figure 2**. Our results show that rms values for the zein films prepared from EtOH solutions were at least 20 times rougher than those from AcOH solutions. In addition, as the concentration of either AcOH or EtOH increases, the rms values increase. Furthermore, such an effect is larger for EtOH than AcOH.

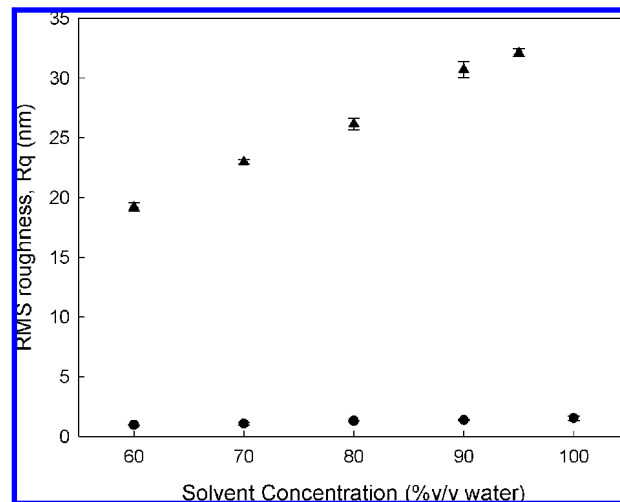


Figure 2. Effects of solvent concentration (v/v) on the surface roughnesses of zein films prepared from the mixtures of water with either AcOH (●) or EtOH (▲) as the solvent.

The surface morphology of zein films depends upon how zein molecules aggregate on the Si wafer surface. In alcohol water solutions, previous small-angle X-ray scattering and viscosity analysis suggested that zein molecules existed as asymmetric particles with the longest dimension of around 15 nm and an axial ratio of around 6:1 (29–31). The structural model of α -zeins as proposed by Matsushima et al. (30) is depicted in **Figure 3A**. Including the N-terminal domains, the total number of motifs would be 10 or 11. If the diameter of the α -helix was assumed to be 12 \AA , then the stacked structure gives a value of $\sim 130 \text{ \AA}$ for the length of one edge (edge *a*). The edge *b* was taken as the diameter of the α -helix (12 \AA), and edge *c* comes from the assumption that each tandem unit has an average of 20 residues (32). Argos et al. proposed that zein molecules tended to self-aggregate through hydrophobic interaction (33). The polar glutamine residues were located on the top and bottom of the structure proposed, while the relatively nonpolar groups were located on the side wall. The isoelectric point (pI) of zein molecules is about 6.2 (7). In acidic pH (i.e., in AcOH solutions), the top and bottom glutamine residues are positively charged. However, the Si wafer surface, which contains mainly silanol groups ($-\text{SiO}^-$), behaves with a negatively charged behavior. Because of the electrostatic attraction, the zein molecules tend to lie down to minimize the interaction energy between the Si wafer and the zein molecules, as shown in **Figure 3B**. Therefore, the resulting surface roughness of zein film is relatively low. However, in EtOH–water solutions, the pH of the zein solution is close to neutral pH. The glutamine residues are only weakly charged, and the whole zein molecules behave negatively charged. On the other hand, the silanol groups ($-\text{SiO}^-$) are also negatively charged. There exists weak electrostatic repulsion between the zein molecules and the Si wafer. The zein molecules tend to perpendicularly line up on the Si wafer surface to achieve minimum interaction energy (high stability), as indicated in **Figure 3C**, resulting in a higher surface roughness for the zein films.

In fact, the pH value may also affect the conformation of protein in solution, because the addition of acid often decreases the degree of ionization of sulfhydryl group and hydroxyl group, while increasing the ionization of the amino group, which would interrupt the S–S bonding and electrostatic interaction. Therefore, the oligomeric structure of zein aggregates as well as the protein conformation can be affected (34). The zein oligomers

