

Contact angle hysteresis of liquid drops as means to measure adhesive energy of zein on solid substrates

L MUTHUSELVI and ARUNA DHATHATHREYAN*

Chemical Lab., Central Leather Research Institute, Adyar, Chennai 600 020, India

*Author for correspondence. E-mail: adhatha@md5.vsnl.net.in

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Abstract. Adhesion of zein to solid substrates has been studied using surface energy profiles as indices and by adhesion mapping using atomic force microscopy (AFM). Different plasticizers like glycerol and sorbitol have been used to form mixed films with zein and properties of these films are studied using surface energy profiles. Comparison of the results from the different mixed samples with those from the pure zein films showed that force mapping could identify areas rich in protein. The adhesion maps produced were deconvoluted from sample topography and contrasted with the data obtained from contact angle measurements. A comparison of the two methods shows that the extent of contact angle hysteresis is indicative of both hydrophobicity of the surface as well as the force of adhesion. Mechanical properties and microstructure of zein films prepared by casting from solutions and using Langmuir–Blodgett film technique have been investigated. Pure zein seemed brittle and exhibited an essentially linear relationship between stress and strain. Films with plasticizer were tougher than these films. In general, mixed films showed better mechanical properties than pure films and had higher ultimate tensile strength and increased per cent elongation. Further, the mixed films of zein showed a higher force of adhesion compared to the pure films.

Keywords. Zein; adhesion; contact angle hysteresis; surface energy.

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1. Introduction

Wetting of solid substrates by liquids is a fundamental phenomenon and it has many applications including lubrication, coating, printing, water proofing and detergency [1–3]. In particular, coatings, i.e., the deposition of a liquid film on a solid substrate, are of significant industrial importance, and they are used in a large number of biocompatible materials. Contact angles of liquid drops in contact with both homogeneous and chemically heterogeneous solid surfaces are analyzed using a 2D lattice Boltzmann model and the changes in the advancing and receding drops related to wetting abilities of different surfaces [4] are studied.

In this regard, proteins, especially hydrophobic proteins from plants [5] in different structural forms, have recently gained increasing popularity in a number

of applications as composite material in combination with polymeric amphiphiles [6,7]. Understanding the wettability of these films is often carried out by measuring the contact angle formed between a liquid drop and a surface and thus assessing the surface energies of different surfaces [8,9]. Often on non-ideal surfaces there is a contact angle hysteresis [10–12] and this has been interpreted as due to various conditions such as existence of metastable states separated by energy barriers, chemical inhomogeneity and possible reorganization or reorientation of large macromolecules at the three-phase contact line [13–15].

Here the superscripts ‘d’ and ‘p’ denote the dispersive and polar components while the subscripts ‘s’ and ‘l’ denote the film surface and liquid used.

Recently a number of excellent reports have appeared in the literature wherein adhesion forces have been analyzed using the atomic force microscope (AFM) [16–18]. These force values have been related to contact angle of liquid drops measured on the protein-coated surfaces in the study.

In the present work, films of pure zein protein and films formed from zein mixed with different plasticizers like glycerol and sorbitol have been transferred on solid substrates. The films thus formed have been analyzed using dynamic contact angle at the three-phase contact line and an adhesion map distribution of the surface are carried out using AFM. The results from the contact angle measurements have been compared with those of the adhesion maps and a correlation between the contact angle hysteresis and the surface hydrophobicity made.

2. Experimental

2.1 Materials and methods

Zein from maize corn (regular grade) was purchased from Sigma Chemicals, USA and defatted using hexane. Definite volume of ethanolic solution of zein was put in a rotatory evaporator and all the ethanol was first evaporated. Then a known volume of HPLC grade hexane was added to it, filtered and the remaining hexane was evaporated again in the rotatory evaporator. This process was repeated at least three times till no fat was detected in the filtrate.

An ethanolic solution of the above protein was prepared using various dry weight amounts ranging from 0.5 to 10%. Deionized water from a Millipore-Mill-Q system (water resistance 18 m Ω) was used. The other plasticizers used in this study were pure glycerol and sorbitol (Sigma Chemicals, USA) and were 99% pure. The pure solution of the protein was prepared by adding the protein in 85% ethanol and 15% water while those with mixed systems with plasticizers contained plasticizers in varying amounts (0.5–5%) keeping the weight of zein constant.

