Polymer 51 (2010) 3653–3659

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Influence of pressure on the state of poly(*N*-isopropylacrylamide) and poly(*N*,*N*-diethylacrylamide) derived polymers in aqueous solution as probed by FTIR-spectroscopy

Matthias Pühse [a], Martina Keerl [b], Christine Scherzinger [b], Walter Richtering [b], Roland Winter [a,*]

[a] Faculty of Chemistry, Physical Chemistry I, TU Dortmund University, Otto-Hahn-Str. 6, D-44227 Dortmund, Germany
[b] Institute of Physical Chemistry, RWTH Aachen University, Landoltweg 2, D-52056 Aachen, Germany

ARTICLE INFO

Article history:
Received 4 May 2010
Received in revised form
1 June 2010
Accepted 3 June 2010
Available online 11 June 2010

Keywords:
High pressure
FTIR spectroscopy
Microgels

ABSTRACT

We investigated the hydration and swelling properties of poly(*N*-isopropylacrylamide) and poly(*N*-isopropylacrylamide)-poly(*N*,*N*-diethylacrylamide) derived microgels by Fourier transform infrared- (FTIR-) spectroscopy in a wide region of the temperature–pressure plane. These systems are known to show a swollen-to-collapsed-transition upon temperature elevation. Our data reveal that pressure favours the swollen, hydrated state over the collapsed state in all systems investigated. A detailed analysis of the fractions of the respective IR sensitive amide-I′-subbands allowed the calculation of ΔG^{o} and ΔV^{o} for the pressure-induced swelling process as well as evaluation of various intra- and intermolecular hydrogen bonding connectivities in the different systems. In fact, considerable differences exist between different polymer or microgel types with regards to their hydrogen bonding pattern as a function of temperature and pressure, and the microgels may even exhibit a biphasic swelling behavior. Notably, the thermodynamic parameters derived reveal to be in the same order of magnitude as measured for the pressure and cold denaturation of proteins.

1. Introduction

Polymers consisting of certain acrylamide derivates like poly(*N*-isopropylacrylamide) (PNIPAM) or poly(*N*,*N*-diethylacrylamide) (PDEAAM) show upon heating a phase transition from a relatively hydrophilic, swollen and stretched conformation to a relatively hydrophobic, compact, globular state, i.e., exhibit thermoresponsivity [1–5]. Above this so-called lower critical solution temperature (LCST, 32 °C in the case of PNIPAM), the polymer precipitates, which manifests itself in an enhanced turbidity or even flock formation (cloud point). The transition is endothermic and hence entropy driven, as the release of bound water around the hydrophobic isopropyl- and/or diethyl-residues causes an increase of the entropy of the total system. These properties render such polymer systems a simplified model system for the cold denaturation of proteins [6]. Proteins from mesophilic organisms generally unfold under the release of heat at subzero temperatures [7,8]. The necessary liquid state of water can only be maintained by means of high hydrostatic pressure (HHP) or by supercooling of small volumes. Polymers like PNIPAM are hence easier to handle and provide the additional advantage that the whole phase transformation can be understood purely in terms of hydrogen bonding patterns, which is not possible in proteins or peptides, as the secondary structure changes hide this information upon IR-analysis [9].

Upon incorporation of a crosslinker, the linear polymers form hydrogels, which can be divided in macro- and microgels [1]. Microgels possess radii from ~10 to ~1000 nm and might be physically or, as in our case, chemically crosslinked. The gels adopt the stimulus-sensitive properties of the linear polymers, and change their physical state according to their monomer composition, by changes in temperature, pH, light exposition or electric fields, which implicates numerous potential applications in microtechnology and biomedicine [10]. Particularly interesting is for example the use of hydrogels for the stimulus-sensitive release of therapeutically active substances [11].

Cross-linking induces some changes in the physical characteristics of the microgels in comparison to the linear polymers. The phase transition temperature (volume phase transition temperature, VPTT) changes only slightly compared to the LCST of linear polymers, whereas the transition itself becomes much broader due to the increasing subchain length inhomogeneity with increasing crosslinker content, as revealed by dynamic light scattering (DLS) analysis, and the swelling capacity decreases drastically with

* Corresponding author.
E-mail address: roland.winter@tu-dortmund.de (R. Winter).

0032-3861/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2010.06.011

Table 1
Assignment of the amide-I′-subbands to the different hydrogen bonding patterns of polyacrylamide polymers. The exact band positions may vary in dependence of the polymer system and the physical conditions.

Band assignment	Wavenumber/cm^{-1}
Intramolecular H-bonds from carbonyl groups to amide-deuterons	1642–1652
Carbonyl groups in mixed hydrophobic/hydrophilic environment[a]	1617–1628
Intermolecular H-bonds from carbonyl groups to D_2O (sometimes two bands)	1580–1612

[a] i.e., where both hydrophobic and hydrophilic groups are present in the direct neighbourhood of the C = O groups.

increasing crosslinker content [12,13]. Small-angle neutron scattering (SANS) experiments also show that the crosslinker density is not equally distributed throughout the microgel particles, it is rather high in the particle core and decreases radially to the periphery [14].

So far, most of the high pressure studies on PNIPAM or PNIPAM-derived hydrogels were performed by measuring the cloud point via turbidimetry under HHP or by DLS and high pressure microscopy [6,15–19]. Although the solution behavior of such temperature sensitive polymers has been investigated by FTIR spectroscopy at ambient pressure [20–23], currently there is only one high pressure FTIR-study for linear PNIPAM, considering the behavior at ambient temperature [9]. Here, we present the first FTIR-spectroscopic investigation of the pressure-temperature behavior of linear PNIPAM and different PNIPAM-PDEAAM-based microgels covering a wide region of the *p*, *T* phase space.

