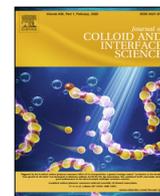




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First events in the coil-to-globule transition of PVME in water: An ultrafast temperature jump – time-resolved elastic light scattering study



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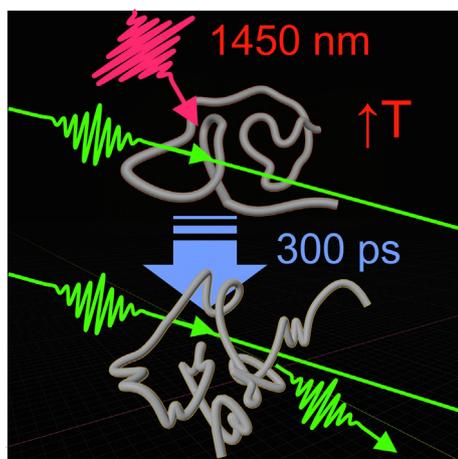
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GRAPHICAL ABSTRACT



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ABSTRACT

Hypothesis: The coil-to-globule transition is an essential phenomenon in protein and polymer solutions. Late stages of such transitions, $>1 \mu\text{s}$, have been thoroughly studied. Yet, the initial ones are a matter of speculations. Here, we present the first observation of a sub-nanosecond stage of the coil-to-globule transition of poly (vinyl methyl ether), PVME, in water.

Experiments: The detection of an early stage of the coil-to-globule transition has been possible thanks to a novel experimental approach – time-resolved elastic light scattering study, following an ultrafast temperature jump. We identified a molecular process active in the observed stage of the transition with use of broadband dielectric spectroscopy.

Findings: In the experiment's time window, from a few ps to around 600 ps, we observed an increase in the light scattering intensity 300–400 ps after the temperature jump that heated the sample above its lower critical solution temperature (LCST). The observed time coincides with the time of segmental

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relaxation of PVME, determined by broadband dielectric spectroscopy in the temperature range of the LCST of the PVME/water mixture. This coincidence strongly suggests that the observed herein stage of coil-to-globule transition is the rapid formation of local nuclei along the polymer chain. Those nuclei may grow and aggregate in later stages of the process, which are out of our experimental time window. © 2021 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

The thermally-induced coil-to-globule transition of a macromolecule in a solution is responsible for scientifically and technologically important phenomena such as the collapse of thermosensitive polymer gels and protein or peptide folding. This kind of transition in polymer solutions has been treated by many researchers as the simplest model of protein folding [1–4] and DNA condensation [5], thus attracting massive interest. Theoretical and experimental studies show that the late stages of such a phase transition may be divided into the formation of “crumpled” and a subsequent “compact” globules followed by their aggregation, which is associated with diffusion of water out of aggregates. [6–8] The observation of earlier stages of the transition was hampered by the limited possibility of rapid heating of the polymer/water mixture and the time resolution of detection. Ye *et al.* measured poly(*N*-isopropyl acrylamide) (pNIPAm) dissolved in water together with a fluorophore with time-resolved fluorescence and light scattering after a temperature jump. The dead time of their detector (20 μs) allowed them to measure relaxation times of 0.11 ms and 0.83 ms which were attributed to nucleation and subsequent merging of “pearls” on a polymer chain, respectively. [9]

On the other hand, the picture of the very initial stages of the transition is somewhat speculative. Some theoretical works pointed out that the transition starts with the dewetting of a polymer chain. [10,11] Other works suggested a strongly cooperative character of the transition, i.e., segmental reorganization of a polymer chain together with the movement of water molecules with just slight dehydration of polymer chains. [12,13] The vast majority of the studies were performed on aqueous pNIPAm solutions and were claimed to be representative for a collapse of flexible non-charged homopolymers in water. For example, Mochizuki and Ben-Amotz recently performed a study of the temperature-triggered transition of pNIPAm/water mixture in a microfluidic channel with a method called “Raman - multivariate curve analysis”. Surprisingly, they observed the onset of the solution cloud point (related to the formation of polymer globules) to appear before the hydration shell’s structural transformation. [14] On the contrary, wide-angle X-ray scattering and dielectric relaxation spectroscopy measurements by Yanase *et al.* [15] on a similar system (pNIPAm/water) pointed at the strongly concerted manner of the polymer dehydration and critical fluctuations of the polymer chain.