The solid surface used here is a microscopic slide made of glass (Erma, Germany; grade 5; dimensions, 35 × 45 × 2 mm). The alkane taken for testing is HPLC grade and was obtained from Lancaster Chemicals, UK. The glass slides were washed in freshly prepared chromic acid, washed repeatedly with deionized distilled water, and then sonicated in n-propyl alcohol for 10 min. Freshly cleaned slides were used (after storing them in desiccators for 3 h) for every measurement.

2.2 Film preparation

The preparation of the film on glass was carried out using dip-coating technique. The protein films were then transferred on to the substrates at a dipping rate of 0.3 mm/min using a NIMA dip coater (model D1L) in a sealed cabinet. The mixed films of zein with different plasticizers were prepared freshly by mixing the plasticizers in defined amounts with zein in ethanolic solution. Film thickness was measured with an electronic digital micrometer (Cole-Parmer Instrument Co., Vernon Hills, IL). Film strips were placed within the micrometer, and the gap was reduced until first contact was noted. Measurements were taken at at least five locations, and the mean thickness was calculated. The approximate thickness of the film coating is about 1 μm . In order to carry out surface characterization of such films, it is important that the experiments are performed on relatively smooth surfaces ($R_a < 10$ nm) in which surface heterogeneity is understood down to the sub-micrometer level. Hence by Nomarski optical interference technique the films prepared were checked for surface homogeneity and the R_a was found to be of the order of 0.5 nm. A Nomarski style differential interference contrast prism (manufactured by Olympus) was used with a Hund Wetzler, Apo 10x objective on the microscope. Half of the coverslip was covered with the protein with defined number of dip transfer. The interference pattern obtained at the edge of the film covered and uncovered surface gave the thickness of the film.

Prior to AFM investigations, all films were rinsed with water. Both topography and force measurements were performed using a Nanoscope III atomic force microscope (Digital Instruments, Santa Barbara, CA). Silicon nitride tips (Digital Instruments) were used in contact mode. Line scan frequencies were between 3 and 5 Hz. The images were unaltered except for slope removal along each scan line to travel the image.

Silicon nitride cantilevers of length 200 μm and nominal spring constant (K) of 0.032 Nm^{-1} , bearing an integrated standard profile tip were used. Since the actual spring constant of an individual commercial cantilever may differ by up to an order of magnitude from the nominal value, K was determined for the cantilever used in this study by a method described earlier [19]. To avoid any errors in the measurements due to variation in tip radii, the same tip was used for all force measurements reported in this paper. Topographic imaging was performed in contact mode, both in air under ambient conditions and in water using a wet cell. Layered imaging was used to obtain force–distance curves over the entire image frame of 20 $\mu\text{m} \times 20$ μm at a resolution of 40 \times 40 pixels (1600 force curves) and a scan rate of 1 μm s^{-1} : in this type of imaging each pixel encodes the measured force between the tip and the substrate, effectively, a ‘slice’ through the force–distance curve. For each layered image acquired, a corresponding topographic image of identical resolution and spatial orientation was also obtained. Layered images were obtained under ambient conditions.

The layered images, as measured, show a combination of topography and adhesion. A custom-built software helped us to obtain sensor response and the spring constant by locating the position of the ‘pull-off’ region and calculating the adhesion force from the measured values of cantilever deflection. The program then allowed the adhesion force values acquired over the entire 40 \times 40 pixel image frame

to be plotted as a gray-scale image. Force maps obtained from this method are similar to those reported on the basis of pulsed force AFM work [20].

