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Elastic Properties of Poly(*N*-isopropylacrylamide) and Poly(acrylamide) Hydrogels Studied by Scanning Force Microscopy

T. R. Matzelle,*,† G. Geuskens,‡ and N. Kruse†

Chimie Physique des Matériaux, CP 243, and Chimie Macromoléculaire, CP 206/1, Université Libre de Bruxelles, B-1050 Bruxelles, Belgium

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ABSTRACT: Poly(*N*-isopropylacrylamide) (PNIPAAm) and poly(acrylamide) (PAAm) hydrogels of varying cross-linker concentration were characterized for their swelling behavior in water. Local elastic properties of these hydrogels were studied using atomic force microscopy (AFM) in a temperature-controlled liquid cell. Elastic moduli (Young's moduli) of both PNIMAAm and PAAm hydrogels were determined at different temperatures and varying cross-linker concentrations. Spherical probes (micrometer sized) as well as sharp conical probes were used in force vs displacement data acquisition. Young's moduli were determined by evaluating the indentation of the gel surface as a function of the probing load. On PNIPAAm hydrogel surfaces a dramatic increase of the stiffness (up to 10 times) was observed when crossing the phase transition at ∼33 °C. The cross-linker concentration has a strong influence on Young's modulus for temperatures above the critical temperature while only small variations were seen for temperatures below 33 °C. PAAm hydrogels show a different behavior: Young's moduli were found to be just slightly dependent on the temperature though the cross-linker concentration again exerts a strong influence.

Introduction

The physical properties of gels may drastically alter when changing the pH, temperature, salt concentration, etc., in aqueous solution. In particular, poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogel has attracted considerable attention as a thermoresponsive gel since it undergoes a reversible volume phase transition at around 33 °C, the "lower critical solution temperature" (LCST) of PNIPAAm homopolymer in water.[1] This behavior may find applications in various areas of biophysics and medicine such as in thermal switches, drug-release systems, micro/nanoactuators, or microvalves/pumps.[2−4] The realization necessitates a thorough understanding of the structural and micromechanical properties of such "intelligent" gels. With this background we have previously studied the friction behavior and the local elasticity of PNIPAAm hydrogel above and below the LCST.[5] Using scanning force microscopy (SFM), we have demonstrated that Young's moduli can be determined from the force-controlled indentation of soft samples (such as PNIPAAM hydrogels at low cross-linker concentration), using micrometer-sized spherical probes rather than conical tips. Values of the order of several kilopascals were found, similar to those known for soft biomaterials such as human platelets and marine bacteria.[6,7]

The present paper is aimed at revealing how different cross-linker concentrations and varying temperatures below and above LCST influence Young's moduli. Moreover, direct comparison will be made with the elastic properties of poly(acrylamide) (PAAm) hydrogel which shows no temperature-sensitive phase transition. To put our results in perspective, we also present measurements of the swelling ratio of both PNIPAAm and PAAm hydrogels. Respective data were published by Shibayama et al. and Muniz et al.[8,9]

Macroscopic measurements of the elasticity of PNIPAAm hydrogel were previously reported by Takigawa et al.[10] More generally, it seems that while a number of macroscopic studies were performed with various hydrogels over the past years, quantitative reports of the microrheology of materials are still quite scarce. Mahaffy et al.[11] have recently measured by SFM the viscoelastic properties of fibroblast cells and PAAm gels using a sinusoidal modulation technique of the indentation of spherical micrometer-sized polystyrene beads. A modified Hertz theory was used by these authors to determine the frequency-dependent storage and loss moduli of their samples.

Experimental Section

NIPAAm monomer was polymerized in aqueous solution in the presence of *N*,*N*′-methylenebis(acrylamide) (MBAAm) as cross-linker for 12 h at 20 °C using ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) as redox initiator system. The concentration of NIPAAm in the pregel solution was always 1 mol L^{-1}, that of APS 10 mmol L^{-1}, and that of TEMED 4 mmol L^{-1}. PAAm gels were prepared under similar synthesis conditions, but the monomer concentration was always 5 mol L^{-1}.

