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The Dependence of the Cloud Point and Hysteresis of Poly(N-isopropylacrylamide) on Experimental Conditions: The Need for Standardization of Thermoresponsive Transition Determinations^a

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^a **Supporting Information** is available online from the Wiley Online Library or from the author.

Abstract

Inappropriately, the critical solution temperature (CST) measured by one single, individual set of conditions of thermoresponsive polymers with LCST- or UCST-type behavior is considered almost exclusively as the LCST or UCST, respectively. These are correctly the minimum or maximum, respectively, of the full phase diagrams. Because the dynamic phase transition depend on the conditions, and no standardized or widely accepted process exist for CST determination, systematic investigations were carried out by turbidimetry with the most widely investigated thermoresponsive polymer, poly(N-isopropylacrylamide) (PNIPAAm) to unveil the effect of data evaluation, measurement conditions on the transmittance-temperature curves, cloud point (T_{CP}) and heating-cooling hysteresis. The unusual dependence of the fundamental hysteresis parameters, i. e. width of hysteresis (X_H) and extent of transmittance recovery (Y_H), of PNIPAAm on a broad range of conditions is revealed for the first time. On the basis of our findings, we propose to use as a standard set of condition the inflection point of transmittance(absorbance)-temperature curves as T_{CP} and T_{CL} , 0.1 wt% polymer solution, 0.2 °C/min heating/cooling steps with 5 min equilibration between the gradual change of temperature, 488 nm wavelength to obtain data comparable to light scattering measurements at this wavelength, and to determine always X_H and Y_H .

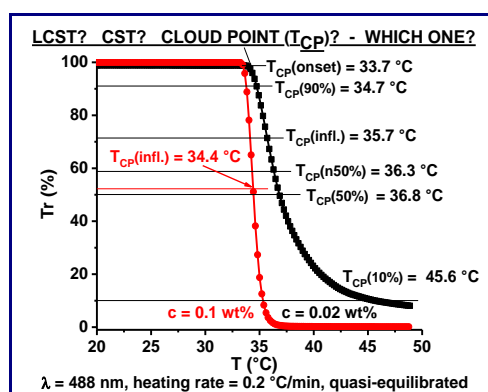


FIGURE FOR ToC_ABSTRACT

1. Introduction

Responsive (smart, intelligent, adaptive) materials are of great scientific and industrial interest, and as a consequence, intensive research and development are taking place with such materials worldwide nowadays. Thermally responsive polymers with either lower critical solution temperature (LCST) or upper critical solution temperature (UCST), due to coil-to-globule or globule-to-coil transitions, respectively, belong to a unique class of smart materials with broad application possibilities ranging from nanotechnologies to biomaterials, tissue engineering scaffolds, intelligent drug release assemblies, sensors, self-healing structures, responsive hybrid materials etc. (see e.g. Refs. ^[1-23] and references therein). Undoubtedly, poly(N-isopropylacrylamide) (PNIPAAm) has been the most investigated temperature-responsive polymer since the first description of its LCST-type behavior.^[24] This is mainly due to its biocompatibility and LCST near to the body temperature in the range of ~32 °C as usually considered (see e.g. refs. ^[1-29] and references therein). However, most of the reported data are not LCSTs, but critical solution temperatures (CSTs) obtained under one single set of experimental parameters. As it has been well-defined for a long time, the LCST and UCST are the minimum and maximum, respectively, of the full phase diagrams, i. e. the CST as a function of polymer/solvent volume (or weight) fraction in the full composition range. The large majority of research reports and patents inappropriately claim a single CST obtained by using only one individually selected polymer concentration and one set of experimental conditions as LCST or UCST. Although these are very valuable data, special care has to be exercised for using the LCST or UCST terms. It has to be mentioned that the reliability of the reported LCST and UCST values are still questionable, even for PNIPAAm,^[5] due mainly to experimental difficulties as discussed by Wu et al.,^[30] who attempted to overcome this problem with a special experimental setup for obtaining phase diagrams of polymers with LCST- and UCST-type behavior recently. We would like to emphasize that on the basis of these aspects of LCST- and UCST-type polymers, it is necessary to strictly distinguish