In this work, we study the initial stages of the coil-to-globule transition of poly(vinyl methyl ether), PVME, in water above the polymer lower critical solution temperature (LCST). PVME is one of the most technologically promising and frequently studied thermo-responsive polymers [16–19], partly due to its LCST temperature located in the vicinity of the body temperature of mammals (35–37 °C) [20,21]. Similar to pNIPAm, PVME is a flexible non-charged homopolymer, yet, of a simpler chemical structure of its monomer unit.

We applied a new experimental approach to study the transition mechanism – a time-resolved optical experiment in which an ultrafast infrared heating pulse (*T*-jump) is followed by a picosecond pulse, which probes Rayleigh scattering of the sample. Steady-state elastic light scattering experiment has been

frequently applied to investigate conformational transitions of various stimuli-responsive polymer systems [22–24], including the PVME solutions [25]. The *T*-jump method was applied in the past in combination with different probe techniques, such as transient infrared [26] and Raman spectroscopies [27,28], time-resolved X-ray [29], or recently with Brillouin light scattering [30], usually on time scales ranging from ns to ms. The already mentioned work by Ye *et al.* [9] is so far the experiment with the best time resolution in the field. To the best of our knowledge, the *T*-jump has never been combined with ultrafast time-resolved Rayleigh scattering experiment.

2. Materials and methods

2.1. Samples

The PVME used in this study – a 50 wt% PVME/water mixture was purchased from Sigma Aldrich. The gel permeation chromatography determined a weight-averaged molecular weight of the polymer $M_w = 58,000$ g/mol with a polydispersity $M_w/M_n = 2.955$. The polymer was further diluted to solutions of different concentrations with ultrapure water (Millipore) of conductivity 18.2 MΩ·cm.

For dielectric spectroscopy studies, the PVME was purified using an ion-transfer resin and freeze-dried in a vacuum before being mixed with pure water to reduce dc conductivity and electrode polarization (EP). The PVME/water mixtures were prepared with PVME concentrations, C_{PVME} , of 10–50 wt% on every 10 wt% of PVME. For the 8 wt% PVME/water mixture, the dielectric relaxation strength of the relaxation process originated from PVME is too small to obtain the correct relaxation time and strength. Therefore, we made dielectric measurements of 10–50 wt% PVME/water mixtures to be able to extrapolate relaxation times values for lower concentrations.

For optical measurements, the prepared solutions (4, 5, 6, 8, 10, 12 wt%) were used without any further purification. Prior to the experiments, PVME solutions were incubated for at least 2 days at a temperature below the LCST for equilibration.

2.2. Methods

2.2.1. Dielectric measurements

Dielectric measurements of 10–50 wt% PVME/water mixtures and pure water were performed in a frequency range of 40 Hz – 50 GHz and at temperatures between –10 °C and 25 °C. We used a Network analyzer (NA, Agilent Technologies N5230C, 100 MHz – 50 GHz), an Impedance Analyzer (IA, Agilent Technologies 4291A, 40 Hz – 110 MHz), and two Impedance/Material Analyzers (IMA, Hewlett-Packard 4291A 1 MHz – 1.8 GHz and Agilent Technologies E4991A, 1 MHz – 3 GHz).

2.2.2. Optical setup

The ultrafast temperature jump – time-resolved elastic light scattering measurements were performed with a reconfigured setup (Fig. 1) that was originally built for femtosecond stimulated Raman scattering (FSRS) and has been described in detail in Ref. [31]. The setup is based on a commercial femtosecond Yb:KGW