The gelation was carried out between two freshly cleaved mica sheets of 20 mm × 20 mm size using a Teflon spacer (thickness about 2 mm). After careful removal of the mica sheets, the gels were subsequently immersed into Millipore-purified water to wash out excess monomers and residual chemicals.

The swelling ratio Q was determined by relating the weight of the water-swollen gel at equilibrium to the weight of the dry hydrogel. The former was measured with a microbalance while the latter was calculated from the total dry monomer content. Samples subjected to measurements of the swelling ratio were subsequently used for SFM. Force vs distance measurements were carried out on small pieces (about 7 × 7 mm in size) cut from samples and fixed by metal clamps on a round metal disk.

The local elasticity of PNIPAAm and PAAm gels was probed with a commercial SFM (TMX 2100 Explorer, TM Microscopes, Sunnyvale, CA). V-shaped Cantilevers (type Ultralever of the same distributor) with a nominal spring constant of 0.4 N m^{-1}

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† Chimie Physique des Matériaux, CP 243.

‡ Chimie Macromoléculaire, CP 206/1.

* Corresponding author: phone +32 2 650 3672; Fax +32 2 650 5708; e-mail matzelle@ulb.ac.be.

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were used for all experiments. The calibration of the Ultralever springs using the “reference-spring method” revealed an accuracy of 0.08 N m^{-1}.[12]

An Ultralever type cantilever with a conically sharp Si_3N_4 tip of about 3 μm length was characterized by field emission scanning electron microscopy (FESEM) to reveal a half opening angle of approximately 12.5° and a radius of about 15 nm. A standard Ultralever was also modified by attaching a small silica sphere (nominally 5 μm in diameter) to their extremity. FESEM has shown the silica sphere surface to be sufficiently smooth for SFM indentation experiments.[5,13]

All force vs displacement measurements were performed in water using a specially designed cell providing temperature control and *xy* positioning with a precision of 200 nm. A Peltier element in the liquid cell allowed to set the temperature to any value between 0 and 50 °C with an accuracy of ±0.05 °C. To avoid evaporation of the liquid, the space between cell edging and the SFM scanner was sealed by a piece of ParafilmM (Pechiney, France). Following this technique, the temperature could be kept constant over hours.

Young’s moduli were evaluated following the method of Radmacher et al.[14] Accordingly, the surface indentation was determined by subtracting the cantilever deflection measured for a soft material, i.e., either NIPAAm or PAAm hydrogels, from that measured for a hard material such as mica (as in our case). Quite generally, the force vs displacement curve of a hard surface shows a linear slope as soon as probe touches the surface so as to result in a cantilever bending proportional to the *z*-piezo movement of the sample surface. On the contrary, the probe indents the surface of a soft material, and thus, the cantilever bending will be smaller and probably not strictly proportional to the *z*-piezo movement. The indentation is best displayed as a function of the load (rather than of the *z*-piezo movement), which can be obtained by multiplying the cantilever deflection by the cantilever force constant. The experimental data plotted in this way allowed to be fitted by the Hertz model[15] which predicts a power law $\delta - f$ relation depending on the geometrical form of the probing body. Using a spherical silica particle (see above) in studies with NIPAAm hydrogel, this relation reads

$$\delta = \left(\frac{3}{4}\,\frac{1-\nu^2}{E}\,\frac{f_{\mathrm{sphere}}}{\sqrt{R}}\right)^{2/3} \qquad (1)$$

where R, ν, and E are the radius of the silica sphere, the Poisson ratio, and the local elastic modulus, in order of mention. For a conical tip the relation modifies to

$$\delta = \sqrt{\frac{2}{\pi}\,\frac{1-\nu^2}{E}\,\frac{f_{\mathrm{cone}}}{\tan\alpha}} \qquad (2)$$

with symbols having the same meaning as in (1) and α being the half opening angle of the tip apex as determined by FESEM (see above). PAAm hydrogel was probed for its elastic properties using Si_3N_4 tips rather than silica spheres. (The higher stiffness as compared to NIPAAm hydrogel necessitated higher local pressures to cause significant indentation.)