2. Materials and methods

2% (w–v) solutions (in D_2O) of linear PNIPAM, crosslinked PNIPAM microgel (1.5% (w–w) BIS), crosslinked NIPAM-*co*-DEAAM copolymer microgel (45 mol-% NIPAM and 55 mol-% DEAAM with 6% (w–w) BIS as crosslinker) were used in this study. The microgel synthesis was performed as described previously [24,25]. Temperature dependent measurements were performed using a Nicolet 5700 FTIR spectrometer (Thermo Fisher Scientific, Waltham, USA) with a liquid-nitrogen cooled MCT detector (HgCdTe). Around 30 μL of the respective polymer solution was filled between CaF_2 windows, which were separated by a 50-μm thick Mylar spacer. For temperature regulation, the cell was placed in a thermostatized jacket with internal temperature measurement. An external water bath was used for temperature control (accuracy: ± 0.2 °C). The sample chamber was purged constantly with dry air. Spectra were recorded from 4000 to 1100 cm^{-1}. To obtain accurate peak positions and to minimize spectral noise, 256 spectra were summed up (spectral resolution: 2 cm^{-1}). Apodization was done

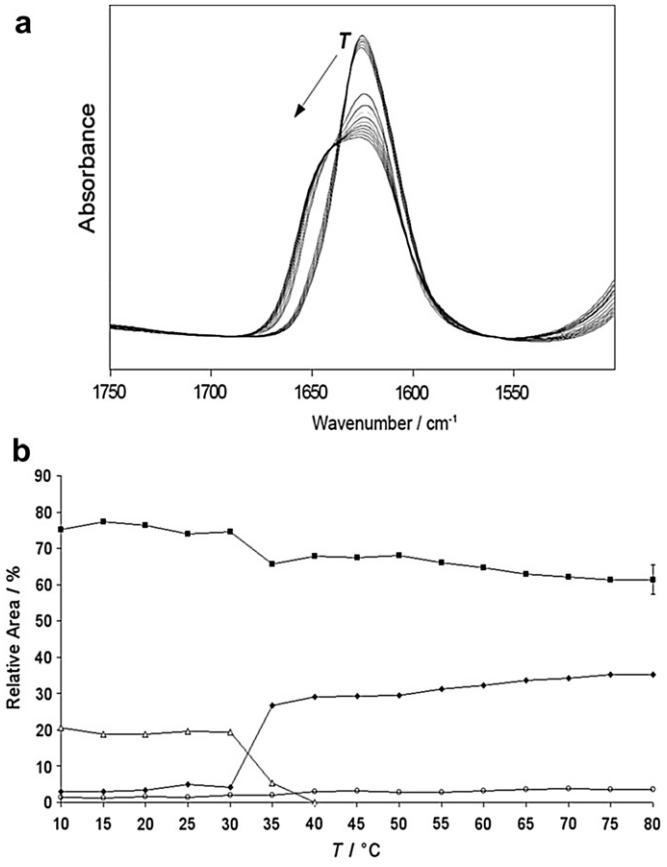

Fig. 1. a) Normalized amide-I′-FTIR-spectra of linear PNIPAM from 10 to 80 °C at ambient pressure. The black arrow indicates the development of spectra with increasing temperature. b) Temperature dependence of the relative areas of the diagnostic amide-I′-bands of linear PNIPAM. Symbol denotation: ◆ intramolecular hydrogen bonds (~1652 cm^{-1}), ■ carbonyl groups in a medium hydrophobic environment (~1626 cm^{-1}), △ hydrogen bonds to D_2O I (~1606 cm^{-1}) and ○ hydrogen bonds to D_2O II (~1583 cm^{-1}). The error bar indicates the fitting uncertainty, as determined from different measurements and is similar for all data points.