between LCST or UCST and the critical solution temperature (CST) of heating or cooling observed under a single set of condition for phase transition determination whatever techniques are used for studying thermoresponsive polymers, including but not limited to UV-visible (UV-vis), IR, NMR and dielectric spectroscopies, DSC, light, X-ray and neutron scattering. The difficulties for LCST and UCST determinations are related especially to the facts that homogeneous polymer solutions (solvent/polymer mixtures) with high concentrations are hard to be prepared (or would require infinitely long times) and due to the dynamics of the thermal phase transition, reaching the thermodynamic equilibrium between the polymer and solvent rich phases for obtaining the CST for every single polymer-solvent mixtures to create a phase diagram would also require very long time and/or extremely low heating/cooling rates. However, it is obvious that both from usual scientific and practical point of views infinitely long dissolution times and/or very low heating/cooling rates cannot be used, especially when the response time is critical. Therefore, widely applicable and technically feasible, if possible standardized, conditions should be used for obtaining reliable and reproducible CST data for comparison and application purposes. However, such conditions are lacking in the existing scientific and technical practice and literature.

A detailed literature evaluation shows that the CSTs, usually but incorrectly claimed as LCSTs, for aqueous solutions of PNIPAAm was reported even as low as 24 °C and as high as around 60-70 °C.^[28,29,31] As shown by a variety of typical examples in **Table S1** (see in Supporting Information), the thermal transition temperatures of PNIPAAm homopolymers lie between these data, and even a quick look at this dataset induce significant uncertainties as to the reliability of the reported so-called LCST values of PNIPAAm in the literature, and other thermally responsive polymers as well, on the one hand. On the other hand, it can also be seen in **Table S1** that a variety of experimental conditions and technics have been applied for the determination of the thermal phase transition of PNIPAAm solutions. These include cloud point (T_{CP}) determination by UV-Vis spectroscopy, static (LS) or dynamic light scattering

(DLS), NMR, IR and dielectric spectroscopies, DSC, viscosity measurements and visual observation. It is important to note that a broad variety of conditions are applied for CST determination in terms of concentration, heating rate and wavelength, especially when UV-Vis spectroscopy or light scattering are used as presented in **Table S1**. However, in the course of our ongoing research in the field of thermally responsive polymers, it has been found that no any clue or proposal exist, which conditions should be applied for obtaining reliable CST values useful for reproduction and comparison purposes. These fundamental uncertainty problems are well reflected in a recent survey by Halperin, Kröger and Winnik on the comparison of existing phase diagrams of the thermal phase transition of PNIPAAm.^[5]

As indicated by the data in **Table S1**, cloud point (T_{CP}) measurements by UV-Vis spectroscopy, available in most of the laboratories worldwide, is far the most frequently applied method for the determination of the phase transition temperature by measuring mainly the transmittance (T_r) as a function of temperature. The absorbance-temperature curves are also used for this purpose in much less cases.^[32-37] Looking at the data and the remarks in **Table S1** indicates that the selection of the conditions for LCST determination is rather individualistic, independent of which method is used, and it is hard if not impossible to make acceptable comparison of not only the CST values but the conclusions based on these data as well. It is also evident from **Table S1** that there is no agreement between the dependence of the CST data on even such parameters as polymer concentration, heating rate and molecular weight of PNIPAAm, not to mention that surprisingly the hysteresis between heating and cooling cycles, which has not only great scientific but practical importance as well, and its dependence on experimental conditions and polymer structure has hardly been investigated.^[38-48] The hysteresis, that is the lower remixing temperature in the cooling cycle compared to the higher CST observed in the course of heating, occurs even in very dilute solutions, i. e. with single chains, due to interchain hydrogen bonding in the case of PNIPAAm as found by Chi et al.^[38,39] Measurements by DSC,^[40-45] FTIR,^[43-46] optical^[47] and

dielectric spectroscopy^[48] have revealed that in addition to interchain H-bonding and aggregation even vitrification^[40] of the polymer takes place at higher concentrations upon heating its solution above the CST. As a consequence, the dissolution (remixing, globule-to-coil transition) temperature is smaller than that of the CST measured during heating. In spite of the significance of this phenomenon, it is rather surprising that with the exception of reports by Zhang et al.^[41,42] and Schönhals and coworkers^[48] on using DSC and dielectric spectroscopy, respectively, for investigating the effect of concentration and heating-cooling rates on the CST of PNIPAAm solutions with relatively low concentrations, the effect of experimental conditions on hysteresis has not been studied in details until now. The influence of a broad range of experimental conditions of cloud point (T_{CP}) determination by UV-vis spectrophotometry (turbidity measurements) on the hysteresis phenomenon of thermoresponsive polymers, including PNIPAAm as well, is completely lacking according to the best of our knowledge.