The accuracy of the E values depends basically on the accuracy of the cantilever force constant, the probe geometry, the loading forces, and the zero point of contact. The uncertainty of the probe geometry plays a minor role as it can be judged with high precision from electron micrograph. The inaccuracy of the point of contact between probe and surface usually increases when probing softer surfaces. However, this zero point of contact could be determined within some 10 nm in the case of our softest hydrogel samples. As cantilevers bend slightly as a result of temperature fluctuations, the loading force may vary over time. Experience shows that the typical drift in the loading force is in the order of some nN/min, depending on the time given to thermally equilibrate the system. In our case the time scale of a measurement was 1 s or less; that is why the influence of the drift can be regarded to be of minor importance. Probably the most crucial point is the inaccuracy of the cantilever force constant. Cantilevers as used for our measurements revealed a force constant of 0.4 ± 0.08 N/m.

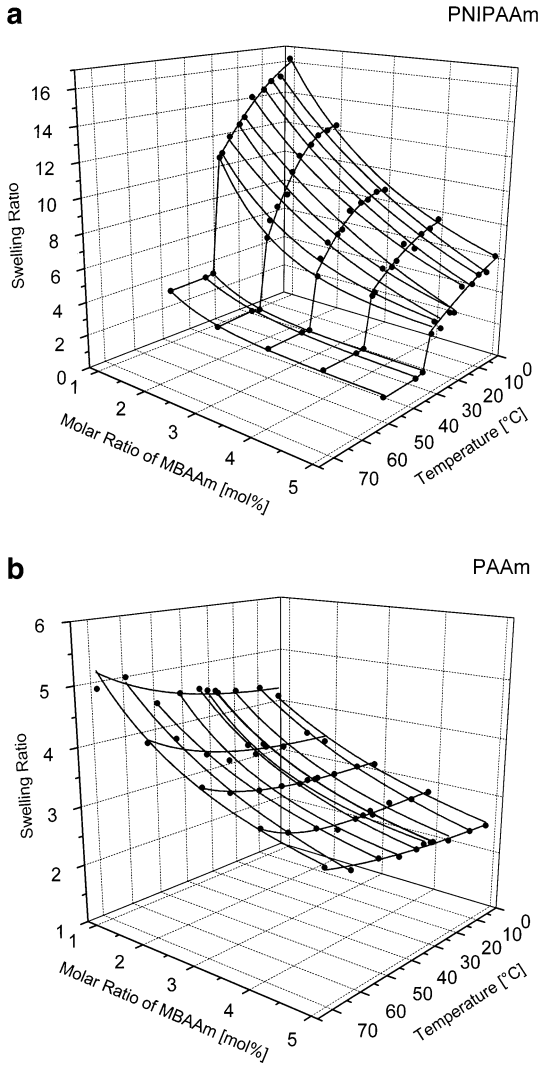

Figure 1. (a) Swelling ratio *Q* of PNIPAAm hydrogel in pure water as a function of the MBAAm content and the temperature. (b) Swelling ratio *Q* of PAAm hydrogel in pure water as a function of the MBAAm content and the temperature.

Results and Discussion

Swelling Ratio of PAAm and PNIPAAm Hydrogels. Figure 1a,b illustrates the temperature dependence of the swelling ratio, *Q*, of PNIPAAm and PAAm hydrogels containing varying concentrations of cross-linker (MBAAm) in the temperature range 2.5−50 °C.