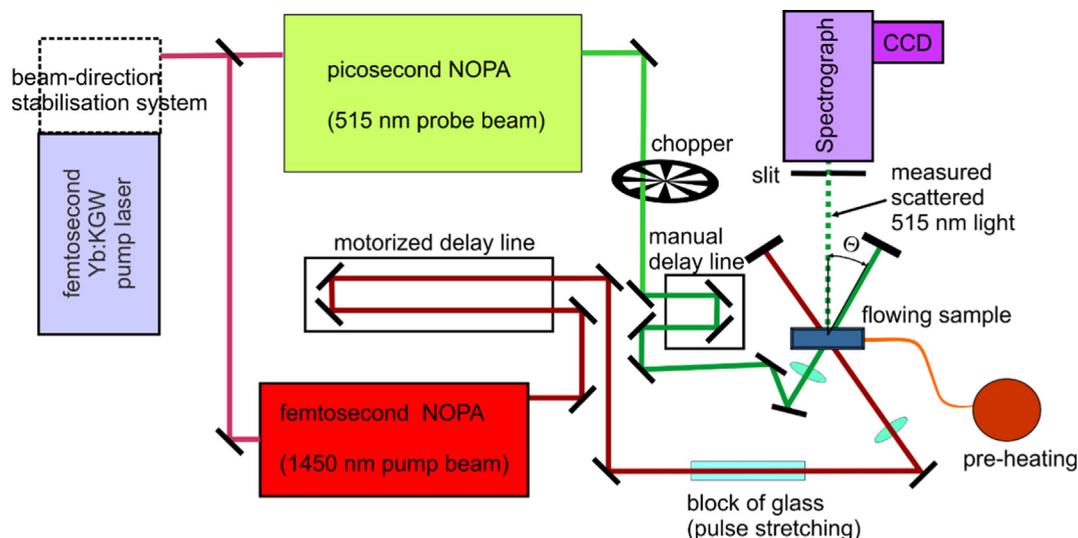


Fig. 1. Schematic representation of the optical setup for temperature jump – time-resolved light scattering experiments, $\theta \approx 5$ deg.

laser system (Pharos, Light Conversion), which produces 200 fs pulses centered at 1030 nm with a repetition rate of 1 kHz. As a probe, we used narrow bandwidth ($\sim 5 \text{ cm}^{-1}$) 2 ps long pulses centered at 515 nm with the energy around 5 μJ , which were generated in the process of frequency mixing of two oppositely chirped copies of the same femtosecond pulse (the method is described in Ref. [32,33]).

To stimulate the temperature-triggered coil-to-globule transition of a polymer in water we used NIR pulse centered at 1450 nm. That pulse was generated as an idler beam in the second stage of a home-built noncollinear optical parametric amplifier (NOPA). The energy of that pulse on the sample was around 6 μJ . The 1450 nm pulse is in resonance with the overtone of the vibrational OH stretching mode of water. Absorption of the energy of that pulse by water in the sample leads to homogenous heating of the sample in the focal spot of the beam within a few picoseconds after pulse arrival. The methodology described here is typical for all the optical temperature-jump experiments [34].

The delay time between the heating pulse and the probe pulse is controlled with the use of a precision optical delay line based on a motorized translation stage (Aerotech PRO165LM). Every second probe pulse was blocked by a mechanical chopper, so the scattering signal intensity was being constantly referenced to the situation without the presence of a probe beam on a sample. The time zero (overlap in time) of pulses used in the experiment was determined by observation of optical Kerr effect (OKE) generated in a sample as described in detail in Supplementary Materials, SM, to Ref. [1].

Since focusing of high-energy NIR pulse in the sample cell lead to the generation of white light in the cell front window and destruction of the cell, the peak power of the NIR pulse was reduced by stretching the pulse in the 100 mm block of glass (SF10). The resultant NIR pulse was ~ 230 fs long.

Both pulses were s-polarized, so the experiment was performed in the VV configuration. The scattered light was collected at the angle $\theta \approx 5$ deg after around 1 m path which allowed for effective beam separation. The scattered light was collected by the spectrometer (spectrograph Andor Shamrock SR 500i with CCD camera Andor Newton U971N). The spectrometer entrance slit was set to 90 μm .

2.2.3. Continuous temperature control

Proper temperature control was an important part of the optical experiment. PVME solution was being pre-heated (either to 32 $^{\circ}\text{C}$

or 34 $^{\circ}\text{C}$) in a 4 ml reservoir placed in a water bath with temperature-controlled to within 0.1 $^{\circ}\text{C}$ (heating stage with thermocouple and a PID controller). Both the water bath and a solution in the sample reservoir were continuously stirred. A sample was transported from the reservoir through isolated Tygon tubes to the optical measurement cell (Harrick Temperature Control Liquid Cell (TCE-S14-3) with Watlow temperature controller). A sample in the chamber was flowing between two thin (around 200 μm) CaF₂ windows separated with a PTFE spacer.

The flow of the liquid sample was forced with a peristaltic pump (Ismatec). The flow speed of 10–12 ml/min was fast enough to refresh sample volume between consecutive measurements (2 ms) but extremely long when compared to the time-scale of observed phenomena (< 1 ns).