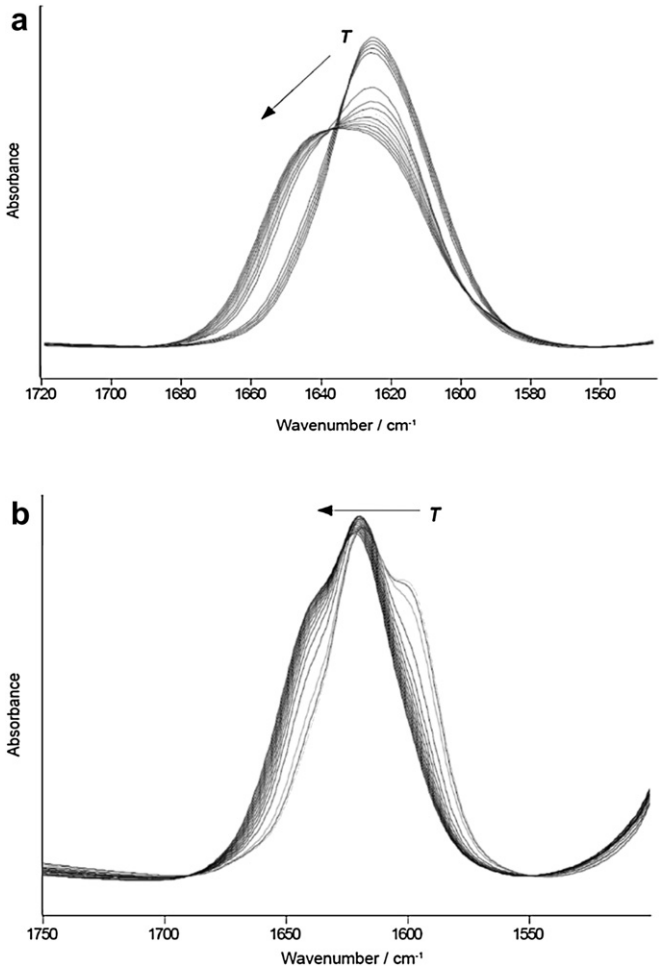

Fig. 2. a) Normalized amide-I′-FTIR-spectra of PNIPAM-microgels from 10 to 80 °C at ambient pressure. The black arrow indicates the development of spectra with increasing temperature. b) Normalized amide-I′-FTIR-spectra of NIPAM-DEAAM (45–55 mol%) copolymer microgels from 10 to 80 °C at ambient pressure. The black arrow indicates the development of spectra with increasing temperature.

with a Happ–Genzel function. Data acquisition was performed every 5 °C from 10 to 80 °C. The temperature equilibration time before spectra recording was 15 min.

The pressure dependent measurements were conducted at 10, 20, 30, 40 and 50 °C with a Nicolet MAGNA 550 FTIR spectrometer (Thermo Fisher Scientific, Waltham, USA). Pressure generation was achieved with a thermostatted High Pressure Diamond Optics P-series diamond anvil cell with type IIa diamonds (High Pressure Diamond Optics Inc., Tucson, USA). A 50 μm thick gasket of stainless steel with a 0.45 mm drilling was placed between the two diamonds holding ~10 nL of the sample. Pressure was determined with co-added $BaSO_4$, which exhibits a characteristic pressure sensitive symmetric sulphate stretching mode around 983 cm^{-1} that increases linearly with pressure [26]. All other technical parameters were identical to the temperature dependent measurements.

Spectral processing and deconvolution of the amide-I′-band was performed with the GRAMS/AI 8.0 software package (Thermo Fisher Scientific, Waltham, USA). After subtraction of noise and the temperature-corresponding background (D_2O), all spectra were normalized to the same area to ensure comparability. Finally, the amide-I′-region was fitted with the number of subbands (which are typical for distinctive hydrogen bonding patterns) detected by the 2nd derivative using a Voigt-function. The subbands could be assigned to the following hydrogen bonding patterns (Table 1 [27]).

The slightly different intensities of amide-I′ subbands at ambient pressure observed for the different sample cells used may derive from the fact that the background correction in the diamond anvil cell is less accurate, but may also be due to the different surface properties and surface-to-volume ratio of the sample cell materials used for the temperature (CaF_2) and pressure (diamond) dependent studies, respectively. As relative changes of conformational states are essentially discussed only, these differences are of minor importance.

3. Results and discussion

3.1. Temperature dependent FTIR-measurements at ambient pressure

As already mentioned above, aqueous solutions of linear PNIPAM have been investigated by FTIR spectroscopy before, and the amide-I′-spectrum of PNIPAM at 20 °C and 1 bar can be

