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Formation of a Mesoscopic Skin Barrier in Mesoglobules of Thermoresponsive Polymers

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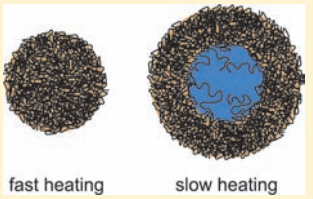
Supporting Information

ABSTRACT: With the combination of molecular scale information from electron paramagnetic resonance (EPR) spectroscopy and meso-/macroscopic information from various other characterization techniques, the formation of mesoglobules of thermoresponsive dendronized polymers is explained. Apparent differences in the EPR spectra in dependence of the heating rate, the chemical nature of the dendritic substructure of the polymer, and the concentration are interpreted to be caused by the formation of a dense polymeric layer at the periphery of the mesoglobule. This skin barrier is formed in a narrow temperature range of ~4 K above T_C and prohibits the release of molecules that are incorporated in the polymer aggregate. In large mesoglobules, formed at low heating rates and at high polymer concentrations, a considerable amount of water is entrapped that microphase-separates from the collapsed polymer chains at high temperatures. This results in the aggregates possessing an aqueous core and a corona consisting of collapsed polymer chains. A fast heating rate, a low polymer concentration, and hydrophobic subunits in the dendritic polymer side chains make the entrapment of water less favorable and lead to a higher degree of vitrification. This may bear consequences for the design and use of thermoresponsive polymeric systems in the fast growing field of drug delivery.



INTRODUCTION

During the last decades, thermoresponsive polymers have been in the scientific focus due to their unique properties, which allow a wide range of applications in fields such as actuation, drug delivery and surface modification.[1−4] When an aqueous solution of a thermoresponsive polymer is heated above its lower critical solution temperature (LCST), the polymer phase separates from its aqueous environment and collapses.[5,6] Except for the case of very dilute solutions,[7,8] the single-chain globules associate to large aggregates which may lead to a macroscopic precipitation of the polymer from the solution. In some cases, however, this association stops and nearly monodisperse, stable globular aggregates with sizes of 50 to several hundred nanometers are formed. Up to date, light scattering,[9−13] fluorescence spectroscopy,[14] light and electron microscopy,[12,15,16] and differential scanning calorimetry[17,18] have been applied to gain a better understanding of the formation and stability of these mesoscopic aggregates.

Various explanations were proposed for the stability of a mesoglobule. In a first approach, the stability of poly(*N*-isopropylacrylamide) (pNiPAAm) dispersions was attributed to electrostatic repulsion between particles, which could either be caused by charged polymer units or by associated salt ions.[19,20] Molecular dynamics (MD) simulations and self-consistent field (SCF) calculations suggest that the more hydrophilic parts of the polymer are located preferably at the periphery of the mesoglobule in direct contact to the surrounding water and provide steric stabilization.[21,22] These theoretical approaches are supported by experimental findings that the copolymerization of the amphiphilic monomer with a small percentage of hydrophilic units leads to an increased stability of the mesoglobules. Examples include pNiPAAm polymers that were grafted with polyethyleneoxide (PEO) chains,[23] p(NiPAAm-vinylpyrrolidone) copolymers,[24] and statistical copolymers with *N*,*N*-diethylacrylamide as thermoresponsive building block and more hydrophilic comonomers *N*,*N*-dimethylacrylamide or *N*-ethylacrylamide.[25]

Wu et al., however, observed that mesoglobules based on NiPAAm are also stabilized by hydrophobic comonomers.[10] According to their suggestion, the hydrophobic units promote intrachain contraction and harden the mesoglobules, thus, slowing down chain motion. Hence, the interaction time between two colliding mesoglobules is not sufficient to induce a permanent chain entanglement and the mesoglobules are protected from further aggregation. This viscoelastic effect was already suggested previously to be one possible reason for the stability of pNiPAAm homopolymer mesoglobules in water[12,27] and poly(ε-caprolactam) dispersions in THF.[26]

The size of the mesoglobules for a given polymer depends on three parameters: the temperature, the polymer concentration, and the rate of heating. For various polymers including the here studied PG2(ET), it was found that the size increases with increasing polymer concentration while it decreases at faster heating rates.[11−13,19] Wu et al. proposed that on fast heating the intrachain contraction dominates the interchain aggregation.[10]