All kinds of opposing conclusions, i.e. decrease, increase and independence of LCST of PNIPAAm on the structural and experimental parameters can be found in the literature (see Table S1 and e.g. refs.^[49-57]). On the basis of these contradictory observations and the largely undefined broad range of conditions, the question arises whether standardized (or at least broadly accepted) experimental conditions can be defined for investigating LCST-type and UCST-type polymers for obtaining reliable and widely comparable results. Due to the fact that cloud point measurements with photometric techniques, such as UV-Vis spectroscopy, is the most widely available and applied method for obtaining the phase transition temperature, this method is selected by us for carrying out systematic investigations on the effect of experimental parameters on the T_{CP} . In order to select a set of well-defined and broadly applicable set of conditions, the dependence of T_{CP} on the heating-cooling hysteresis, on the major experimental parameters, that is, polymer concentration, heating and cooling rates, wavelength of the incident light and the number of the heating-cooling cycles, should be

available. However, beyond the effect of concentration on cloud point only sporadic reports exist on the effect of the other factors, and such detailed investigations including all these parameters and a proposal for a standardized T_{CP} measurement do not exist at all according to our knowledge. Herein, we report on the systematic investigation of the T_{CP} dependence of PNIPAAm, as the most typical thermoresponsive polymer, by transmittance determination in aqueous solution on polymer concentration, heating rate and the way of heating, wavelength of the visible light, heating and cooling, that is hysteresis, and the number of heating-cooling cycles. We also propose a widely applicable set of conditions which may serve as a standard method resulting in CST values providing well comparable data for polymers with LCST- or UCST-type thermoresponsive behavior.

2. Experimental Section

2.1. Materials

N-isopropylacrylamide (NIPAAm, 97%, Sigma-Aldrich) and 2,2'-azoisobutyronitrile (AIBN, 98%, Sigma-Aldrich) were recrystallized from hexane and methanol twice respectively. Tetrahydrofuran (THF, >99%, Molar Chemicals) was refluxed over $LiAlH_4$, distilled and was kept under nitrogen until its use. Diethyl ether (>99%, Molar Chemicals) was used without further purification.

2.2. Preparation of a poly(N-isopropylacrylamide) (PNIPAAm)

Poly(N-isopropylacrylamide) (PNIPAAm) was obtained by free radical polymerization. NIPAAm (2 g, 0.018 mol) was dissolved in 16 ml of THF in a round bottom flask. The solution was degassed by bubbling with argon for 20 min. The reaction mixture was warmed to 60 °C and AIBN (0,029 g, 0.177 mmol) was then added. After 18 hours, the resulting polymer was precipitated twice from THF in diethyl ether and filtered. Finally, the product was dried up to the constant weight in vacuum at 60 °C. The yield was 79%.

2.3. Measurements

Number average molecular weight (M_n) and polydispersity ($PDI = M_w/M_n$) was measured by a gel permeation chromatographic (GPC) system equipped with a Waters 515 HPLC pump, and Mixed C separation columns. THF was used as eluent with a flow rate of 1 ml/min. The molecular weight was determined on the basis of calibration with polystyrene standards of narrow molecular weight distribution. $M_n = 6200$ g/mol, $M_w/M_n = 2.9$ for the applied PNIPAAm sample. The transmittance versus temperature curves for obtaining the cloud point (T_{CP}) was measured by an UV-Vis spectrophotometer (Jasco V-650) equipped with Jasco MCB-100 mini Circulation Bath and Peltier thermostat heating and cooling system. Standard cuvettes with 1 cm optical length were used. The reference was deionized water. The sample was dissolved in deionized water and was diluted to the predetermined concentrations. The polymer solutions were heated and then cooled from 20 to 45 °C and 45 to 20 °C. Every measurement was repeated at least twice.