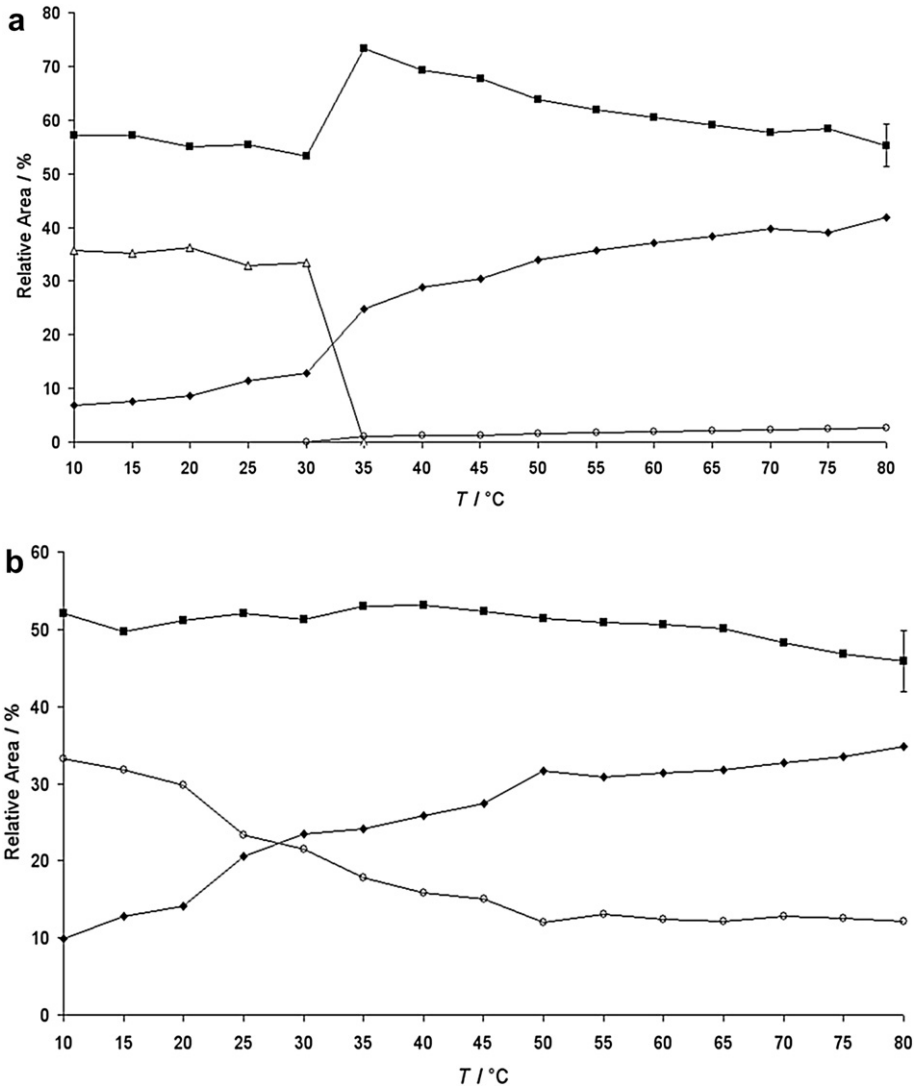

Fig. 3. a) Temperature dependence of the relative areas of the diagnostic amide-I′-bands of PNIPAM-microgels. b) Temperature dependence of the relative areas of the diagnostic amide-I′-bands of NIPAM-DEAAM (45–55 mol%) copolymer microgels. For symbol denotation see Fig. 1. The error bar indicates the fitting uncertainty, as determined from different measurements and is similar for all data points.

deconvoluted to obtain the main diagnostic bands located at ~1652 cm^{-1}, ~1626 cm^{-1}, ~1606 cm^{-1} and ~1583 cm^{-1}, which, in accordance to Table 1, can be assigned to intramolecular hydrogen bonding of carbonyl groups to amide deuterons, carbonyl groups in a medium hydrophobic environment and to hydrogen bonding of carbonyl groups to the solvent D_2O (the latter both), respectively. The bands indicative for hydrogen bonding to the solvent and for carbonyl groups in a medium hydrophobic environment are dominant, as PNIPAM is in the swollen, coil-like state below 32 °C. As reference to the pressure dependent spectra that will be discussed below, Fig. 1a illustrates the development of the normalized amide-I′-spectra upon heating, whereas the relative areas of the detected subbands, which represent the respective relative parts of the total hydrogen bonding pattern, in dependence of temperature at ambient pressure are displayed in Fig. 1b. As mentioned above, the bands at ~1626 cm^{-1} and at ~1606 cm^{-1} are dominant below 30 °C, indicating a rather hydrophilic, coil-like state. Between 30 and 35 °C, the band at 1606 cm^{-1} shows a steep decrease and eventually vanishes at ~40 °C. Also, the relative fraction of the band at ~1626 cm^{-1} decreases, whereas the band at ~1652 cm^{-1}, representing intramolecular hydrogen bonds, shows a steep increase. The midpoint of the transition appears at 32–33 °C, in excellent agreement with the location of the cloud point. The positions of the subbands change only slightly with increasing temperature. Hence, the spectral data reflect nicely the coil to globule transition and confirm previous results [9,27].

In contrast to the nearly discontinuous phase transition of linear PNIPAM, PNIPAM-microgels show a different behavior (Figs. 2a and 3a). The swollen-to-collapsed state transition, which is reflected by the increase of the subband-fraction indicative for intramolecular hydrogen bonds, is less steep and more continuous, whereas the subband responsible for intermolecular hydrogen bonds to the solvent shows a similar steep decrease as in the case of the linear PNIPAM. Such behavior is known from DLS-measurements and due to the well known greater inhomogeneity of the subchains in crosslinked gels which leads to a broadening of the phase transition that becomes more accentuated when the crosslinker content increases (see above). This effect can be nicely seen in the microgel system consisting of 45 mol-% NIPAM and 55 mol-% DEAAM with 6% (w–w) BIS as crosslinker (Figs. 2b and 3b). Here, only a broad, continuous transition between 10 and 50 °C is visible, although DLS-measurements report a remarkably low VPTT of 21 °C, presumably due to strong hydrogen bonding between the two different repeating units at nearly equimolar composition [25,27,28].

By comparing the general hydrophilic/hydrophobic properties of the linear PNIPAM with the microgels, a considerably greater hydrophilicity of the microgels could be seen below the VPTT in the case of the PNIPAM-microgels and also above the VPTT in the case of the NIPAM-DEAAM copolymer gel. A potential reason for this observation is the presence of the hydrophilic BIS-crosslinker, which mediates additional hydrogen bonds to the solvent, as well as the generally reduced shrinking capacity of crosslinked gels in comparison to linear polymers.

3.2. Pressure dependent FTIR-measurements

Upon application of high hydrostatic pressure at constant temperature, all investigated polymer systems show an inverse behavior when compared to temperature elevation. Fig. 4 a,b shows representative FTIR-spectra of the pressure dependent measurements of linear PNIPAM at 20 °C (below the LCST) and at 50 °C (above the LCST), respectively. The corresponding hydrogen bonding pattern analyses after deconvolution are displayed in Fig. 5 a,b. At 20 °C, no changes occur up to ~1500 bar. Above this pressure, the fractions of the subbands representing hydrogen bonds to D_2O increase slowly, whereas the relative area of the band indicative for carbonyl groups in a medium hydrophobic environment decreases concomitantly up to ~6000 bar, and both stay nearly constant up to the maximal pressure of ~9000 bar (ice VI-formation in bulk water occurs above ~8500 bar [29]). The relative fraction of the subband for intramolecular hydrogen bonds remains essentially constant. Measurements at 10 and 30 °C exhibit similar results (not shown).