The surface-free energies of the coated samples were determined by dynamic contact angle measurements in an enclosed, thermostated cell using a NIMA DST 9005 dynamic contact angle meter using a Wilhelmy balance method. Both advancing (θ_a) and receding (θ_r) contact angles of deionized water, hexadecane and DMSO (HPLC grade, Merck, India) were measured. Different rates of advancing contact angles from 0.1 to 5.0 mm/min and receding angles from 0.2 to 5.0 mm/min were used and repeated measurements carried out. The results reported were the mean of five measurements each. Further, the θ values were identical (for any rate). Thus the consistency of the contact angles from experiment to experiment indicated there was no solvent sorption and solid surface of good quality was obtained.

Thus if the chemical or physical structure of the surface did not change on contact with different solvents, the contact angle hysteresis could be attributed to partial liquid retention in the film. θ_a values are fairly constant and the three phase contact line is always moving over a region where liquid penetration and surface swelling have not taken place. Thus all the discussion pertain to use of θ_a for surface energy estimation.

Hysteresis (H) was recorded as $(\theta_a - \theta_r)$. Surface energies were evaluated using the Good–Girifalco–Young’s surface tension component theory, as described elsewhere [21,22]. On the basis of Good–Girifalco in combination with Young’s equation,

$$(1 + \cos \theta)\gamma_l = 2[(\gamma_s^d \gamma_l^d)^{1/2} + (\gamma_s^p \gamma_l^p)^{1/2}],$$

where γ_l is the surface tension of the liquid, γ_s is the surface energy of the solid and the superscripts ‘d’ and ‘p’ correspond to the dispersive and polar components, the total surface energy of the films was evaluated. Theta values for two different liquids like water and octadecane on the zein-coated surfaces were measured. Knowing the γ_l^d and γ_l^p values for water (21.2 and 51.0 mN/m respectively) and γ_l^d value for octadecane (27.87 mN/m), the values of γ_s^d and γ_s^p were determined. The interfacial energy γ_{sl} between the liquid and the film surface can be determined by using the following equation:

$$\gamma_{sl} = \gamma_s + \gamma_l - 2[(\gamma_s^d \gamma_l^d)^{1/2} + (\gamma_s^p \gamma_l^p)^{1/2}].$$

Clean bare glass slides (cleaned with acid) gave θ values of about $7 \pm 0.4^\circ$ (repeatedly cleaned glass slides gave values of about 27°) for water. Fresh cleaned slides were used for all the measurements.

The changes in morphology of films spread as Langmuir films on water surface and as dip-coated films on glass surface were studied using Brewster angle microscopy (BAM).

3. Mechanical strength of films

In order to test the mechanical strength of the films formed, cast films of zein and zein with the plasticizers were made and films were allowed to dry at room temperature and ambient relative humidity and peeled off after 24 h. Tensile measurements

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Table 1. Contact angles for pure zein and zein with plasticizers.

Sample (lowest conc.)	Solvent	θ_a	θ_r
Zein	Water	70.1±0.2	37.3±0.1
	Hexadecane	68.6±0.1	68.0±0.1
	DMSO	60.2±0.3	62.2±0.2
Zein+glycerol	Water	50.9±0.7	44.4±0.8
	Hexadecane	67.4±0.2	68.1±0.1
	DMSO	62.1±0.4	59.5±0.3
Zein+sorbitol	Water	44.1±0.2	40.4±0.3
	Hexadecane	66.2±0.3	65.9±0.3
	DMSO	62.8±0.1	62.6±0.3
Sample (highest conc.)			
Zein	Water	69.2±0.1	64.6±0.2
	Hexadecane	67.5±0.1	67.0±0.3
	DMSO	59.2±0.3	61.8±0.2
Zein+glycerol	Water	53.9±0.7	47.8±0.2
	Hexadecane	66.2±0.3	67.8±0.1
	DMSO	61.1±0.4	60.5±0.2
Zein+sorbitol	Water	40.2±0.1	30.1±0.5
	Hexadecane	65.4±0.2	64.3±0.3
	DMSO	60.48±0.1	61.6±0.3

for tensile strength, elongation, Young's modulus, and toughness were performed with samples from the above method with an Instron testing system (model 1011, Instron Engineering Corp., Canton, MA). The plasticizers used were in the same mass ratio as zein and the films were subjected to stress-strain studies.