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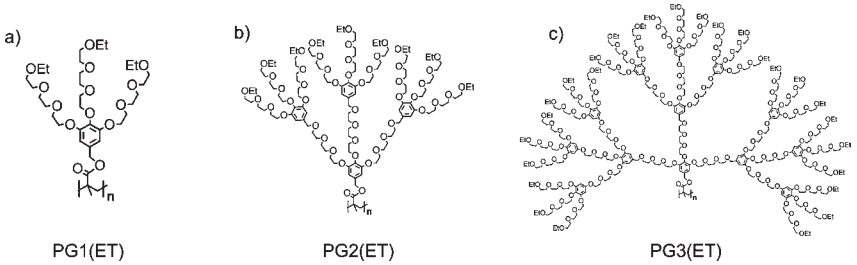
Scheme 1. Chemical Structures of the Thermoresponsive Dendronized Polymers



It is commonly assumed that a faster heating leads to a higher degree of vitrification of the mesoglobule's core,[11,13,14] but only Van Durme et al. found conclusive experimental evidence from modulated temperature DSC for partial vitrification of the polymer-rich phase.[17] However, this method does not provide information on which region of the mesoglobule is vitrified.

It is evident that local nanoscopic information is required in addition to the wealth of existing data on the mesoscopic length scale to gain a more detailed insight into the collapse of thermoresponsive polymers, their assembly into mesoglobules, and the structure and stability of the aggregates. In such disordered macromolecular systems, magnetic resonance spectroscopy can be used to obtain local information about structure and dynamics.[28−30] Though many sophisticated NMR and pulse electron paramagnetic resonance (EPR) techniques have been established to tackle these questions,[31,32] conventional continuous wave (CW) EPR spectroscopy on nitroxide radicals as paramagnetic tracer molecules (so-called spin probes) has proven to be a particularly simple and illuminating method to study the molecular environment of systems undergoing a thermal transition.[33,34] The electronic structure and the spectral parameters of nitroxide radicals are affected by their immediate molecular environment, specifically by the viscosity and by the polarity/hydrophilicity of their surrounding medium.[30,33−36] Indeed, the large polarity contrast between hydrated hydrophilic and collapsed hydrophobic polymer regions upon the thermal transition of thermoresponsive polymers allows for the distinction of the spin probes residing therein. The amphiphilic spin probe 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) was found to be best suited to probe these two fundamentally different regions simultaneously due to its favorable partition coefficient.

In two recent studies, we focused on the local, nanoscale characterization of dehydration processes above and below the critical temperature (T_C) that are connected with the thermal transition and mesoglobule formation of thermoresponsive dendronized polymers based on oligoethyleneglycole (OEG).[34,37] These polymers (PG1−3(ET) (Scheme 1) exhibit fast, fully reversible, and macroscopically sharp phase transitions in the physiologically interesting temperature regime between 30 and 36 °C and were thus deemed particularly suitable model systems.[38,39] We could show that the dehydration of the dendronized polymers starts already at least 20 K below T_C and proceeds over a temperature interval of at least 50 K. In the course of the dehydration, structural heterogeneities on the nanometer length scale are formed, which trigger aggregation of different polymer chains at the critical temperature. In these preceding studies, the polymer solution was ramp heated to a maximum temperature and the measurements were conducted while stepwise cooling the solution.

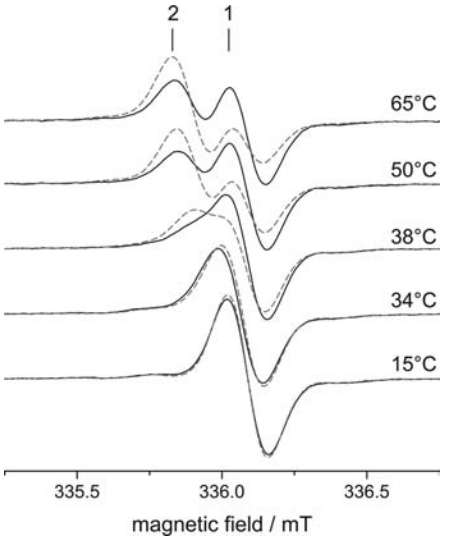


Figure 1. High field lines ($m_I = -1$) of the CW EPR spectra for 0.2 mM TEMPO in an aqueous solution of 10 wt % PG2(ET) at selected temperatures. The black, solid spectra were obtained at a slow heating rate (<1 K min^{-1}); the gray, dashed spectra were obtained after ramp heating the polymer solution (>30 K min^{-1}). The spectral contribution from TEMPO molecules in the aqueous phase is denoted 1. TEMPO molecules in the hydrophobic polymer-rich gel phase give rise to contribution 2.