At 50 °C, i.e., above the LCST, the spectral changes are much more pronounced and start already between 1 and 500 bar. Up to ~5400 bar, the relative fraction of the subband for intramolecular hydrogen bonds has decreased from ~50 to 17% and the relative fraction of the subband for carbonyl groups in medium hydrophobic environment has increased from ~50 to ~79%. Above ~5400 bar, a new band for hydrogen bonding to D_2O emerges at 1612 cm^{-1} and starts to increase together with the other band for hydrogen bonding to the solvent (at ~1595 cm^{-1}), up to the maximal pressure of 11 400 bar. In this upper pressure range, a further slight pressure-induced increase in swelling of the microgel is observed, only. Similar results are observed for 40 °C (data not shown). Altogether, according to the FTIR-data, HHP favours the formation of a more hydrated, coil-like state.

Assuming a two-state-model for the transition at 50 °C between 1 and ~5400 bar, the associated Gibbs energy changes for the swelling process can be calculated via the equation

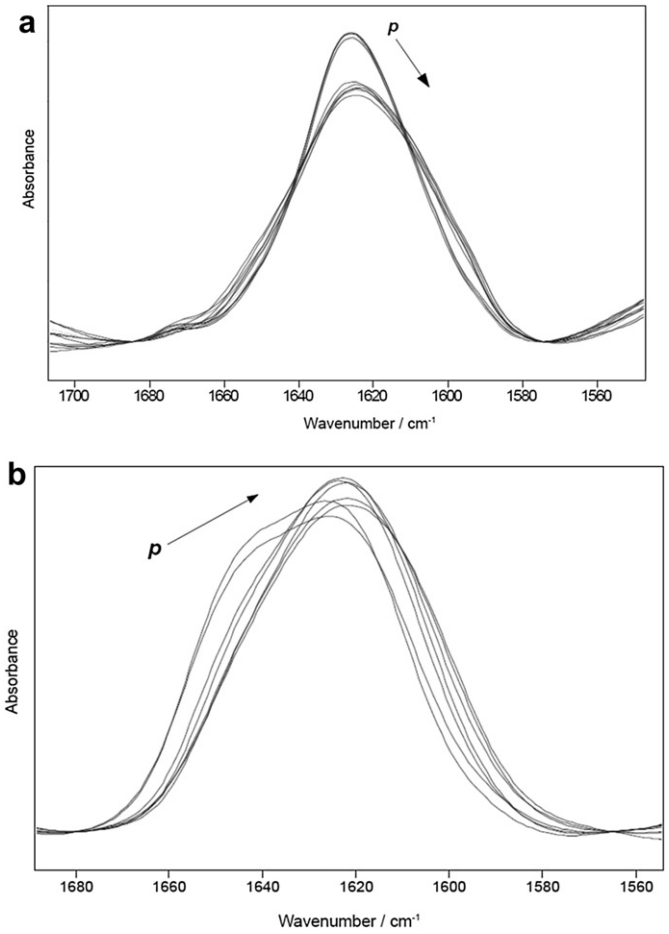

Fig. 4. a) Normalized amide-I′-FTIR-spectra of linear PNIPAM from 1 to ~9000 bar at 20 °C. The black arrow indicates the development with increasing pressure. b) Normalized amide-I′-FTIR-spectra of linear PNIPAM from 1 to ~11400 bar at 50 °C (for better visibility not all spectra are shown). The black arrow indicates the development with increasing pressure. All pressure-induced changes are completely reversible (data not shown).