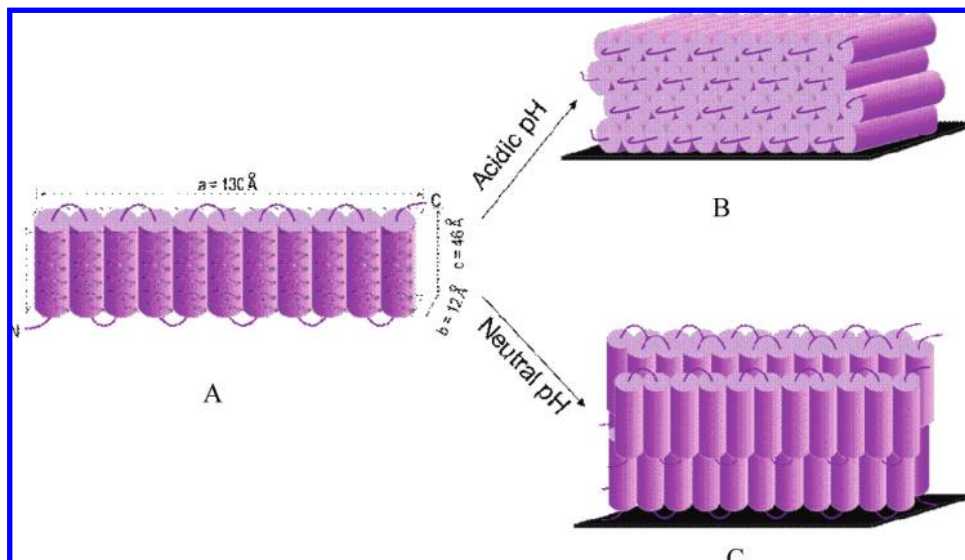


Figure 3. Scheme of zein film formation at different solvent conditions. Part **A** is the conformation of zein molecules in solution proposed by Matsushima et al. (30), part **B** is the possible organization of zein molecules on a Si wafer cast from AcOH and its mixtures with water, and part **C** is the possible organization of zein molecules on a Si wafer cast from EtOH/water mixtures.

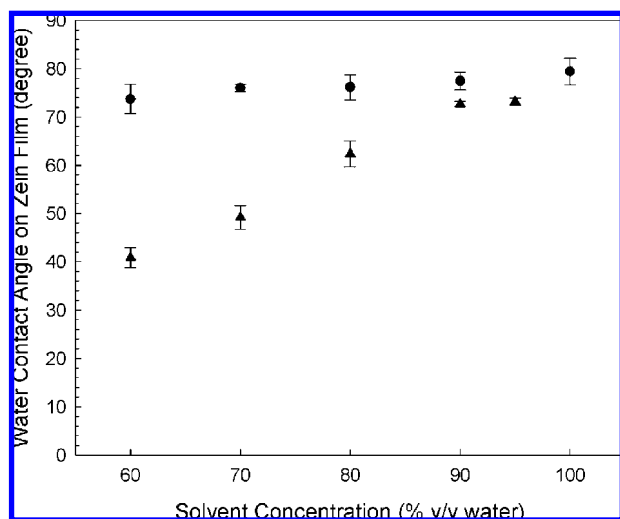


Figure 4. Effects of solvent concentration (v/v) on the water contact angles of zein films prepared from the mixtures of water with either AcOH (●) or EtOH (▲) as the solvent.

tend to dissociate, and the protein chains tend to extend, which could later assemble into an organized manner during film-forming processes. As we discussed above, the conformation of zein in EtOH solutions has been reported by Matsushima et al. (30); however, zein conformation in acetic solution still remains unclear and is a subject of our ongoing research.

To better understand the solvent effect, we analyze the surface hydrophilicity of the spin-coated zein films using contact angle measurements. **Figure 4** shows the effects of solvent concentration on the static contact angles of zein films prepared from either AcOH water solutions or EtOH–water solutions with different AcOH/water or EtOH/water ratios. The water contact angles of zein films from the EtOH solutions are consistently lower than those from AcOH solutions, suggesting that the zein films cast from AcOH solutions are more hydrophobic than those from the EtOH solutions. Surprisingly, zein films from EtOH were shown to be much more hydrophilic when the concentration of EtOH decreased.