In this study, we examine heating rate dependent effects which influence the mesoglobule formation of the thermoresponsive polymer. To this end, the temperature of the polymer solutions is raised at a considerably slower rate and the results are compared to those obtained from ramp-heating the dendronized polymers. These EPR-spectroscopic data could not be explained with the structural models of mesoglobular aggregates cited above. Therefore, a different model is proposed, which is in agreement with the obtained EPR data and with the vast majority of published results on the formation of mesoglobules.

RESULTS

CW EPR Spectra. The amphiphilic nitroxide radical 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) is particularly suited to study both hydrophilic (hydrated) and hydrophobic (collapsed) regions of a polymer.[33,34] The high field lines of the TEMPO CW EPR spectra in an aqueous solution of 10 wt % PG2(ET) at selected temperatures are shown in Figure 1. At elevated temperatures,

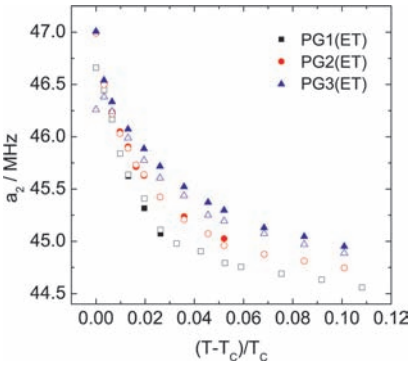


Figure 2. Hyperfine coupling constants a_2 above T_C as function of the reduced temperature for 0.2 mM TEMPO in 10 wt % aqueous solutions of PG1(ET), PG2(ET), and PG3(ET). Closed symbols represent data points obtained by ramp heating (>30 K min^{-1}); open symbols correspond to a slow heating rate (<1 K min^{-1}).

the high field line consists of two spectral contributions, arising from distinct nitroxide species 1 and 2 that are located in environments with different polarities. Species 1 resides in a strongly hydrated, hydrophilic environment, while species 2 is surrounded by a more hydrophobic medium (comparable to chloroform or *tert*-butylalcohol).[40] The different spectral positions are mainly caused by different isotropic hyperfine coupling constants a_{iso} of the electron spin to the ^{14}N nuclear spin. A smaller a_{iso} value indicates a more hydrophobic environment.

At temperatures above T_C, the aqueous solution of a thermoresponsive polymer phase separates into a concentrated gel phase (the mesoglobule) and a diluted water-rich phase. The spectral species 1 accounts for the TEMPO molecules in the dilute phase while species 2 stems from those spin probes in the gel phase. At intermediate temperatures, the gel phase is still highly swollen with water and species 2 dynamically exchanges between regions of different polarities. Its hyperfine coupling constant is an effective, weighted average between the two extreme values of spin probes in purely hydrophilic and purely hydrophobic regions (as observed at 65 °C). Since this exchange is locally restricted to a few nanometers, the effective coupling constant a_2 is a quantitative measure of the fraction of hydrated and collapsed polymer regions in the immediate vicinity of the spin probe at a given temperature. This is discussed in detail in an earlier publication.[34]

In this paper, we focus on the differences between the spectra recorded for different heating rates. The solid spectra in Figure 1 were obtained at a slow heating rate (<1 K min^{-1}), while the dashed spectra were obtained after the polymer solution was ramp heated (>30 K min^{-1}) to 65 °C. Hardly any heating rate dependence on the spectra is observable up to the critical temperature of 34 °C. However, a further raise of the temperature by only 4 K causes dramatic differences in the spectra. With a slow heating rate, a considerably higher fraction of spectral species 1 (x_1) residing in a more hydrophilic environment is retained. This deviation between the spectra recorded at different heating rates grows as the temperature is further increased.

Spin probe species 1 stems from TEMPO molecules placed in bulk water and swollen, predominantly hydrophilic polymer regions. The fraction x_1 of this species depends on the volumetric ratio of these regions to the collapsed/collapsing regions in the polymer. This is a direct consequence of the amphiphilicity of the spin probe that chooses its environment depending on its