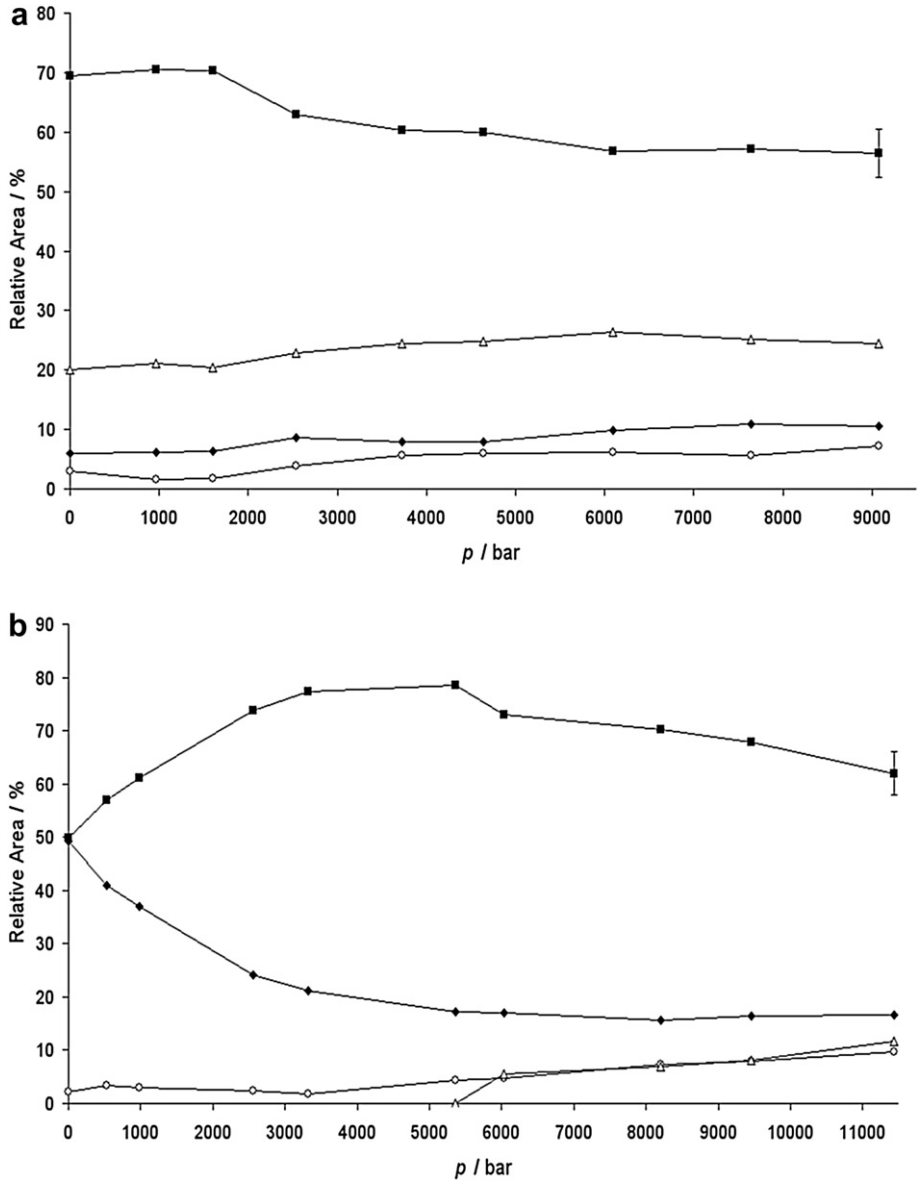

Fig. 5. a) Pressure dependence of the relative areas of the diagnostic amide-I′-bands of linear PNIPAM at 20 °C. b) Pressure dependence of the relative areas of the diagnostic amide-I′-bands of linear PNIPAM at 50 °C. For symbol denotation see Fig. 1.

$$\Delta G^{o} = -RT\ln K_{eq} = -RT\ln\left[\left(I_{collapsed} - I_p\right)\Big/\left(I_p - I_{swollen}\right)\right] \quad (1)$$

where $I_{collapsed}$ denotes the fractional band intensities in the collapsed state (at 1 bar), I_p at pressure p, and $I_{swollen}$ in the swollen state (at ~5400 bar), respectively.

The associated volume change can be obtained via the relation

$$\left(d\Delta G^{o}/dp\right)_T = \Delta V^{o} \quad (2)$$

By plotting ΔG^{o} vs. p, the volume change ΔV^{o} can be obtained from the slope and $\Delta G^{o}{}_{1\ bar}$, which refers to the thermodynamic stability of the swollen state with respect to the collapsed state at atmospheric pressure, from the y-axis intercept. The calculated values for the HHP-induced globule → coil transformation of linear PNIPAM are $\Delta G^{o}{}_{1\ bar} = 4.2 \pm 0.24$ kJ/mol and $\Delta V^{o} = -29 \pm 1.09$ mL/mol at 50 °C. Notably, the volume change is of similar magnitude as that for pressure-denaturation of proteins like SNase or RNase A at ambient temperature [7,30,31], only $\Delta G^{o}{}_{1\ bar}$ is considerably smaller, which is not surprising, as the polymer is near its "cold-unfolding temperature". The negative ΔV^{o} indicates a release of void volume in the collapsed state during the swelling process, as charged or polar side-groups are not present in the polymer, whose hydration would also lead to a decrease of the system volume [7].

The investigated microgel systems exhibit a similar behavior to that observed for the linear PNIPAM, i.e. no or only slight swelling under pressure below the respective VPTT (data not shown) and a rather pronounced collapsed to swollen transition above the VPTT. In comparison to the linear PNIPAM, the PNIPAM-microgels exhibit a significantly smaller swelling capacity at 50 °C, as can be deduced from the relative amide-I′-subband areas (Fig. 6a). Even more pronounced as in the case of the linear PNIPAM, a biphasic swelling is visible with a first, partial swelling between 1 and ~1800 bar, followed by a plateau and a second swelling phase between ~5000 and ~9500 bar. The reduced swelling capacity in the collapsed state is also observed in the other microgel system, most likely due to the elastic resistance imposed by the crosslinker