2.2.4. Temperature jump

The laser temperature jump was realized with the use of the standard procedure thoroughly described in Ref.[34]. The magnitude of the temperature jump (around 2.3 K) was calculated with the use of the following formula:

$$\Delta T = \frac{k}{\rho C_p} \left(\frac{I}{A} \right)^{-kz} \quad (1)$$

where $k = 15 \text{ cm}^{-1}$ is the absorption coefficient of water at 1450 nm, $z = 0.02 \text{ cm}$ is the sample thickness, $A = 7.069 \times 10^{-6} \text{ cm}^2$ is the area of the sample irradiated by the infrared pulse (the 1450 nm beam diameter is 0.003 cm), the pulse energy $I = 6 \times 10^{-6} \text{ J}$, $C_p \approx 4.18 \text{ [J/g} \cdot \text{K]}$ is the heat capacity of water and $\rho \approx 1 \text{ g/cm}^3$, is the density of the aqueous solution. We assumed that the density and C_p of the 8 wt% PVME aqueous solution is very similar to these of pure water and we used water values in our calculations.

It is well known from the time-resolved Raman and infrared spectroscopy experiment that the relaxation of vibrational excitations of water takes hundreds of femtoseconds [31,35] and the deposited energy goes into thermal modes within a few picoseconds [36,37]. Hence, we may assume that approximately 20 ps after the arrival of the heating pulse, the irradiated sample volume is homogeneously heated. After each temperature jump, a sample was returned to the reservoir to be cooled down to the base temperature of the solution (32 $^{\circ}\text{C}$ or 34 $^{\circ}\text{C}$).

3. Results and discussion

An example of the steady-state (at the equilibrated state of the sample, at different temperatures) elastic light scattering measurement for 4 wt% and 8 wt% PVME in water, acquired in our setup, are shown in Fig. 2. Giving that the critical concentration of PVME in water is $c^* \approx 5$ vol%, [38] the herein studied concentrations are close to or already in a semidilute region. It is clear from the figure that the light scattering from both samples increases significantly at the transition onset temperature (around 35 °C). As it was shown by Zhang et al. “Rayleigh scattering is proportional to the number of scattering particles per unit volumes and the volume of one aggregate” [25] and thus can be used to follow the extension of macromolecular chains during a phase separation process. Consequently, the results shown in Fig. 2 can be explained by phase separation of the homogenous polymer solution. An increase in the scattered light’s intensity is thus associated with growing domains of that polymer fraction, which separates from the solvent. Such measurement of scattered light by an equilibrated sample vs. temperature provides, however, no information on the kinetics of the process. To study the kinetics of the process, one needs a time-resolved experiment.

In our experiment, we achieve the temperature jump of the magnitude of 2.3 K in the irradiated sample volume with the ~ 230 fs pulse centered at 1450 nm, which is in resonance with the overtone of the stretching mode of water molecules. The heating pulse is followed after a variable delay time t with a picosecond narrow-bandwidth pulse centered at 515 nm, which probes temperature-induced changes in the elastic light scattering of the sample. It is important to stress that while the sample is already heated by 2.3 K, it has still not reached a thermodynamic equilibrium in the time frame under study. This means that the sample responds to the heating pulse in molecular motions following the temporal and spatial hierarchy - the solvent molecule may move first, then polymer side groups, polymer segments, whole chains, etc. Hence, in the experiment, we may observe only initial (from a few ps to 600 ps) phases of the coil-to-globule transition, and

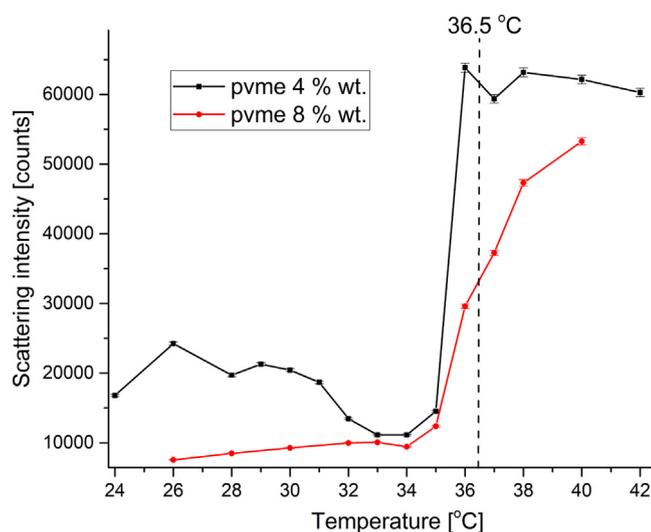


Fig. 2. Stationary Rayleigh scattering experiment on 4 wt% (squares) and 8 wt% (circles) PVME in water against temperature, measured in our setup for ultrafast spectroscopy, the LCST temperature of PVME /water mixture is marked with dashed line. Lines connecting points are for eye-guidance. The intensity of the light scattering on a detector was adjusted with neutral density filters such to use the full dynamic range on the detector. Hence, the scattering intensities should not be compared between samples. The error bars are related to the error of the measurement setup.

the full collapse of polymer coil is never achieved in the presented time-resolved study.