Figure 5 shows the ATR-FTIR spectra of zein films prepared from AcOH (solid line) and 95% EtOH–water solution (dashed

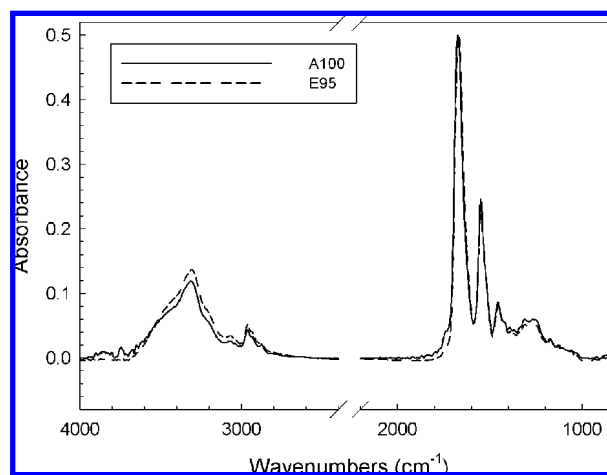


Figure 5. ATR-FTIR spectra of zein films prepared from either acidic acid (A100, solid line) or 95% EtOH/water mixture (95:5 v/v) (E95, dashed line).

line). No significant difference in functional group profiles was found in these two samples, suggesting that the solvent type has negligible effect on the functional groups of the bulk zein films. In another word, no chemical reaction occurred between zein molecules and either AcOH or EtOH. Therefore, the changes in surface properties of zein films are mainly due to the conformational difference of the proteins.

Very often, the surface hydrophilicity of a film is affected more by its functional group or elemental composition on the surface rather than those in bulk materials. Therefore, XPS was used to investigate the surface elemental compositions of different zein films. Our XPS survey scans of binding energy between 0 and 1200 eV indicated that, similar to many proteins, the surfaces of all of the zein films were mainly composed of carbon, nitrogen, and oxygen, with a negligible peak from sulfur (results not shown). The effect of solvent on the surface elemental composition of zein was summarized in **Table 1**, where the ratio of elemental oxygen to elemental carbon of zein film prepared from 95% EtOH–water solution is slightly higher than from pure AcOH. However, this oxygen to carbon ratio is significantly higher for zein film prepared from 60% EtOH/

Table 1. Surface Elemental Compositions of Zein Films Prepared from EtOH/Water Mixtures and AcOH/Water Solutions Obtained from XPS Survey Scans^a

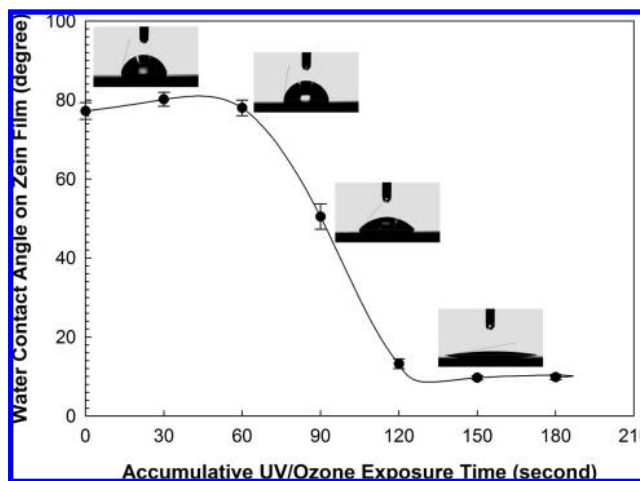
zein film	elemental composition				rms roughness (nm)	water contact angle (degree)
	C (%)	N (%)	O (%)	O/C		
A60	56.8	13.7	29.5	0.519	0.95 ± 0.07	73.7 ± 3.0
A100	63.1	13.4	23.5	0.372	1.51 ± 0.20	79.4 ± 2.7
E60	27.2	5.94	66.9	2.455	19.2 ± 0.4	40.9 ± 2.0
E95	57.4	14.4	28.2	0.491	32.1 ± 0.3	73.2 ± 0.7

^a For comparison, the surface roughnesses from AFM and water contact angles from contact angle measurements were also provided.

water (E60) solution than from 60% AcOH solution (A60). Such a difference in surface elemental composition correlates very well with the water contact angle results, which indicates that E60 is more hydrophilic than A60. This relationship further proves the mechanism that we proposed in **Figure 3**, which suggests the different conformation reorganizations of zein molecules during the different solvent evaporation processes: When the AcOH/water mixture is used as the solvent, zein molecules tend to lie down on the Si wafer with the hydrophobic side wall in contact with the air; thus, the water contact angle of the zein surface is more hydrophobic; however, when EtOH/water mixture is used as the solvent, the zein molecules tend to line up perpendicularly to the Si wafer surface with the polar amino acid residues on the top; therefore, the zein film surface behaves more hydrophilic. The existence of higher elemental composition of oxygen originating from $-\text{COOH}$ for E60 zein film than A60 zein film further demonstrated that a larger amount of polar amino acid residues located on the top of E60 zein film.