Each data point represents the average of at least three measurements. The values for *Q* were plotted as fitted scatter graphs depending on both parameters, the temperature and the cross-linker content. As expected, a higher cross-linking density in both the PNIPAAm and the PAAm network results in a decrease of *Q* because the polymer chains are more strongly prevented to move apart in water. It should be noted that below the LCST of PNIPAAm the swelling ratio of PNIPAAm hydrogels is always larger than that of PAAm hydrogels with the same cross-linking density. This is mainly because the concentration of NIPAAm monomer in the pregel solution was lower than that of AAm monomer (5 mol L^{-1}). For solubility reasons the concentration has to be kept at ≤1 mol L^{-1}. It was shown previously[16] that the

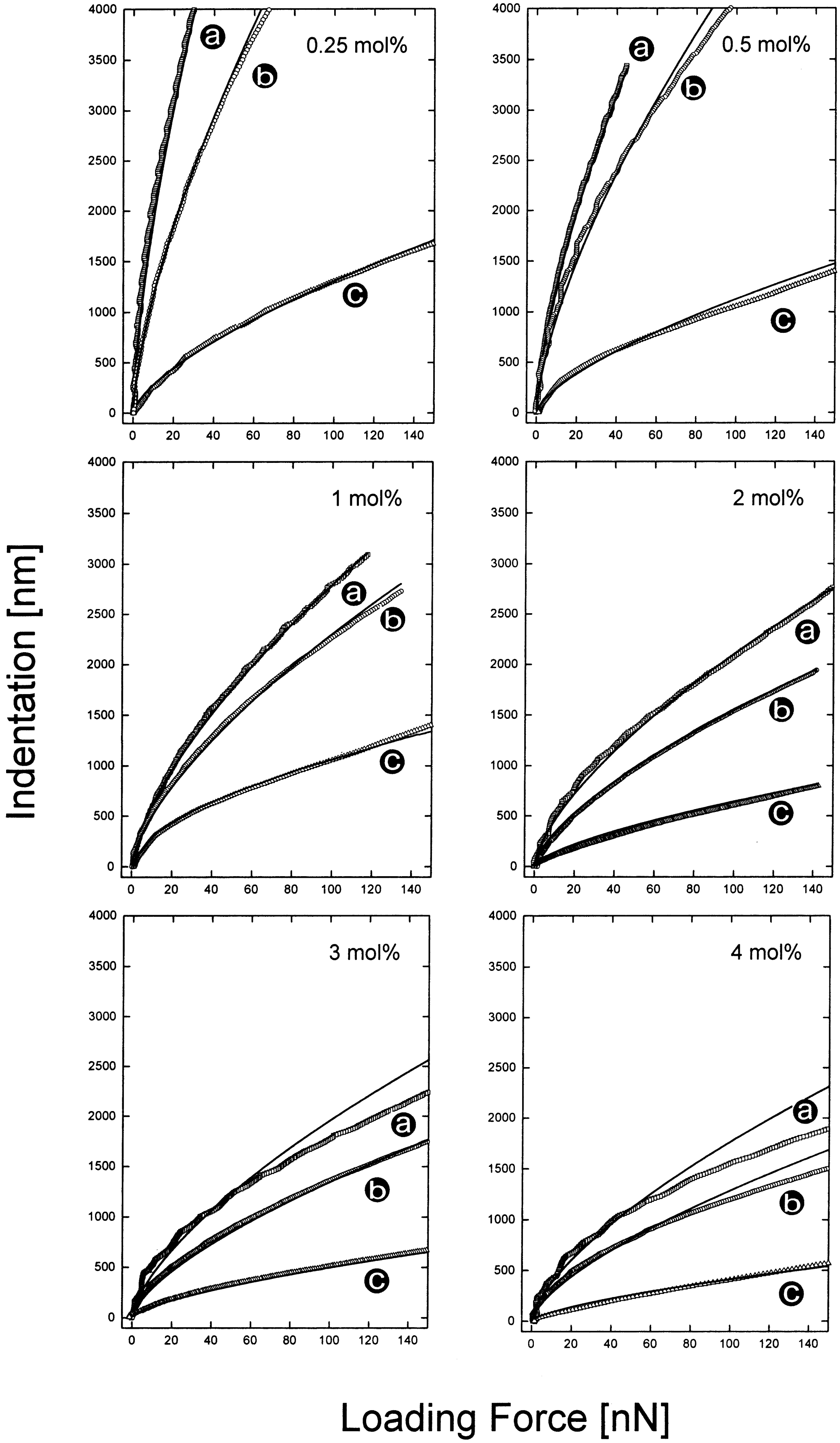

Figure 2. Indentation in PNIPAAm hydrogel as a function of the load in dependence of the temperature at different cross-linker concentrations. Experimental data are represented by symbols whereas solid lines correspond to the Hertz model assuming a sphere indenting a flat surface.

swelling ratio is lower when preparing the hydrogel from a more concentrated monomer solution because chain transfer to the polymer during polymerization is more likely in a more concentrated solution. Chain transfer to the polymer first results in branching but subsequently in cross-linking (in addition to the effect of MBAAm). Moreover, intramolecular reactions leading to cyclization are more likely than competing cross-linking in less concentrated solutions. As already observed previously by Muniz et al.,[9] the swelling ratio of PAAm hydrogels increases slightly with temperature because of the increasing mobility of polymer chains. On the other hand, the swelling ratio of PNIPAAm hydrogels below the LCST decreases slowly at first and abruptly when reaching this temperature. This is a consequence of the progressive destruction of the hydrogen bonds between polymer and water, finally resulting in a phase transition at the LCST of PNIPAAm. As can be seen from Figure 1**,** both the decrease and the subsequent drop of *Q* values are more pronounced for lower cross-linker densities. Above the LCST the influence of the temperature on the swelling ratio of PNIAAm hydrogel is rather weak. The cross-linker concentration still plays a role that is, however, less strong than that observed below the LCST.