Samples were cut into dumbbell-shaped specimens from the central region of zein films and preconditioned for 48 h at 25°C and 50% RH inside desiccators containing saturated solutions of calcium nitrate. Averages of 10 measurements were recorded. Testing protocols followed ASTM Standard D638- 91, type I (ASTM 1994). At least 15 specimens for each treatment were tested.

4. Results and discussion

Table 1 gives the contact angle values for the lowest and highest concentration of zein with plasticizers. Though zein is known to contain hydrophobic residues, once the films are formed the protein does not react with water or with hexadecane or DMSO.

Figures 1a–1c show the plots of contact angle hysteresis $\Delta\theta$ obtained with water and the dispersive component of solid/liquid interface energy γ_{sl}^d of the film as a function of % concentration of pure protein and protein with glycerol and sorbitol respectively. It is seen from the plots that there exists a direct relationship between $\Delta\theta$ and the γ_{sl} values with concentration. The extent of contact angle hysteresis or

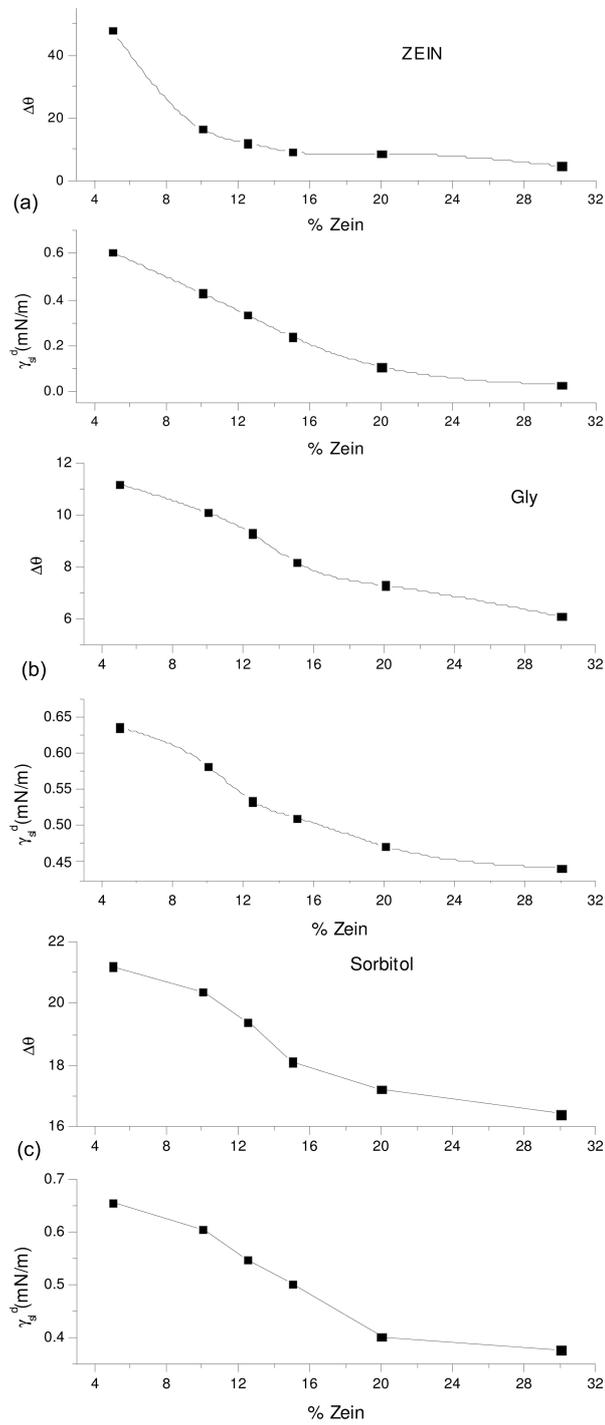


Figure 1. Plots of γ_{sl}^d and $\Delta\theta$ (contact angle hysteresis) vs. protein concentration for (a) pure zein, (b) zein + glycerol and (c) zein + sorbitol films.