UVO Treatment To Modify the Surface Hydrophilicity of Zein Film. Although the use of different solvents may be used to engineer zein films of different hydrophilicities, the variation of water contact angle is only about 30°. Recently, a simple and convenient method, UVO treatment, has been developed to achieve zein films with controlled hydrophilicities. UVO treatment can cause oxidation to the surface groups via a sequence of reactions. Molecular oxygen excited by UV light can dissociate to form atomic oxygen, O^{1D} , and ozone, O_3 , which are very reactive and can further generate hydroxyl radicals, $\cdot\text{OH}$. UV light may also excite the zein film and generate free radicals. All of these reactive radical species will subsequently react with the film surface and eventually oxidize the surface. Through the reactions, different kinds of polar functional groups may be generated, including hydroxyl, peroxy, carboxyl, and ester groups, depending on the extension of oxidation (25), which can eventually modify the surface hydrophilicity.

In our experiment, zein films prepared from glacial AcOH solutions with the zein concentration of 4% were exposed to UVO treatment because they exhibited the most hydrophobic surface in previous experiments. Surface water contact angles were measured to monitor the modification to the surface hydrophilicity, and the results are displayed in **Figure 6**. Our data clearly indicated that UVO treatment was able to quickly convert the zein protein film surfaces from highly hydrophobic to highly hydrophilic over a very short period time (~ 2 min), as evidenced by the decrease of the water contact angle of zein films to less than 10°. There exist three stages during the UVO treatment: (1) When the UVO exposure time is less than 60 s, there is a slight increase in the water contact angle of the zein film, which may be due to the change of surface morphology resulting from the cross-linking reactions in protein molecules

**Figure 6.** Effect of UVO exposure time on the water contact angles of zein film prepared from 100% AcOH.**Table 2.** Surface Elemental Compositions of Zein Films Prepared from Pure AcOH during Different UVO Exposure Times Obtained from XPS Survey Scans^a

UVO exposure time (s)	elemental composition				water contact angle (degree)
	C (%)	N (%)	O (%)	O/C	
0	71.4	12.0	16.6	0.23	82.4
90	65.1	12.7	22.2	0.34	41.9
180	62.2	14.2	23.6	0.38	11.1

^a For comparison, the water contact angles from the contact angle measurements were also provided.

(25); (2) when the UVO exposure time is between 60 and 120 s, a dramatically decreased water contact angle from ~ 78 to $\sim 10^\circ$ is observed; and (3) a further increase in UVO exposure time has negligible impact on the contact angle of zein films. The surface elementary compositions of zein films at different stages were also studied by XPS, and the results are summarized in **Table 2**. One notes that during UVO treatment, the carbon content decreased, whereas the oxygen content increased with increasing UVO exposure time. To further clarify the mechanism of the increase in oxygen content as well as the surface hydrophilicity, XPS high-resolution scans were carried out for carbon 1s of zein films. By decomposing the high-resolution carbon 1s spectra collected from zein films at each UVO exposure stage, the chemical structure of the oxidized functional groups as well as the oxidation mechanism on the zein film surface can be elucidated, as shown in **Figure 7**. There are three components in all of the carbon 1s high-resolution spectra: (1) Peak 1, which is located at around 284.1 eV, has the binding energy characteristic to aliphatic carbons (C–H and/or C–C) and shows a slight shift toward the higher energy level after UVO treatment; (2) peak 2, which is located at around 285.6 eV, originates from the carbon associated with either C–O–C or C–OH; and (3) peak 3, which is located in the binding energy ranging from 287.1 to 287.4 eV, corresponds to the carbon related to $-\text{COOH}$. The area for each peak, which corresponds to the composition of each relevant carbon peak, was calculated by fitting to a Gaussian function and is summarized in **Table 3**. High-resolution C 1s results indicate that, during the UVO treatment, the peak composition of $\text{C}_{3-\text{COO}}$ increases from 19.5% at 0 s to 30.0% at 180 s, whereas the peak composition of $\text{C}_{1-\text{C-H}}$ decreases from 68.1% at 0 s to 56.9% at 180 s, suggesting that the increase in oxygen content is mainly due to the formation of $-\text{COOH}$ on the surface of zein films. This XPS data further support our previous water contact angle