Fig. 3a presents scattering spectra at different delay times between the pump (heating) and the probe pulses for 8 wt% aqueous solution of PVME. The solution was continuously pre-heated to 34 °C. Thus, the pump pulse produced 34 °C \rightarrow 36.3 °C T-jump, i.e., just above the LCST of PVME (the LCST of 8 wt% PVME solution determined with small angle laser light scattering is around 34.7 °C [20]). The shape of the scattered light spectrum replicates the spectrum of the 515 nm probe. We observe an increase in the intensity of scattered light vs. time for both the side peak (at around 517 nm, Fig. 2a) and the central 515 nm line, for the probe spectrum, see Suppl. Fig. 1, an example of an increase in the intensity of scattered light in central peak see Suppl. Fig. 2a).

In Fig. 3b, we show the time traces of the integrated Rayleigh scattering spectrum of the 8 wt% PVME solution. The experiment was performed three times on different days with a fresh sample each time, giving similar results. Fig. 3b presents one of the 3 results, for other scans see Suppl. Figs. 3 and 4. We performed similar experiments for other (4 wt%, 5 wt%, 6 wt%, 10 wt%, 12 wt%) concentrations of PVME in water. Due to very large noises, we

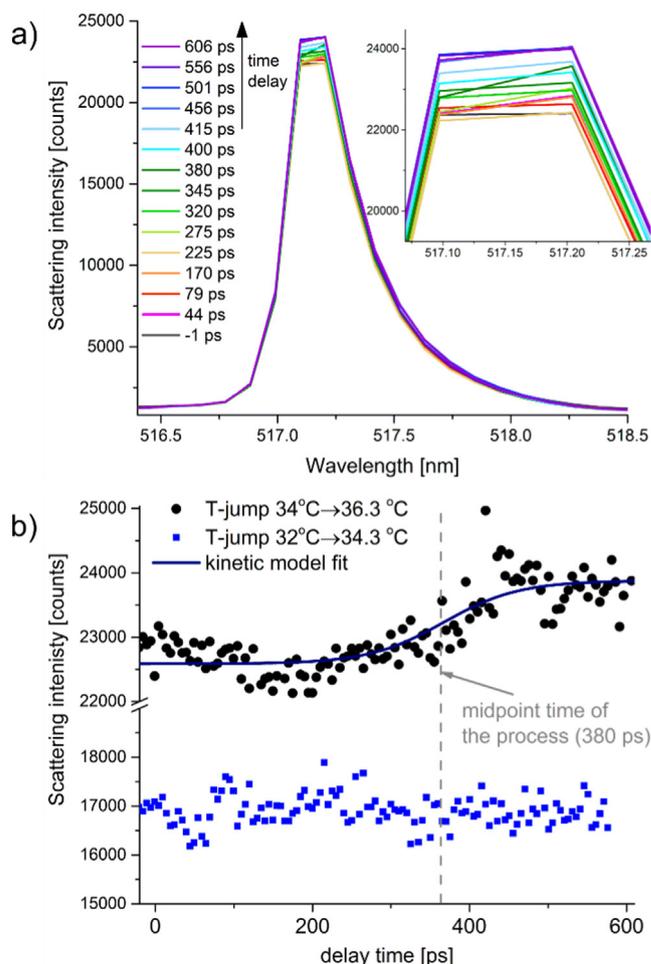


Fig. 3. a) spectra of Rayleigh scattered light by a 8 wt% solution of PVME in water in the 34 °C \rightarrow 36.3 °C T-jump experiment, for different delays between the heating and the probe pulses, inset: zoom-in of the maxima of Rayleigh scattering peaks b) time-traces of maximum intensity of Rayleigh spectra for both 34 °C \rightarrow 36.3 °C T-jump (black circles) and 32 °C \rightarrow 34.3 °C T-jump (blue squares). The midpoint of the transition in the former dependence is marked with a dashed line. The kinetic model fit to the 34 °C \rightarrow 36.3 °C T-jump is marked with the solid navy line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