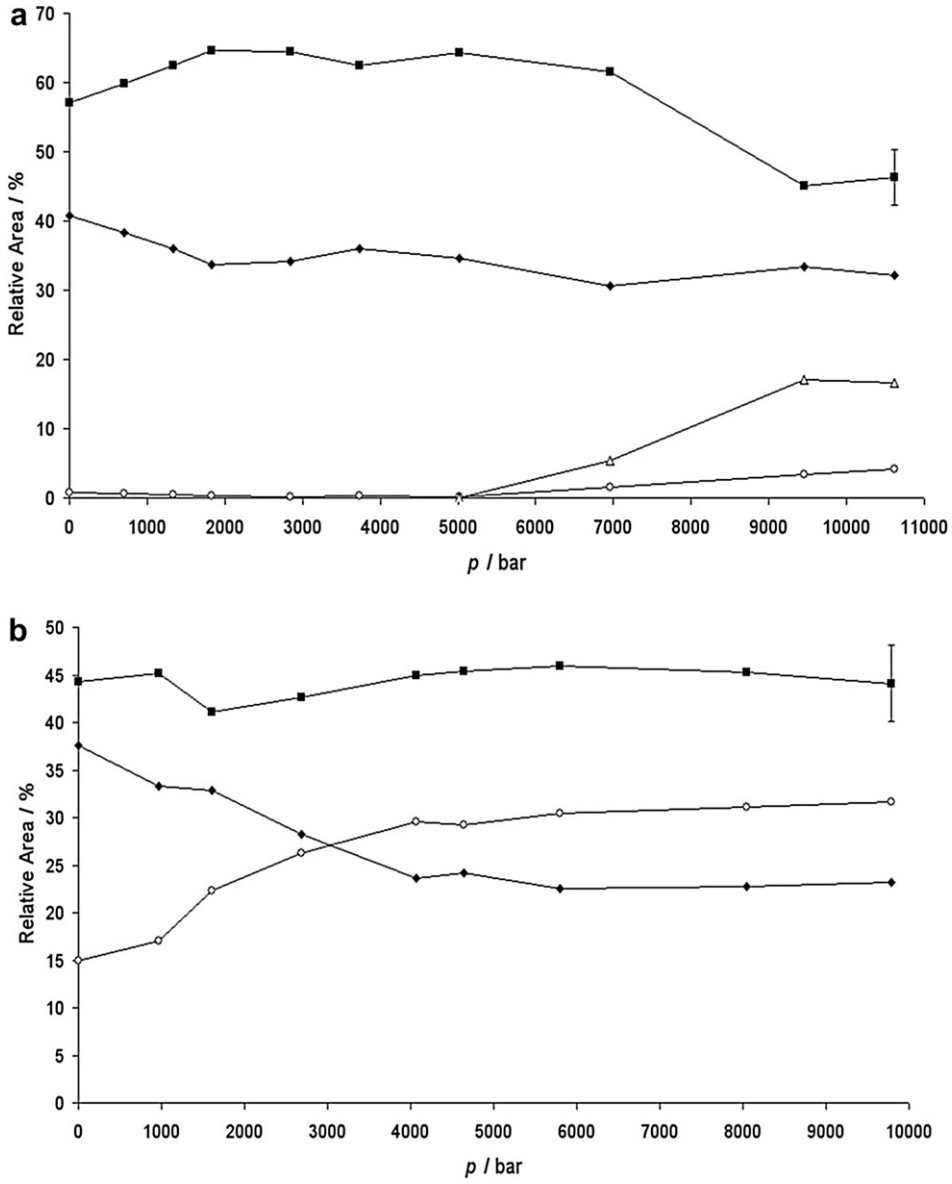

Fig. 6. a) Pressure dependence of the relative areas of the diagnostic amide-I′-bands of PNIPAM-microgels at 50 °C. b) Pressure dependence of the relative areas of the diagnostic amide-I′-bands of the NIPAM-DEAAM (45–55 mol-%) copolymer microgels at 40 °C. For symbol denotation, see Fig. 1. The error bar indicates the fitting uncertainty, as determined from different measurements and is similar for all data points.

on the gel network. The pronounced biphasic swelling of the PNIPAM microgel might be attributed to an inhomogeneous distribution of the BIS-crosslinker, which leads to an equally inhomogeneous distribution of packing defects.

By examining the HHP-behavior of the PNIPAM-PDEAAM copolymer microgels at 40 °C, a pronounced swelling is visible above atmospheric pressure, which reaches a final plateau at ~5 kbar (Fig. 6b). Upon application of a two-state process for the collapsed → swollen transition, one estimates $\Delta G^{o}_{1\ bar} = 4.8 \pm 1.4$ kJ/mol and $\Delta V^{o} = -23.6 \pm 7.5$ mL/mol (owing to missing extended plateau regions below and above the transition, these values should be considered approximate, only; here we are interested in the order of magnitudes, only). The value of $\Delta G^{o}_{1\ bar}$ is only slightly higher than the one found for the pressure-induced-transition of linear PNIPAM at 50 °C, and ΔV^{o} is even smaller, although the uncertainty is rather high.

All experiments show that pressure induces increased solvation and thus pressure is antagonistic to temperature as schematically illustrated in Fig. 7. When comparing the different behavior of the copolymer microgel, the homopolymer microgel and the linear PNIPAM, two aspects come into play: (i) the different hydrophobicity and substitution pattern of the DEAAM and NIPAM repeating units and the different chemical structure of the crosslinker as well

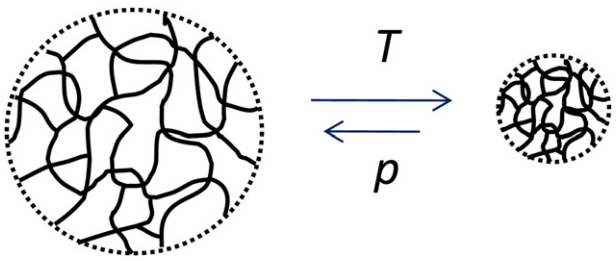

Fig. 7. Schematic drawing of the opposite effect of temperature and pressure on the swollen-to-collapsed-transition of the polymers.

as (ii) the topological constraints of the network as compared to a linear chain. To this end, these two aspects cannot yet be separated, nevertheless the data clearly reveal that both “chemical” and topological properties influence the behavior at high pressure and thus will allow preparing microgels with well-defined pressure sensitivity.

4. Conclusions

Altogether, the data obtained show that all investigated polymer-/microgel-systems are getting more hydrated under HHP-conditions above and in most cases, though less pronounced, also below their LCST/VPTT, which means that pressure generally favours the swollen or coil-like state over the collapsed state, not only above the cloud point temperature. This effect is - at least in part - probably due to a weakening of hydrophobic interactions upon pressurization, in agreement with theoretical considerations [32]. The negative ΔV^{o} indicates the presence of void volumes in the collapsed state. These results are also in excellent agreement with previous high pressure FTIR-measurements on linear PNIPAM at ambient temperature, which also indicate the formation of a more hydrated conformation under pressure [9], i.e., pressure and temperature have an antagonistic effect on the hydration, and it will be interesting to investigate how pressure affects the interaction of PNIPAM with additives that change the LCST [33]. Interestingly, for the PNIPAM microgel, a biphasic swelling behavior has been observed.

