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Photo-Cross-Linkable PNIPAAm Copolymers. 5. Mechanical Properties of Hydrogel Layers

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Atomic force microscopy (AFM) was used to study the mechanical properties of photo-cross-linked, temperature-responsive hydrogel layers in water. Photo-cross-linkable linear polymers based on *N*-isopropylacrylamide and 2-(dimethylmaleimido)-*N*-ethyl-acrylamide were spin-coated to produce uniform thin films of cross-linked responsive hydrogels. These films were imaged using AFM, and force–distance curves were used to measure the temperature-dependent elastic modulus. The volume phase transition of the hydrogel layers is constrained by the presence of a fixed substrate, and the length scale of these effects is related to the modulus. These materials were also studied with surface plasmon resonance and optical waveguide spectroscopy to determine the polymer volume fraction as a function of the temperature. The modulus varies as a function of the polymer volume fraction and is in good agreement with previous measurements on bulk hydrogels. The effect of the cross-linking density and degree of ionization on the modulus was investigated, and a comparison of these results to rubber elasticity theory for a swollen network was used to study the hydrogel morphology. The concentration of elastically active network chains is lower than expected but increases as the network collapses at temperatures above the volume phase transition temperature.

Introduction

Atomic force microscopy (AFM) has been widely used for imaging and mechanical measurements of soft materials.[1–3] The materials range from polymer films above or below their glass transition temperatures[3,4] to highly swollen polymer networks.[5,6] Force–distance curves can be generated, and the resulting indentation of the cantilever under a given load is used to calculate the surface elastic modulus.[7] The resulting elastic modulus tends to be high, compared to bulk values, and substrate effects can be significant for thin polymer films. However, the results from different models are in good agreement when the films are sufficiently thick and the indentation is small relative to the total film thickness.[1,4] Substrate effects can be minimized by using reference experiments that measure the modulus as a function of both the film thickness and the indentation, and the Hertz model has been used to obtain elastic modulus values as low as 2 kPa.[7] AFM imaging of hydrogel networks has revealed domains on the submicrometer length scale that are related to inhomogeneous gel network structures.[8–10] AFM has also been used to observe the swelling behavior of micropatterned pH-responsive polymers,[11] and studies of responsive hydrogels range from single-chain experiments[12] to tribology[13] and modulus[6,14] measurements.

Responsive polymers and hydrogels have been studied extensively for a wide range of applications,[15] and a number of techniques have been used to make films, membranes, and responsive surfaces from these materials.[16–18] One of the most intensively studied systems is poly(*N*-isopropylacrylamide) (PNIPAAm) gel, which exhibits a temperature-induced volume phase transition in water upon heating above 32 °C.[15,19] The change in the transition temperature with applied stress often complicates the mechanical measurements of responsive hydrogels near the transition temperature,[20] but the mechanical properties of bulk hydrogels can be measured by indentation,[21] simple tension and compression,[22] small-

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angle neutron scattering,[23] and dynamic mechanical analysis.[24] In the swollen state, the modulus decreases with increasing ionic content and increases with increasing cross-linking density as the resulting swelling ratio and network chain concentration change, which can be described by rubber elasticity theory of a swollen cross-linked network.[25] The ionic content does not affect the modulus in the collapsed state, which is determined primarily by the cross-linking density.[26]

We have studied PNIPAAm hydrogel layers with a combination of surface plasmon resonance (SPR) and optical waveguide spectroscopy (OWS) and have found the resulting swelling ratio and transition temperature to be dependent on the film thickness, degree of ionization, and cross-linking density.[27,28] These materials have been patterned and used as actuators in microfluidic devices, and the performance of the actuator is closely related to its mechanical properties.[29] The hydrogel mechanical properties can be measured with AFM, but the effect of ionizable comonomers has not been studied previously on this length scale. The volume phase transition of the hydrogel layer is constrained by the presence of a fixed substrate, and the modulus has been correlated with the length scale of these effects.[14] The modulus can also be modeled with rubber elasticity theory and used to learn more about the gel morphology. In the present study, we extend this work to investigate the influence of the cross-linking density and degree of ionization on the temperature-dependent elastic modulus of PNIPAAm hydrogel layers.