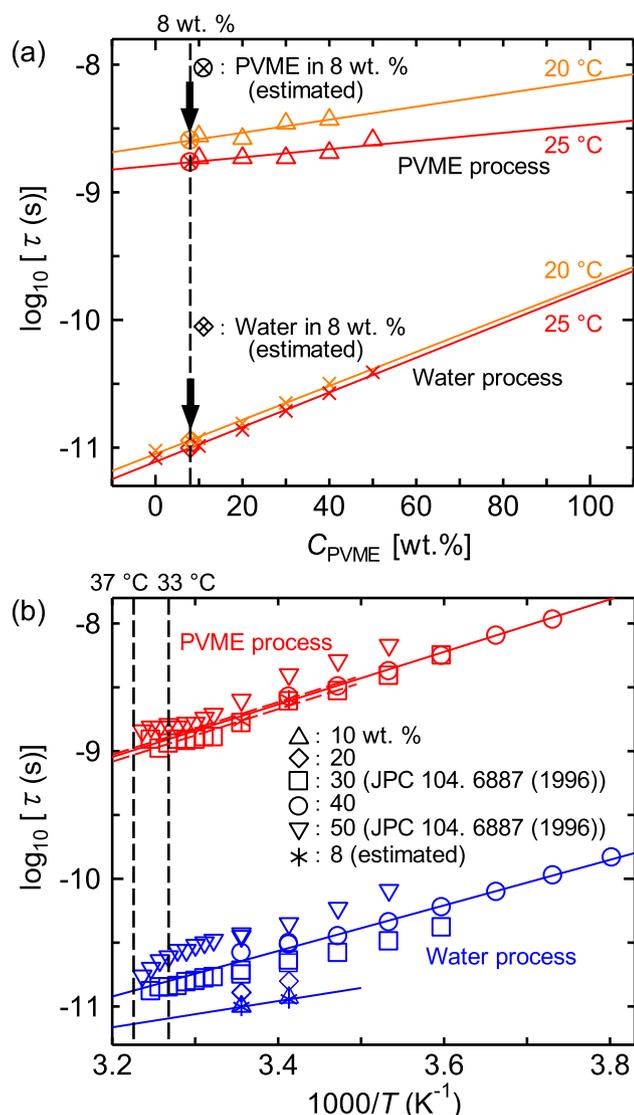


Fig. 4. a) Plots of logarithm of relaxation time τ of the PVME process (Δ) and the water process (\times) against the PVME concentration at 20 and 25 °C. b) Plots of logarithm of relaxation time τ of the PVME process and the water process against reciprocal temperature with various concentration. The dot line plot is relaxation time τ of indefinite loss peak. The data for concentrations 30 and 50 wt% of PVME are from ref. [33].

observed no time-dependence of light scattering for higher polymer concentrations. The noises, already noticeable for the 8 wt% sample, were much higher for 10 wt% and 12 wt% samples and originated probably from strong light scattering on some associated polymer coils what masked light scattering from local processes. For concentrations lower than 8 wt%, the magnitudes of light scattering changes after a T -jump were much smaller, and hence the results were difficult to interpret. The best result with polymer concentration close to c^* was obtained for 6 wt% solution – see Suppl. Fig. 2.

It is clear from Fig. 3b that the scattering signal coming from the sample raises significantly between 200 ps and 450 ps and afterward stays rather constant (in the time range of the experiment, i.e., 600 ps after the T -jump). Since one can imagine various artifacts resulting in an effect similar to that in Fig. 3, the proper choice of the reference experiment was crucial to let us assign our observation to the phase transition in the system studied. The reference measurement was performed on the same sample (8 wt% of PVME

in water) on the same day, with the same experimental setup settings. The only difference between the measurements was that in the reference one, the sample was being pre-heated to 32 °C, instead of 34 °C. Thus, the heating pulse resulted in the temperature jump from 32 °C to around 34.3 °C – just below the LCST of the polymer.

One may compare the curves coming from both measurements in Fig. 3b. It is apparent from the time trace of the reference measurement that no increase in the sample's scattering takes place in the time window of the experiment. Hence, the changes in the scattering that we observe in the 34 °C \rightarrow 36.3 °C T -jump experiments should be exclusively related to some evolution in the sample at the temperature range 35–36 °C, i.e., at the temperature of LCST of PVME/water system. The second observation is that the overall level of scattering signal (“baseline”) in the reference experiment is significantly lower than in the case of 34 °C \rightarrow 36.3 °C T -jump. It is well known [19,25] and clear from Fig. 2 that some polymer chains change their conformation into globular or even aggregate [39] at temperatures well below the mid-point of the coil-to-globule transition. Consequently, long-time annealing of the sample at 34 °C (in the reservoir) produces a significantly larger fraction of coiled chains compared to the same sample annealed at 32 °C. That additional coiled polymers are most likely responsible for the scattering “baseline” in the time-trace of 34 °C \rightarrow 36.3 °C T -jump experiments. Moreover, these coils could be a source of an additional measurement noise.