$\Delta\theta$ value changes from pure protein to the mixed films. In the case of mixed films of zein with glycerol the lowest hysteresis is observed while for the pure protein there is maximum hysteresis. Further, from the γ_{sl} values it is seen that the surface of pure protein shows slightly smaller interfacial energy values than the mixed films. Thus even though the zein protein contains many non-polar amino acid residues and is considered a hydrophobic protein, the surface energy shows a more polar nature. Such changes in overall surface properties have been observed and it has been reported that when a hydrophobic protein surface is exposed to an aqueous or highly polar environment, such as water, if given sufficient mobility, it orients its polar chemical components towards the interface [23–25]. Such restructuring has been used to predict polarity of amino acids and predict surface dipoles in LB films [26,27].

This reorientation has the effect of creating a surface that is more hydrophilic and higher surface free energy. This could be due to restructuring of water due to the presence of plasticizer molecules at the interface and thereby lowering the number of residues exposed to the solvent. This two-step reorientation process of change in the surface conformation may be explained as follows. In the first step, movements of the macromolecular chains occur. Then, as a second step, the side chains move and orient at the surface.

Reorientation is particularly pronounced in well-networked solutions with strong H-bonds, where the polarity is such that a high interfacial free energy driving force is provided to the surface. This driving force can be strong enough for the diffusion or reorientation of polar segments or side chain, thus creating a more hydrophilic surface.

The contact angles for hexadecane and DMSO in contact with the films showed similar trends and reflected the more hydrophilic nature of the film surface.

A number of recent papers on spreading of liquid drops on solid surfaces have discussed the interesting aspect of reorientation of functional groups on the surface, contact angle hysteresis in relation to heterogeneity of the surface and the relationship between contact angle hysteresis and surface hydrophobicity [28,29].

In this work the adhesion map and the distribution of force of adhesion seem to show a clear correlation to the contact angle hysteresis.

The topographic image with the adhesion map is presented in figure 2 for the pure protein and the direct relationship between the topographic features and the relative force of adhesion associated with each domain is seen clearly; the pits in the topography image (light colour) correspond to areas of high adhesion.

Histograms of adhesion values obtained for samples of the zein films and those of zein with glycerol and sorbitol are presented in figure 3. The bars correspond to a force interval of 2.5 nN in the main plot and 0.5 nN intervals in the inset.

The plots demonstrate clearly that the average force of adhesion is much higher in the case of mixed films of zein with glycerol and sorbitol than with pure protein. This indicates clearly that as the surface hydrophobicity increases, the force of adhesion also increases. Thus it can be inferred that extent of contact angle hysteresis can be related to the corresponding force of adhesion.

The low value of θ_r seen for low concentration of zein could definitely be partly due to low coverage of zein. However, as the concentration increases and also with plasticizers, $\Delta\theta$ values get smaller (θ_r is non-zero).