3. Results and Discussion

Figure 1A shows a typical transmittance versus temperature curve of a PNIPAAm solution. By exploring the literature, we have found that various ways exist to evaluate such curves, including absorbance-temperature curves as well, for the determination of the cloud point (T_{CP}). Here, it has to be noted that an extremely large number of reports exists in the literature, which incorrectly considers a selected transition point on such a curve as the LCST or UCST for the given polymer system by applying only one single set of experimental conditions. As already mentioned the LCST or UCST are defined as the minimum or maximum values, respectively, of the phase diagram, i.e. the phase transition temperature as a function of the polymer/solvent volume (or weight) fraction in the full composition range. Therefore, in our opinion, it is better to refer to the phase transition temperature determined under selected conditions by turbidity measurements with photometric methods as cloud point (T_{CP}). It is also noteworthy to mention that for such a transition point of a given polymer

solution all the experimental parameters should be fully provided. As shown in **Figure 1A** and for a variety of examples in **Table S2** (see Supporting Information), there is no broadly accepted agreement on which point should be taken into account on the transmittance-temperature (or absorbance-temperature) curves as the cloud point (T_{CP}) for polymer-solvent mixtures with LCST or UCST. As indicated in **Table S2**, the onset temperature of the transmittance (absorbance) change ($T_{CP}(\text{onset})$), the temperature belonging to 90%, 50% or 10% of the transmittance, and surprisingly rarely the temperature at the inflection point of the S-shaped transmittance-temperature curves are all reported as the cloud point. Here, we would like to note again that the cloud point (T_{CP}) and LCST are inappropriately used as identical terms in the vast majority of the literature. It is evident from the data in **Figure 1A** that the cloud point values range from 34.3 °C to 46.6 °C depending on which data point is selected as T_{CP} on the displayed transmittance-temperature (T_r - T) curve. One of the most unreliable choices is the onset temperature ($T_{CP}(\text{onset})$), especially when the transmittance decrease with increasing temperature has an initial curvature with relatively large radius. The $T_{CP}(90\%)$ is meaningless when the investigated polymer solution has lower than 90% initial transmittance at lower than the thermal phase transition temperature. Taking the temperature at 10% transmittance as cloud point ($T_{CP}(10\%)$) has similar problem, especially when the transmittance does not reach this value. Another selection for the cloud point temperature is the transmittance at 50% ($T_{CP}(50\%)$). However, in cases when the transmittance does not reach 50%, this is meaningless, or when the transmittance does not change between 100% and 0% and/or when an asymmetric curve is obtained, serious uncertainties may arise with the $T_{CP}(50\%)$ values as a reliable choice for cloud point. In order to overcome this problem, there have been reports^[47,52,58,59] in which the transmittance-temperature or absorbance-temperature curves are normalized between 0 and 1 by selecting the minimal value as zero and the measured maximum value as 1, and the temperature value at 0.5 is selected as $T_{CP}(n50\%)$ or the temperature at 1% change of the normalized absorbance is considered as the cloud

point.^[37] However, significant distortion of the measured curves may occur with this normalization. As the data indicate in **Figure 1A**, there is a range of more than 12 °C difference between the lowest ($T_{CP(\text{onset})}$) and highest ($T_{CP(10\%)}$) values for cloud points, and even 3.1 °C difference is obtained between $T_{CP(\text{onset})}$ and $T_{CP(50\%)}$. These observations clearly show that this broad range of individualistic selection of the evaluation method for obtaining T_{CP} values results in unreliable data which are not suitable to make acceptable comparisons between a large number of literature data obtained by cloud point measurements. Taking into account all these uncertainties with the applied evaluation methods, and the fact that the inflection point is the most characteristic point of any S-shaped transition observed in sciences and engineering, we propose to use the inflection point in the transmittance (absorbance)-temperature curves as the cloud point (T_{CP}) for polymer solutions with LCST or UCST behavior as displayed in **Figure 1A**. As shown in **Figure 1B**, the inflection point for obtaining T_{CP} can be easily determined as the minimum or maximum values of the derivatives of the transmittance-temperature or absorbance-temperature curves, respectively.