Experimental Section

Synthesis of the Photo-Cross-Linkable Polymers. The 2-(dimethylmaleimido)-*N*-ethyl-acrylamide (DMIAAm), 3-acryl-oylamino-propionic acid (AAmPA), and *N*-(2-(dimethylamino)-ethyl)-acrylamide (DMAAAm) monomers were prepared according to the literature.[28,30] The PNIPAAm copolymers were obtained by free-radical polymerization of *N*-isopropylacrylamide (NIPAAm) as the central component, DMIAAm as the photo-cross-linkable component, and acidic AAmPA or basic DMAAAm as the ionizable component. The details of this synthesis and characterization of these materials have been published previously.[28,31] The name of each sample and its corresponding composition can be found in Table 1.

Responsive Hydrogel Layers. Photo-cross-linked polymer films were prepared by spin-coating on different substrates from a cyclohexanone solution containing 0.1–10 wt % polymer and 2 wt % thioxanthone sensitizer with respect to the polymer. The resulting dry film thicknesses ranged from 10 to 600 nm. The substrates used for SPR and OWS were LaSFN9 glass slides coated with a 50-nm gold film, which was deposited by evaporation with an Edwards 306 Autocoater. Silicon wafers were used as substrates for the AFM measurements. The films were dried under a vacuum and cross-linked by UV irradiation, using a 75-W high-pressure Hg lamp at a wavelength $\lambda > 300$ nm, for at least 60 min.

AFM Measurements. The mechanical properties of the hydrogel layers were measured using AFM. Standard AFM

Table 1. Composition of Photo-Cross-Linkable Linear Polymers

sample	DMIAAm [mol %]	AAmPA [mol %]	DMAAAm [mol %]
NIPAAm2	2.4		
NIPAAm5a	4.5		
NIPAAm5b	4.8		
NIPAAm10	7.2		
AAmPA2	4.3	2.5	
AAmPA5	5.3	4.9	
AAmPA10	4.7	10.1	
DMAAAm2	5.0		1.2
DMAAAm5	4.3		3.9
DMAAAm10	5.9		9.8

cantilevers as well as cantilevers modified with a 5-μm colloidal silica sphere[32] were used to generate force–distance curves.[1] The choice of cantilever is important to create a stable image with minimal damage to the sample.[3] Therefore, a higher spring constant (0.39–0.43 N/m) was used for measurements with colloid-modified cantilevers, while a lower spring constant (0.09–0.19 N/m) was used for measurements with standard cantilevers. The spring constants were calibrated using established procedures,[33,34] and the samples were mounted on the AFM scanner together with a liquid cell and a custom-built heating stage. The heating stage used a Peltier element[35] to provide heating and cooling along with a water-cooled aluminum heat sink to maintain the piezo-electric scanner at a constant temperature. The scanner was also recalibrated to allow for the additional height of the heat sink and the heating stage. A thermocouple was used to determine the temperature in the fluid cell; stable images could be achieved at temperatures ranging from 5 to 50 °C. Force–distance curves were also generated, and the indentation of the cantilever under a given load was fit to the Hertz model and used to calculate the elastic modulus of the hydrogel layer.

There was some variability in the modulus measurements, indicated by error bars in the corresponding figures, and each modulus value is an average of 10 measurements at different points on the same sample. Substrate effects can be significant when the modulus is measured by indentation.[1,7] To minimize these effects, the modulus was measured as a function of the indentation and dry film thickness, and the modulus values were found to be consistent for dry film thicknesses greater than 500 nm with indentations less than 20% of the film thickness. Thus, films on the order of 500-nm dry-film thickness were used for the modulus measurements presented here. Nanorheological effects are also possible for AFM measurements of viscoelastic surfaces, but there are few reports of these effects in the literature.[13] All the data reported here were taken at 1 Hz, but additional measurements at 0.1 and 10 Hz gave similar modulus values. Therefore, it does not appear that the rate of indentation affects the modulus measurements in this range of frequencies.