Having attributed the time-dependent increase in the light scattering of the sample to the LCST of the PVME/water mixture, we need to identify the molecular process active in the initial stage of the phase transition. To this end, we use broadband dielectric spectroscopy (BDS). The midpoint of the curve in Fig. 3b is at the time $\tau \approx 380$ ps, and in the time-trace in Suppl. Fig. 3, the midpoint is at around 310 ps. The BDS studies on PVME/water systems show the following processes: i) dielectric relaxation process of water at 10 GHz is the well-known primary relaxation process of water affected by the presence of PVME molecules; ii) the relaxation process observed at 100 MHz originates from the local chain motion, i.e., the segmental motion of PVME which correspond to the structural relaxation generally called the alpha-relaxation. [28,40] At the temperature range (32–36 °C) studied herein the rotational reorientation time of water is of the order of 10 ps and we cannot distinguish any other kind of local motion of PVME from the segmental relaxation of the PVME chain [40] (see Suppl. Figs. 5–11). For the 8 wt% PVME-water mixture, the dielectric relaxation strength of the relaxation process originated from PVME is too small to obtain the correct relaxation time and strength. Therefore, we performed systematic dielectric spectroscopy studies of different concentrations of PVME/water mixture at different temperatures. Next, from the obtained relaxations maps we extrapolated the segmental relaxation process in the 8 wt% PVME solution at 32–36 °C temperature range to be of the order of 900–1100 ps. (see Fig. 4 and section 2 of SM, for details). This is roughly three times longer than the 300–400 ps observed in the time-resolved light scattering experiment. We should keep in mind that dielectric spectroscopy probes the first-order correlation function, proportional to the first-order Legendre polynomial, $P_1(t)$, and the time-resolved light scattering depends on the second-order correlation function, proportional to the second-order Legendre polynomial, $P_2(t)$. From the theoretical considerations [41] (see also SM to our paper, section 3) it comes that the ratio of the time τ_1 (derived from the first-order correlation function) and τ_2 (derived from the second-order correlation function) is equal to 3. Hence, the time $\tau_2 = 300$ –400 ps determined with our time-resolved scattering experiment corresponds well to the segmental relaxation time of PVME at the same temperature and concentration in water. Similarly, $\tau_2 \approx 215$ ps for 6 wt% PVME (Suppl. Fig. 2) goes along above

considerations and our extrapolation of relaxation time of PVME segmental relaxation process.

From Rayleigh's theory of light scattering, an increase in the intensity of the elastic light scattering of a material may be related either to an increase in the number or of the volume of scatterers. The coil-to-globule transition studied in this work should ultimately lead to the formation of hydrophobic polymer globules. In the theoretical works of Byrne *et al.* [42] and later of Klushin [43] that process is postulated to start with rapid nucleation – formation of small “defects” along the chain followed with slow ingrowth of clusters around these nuclei. The clusters ultimately merge into large aggregates and the macroscopic demixing into polymer-rich phase and solvent-rich phase is observed afterward. An early stage of the process, which we observed, happens in a time comparable to that of segmental relaxation of the polymer chain. This means that it cannot be assigned to any process taking multiple steps of polymer segments and should be assigned to the “nucleation step”. Ye *et al.*, in their excellent study on pNIPAm in water with microsecond time-resolved light scattering after a temperature jump, measured relaxation times of 0.11 ms and 0.83 ms, which they assigned respectively to nucleation and subsequent merging of clusters–“pearls” on a polymer chain. [9] Given our results, the relaxation time presented by them should be rather attributed to the formation of smaller clusters and their merging into larger ones.