Local Elastic Properties of PNIPAAm Hydrogels. Figure 2 shows indentation vs load curves obtained below and above the LCST of PNIPAAm, i.e., in the swollen ($a = 10$ °C, $b = 20$ °C) and collapsed ($c = 40$ °C) state at different mole ratios of the cross-linking agent. Plots were calculated from force measurements as described in the Experimental Section. All measurements refer to samples equilibrated thermally during several hours after each temperature increment and are fully reproducible when probing different surface regions under identical conditions. When plotting the probe indentation against the loading force on the cantilever, a nonlinear response is observed which is due to an increase in surface compliance under higher loads leading to larger contact areas of the probe−surface interaction. The data were fitted to the Hertz model for a hard spherical probe indenting a flat surface (eq 1).

The presence of adhesion forces between the indenting sphere and the gel surface was neglected in view of the loads which amounted up to 300 nN. For each indentation curve Young's moduli were determined from the best fit to the experimental data using eq 1. Parameters of the Hertz equation were $R = 3$ μm and $\nu = 0.5$ along with a cantilever force constant of 0.4 ± 0.08 N/m in each case. As can be seen in Figure 2, the agreement between model and experiment is excellent except when probing the more highly cross-linked hydrogels at high loads. Under these conditions the samples tend to be more rigid than predicted. This behavior is reminiscent of "strain hardening" in rubbers where large strains can result in partial and reversible crystallization as the polymer chains are forced close together. Of course, in hydrogels no crystallization can occur, but large loading forces in the more highly cross-linked hydrogels could increase physical interactions through hydrogen bonds between pendant groups, leading to higher moduli.

In the case of the PNIPAAm hydrogel cross-linked with 0.25 mol % MBAAm, Young's moduli obtained according to the above procedure are 0.33 kPa in the swollen state at 10 °C and 13.9 kPa in the collapsed gel at 40 °C. Thus, the local stiffness is about 42 times higher in the collapsed state than in the swollen state at this temperature. All experimental *E* values are shown in Figure 3. Uncertainties due to the force constant of the cantilever and due to the zero point of contact are taken into account by error bars on the graph.