SPR and OWS Swelling Experiments. The refractive index and film thickness of each hydrogel layer were measured simultaneously, using a combination of SPR and OWS in the Kretschmann configuration, as described previously.[28,31,36] The swelling behavior of the photo-cross-linked gel films was observed as a function of the temperature by placing the samples in a flow-through cell that was connected to a peristaltic pump and a temperature controller. The refractive index and film thickness can be used to calculate the swelling ratio and polymer volume fraction of the hydrogel layers, and these parameters can be compared to the change in the degree of swelling and the mechanical properties, as observed by AFM.

Results

Swelling of Hydrogel Layers. AFM was used to characterize the mechanical properties and swelling

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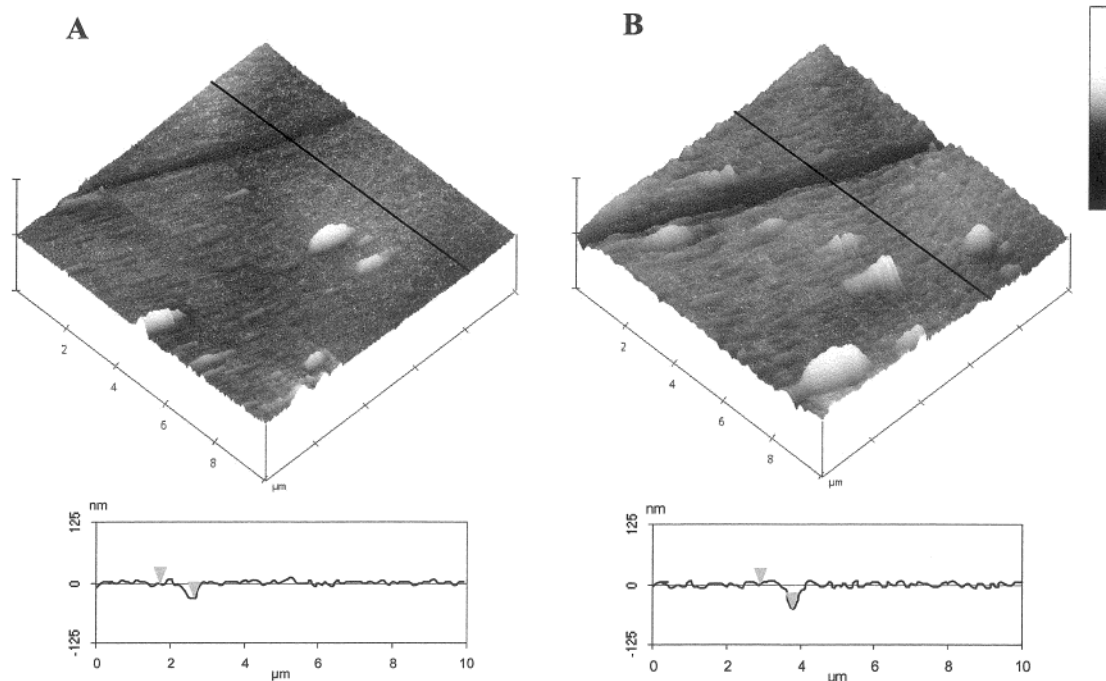
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Figure 1. AFM image (10 μm $\times$ 10 μm) of the area around a scratch on a NIPAAm5b hydrogel surface in water. The depth of the scratch was determined by taking a cross-section of these images, shown below, with the vertical distance between the arrows being (A) 32 nm at 34 °C and (B) 47 nm at 26 °C. The transition temperature is around 29.5 °C, and the degree of swelling changes as a function of the temperature. The scale bar ranges 250 nm from black to white.