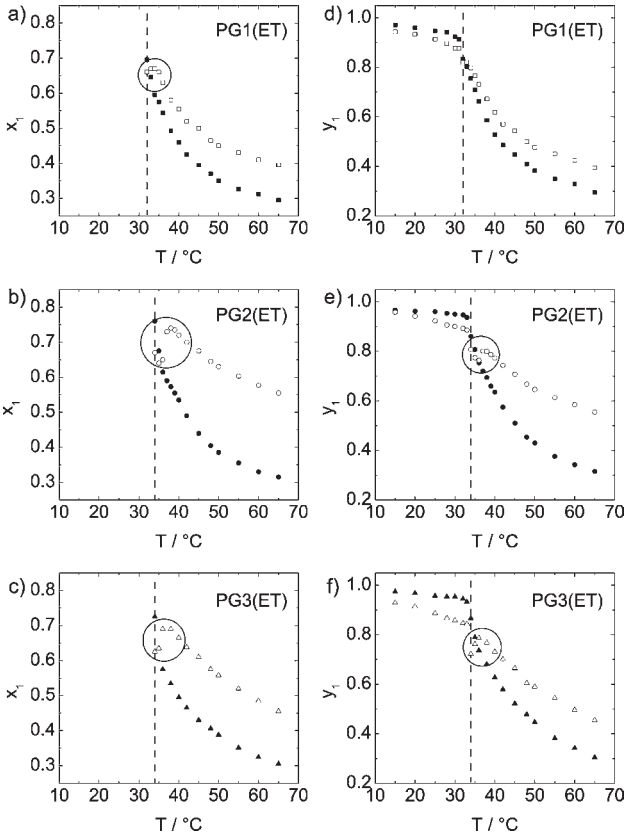


Figure 3. (a−c) Fraction x_1 of the hydrophilic spectral component S_1 for 0.2 mM TEMPO in 10 wt % solution of PG1−3(ET) upon fast heating (closed symbols) and slow heating (open symbols). (d−f) Fraction of TEMPO in hydrophilic regions y_1 as a function of temperature. The critical temperature T_C is marked by a dashed line. The local increase of x_1 and y_1 at temperatures slightly above T_C and at a slow heating rate is highlighted by circles.

hydrophobicity and its availability. For example, an increase of the polymer concentration leads to a linear increase of the spectral species 2 at temperatures above T_C (data not shown). An increased fraction x_1 can be interpreted as an increased fraction of hydrophilic regions in the sample and a decreased fraction of collapsing, more hydrophobic regions. Thus, slowly heating the polymer solution to a maximum temperature of 65 °C apparently results in a larger fraction of hydrophilic domains.

Strikingly, the spectral position (and hence the apparent hyperfine coupling constant) of species 2 is not affected by the heating rate. This is illustrated in more detail in Figure 2, where the extracted hyperfine coupling values of the hydrophobic component a_2 are displayed for the slowly heated and ramp heated solutions of all dendronized polymers in this study. Besides small deviations for the third generation polymer PG3-(ET), hardly any dependence on the heating rate of a_2 is observed for all dendronized polymers. As explained above, this species is in fast dynamic exchange between hydrated and collapsed regions and its apparent hyperfine coupling constant is a measure for the fraction of hydrophilic and hydrophobic regions in its immediate surrounding. It is remarkable that the rate of heating does not affect the immediate *nanoscopic* environment of the spin probes, while the substantially different fractions of spin probes 1 and 2 (x_1 and x_2) strongly suggest that the *macroscopic* sample contains a higher fraction of hydrophobic

regions when heated with a faster rate. These seemingly opposing observations on different length scales provide the cornerstones of a new model for the mesoglobule formation that is proposed in the Discussion section. We note that, irrespective of the heating rate, the obtained EPR spectra do not change with time. They are stable for at least 1 h as checked at a temperature of 40 °C.

Quantitative Analysis of Hydrated and Collapsed Polymer Regions. Above the critical temperature, the CW EPR spectra comprise signatures from two distinct TEMPO species. Species 1 stems from spin probes in the aqueous, diluted phase and species 2 from probes in the concentrated gel phase. The two species contribute to the composite CW EPR spectra with fractions x_1 and x_2, respectively. The fraction x_1 of the spectral component in the aqueous phase is illustrated in Figure 3a−c as a function of temperature and heating rate. For all polymers PG1−3(ET) at temperatures far above T_C, the fraction of spin probes in the aqueous phase is significantly higher when the solutions are heated with a slow rate. This discrepancy is far more pronounced for the second and third generation dendronized polymers, while only small changes are observed for PG1(ET).

Remarkably, the shape of the x_1 curves of the ramp heated polymer solutions coincide with the corresponding curves of the hyperfine coupling constant a_2 (Figures 2 and 3a−c, closed symbols). Thus, in this case, the nanoscopic environment of the spin probes in the gel phase, represented by a_2, reflects the macroscopic distribution of the spin probes in the gel and the diluted phase as given by x_1.