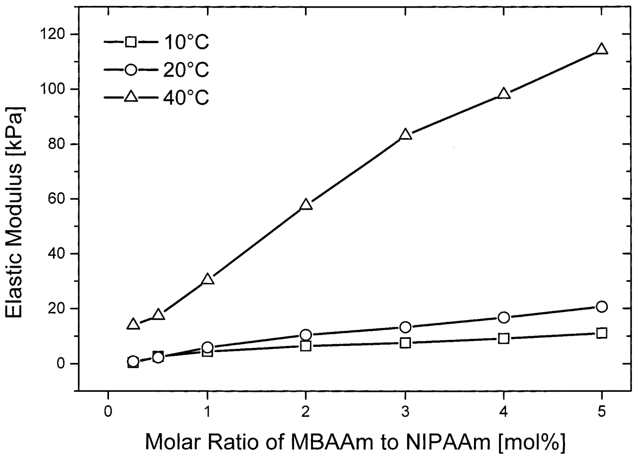

Figure 3. Young's moduli *E* of water-swollen PNIPAAm gel in dependence of the cross-linker content at three different temperatures as revealed by SFM using a glass sphere as probe.

It is noteworthy that the influence of cross-linking density on the modulus is much larger in the collapsed state than in the swollen state. This could be related, as already suggested above, to increasing physical interactions between pendant groups as the cross-linking density increases, resulting in additional physical cross-links.

Local Elastic Properties of PAAm Hydrogels. Water-swollen PAAm gels were observed to be much stiffer than PNIPAAm gels. This, in agreement with a lower swelling index, is mainly due to the fact that the concentration of AAm monomer in the pregel solution was larger than that of NIPAAm. With this background it is evident that a significant indentation could not be reached using a silica particle sphere with loading forces up to 300 nN. Instead, a Si_3N_4 tip was employed in order to increase the local pressure for indentation of the PAAm gel. Again, experimental data were fitted using the Hertz theory, and *E* was adjusted to give the best fit.

A series of indentation plots were obtained after swelling the sample to equilibrium in a water bath. The results are shown in Figure 4.

Young's moduli determined according to the above procedure range from 90 to 465 kPa for cross-linker concentrations between 1 and 5 mol %. This increase corresponds to a factor of about 5. For comparison, a similar influence is found for PNIPAAm hydrogels in the collapsed state (see above): for the same variation in the cross-linker concentration an increase by a factor of about 4 is obtained while keeping the sample at 40 °C. The influence is less strong for the same sample in the swollen state at 10 °C but increases as the temperature rises. Again, increasing physical interactions through hydrogen bonds between pendant groups can be invoked to explain the result. All the results are collected in Figure 5. The error bars in the graph reflect the uncertainty in the cantilever force constant and the zero point of contact.

We have also investigated the temperature dependence of Young's modulus for various MBAAm concen-

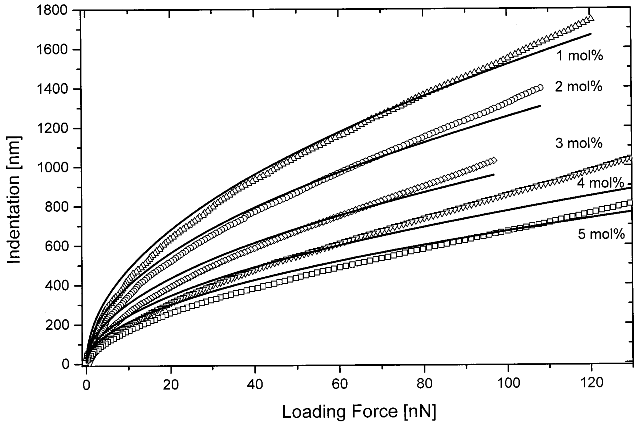

Figure 4. Indentation in PAAm hydrogels as a function of the load in dependence of the cross-linking density ($T = 20$ °C). Experimental data are represented by symbols whereas solid lines correspond to the Hertz model assuming a cone indenting a flat surface.