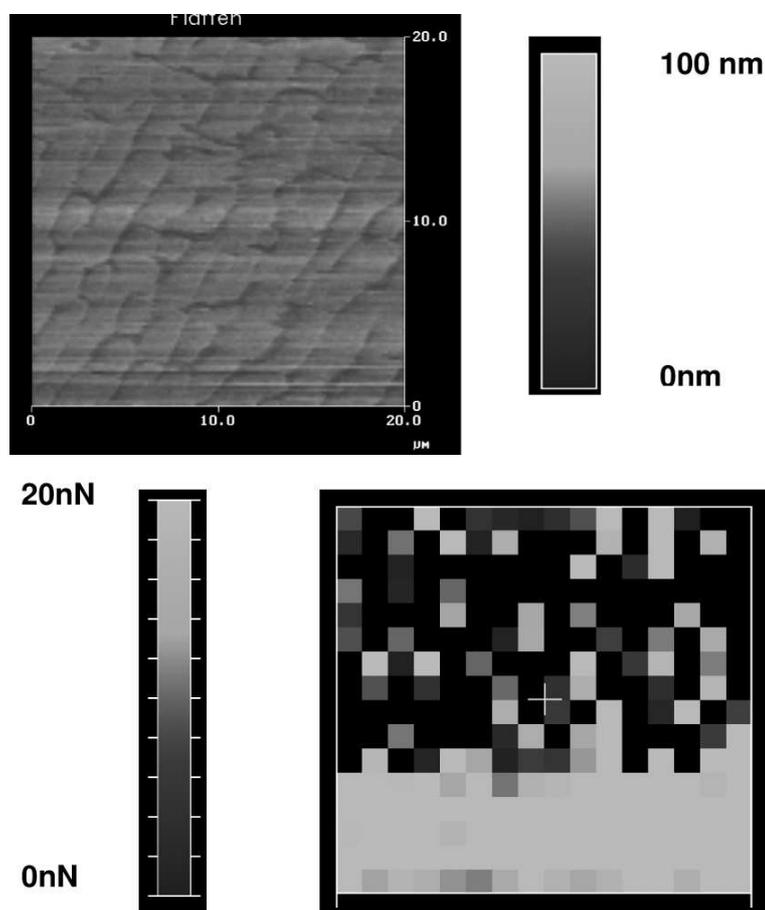


Figure 2. Topography and adhesion map of pure zein film (both images are reflective of the same area).

Figures 4a and 4b show the BAM micrographs of Langmuir films of pure zein on water and as dip-coated film on glass. Since the protein is fairly hydrophobic in nature, it seems to form domains or clusters on water surface while on glass the surface appears nearly uniform (as seen by the lack of any contrasting domains). The films on glass with and without the plasticizers are fairly homogeneous in nature indicating the possible reorientation of the protein films on glass surface.

Table 2 gives the data on mechanical strength and the stress-strain characteristics of pure zein and zein with glycerol and sorbitol.

Pure zein seemed brittle and exhibited an essentially linear relationship between stress and strain. Films with plasticizer were tougher than these films. Significant differences ($P < 0.05$) were found between pure zein and zein mixed with plasticizers for tensile strength, strain at break, elastic modulus, and material toughness. In general, mixed films showed better mechanical properties than pure films and had higher ultimate tensile strength and increased per cent elongation. The elastic