When the samples are heated at a slow rate, however, the x_1 curves deviate considerably from the corresponding a_2 curves indicating apparent differences between the nanoscopic and macroscopic structure. Moreover, at temperatures only slightly above T_C, a peculiar effect is observed, which is most pronounced for PG2(ET). Up to 35 °C (1 K above T_C), a slow heating rate leads to slightly decreased fractions x_1, that is, more spin probes are located in the vicinity of collapsing polymer regions. While a fast heating rate causes x_1 to decrease monotonously with increasing temperature, a significant increase from 0.64 (35 °C) to a local maximum of 0.74 (38 °C) is observed in the case of slow heating. Only at higher temperatures the expected decrease is observed. Such a local maximum appears for all dendronized polymers PG1−3(ET). The magnitude of the heating rate dependent deviation of x_1 at high temperatures markedly depends on how strongly this characteristic maximum is observed. The mere occurrence of a local maximum is very surprising since the hyperfine coupling constant a_2 suggests a steady formation of hydrophobic regions that are in exchange with hydrophilic regions. This seemingly paradox phenomenon is discussed in the next section.

The variable x_1 is a measure for the macroscopic distribution of the spin probes in polymer-rich and polymer-poor regions of the sample. However, x_1 does not yield information about the macroscopic distribution of hydrated, hydrophilic and collapsed, hydrophobic polymer regions through the thermal transition. First, two distinct spectral species, and thus information about x_1, are only present at temperatures above T_C. Second, most pronouncedly at temperatures close to T_C, both types of spin probes dynamically exchange between hydrated and collapsed polymer regions. This exchange on the nanoscopic scale is encrypted in the hyperfine coupling constants a, which are intermediate between the extreme values of a purely aqueous environment and the (hydrophobic) environment of a fully collapse polymer chain. The fractions of spin probes in hydrophilic regions (denoted y_1) and hydrophobic regions (denoted $y_2 = 1 - y_1$) are related to the spectral contributions x_1 and x_2 and are obtained by relating the apparent population of each spectral component x_i to the fraction of hydrated and collapsed regions in their local environment (details can be found in the Supporting Information). Up to T_C, the hydrophilic fractions y_1 are not affected strongly by the heating rate. Above T_C, plots of y_1 versus the temperature bear all features of the x_1 curves as the hyperfine coupling constants (containing nanoscopic information about the spin probes' environment) are not affected by the heating rate (Figure 3d−f). Thus, the slow and fast heating y_1 curves exhibit a strong deviation in a narrow temperature interval of only 4 K. A steep monotonous decrease of y_1 is observed for a fast heating rate while the fraction of hydrophilic regions increases to a local maximum upon slow heating. At higher temperatures, both fast and slow heating curves steadily decrease with the fast heating curve exhibiting a slightly steeper slope. At temperatures far above T_C, this leads to a dramatic difference. While 70% of all spin probes are located in the collapsed, hydrophobic regions of PG2(ET) at a fast heating rate, this value drops by one-third to 45% when the polymer solution is heated slowly up to the maximum temperature of 65 °C.

DISCUSSION

Thermodynamically, the separation of an aqueous solution of a thermoresponsive polymer into a concentrated gel phase (the mesoglobule) and a diluted phase is determined by temperature and polymer concentration. The ratio of the amphiphilic spin probes in the gel phase (species 2) and the dilute phase (species 1) is governed by the volumetric ratio of these regions and should not depend on the annealing history. As already noted in our first report on these systems,[34] the collapse cannot be described as a thermodynamic phase transition. While thermodynamics require a single deswelling process to be responsible for the dehydration of the mesoglobules, the dehydration takes place in at least two steps over a broad temperature interval of 30 K. Thus, the path-dependent observations reported here are in complete agreement with the earlier findings and offer more insight into such structures that are formed by a molecularly controlled non-equilibrium process.

When the dendronized polymer is heated slowly to high temperatures, far more TEMPO molecules remain located in the diluted, water-rich phase than in the case of fast heating. Apparently, a slow formation of the mesoglobule triggers the expulsion of more spin probes from the polymer-rich gel into the aqueous phase. Under equilibrium conditions, such excess of expelled spin probes would diffuse back into the mesoglobule to restore the thermodynamic partition coefficient. At least after a certain diffusion time, the ratio between spin probes in hydrophilic and hydrophobic regions would equilibrate again and show a heating-rate independent behavior. In contrast to such a scenario, the CW EPR spectra of our thermoresponsive polymer systems remain unaffected, irrespective of the heating rate, for at least 1 h once the mesoglobules are formed.