Furthermore, we want to associate the process visible in Fig. 3b with a suitable kinetic model. Having tested a number of models, we achieved the best fit to the experimental data with the one characteristic for an autocatalytic process:



where k_1 , k_2 are kinetic rate constants, A is a concentration of a substrate, and B is a concentration of a product (see the fit to the data in Fig. 3b and additional information on the model in section 4 of SM). Autocatalysis is known to describe well non-equilibrium conformational phase transitions. [44] Such kinetics goes along with the molecular interpretation of the here presented results – temperature-activated molecular dynamics of polymer segments leads to the formation of some “defects” – nuclei on a polymer chain (the first step of the reaction), and these defects grow in volumes in the second step of the reaction. The time-traces in Fig. 3b and in Suppl. Figs. 2-4, 12-14 were fitted with the equation S9. From the fits, we may obtain the characteristic rate constant k_1 of the first process, $A \rightarrow B$, but the rate constant of the second step of the proposed mechanism, k_2 , cannot be decoupled from the initial concentration of the “reactive moiety” of the first step, $[A](0)$, so we may determine $k'_2 = k_2 \cdot [A](0)$. Our fit to the data was achieved with the following rate constants: $k_1 = (1 \pm 1.4) \cdot 10^{-5} \text{ ps}^{-1}$ ($1/k_1 = 100 \text{ ns}$), $k'_2 = (2.08 \pm 0.45) \cdot 10^{-2} \text{ ps}^{-1}$ ($1/k'_2 = 48 \text{ ps}$). Considering these values and the values obtained from fits to other sets of data (Suppl. Figs. 2-4) it is apparent that $1/k'_2$ is of the order of tens of ps, while $1/k_1$ is 3–4 orders of magnitude slower and has a large variability. Concerning the first step, molecular dynamics simulations imply that rearrangements of oligomeric regions of a polymer above its LCST can occur within tens of ns [45]. Concerning the second step, we can suppose a diffusion-controlled bimolecular process, which is the fastest bimolecular process in solution. The value of the second-order rate constant for a diffusion-controlled bimolecular process in water is of the order of $10^{10} \text{ M}^{-1} \text{ s}^{-1}$. In the 8 wt% PVME/water mixture, the concentration of PVME residues is about 1.4 M. The resulting $1/k_2 \cdot [A](0)$ value is about 70 ps, compatible with the k'_2 values determined herein.

In future studies on the aqueous solution of other polymers with well-defined molecular weights, we plan to incorporate a dis-

persion of segmental relaxation times distribution into the kinetic model. We expect that the broader range of samples with narrow polydispersity indices and a more advanced kinetic model should clarify the early mechanism of polymer coil-to-globule transition.

4. Conclusions

In this work, we applied for the first time the ultrafast temperature-jump – time-resolved light scattering method to study the initial stages of the coil-to-globule transition in the thermo-responsive PVME/water system. In our experiment's time window – from a few ps to 600 ps, we were able to identify just one process with a midpoint time $\tau_2 \approx 300 - 400 \text{ ps}$. Such time coincided with the relaxation time of the segmental relaxation of PVME in 8 wt% aqueous solution at 32–36 °C temperature range seen by dielectric spectroscopy. This agreement strongly suggests that the observed herein initial stage of coil-to-globule transition is the rapid formation of local defects – nuclei for further growth of clusters along the polymer chain. The formation of such defects in the course of coil-to-globule transitions of homopolymers is in accordance with recent theoretical findings. [4,46] The nucleation and growth of the defects also goes along with the autocatalytic kinetic model we used to fit the time-resolved light scattering data. With the sensitivity of our experiment, we have not observed any other stages of the polymer collapse, which involves a change in the number or in a volume of scatterers in the solution studied.

We expect that the sensitivity of the method could be increased by providing a more energetic heating pulse and so, producing a higher T-jump, e.g., from 28 °C to 36 °C. It is known that some chains fold in lower temperatures than nominal phase transition temperature, as low as at 32 °C in PVME/water system [25]. Circulation of this fraction of polymer chains in a system is the most probable source of noise in the presented experiment. We hope that a similar experiment of better signal-to-noise ratio would also reveal other initial stages of the coil-to-globule transition and bring us closer to understanding the exact mechanism of the process.

CRediT authorship contribution statement

Marcin Pastorczak: Conceptualization, Funding acquisition, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft. **Michał Nejbauer:** Methodology, Software, Writing – review & editing. **Naoki Shinyashiki:** Resources, Formal analysis, Supervision, Investigation, Writing – review & editing. **Masanobu Takatsuka:** Investigation, Visualization. **Gonzalo Angulo:** Formal analysis, Validation, Data curation, Writing – review & editing. **Yuriy Stepanenko:** Resources, Supervision, Writing – review & editing. **Czesław Radzewicz:** Funding acquisition, Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcis.2021.10.158>.

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