behavior of the temperature-responsive hydrogel layers. All measurements were performed in water, and a heating stage was used to control the temperature of the fluid cell. The volume phase transition of the responsive hydrogel layers is confined to one dimension, perpendicular to the substrate. This constraint is known to affect the transition temperature and degree of swelling.[28] AFM has been used to measure the temperature-dependent elastic modulus of responsive hydrogel layers,[13] and the mechanical properties of responsive hydrogel layers have been correlated to the length scale of the confinement effects.[14] A more detailed understanding of the mechanical properties of hydrogel layers should, therefore, improve the understanding of these effects. Here, we extend this technique to investigate the effects of both the cross-linking density and the degree of ionization.

The hydrogel layers were prepared by the photo-cross-linking of linear polymers based on NIPAAm as the temperature-responsive component and DMIAAm as the photo-cross-linkable component. AFM was used to image the hydrogel layers in water as a function of the temperature, and the collapse of the hydrogel layer at temperatures above the volume phase transition temperature could be observed directly, as shown in Figure 1. The swelling as a function of temperature is indicated by the step height increasing from 32 to 47 nm as the temperature decreases from 34 to 26 °C. This indicates a volume change that is much smaller than that of the corresponding bulk hydrogels, which can have a total volume change as large as 100-fold.[19] However, similar trends have been reported for photo-cross-linked hydrogel layers studied with SPR and OWS, and while a 300-nm film has a 5-fold volume change, the corresponding 100-nm film swells to only 2.5 times the collapsed film thickness.[28] A theoretical treatment of the volume phase transition in constrained hydrogel layers has also shown a reduction in the total volume change as the volume change is confined to one dimension, perpendicular to the substrate.[37] SPR and OWS cannot be used for films thinner

than 100 nm, and the results presented here provide verification that this trend continues for thinner films of responsive hydrogels.

Because of the thermal drift in the piezo-electric stage, we cannot be sure that we are imaging the exact same spot on the surface. However, the general appearance of the surface can be observed as a function of the temperature. Previous AFM studies of responsive hydrogels have reported inhomogeneous surfaces with larger domains ranging in size from 100 nm to 1 μm, varying as a function of the degree of swelling as well as the cross-linking density and polymerization conditions.[8–10] We have observed similar features (larger bumps in Figure 1) that range from 200 nm to 1 μm and increase in size with increased degree of swelling, which is consistent with previous measurements of responsive hydrogel surfaces.[8,10] A more quantitative measure of the surface is the root-mean-square roughness, and for the images in Figure 1, the roughness increases from 9.5 to 18.6 nm as the temperature decreases from 34 to 26 °C.

Mechanical Measurements. Thicker hydrogel layers were used for the measurement of hydrogel mechanical properties, and a force−distance curve was measured at each temperature. Figure 2 shows the deflection of the cantilever as a function of the piezo z distance, and the change in the slope demonstrates the effects of the temperature-dependent elastic modulus. For soft samples, the deflection is smaller as a result of the sample indentation, and the indentation of the tip can be calculated from the difference between the curve for the soft sample and the curve for a rigid surface (clean Si wafer). The loading force is given by the cantilever deflection multiplied by the cantilever spring constant, and the force−distance curves are calculated from the data presented in Figure 2. All the measurements are from the repulsive region of the force−distance curve.