In this context, we would like to refer also to a study on hydrogels consisting of the PNIPAM-isomer poly(*N*-vinyl-isobutyramid) (PNVIBA), where collapsed, optical intransparent gels have been found to regain their transparency when subjected to pressure treatment, i.e. the collapsed, insoluble polymer chains are regaining their solubility by hydration [19]. Hence, such polymers and microgels can not only serve as simplified model systems for the cold denaturation process of proteins, but also for the pressure-induced protein unfolding and denaturation process, which is analogous to the globule → coil transition.

Generally, pressure sensitivities and the values of the thermodynamic parameters vary between linear PNIPAM and the microgels. Owing to their inherent complexity, a detailed molecular-level picture of the differential behavior of these linear polymer and microgel systems is not possible at the moment, however. Possible influencing factors comprise the crosslinker density and the monomer composition, which most likely influence the formation of packing defects in the collapsed state and the general H-bonding pattern. The occurrence of a biphasic swelling behavior might be due to an inhomogeneous distribution of the crosslinker. Future studies might target these questions using additional suitable techniques, such as small-angle X-ray scattering (SAXS) and NMR-spectroscopy.

All temperature- and pressure-induced changes are completely reversible, which is not only a prerequisite for obtaining thermodynamic properties of the system, it is also an important property for putative technical applications, like the stimulus-sensitive encapsulation of chemical compounds, enzymes, catalysts, etc. [1,10,34–36], which certainly still needs further exploration.

Acknowledgements

Financial support from the Deutsche Forschungsgemeinschaft (DFG) is gratefully acknowledged.

References

[1] Lyon LA, Nayak S. Angew Chem Int Ed Engl 2005;47:7686–708.
[2] Liu R, Fraylich M, Saunders BR. Colloid Polym Sci 2009;287:627–43.
[3] Kuckling D. Colloid Polym Sci 2009;287:881–91.
[4] Tanaka T, Sato E, Hirokawa Y, Hirotsu S, Peetermans J. Phys Rev Lett 1985;55:2455–8.
[5] Hirokawa Y, Tanaka T. J Chem Phys 1984;81:6379–81.
[6] Kunugi S, Tanaka N. Biochim Biophys Acta 2002;1595:329–44.
[7] Winter R, Lopes D, Grudzielanek S, Vogtt K. J Non-Equilib Thermodyn 2007;32:41–97.
[8] Mishra R, Winter R. Angew Chem Int Ed Engl 2008;120:6618–21.
[9] Meersman F, Wang J, Wu Y, Heremans K. Macromolecules 2005;38:8923–8.
[10] Chaterji S, Kwon IK, Park K. Prog Polym Sci 2007;32:1083–122.
[11] Kavanagh CA, Rochev YA, Gallagher WM, Dawson KA, Keenan AK. Pharmacol Ther 2004;102:1–15.
[12] Wu C, Zhou S. Macromolecules 1997;30:574–6.
[13] Kratz K, Hellweg T, Eimer W. Polymer 2001;42:6631–9.
[14] Stieger M, Richtering W, Pedersen JS, Lindner P. J Chem Phys 2004;120:6197–206.
[15] Kato E. J Chem Phys 1997;106:3792–7.
[16] Kato E. J Chem Phys 1994;101:468–72.
[17] Otake K, Karaki R, Ebina T, Yokoyama C, Takahash S. Macromolecules 1993;26:2194–7.
[18] Yamazaki Y, Tada T, Kunugi S. Colloid Polym Sci 2000;278:80–3.
[19] Kunugi S, Kameyama K, Tada T, Tanaka N, Shibayama M, Akashi M. Braz J Med Biol Res 2005;38:1233–8.
[20] Maeda Y, Nakamura T, Ikeda I. Macromolecules 2002;35:10172–7.
[21] Katsumoto Y, Tanaka T, Ozaki Y. J Phys Chem B 2005;109:20690–6.
[22] Maeda Y, Higuchi T, Ikeda I. Langmuir 2000;16:7503–9.
[23] Maeda Y, Yamamoto H, Ikeda I. Colloid Polym Sci 2004;282:1268–73.
[24] Senff H, Richtering W. J Chem Phys 1999;111:1705–11.
[25] Keerl M, Richtering W. Colloid Polym Sci 2007;285:471–4.
[26] Wong PTT, Moffat DJ. Appl Spectrosc 1989;43:1279–81.
[27] Keerl M, Smirnovas V, Winter R, Richtering W. Angew Chem Int Ed Engl 2008;47:338–41.
[28] Maeda Y, Yamabe M. Polymer 2009;50:519–23.
[29] Bridgman PW. J Chem Phys 1935;3:597–605.
[30] Panick G, Vidugiris GJA, Malessa R, Rapp G, Winter R, Royer CA. Biochemistry 1999;38:4157–64.
[31] Panick G, Winter R. Biochemistry 2000;39:1862–9.
[32] Hummer G, Garde S, García AE, Paulaitis ME, Pratt LR. Proc Natl Acad Sci USA 1998;95:1552–5.
[33] Hofmann C, Schonhoff M. Colloid Polym Sci 2009;287:1369–76.
[34] Kabanov AV, Vinogradov SV. Angew Chem Int Ed Engl 2009;48:5418–29.
[35] Ballauff M, Lu Y. Polymer 2007;48:1815–23.
[36] Welsch N, Wittemann A, Ballauff M. J Phys Chem B 2009;113:16039–45.