Thus, one has to consider a structure of the collapsing mesoglobule that prohibits the back-diffusion of the spin probes into the gel phase. In the literature of responsive hydrogels, the so-called *skin barrier effect* is well-known.[5,41,42] The collapse of such a macroscopic gel results in the densification of its surface due to dehydration of the polymer chains. This collapsed surface becomes impermeable for the residual water that is still

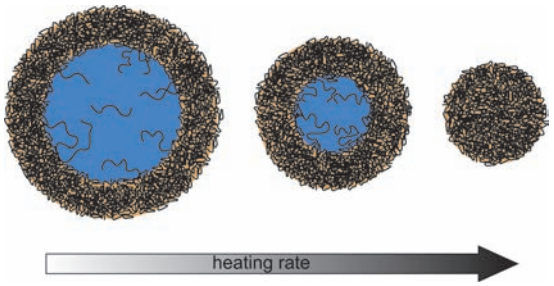


Figure 4. Sketch of the skin barrier effect in mesoglobules of different sizes. When increasing the temperature above a certain threshold, an impermeable outer polymeric layer is formed as the swollen polymer is dehydrated and densified (orange). The amount of water entrapped in the core (blue) in large polymer aggregates which are formed at a small rate of heating increases with the size of the mesoglobules. Fast heating results in smaller aggregates with a higher surface to volume ratio and lead to the entrapment of less or no water. Note that, for clarity, the figure is simplified and depicts the polymer enriched phase outer layer as being purely polymeric and the polymer-depleted phase in the core as purely aqueous.

incorporated in the core of the gel. Thus, the hydrogel is kinetically trapped in a semicollapsed state. In the next paragraphs, we rationalize how the skin barrier effect could account for the observed heating rate dependent effects in the mesoglobule formation, the impact of the polymer concentration, and the molecular weight of the formed aggregates.

It is a general phenomenon that, upon slow heating and at higher polymer concentration, larger mesoglobules are formed.[10−12] These dependencies were confirmed in a recent light scattering study on PG2(ET).[13] Upon slow heating and at higher polymer concentration, larger mesoglobules are formed since interchain aggregation of the partially collapsed polymers is promoted.[10−13] Let us now assume that a dense, impermeable outer layer is formed in the early stages of mesoglobule formation. Even if the formation of an impermeable outer polymeric layer were not affected by the heating rate, the skin barrier effect would be more pronounced in the bigger mesoglobule since the volume to surface ratio of a sphere increases linearly with its radius R. In other words, a larger mesoglobule possesses a larger core which is not able to collapse once a dense outer layer is formed.

In this framework, the increased fraction of spin probes in the aqueous phase upon slow heating can be readily explained. On the one hand, the diffusion of TEMPO from the diluted phase into the mesoglobule is prohibited due to the impermeable outer polymeric layer. This is true for both fast and slow heating, and should not affect the ratio of the spin probes. On the other hand, the remaining spin probes in the gel phase are trapped in the aggregates, which form a closed system. In case of large aggregates, this system contains a higher volume fraction of water, which leads to a higher fraction of spin probes in a hydrophilic environment. For small aggregates, the fraction of entrapped water is much lower and more spin probes are located in nanoscopic hydrophobic regions (Figure 4). In the present study, the size of the mesoglobules was varied by applying different heating rates. Small aggregates are formed at fast heating rates, while large aggregates are formed at small heating rates. For example, an increase of the heating rate from 0.1 to 1 K min^{-1} leads to a decrease of the hydrodynamic radius of PG2(ET) from ∼600 to ∼180 nm (at 40 °C).[13] A similar de/increase of the size of the mesoglobules can be achieved by a de/increase of the polymer concentration. Hence, the effects of the heating rate on the morphology of the mesoglobule, as discussed in this paper, should be similar to the influence of the polymer concentration of the aggregates.

In the following, we concentrate on two spectral details which allow inferring more details about the proposed dehydration mechanism. First, the hyperfine coupling values of the hydrophobic spectral component a_2 are unaffected by the heating rate and hence by the amount of entrapped water. The hyperfine coupling values provide a direct measure of the hydrophilic and hydrophobic fractions in the direct surrounding of the spin probe (∼5 nm).[34] Hence, the nanoscopic hydration of the polymer chains is not affected by the heating rate. This apparent independence suggests that a microphase separation takes place inside the mesoglobule. The spin probes at the mesoglobule's periphery experience the environment of polymer chains that are (in the process of being) dehydrated and are not affected by the entrapped water (species 2). The spin probes in the mesoglobule's core, on the other hand, are placed in fully water swollen polymers in a range greater than their mean free pathway (species 1, cf. Figure 4). Second, the fraction of spin probes in the gel phase grows larger at higher temperatures. With increasing temperature, there is a stronger tendency for the OEG chains to become more hydrophobic, which may lead to a larger volumetric ratio of hydrophobic polymer-rich regions inside the mesoglobule. Further, the larger hydrophobicity of the polymer chains at higher temperatures leads to more favorable partition constants of the spin probe toward these regions.[43] This may additionally contribute to the increasing fraction of the spin probe in a hydrophobic environment with increasing temperature. Any change in temperature also leads to a reorganization of the polymers in the mesoglobules and affects the surrounding of the spin probes. In the course of this reorganization, small amounts of water may be released from the mesoglobule and spin probes may diffuse into the mesoglobule from the diluted phase. These assumptions are corroborated by light scattering studies that observed a notable shrinking of the mesoglobules above T_C.[11,13] At a given temperature, however, the system remains in a metastable state.