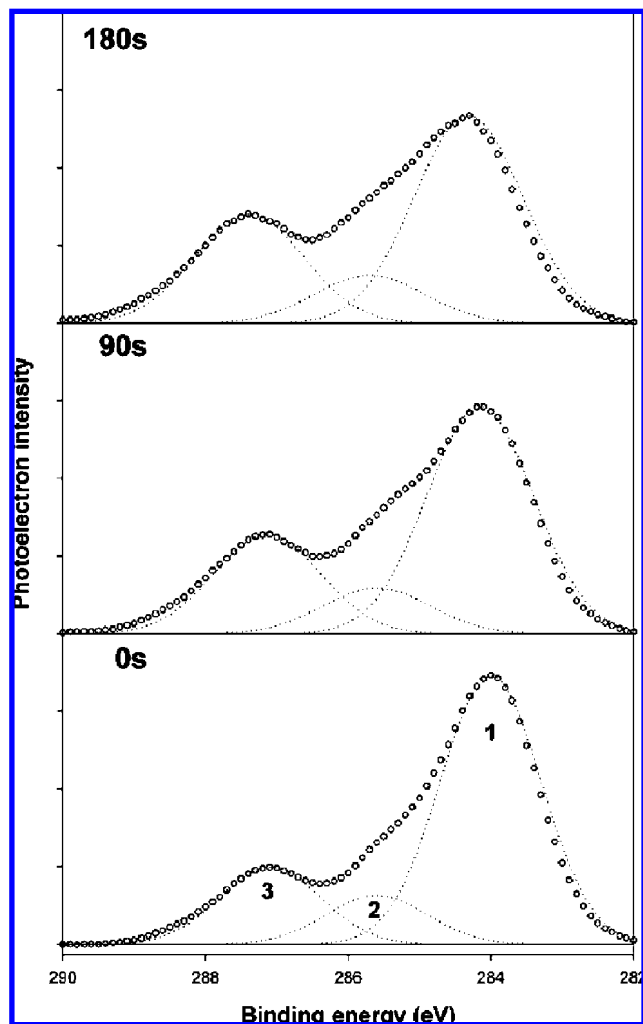


Figure 7. High-resolution XPS carbon *1s* spectra of zein films treated by using 0, 90, and 180 s UVO exposure times. The circles represent original XPS data, and dots represent deconvolution peaks fitted from three Gaussian functions.

Table 3. Compositions of Carbon *1s* of Zein Films at Different Chemical Environments When Treated by Using 0, 90, and 180 s UVO Exposure Times^a

UVO exposure time (s)	composition and binding energy of C <i>1s</i> signals		
	C–C, C–H	–C–OH	–COOH
0	68.1% (284.0 eV)	12.4% (285.6 eV)	19.5% (287.1 eV)
90	61.0% (284.1 eV)	12.3% (285.6 eV)	26.7% (287.1 eV)
180	56.9% (284.3 eV)	13.1% (285.7 eV)	30.0% (287.4 eV)

^a The results were fitted from the high-resolution XPS carbon *1s* spectra of zein films using three Gaussian functions.

results, which show a lower contact angle at an UVO exposure time of 180 s than at 0 s. It should be emphasized that the UVO-induced oxidation occurred only at the very top surface of the zein protein film, and –OH or –COOH groups formed have no obvious detrimental side effects. Therefore, this surface modification method shows tremendous promise for food applications as compared with many other chemical modification methods.

CONCLUSION

In summary, we have demonstrated the efficacy of our new approach to control the surface morphology and surface hy-

drophilicity of zein films through the characterization of their surface properties using a combination of AFM, water contact angle, and XPS. Alternation of the solvents between EtOH and AcOH to spin-cast zein films leads to a distinct surface morphology and hydrophilicity. Zein films prepared from EtOH solutions are more hydrophilic due to their higher percentage of polar functional groups on the surface. The different surface morphologies for zein films cast from different solvents originate from the difference in the interactions between zein molecules and Si wafers, resulting in different organization patterns of zein molecules during the film formation process. The oxidation of zein film using UVO treatment has been monitored through a combination of water contact angle and XPS. Our results show that UVO treatment can efficiently control the surface hydrophilicity by converting some of the –C–H groups into –COO[–] groups. With controllable surface properties, many potentials of zein in food/drug delivery, coating, and packaging applications can be readily exploited.

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