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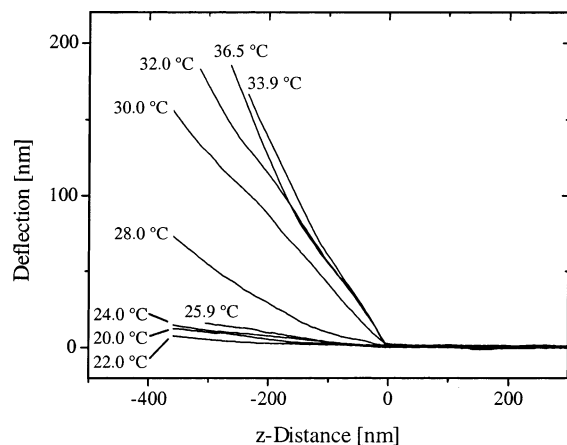
Figure 2. Deflection of the AFM cantilever as a function of the piezo *z* distance for the NIPAAm5b sample in water as a function of the temperature. A standard AFM cantilever is used, and the differences in the slope are an indication of the temperature-dependent elastic modulus. The dry film thickness is around 500 nm, but the total thickness of the film varies as a function of the temperature.

Because these measurements are carried out in a liquid cell, there is also no significant attractive region close to the surface.[38]
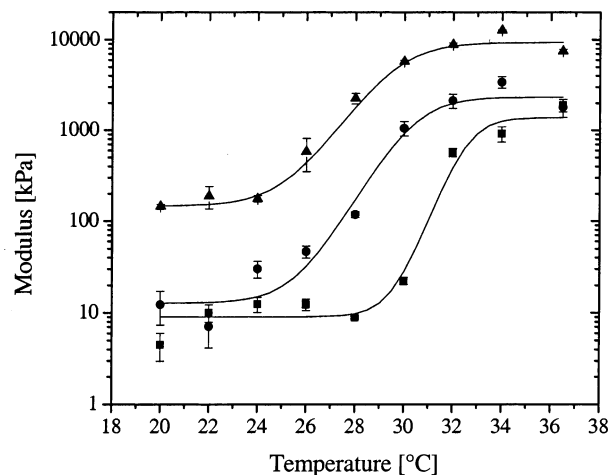
The Hertz model predicts the indentation of a cone or sphere on an elastic sample, and the loading force (F) is a function of the indentation (δ), as shown in eqs 1 and 2. The elastic modulus can be calculated from the slope of linear fits to these curves, and the analysis was performed in the region of piezo movement from -37 to -149 nm (*z* distance in Figure 2).[1] The resulting indentation is sufficiently large that the Hertz model for a sphere does not apply for standard AFM cantilevers. The Hertz model is written as[6]

$$F_{\text{cone}} = \frac{\pi}{2} \frac{E}{(1 - v^2)} \tan(\alpha)\delta^2 \quad (1)$$

$$F_{\text{sphere}} = \frac{4}{3} \frac{E}{(1 - v^2)} \sqrt{R}\delta^{3/2} \quad (2)$$

where E is the elastic modulus, v is the Poisson ratio of the soft material, δ is the indentation, α is the opening angle of the cone, and R is the radius of the sphere. The parameters used for the calculations are an opening angle of 30° and a Poisson ratio of 0.5, although anomalies such as negative Poisson ratios have been found at temperatures near the volume phase transition temperature.[20] The Hertz model for a cone is used for standard AFM cantilevers, while the model for a sphere is used for the colloid-modified cantilevers. The results of the calculation using a standard AFM cantilever are shown in Figure 3.

The same measurements were repeated with a colloid-modified cantilever, and the results are summarized in Table 2 for hydrogel layers with different cross-linking densities and ionizable comonomer concentrations. In the swollen state, the modulus is a function of both the cross-linking density and the ionizable comonomer concentrations, while the modulus in the collapsed state is primarily a function of the cross-linking density. Similar effects of the cross-linking density on the modulus have been seen for responsive hydrogel layers that are cross-linked in the swollen state by free-radical polymerization.[39] Bulk

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Figure 3. Temperature-dependent elastic modulus in DI water for PNIPAAm hydrogel layers with different degrees of cross-linking (■, NIPAAm2; ●, NIPAAm5b; ▲, NIPAAm10). The solid lines are to guide the eye.