The skin barrier effect proposed here explains why the mesoglobules are stable in size albeit experiencing nonequilibrium conditions. At a given temperature and irrespective of the heating rate, the size of the mesoglobule remains constant, even if the polymer solution is diluted to substantially lower concentrations.[11,12] It also accounts for the EPR spectra being time-independent at a certain temperature as the mesoglobules do not undergo structural changes and spin probe diffusion in and out of the aggregates is prohibited.

Comparison with Other Characterization Techniques on Similar Mesoglobular Systems. In the introduced framework of the skin barrier and its increasing effect on larger mesoglobules, a large aggregate would approach the characteristics of a water-filled (hollow when looking at the polymer content) sphere. The collapsed chains give rise to a high polymer density at the periphery of the sphere, while the swollen polymers in the core exhibit a significantly decreased density. This hypothesis can be tested by light scattering since the ratio of the radius of gyration and the hydrodynamic radius R_g/R_h assumes characteristic values depending of the shape of the mesoglobule. For a uniform sphere, a value of 0.775 is expected, while an infinitely shallow hollow sphere gives rise to a characteristic ratio of $R_g/R_h = 1$.[44] Hence, a mesoglobule with a denser outer part should exhibit an R_g/R_h between these two values.

Opposing the picture of a hollow sphere, a ratio of ~0.7 was observed for PG2(ET).[13] Kujawa et al., studying fluorescence labeled pNiPAAm mesoglobules, noted that values below 0.78 are obtained, characteristic of a molten globule with a denser core.[14] However, of all seven reported R_g/R_h ratios in their paper, only two values fall below a threshold of 0.77. Aseyev et al. stated that the R_g/R_h ratio of the pNiPAAm mesoglobules in their study fluctuates around a value of 0.775, but a closer inspection of the data reveals a clear linear increase of R_g/R_h with an increasing molecular weight of the formed aggregates (Figure 9 in ref 11). In fact, one can find clear support for the proposed hollow sphere formation of large aggregates in this most detailed study with 20 different values for a variety of molecular weights. For small aggregates with low molecular weights (~1×10^8 g mol^{-1}), the obtained values indeed fluctuate around the characteristic value of a uniform sphere while values around 0.9 are found for large aggregates (>1.5×10^9 g mol^{-1}). Thus, the approach to explain our experimental CW EPR findings in terms of a skin barrier effect is in agreement with almost all light-scattering data on similar systems.

In the previous paragraphs, it was explained and reasoned how the skin barrier effect could account for an entrapment of water and how this would lead to a higher spin probe fraction in a hydrophilic surrounding. The next paragraphs will focus on a peculiar detail of the EPR collapse curves that was mentioned above: A slow heating rate causes the fraction of the spin probe species 1 in the diluted phase to *increase* to a local maximum at temperatures slightly above the critical temperature (cf. Figure 3).

Utilizing nonradiative energy transfer, Kujawa et al. observed that, in a narrow temperature window of a few Kelvin in the vicinity of T_C, fluid pNiPAAm mesoglobules were formed that merged and grew.[14] Upon further heating, these mesoglobules underwent a conversion from fluid particles to rigid spheres that were unable to merge or undergo chain exchange. Further, they observed a broad distribution of relaxation times, which they attributed to heterogeneities in the mesoglobule. In their light-scattering study of the mesoglobule formation of PG2(ET), Bolisetty et al. observed a transition from reaction limited colloidal aggregation to diffusion limited colloidal aggregation at 38 °C.[13] This is the exact temperature, where the local maximum of the spin probe fraction in the diluted phase x_1 is observed for PG2(ET). All these observations support the proposed skin barrier effect and indicate that a dense polymer layer is formed in the same narrow temperature regime in which the counterintuitive increase of x_1 is observed. Further, FTIR studies on pNiPAAm revealed that loosely bound water molecules are expelled from the polymer chains into bulk water in this small temperature range.[45−47] During this expulsion, it is likely that a fraction of the amphiphilic spin probes at the periphery of the mesoglobule is expelled into the dilute phase. In our CW EPR spectra, this manifests itself as an increase of the fraction x_1.