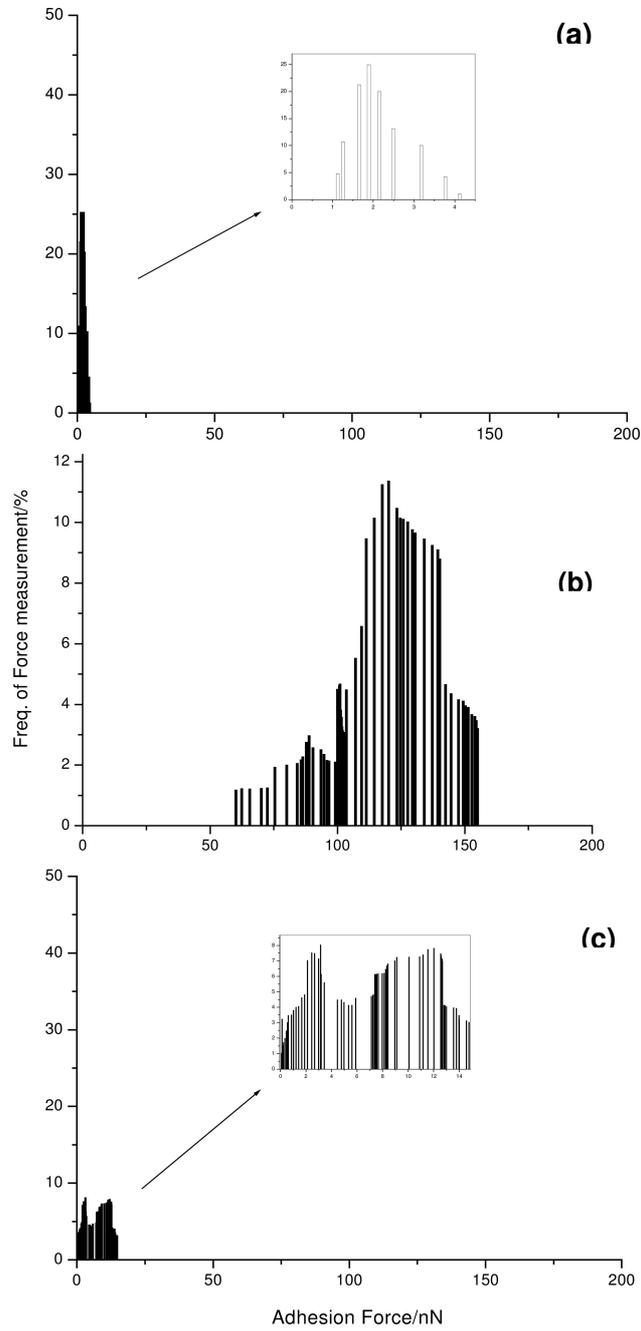


Figure 3. Histograms corresponding to (a) pure zein and mixed films of zein with (b) sorbitol and (c) glycerol.

Table 2. Tensile properties of zein films.

Sample	Tensile strength (mPa)	Strain break (%)	Elastic modulus (mPa)	Toughness (mPa)
Zein	5.71 ± 1.01	2.76 ± 0.24	270.11 ± 5.61	0.071 ± 0.03
Zein + glycerol	6.17 ± 1.1	3.22 ± 0.12	297.23 ± 4.34	0.089 ± 0.01
Zein + sorbitol	7.92 ± 0.99	8.12 ± 0.11	243.14 ± 7.11	0.756 ± 0.01

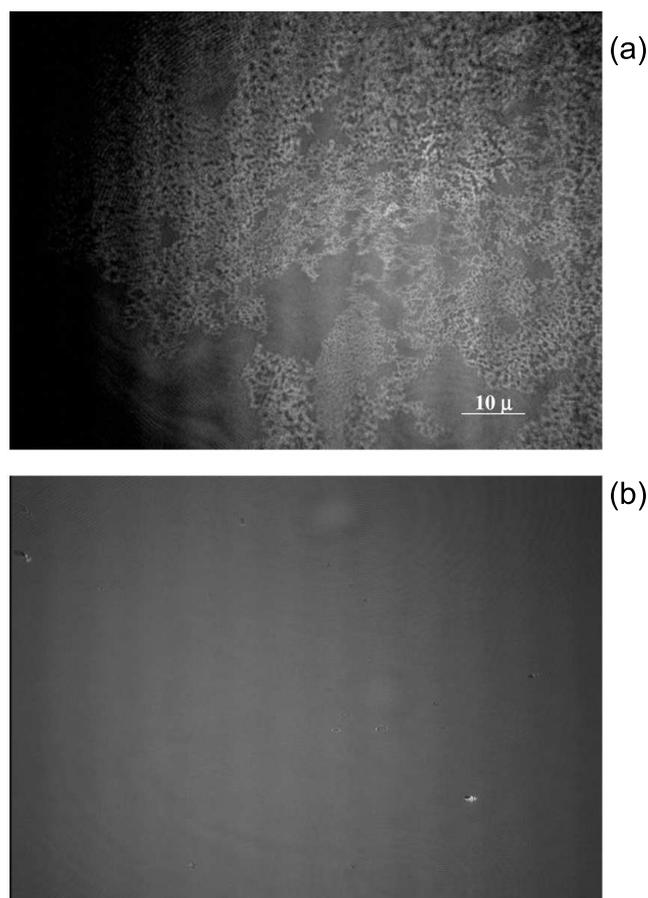


Figure 4. BAM micrograph of (a) Langmuir films of pure zein on water and (b) as dip-coated film on glass.

modulus was determined from the slope of the stress-strain curve, for which only the linear region was considered. Elastic moduli of mixed films were lower than those of pure films. Toughness, calculated as the energy required for breaking divided by the volume of the specimen tested, was much higher for mixed films due to increased tensile strength and per cent elongation.

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In conclusion, plasticizers like glycerol and sorbitol increase the mechanical strength of the zein films. Further, there is a strong correlation between the contact angle hysteresis of liquid drops on zein film and adhesion of these films. Topographic imaging and adhesion mapping have been used to study surface hydrophobicity of such films. However, in order to quantify the adhesive energy and correlate it with the hysteresis at molecular level, further work is required.

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