Table 2. Modulus Values for Hydrogel Layers in DI Water

	modulus [kPa]	
	swollen (15 °C)	collapsed (42 °C)
NIPAAm2	4.5	490
NIPAAm5b	16	950
NIPAAm10	20	1300
AAmPA2	10	470
AAmPA5	12	470
AAmPA10	17	480
DMAAAm2	11	470
DMAAAm5	18	500
DMAAAm10	29	550[a]

[a] Because T_c for this sample is around 45 °C, the modulus in the collapsed state was measured at 50 °C.

modulus values for the photo-cross-linked hydrogels are not available, but the modulus is generally expected to decrease with increasing ionization.[25] This is an important factor when considering the effect of cross-linking and ionization on constrained hydrogel layers. The length scale of constraint has been shown to increase with both the increasing cross-linking density and the degree of ionization,[14] and the fact that both parameters result in an increased modulus for these materials helps explain these results. The effect of ionization on the hydrogel modulus can be explained by the samples being highly swollen relative to the reference state,[40] and these results have been described in greater detail in ref 14.

The modulus values are in good agreement with measurements on the same system using standard AFM cantilevers with a lower spring constant (see Figure 4). Previous reports have suggested that the sharp tip of an AFM cantilever will damage a soft hydrogel layer and that a colloid-modified cantilever should be used to minimize wear to the sample.[6] However, we have found that as long as the cantilever spring constant is chosen appropriately, both give comparable results. The measurements with the standard AFM cantilever tend to have more scatter, but the modulus values are in good agreement for repeated measurements on different spots on the sample surface. This is probably related to inhomogeneous network structures, relative to the size of the probe,[8−10] and the larger colloidal tip gives more reproducible results because it samples a larger area of the hydrogel surface.

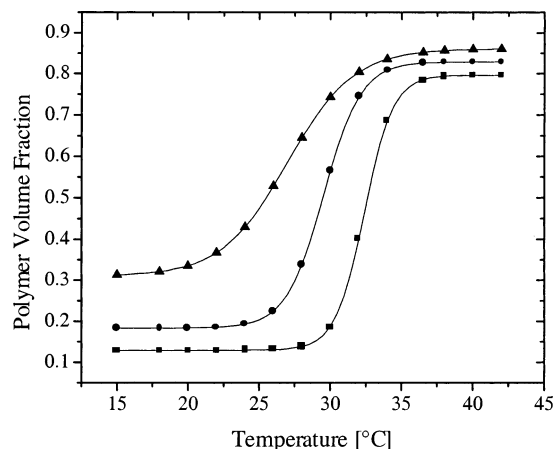(40) Ilavsky, M. *Polymer* **1981**, *22*, 1687−1691.
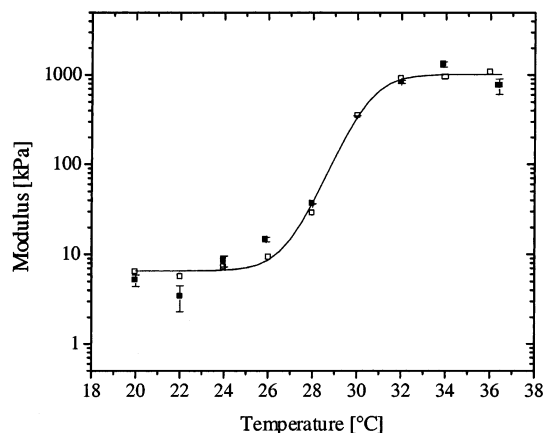
Figure 4. Comparison of modulus values measured with a standard AFM cantilever (■) with a lower spring constant and a colloidal cantilever (□) with a higher spring constant for the NIPAAm5a sample in water.