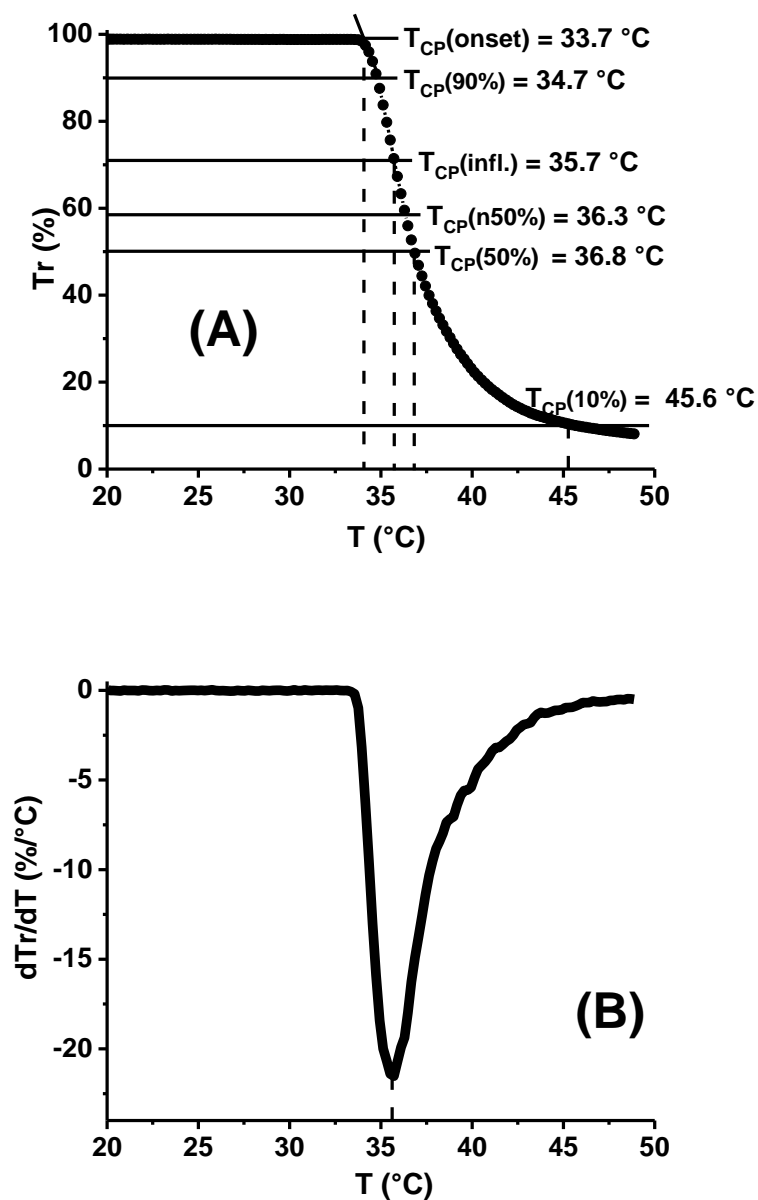


Figure 1. The cloud point (T_{CP}) values by various evaluation methods of the transmittance-temperature (Tr-T) curve (A) and the derivate of the Tr-T curve of a PNIPAAm solution (conditions: 0.02 wt% PNIPAAm, 0.2 °C/min heating rate with 5 min equilibration, 488 nm wavelength).

As well reflected by the data in **Tables S1** and **S2**, experiments for T_{CP} determination have been carried out with a broad range of heating rates ranging from 0.0003 °C/min^[60] up to

even 15 °C/min.^[61] Considering that the coil-to-globule transition and the subsequent aggregation of polymer chains, resulting in transmittance change as a function of temperature, are dynamic processes, it is obvious to assume that extremely long experimental time is needed to reach equilibrium or near to equilibrium conditions.^[30] Alternatively, the transition temperature at thermal equilibrium can be approximated by either determining T_{CP} with selected equilibration time at each point of data acquisition or by measuring the TCP at various heating rates and extrapolating the resulting values to zero heating rate. In spite of these, only few reports exist on the effect of heating rate on the Tr-T curves and on the observed T_{CP} values.^[56,60,62-64] The effect of heating rate on the dependence of transmittance as a function of temperature is shown in **Figure 2**. The curves in this Figure clearly show that the heating rate has significant effect on the transmittance-temperature curves. Increasing the heating rate increases both the width of the transition and T_{CP} as well. Increasing the heating rate from 0.2 °C/min to 1.5 °C/min increases the T_{CP} from 34.6 °C to 35.9 °C. When subsequent to every 0.2 °C/min temperature increase increment 5 minutes waiting period was applied, three observations can be made: (i) the decrease of the transmittance starts at higher temperature than that in the cases of 0.2 °C/min and even 0.5 °C/min heating rates, (ii) the temperature range of the thermal transition is narrower than without equilibration, and (iii) the T_{CP} value is even lower than in the case of 0.2 °C/min heating rate. It is interesting to note that linear extrapolation of the T_{CP} data to zero heating rate provides a T_{CP} in good agreement with that observed with 5 min equilibration time. This indicates that even such a short equilibration time may result in cloud points near to that expected under thermal equilibrium. Thus, it can be concluded that certain time of equilibration is necessary between detecting transmittance values after every temperature increase/decrease increments. On the basis of these findings, it can be proposed to apply at least practically feasible equilibration time between every temperature increase or decrease for T_{CP} determination. In order to carry out such experiments in an acceptable and broadly applicable time frame, not only for T_{CP} determination but critical