Picture of Mesoglobule Formation. Combining all these pieces of information, one can construct a detailed model of the mesoglobule formation in the framework of the skin barrier effect: In a narrow regime above T_C (~4 K), a dense, impermeable polymeric layer is formed by the dehydration of peripheral dendrons/polymers. During this dehydration process, one part of the incorporated spin probes is released from the mesoglobule into the bulk water phase. Once expelled from the mesoglobule, they are unable to re-enter due to the skin barrier. For the same reason, entrapped water is forced to stay inside the aggregate. As the temperature increases, interactions between polymer and water become less favorable and the entrapped water is microphase-separated from the polymer chains in the formed mesoglobule. Larger aggregates with a high amount of entrapped water are formed at a slow rate of heating or at a large polymer concentration and contain a higher amount of incorporated water than small aggregates. At high temperatures, they assume a hollow, sphere-like structure with a dense polymer corona and a core that—in a simplified picture—predominantly consists of bulk water.

Having introduced the general model of a skin barrier formation, the dependence of the thermal transition on the different dendron architectures of the polymers needs to be addressed. Only slight spectral changes are observed for PG1(ET) as a function of the heating rate while the spectra of TEMPO in PG2(ET) and PG3(ET) exhibit considerable deviations. The dendrons of each polymer in this study possess terminal ethoxy groups, which determine the hydrophobicity of dendritic periphery and determine the critical aggregation temperature of the polymer.[38] PG2(ET) and PG3(ET) additionally possess a more hydrophilic triethyleneoxide core that efficiently binds water molecules. This internal water reservoir counteracts both the dehydration of single polymer chains below the critical temperature and the dehydration of the mesoglobular aggregates at high temperatures.[34,37] Hence, PG2(ET) and PG3(ET) contain considerably more associated water when the impermeable polymer skin is formed and are stronger affected by the heating rate. This assumption is supported by the fact that the EPR spectra of the dendronized polymer PG2(ETalkyl) only exhibit a weak dependence of the rate of heating (see Supporting Information). In this polymer, the triethyleneoxide core was replaced by a hydrophobic octane unit.[34] In contrast to PG2(ET) and PG3(ET), PG2(ETalkyl) possesses a core which exhibits a higher hydrophobicity than the periphery of the dendron and does not lead to the entrapment of water in the mesoglobule. We note that the effect of the heating rate on the EPR spectra is more pronounced for PG2(ET) than PG3(ET), although PG3(ET) possesses an extended hydrophilic core and thus a larger water reservoir. A decrease of the heating rate from >30 to <1 K min^{-1} leads to 25% more spin probes being located in a hydrophilic environment for PG2(ET) at 65 °C, while an increase of spin probes in hydrophilic regions of only 15% is observed for PG3(ET) (cf. Figure 3). This counterintuitive behavior may be due to the low degree of polymerization of PG3(ET) of DP_n = 16 (compared to DP_n > 130 for all other dendronized polymers in this study, cf. Supporting Information).

CONCLUSIONS

EPR spectroscopy yields unprecedented insights into the structure formation during the thermal transition of thermoresponsive dendronized polymers. This molecularly controlled nonequilibrium process is characterized by heating rate dependent changes of spin probe fractions in hydrophilic and hydrophobic environments. The presented EPR spectroscopic data, in combination with complementary data from other characterization techniques, indicate the formation of a dense polymeric layer at the periphery of the mesoglobule, which is formed in a narrow temperature range of ~4 K above T_C and prohibits the release of molecules that are incorporated in the polymer aggregate. This skin barrier causes the entrapment of considerable amounts of water in large aggregates, which are formed at low heating rates and at high polymer concentrations. The entrapment of water is

facilitated by dendronized polymers with hydrophilic cores. These detailed insights into the impact of the heating rate, the polymer concentration, and its molecular composition on the processes triggering the mesoglobule formation provide important knowledge for the control of the mesoglobule structure and for tailoring its function for various applications. As an example, the uptake and transport of hydrophilic guest molecules is facilitated by a slow heating rate leading to an aqueous environment inside the mesoglobule. On the contrary, a hydrophobic guest is most efficiently incorporated in small and completely collapsed aggregates formed in a dilute polymer solution.

■ ASSOCIATED CONTENT

Supporting Information. Experimental section, detailed calculation of spin probes in a hydrophilic environment y_1 and heating rate dependent EPR spectra of PG2(ETalkyl). This material is available free of charge via the Internet at http://pubs.acs.org.

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