Discussion

Hydrogel Modulus. The modulus of a hydrogel layer can be related to the degree of swelling of the cross-linked network. By exceeding the volume phase transition temperature, the network collapses and the local stiffness increases by nearly 2 orders of magnitude (see Figure 3). The differences among the three samples depend on the polymer volume fraction as a function of temperature, and this will be discussed in greater detail in the following sections. As described by rubber elasticity theory of a swollen cross-linked network, the modulus of the network increases with the volume fraction of polymer and the concentration of elastically active network chains.[25] The working range of temperatures for the AFM heating stage is 5–50 °C, and a combination of SPR and OWS can be used to measure the refractive index and film thickness over this same range of temperatures. The refractive index is then used to calculate the polymer volume fraction,[41] and the degree of swelling is defined as a function of the film thickness.[31]

The transition temperature can be seen from the change in the modulus as a function of the temperature, as shown in Figure 3. The transition temperature decreases with increased photo-cross-linking content, as the DMIAAm chromophores make the network more hydrophobic. The logarithmic scale of the modulus values in Figure 3 makes it difficult to compare the width of the transition as a function of the DMIAAm content. However, the transition does become broader with increasing cross-linking density, which is predicted by Flory–Rehner theory for bulk hydrogels.[19] An analogous one-dimensional model has been used to describe the degree of swelling of constrained hydrogel layers in the swollen and collapsed states but fails to describe the discontinuity of the resulting volume phase transition.[37] However, the trend seen here is consistent with previous measurements using SPR and OWS.[31] The polymer volume fraction as a function of the temperature is calculated from the SPR and OWS data (see Figure 5) and also shows decreasing transition temperatures and broader transitions with increasing DMIAAm content. The volume fraction data in Figure 5 corresponds to the modulus measurements in Figure 3, and the differences in the modulus values for the three samples correspond to the polymer volume fraction as a function of the temperature. The modulus increases with

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Figure 5. Polymer volume fraction calculated from SPR and OWS measurements on the same system. The polymer volume fraction changes as a function of the temperature in DI water for PNIPAAm hydrogel layers with different degrees of cross-linking (■, NIPAAm2; ●, NIPAAm5b; ▲, NIPAAm10).

increasing polymer volume fraction, and this relationship can also be used to calculate the theoretical network modulus.

Comparison with Theory. The elastic modulus (E) of a swollen cross-linked network can be expressed through rubber elasticity theory as[25]

$$E = \left(\frac{RTn_e}{V_0}\right)\phi_2^{1/3} \tag{3}$$

where R is the gas constant, T is the temperature, n_e is the number of moles of elastically effective network chains in volume V_0, and ϕ_2 is the volume fraction of polymer. This approach compares the gel swelling to a reference state where the polymer chain conformation is assumed to be unperturbed. The reference state is assumed to be where the cross-links are formed, in this case the dry state. The volume fraction of polymer can be calculated from the refractive index, and the cross-linking density can be estimated from the mole fraction of the DMIAAm chromophore.

The temperature-dependent elastic modulus calculated from eq 3 was compared to that measured with the AFM force–distance curves. The values are of the same order of magnitude, but the measured values are consistently lower than those calculated from theory. The most likely difference lies in the calculation of n_e from the DMIAAm content of the linear polymers. The theoretical network assumes that every chromophore acts to form a cross-link, every network chain ends in a cross-link, and no loops or trapped entanglements exist. This is clearly not the case for hydrogel layers formed from photo-cross-linkable linear polymers because the free-radical polymerization of the linear DMIAAm copolymers determines the distribution of the cross-links. The cross-linking reaction also takes place below the glass transition temperature of the polymer, and the photo-cross-linking reaction may be inefficient under these conditions. Therefore, there is likely a high concentration of dangling chain ends in the actual network as well as other nonideal effects.