solution temperature (CST) measurements by other techniques, such as DSC, scattering methods, IR, NMR and dielectric spectroscopies, we suggest to use 0.2 °C temperature increments and 5 minutes equilibration at constant temperature before increasing (decreasing) the temperature again by 0.2 °C. Although this period of time is most likely sufficient to obtain only a close to the thermal equilibrium state, this quasi-equilibration is suggested to be used in order to avoid larger errors in T_{CP} and in general CST determinations as a consequence of heating/cooling without taking into account the dynamics of demixing/remixing.

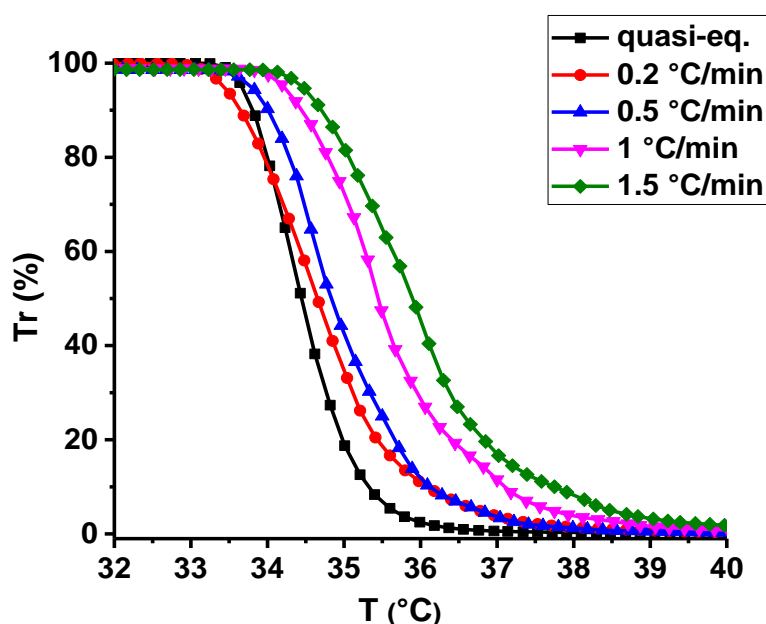


Figure 2. Transmittance-temperature curves of a PNIPAAm solution at different heating rates including 0.2 °C/min heating followed by 5 min thermal equilibration time (quasi-eq.) (conditions: 0.1 wt% PNIPAAm solution, 488 nm wavelength).

Unless attempts made to obtain the full scale phase diagram, authors almost exclusively use polymer solutions with one, individually selected concentration for T_{CP} determination in a broad range according to literature reports, i.e. from about 0.00007% up to