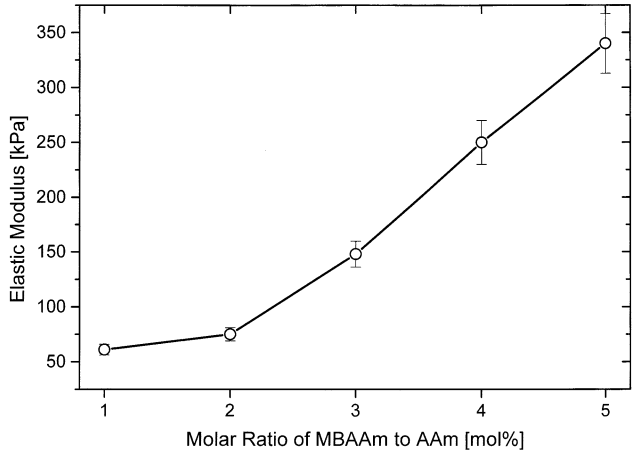

Figure 5. Young's moduli E of water-swollen PAAm gel surfaces at 20 °C in dependence of the cross-linker concentration.

trations in PAAm hydrogels. As to be expected, this dependence proves to be much less pronounced due to the absence of a phase transition in PAAm hydrogel. Moreover, rather than to increase the local elasticity is found to decrease slightly from 93 to 84 kPa as the temperature rises from 10 to 50 °C. For the sake of clarity, we have not included these data in Figures 4 and 5. In macroscopic measurements the influence of the temperature is somewhat more distinct (but of the same sign): the elastic modulus varies between 89 and 75 kPa for temperatures ranging from 25 to 40 °C, respectively.[9] We do not consider important the difference in the absolute E values obtained in macroscopic and microscopic rheological measurements of PAAm hydrogels. Slight variations in the sample preparation can easily result in such deviations. On the other hand, one might have expected local variations in the moduli when probing different areas of the surface with a conical tip. Since this is not the case, we conclude that the PAAm sample response to stress is not determined by the local structure of the polymer network but rather involves "collective" properties of the sample. This point needs, however, more detailed experimental consideration as, for example, by using a dynamic nanorheological method similar to the microrheological method proposed by Mahaffy et al.[11]

Finally, we mention that it is difficult to compare our (local) measurements with those of Takigawa et al.[10] Although the trend in Young's moduli of PAAm hydrogels is similar to our measurements, differences remain with regard to the temperature effect. This may originate from the very different molar ratio of monomer and cross-linker during their gel synthesis. As shown in the present paper, the elastic moduli vary quite strongly with the gel preparation conditions. For the same reasons a comparison with the data provided by Mahaffy et al.[11] is not possible.

Conclusion

SFM force vs displacement measurements were carried out on PNIPAAm and PAAm gels in dependence of the water temperature and the concentration of cross-linking molecules in the synthesis solution. The elastic properties of thermosensitive PNIPAAm gel were compared with those of the PAAm gel in terms of Young's moduli. The results show that SFM allows to evaluate quantitatively Young's moduli of gels with high precision. Indentation vs load curves confirm in good agreement with macroscopic measurements that the phase transition occurring at $T_{\mathrm{LCST}} \sim 33$ °C exerts a strong influence on the mechanical properties of the PNIPAAm network. The E values of both PNIPAAm and PAAm hydrogels show a significant dependence on the concentration of the cross-linking agent. Thus, the cross-linking density is considered to have always a major contribution to the gel elasticity.

The methodological approach by SFM has thus been proven to be reliable, allowing micro- or even nanorheological measurements of delicate materials such as soft hydrogels for which the elastic moduli are comparable or even lower than those of biological cells. An extension to frequency-modulated indentation measurements is considered an interesting perspective for the local probing of nonlinear dynamic effects of polymer chains in hydrogels with varying cross-linker concentration.

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