We can compare the calculated modulus to the actual modulus of the network to get an estimate of the fraction of dangling chain ends. The concentration of elastically active network chains (n_e) was, therefore, estimated from a fit to the modulus values measured with AFM. This is shown in Figure 6 for several different samples in deionized (DI) water at 15 °C; the solid line indicates the
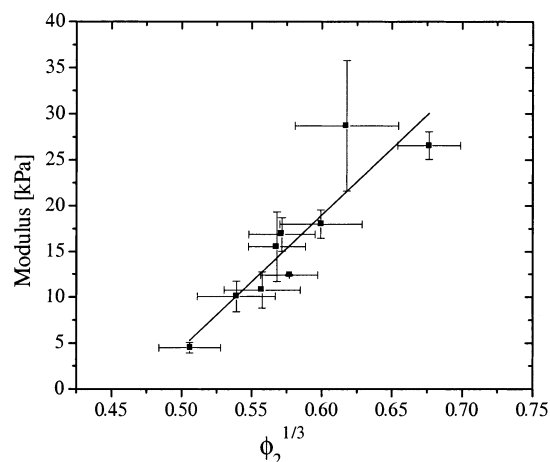
Figure 6. Elastic modulus of PNIPAAm hydrogel layers in water at 15 °C, which is well below the transition temperature for each of the samples. The samples have different cross-linking densities and ionizable comonomer concentrations. The linear fit is the modulus predicted by rubber elasticity theory for a swollen cross-linked network using 23% of the predicted concentration of elastically active network chains.

results from rubber elasticity theory with 23% of the calculated concentration of elastically active network chains. The samples have different cross-linking densities and degrees of ionization, and $23 \pm 3\%$ appears to be a reasonable estimate for all the samples in the swollen state, at temperatures well below the transition temperature of each sample. Because the modulus increases linearly with the concentration of elastically active network chains, this also corresponds well to the modulus values for the swollen network being about 23% of the modulus values calculated from theory. The fact that all the data points in Figure 6 fall on a single line demonstrates that rubber elasticity theory does describe the modulus of the photo-cross-linked network. Therefore, the difference between the two calculations shows that the cross-linking density of the network cannot be calculated directly from the DMIAAm content of the corresponding linear polymers. Instead, this technique provides a means to determine the effective cross-linking density and can be used to study the morphology of the hydrogel layer.

This same approach was used for the hydrogel layers in the collapsed state, and the modulus values are again lower than those predicted by theory. However, the data points do not all fit on one line, and the estimated concentration of elastically active network chains is different for the neutral and ionized hydrogel layers. The neutral networks are best fit with $89 \pm 9\%$ of the calculated concentration of elastically active network chains, while the ionized networks are best fit with $47 \pm 5\%$ of the calculated concentration of elastically active network chains (data not shown). Again, this corresponds well to the modulus values for the collapsed network being about 89 and 47% of the modulus values calculated from theory for the neutral and ionized hydrogel samples, respectively. The difference between the neutral and ionized hydrogel layers may be due to hydrophobic aggregation in the collapsed state that is disrupted by the presence of ionized groups. These aggregates can act as additional cross-links, increasing the effective cross-linking density and the resulting modulus of the hydrogel network.[42]

Conclusion

AFM was used to characterize photo-cross-linked hydrogel layers on the basis of NIPAAm and DMIAAm. The degree of swelling could be directly observed with AFM imaging, and the temperature-dependent elastic modulus was measured using force−distance curves. By choosing cantilevers with the appropriate spring constants, standard AFM cantilevers could be used with minimal damage to the swollen hydrogel layers. A colloid-modified cantilever was also used for modulus measurements, and the improved resolution is likely due to the inhomogeneous network structure, relative to the size of the tip. A combination of SPR and OWS was used to measure the polymer volume fraction as a function of the temperature, and the modulus increased with increasing polymer volume fraction. These results were compared to rubber elasticity theory for a swollen network, which was used to study the hydrogel morphology. The network appears to have a high concentration of dangling chain ends, which is to be expected from the photo-cross-linking of linear polymers, and hydrophobic aggregation makes a significant contribution to the mechanical properties of the collapsed gel network.

(42) Lowe, T. L.; Benhaddou, M.; Tenhu, H. *Macromol. Chem. Phys.* **1999**, *200*, 51−57.