20%, that is in a range of more than 5 orders of magnitude (see Table S1). The T_{CP} value obtained by this kind of single-point T_{CP} determination is almost exclusively but misleadingly reported as LCST (or UCST). Due to the fact that T_{CP} is obviously depends on polymer concentration, for reliable comparison purposes, the choice of the concentration for single-point determination of T_{CP} should be broadly agreed on, that is this factor of such measurements should also be standardized. The effect of concentration of PNIPAAm on the transmittance-temperature (Tr-T) curves and their derivatives as a function of temperature in the heating cycle is displayed in **Figure 3A** and **3B**, respectively. In order to avoid any effect of sample preparation, the second heating and cooling scans were used in every measurement. However, it has to be noted that remarkable differences in the Tr-T curves of the first and second scans were not observed. As shown in **Figure 3**, the region of the thermal transition, the shape of the Tr-T curves and even the initial transmittance value strongly depend on concentration. The phase transition at high dilutions (0.01 and 0.02 wt%) is broad. The transmittance does not reach zero in the investigated temperature range, i.e. up to 45 °C. With increasing concentration, the transition between 100% and 0% transmittance becomes narrower and it is shifted towards lower temperatures, that is, the cloud point (T_{CP}) decreases with increasing polymer concentration. It can also be observed in **Figure 3A** that the transmittance is lower than 100% below T_{CP} for solutions with concentrations of 1 wt% or higher, and it even drops below 90% in the case of polymer solutions with higher than 10 wt% concentration. It has to be noted that visually homogeneous solutions with such high PNIPAAm concentrations were obtained with stirring and shaking only after long dissolution times, even several weeks. This indicates that from practical point of view low polymer concentration with relatively sufficiently low dissolution times should be selected for rapid and reliable T_{CP} determination. We propose to select 0.1 wt% polymer concentration, which does not require long dissolution time and provides sharp phase transition with well-defined T_{CP} value. Unless otherwise stated, this concentration is applied for investigating the heating-

cooling hysteresis and the effect of wavelength and the number of heating-cooling cycles on the cloud point of the PNIPAAm solution in the rest of this study.

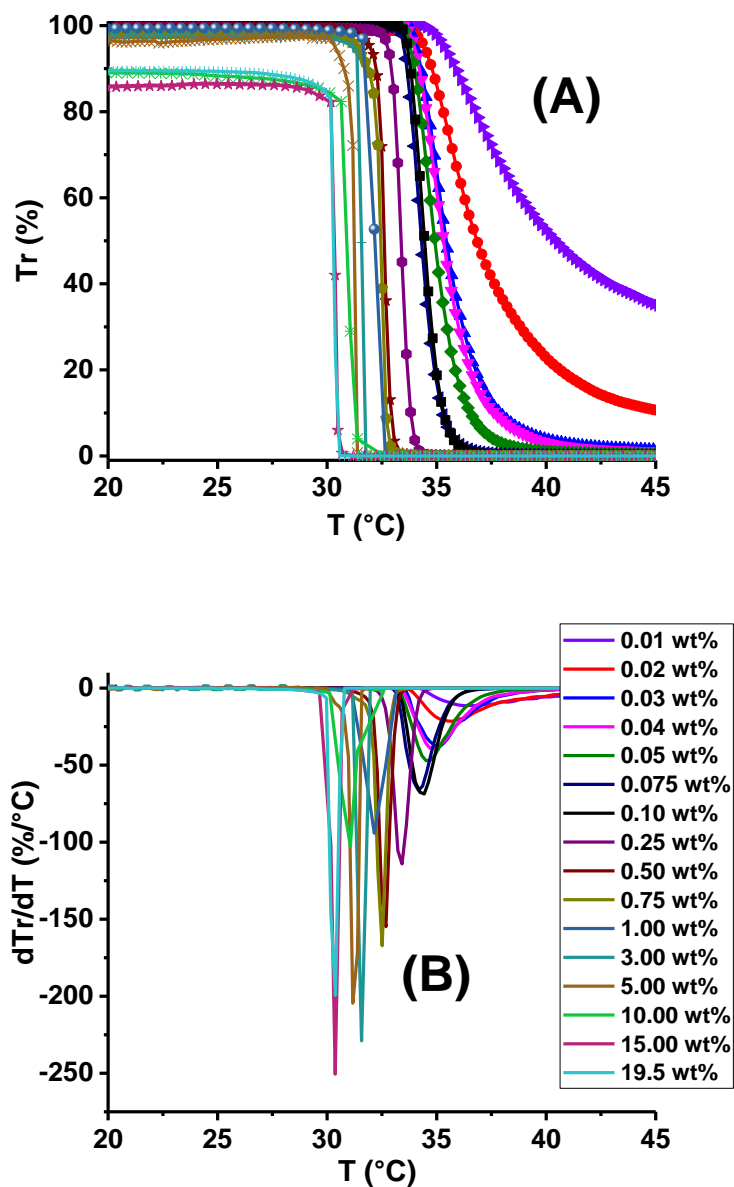


Figure 3. Transmittance-temperature curves (A) and their derivatives (B) during heating of a PNIPAAm solution at different concentrations (conditions: 0.2 °C/min heating rate with 5 min equilibration, 488 nm wavelength).

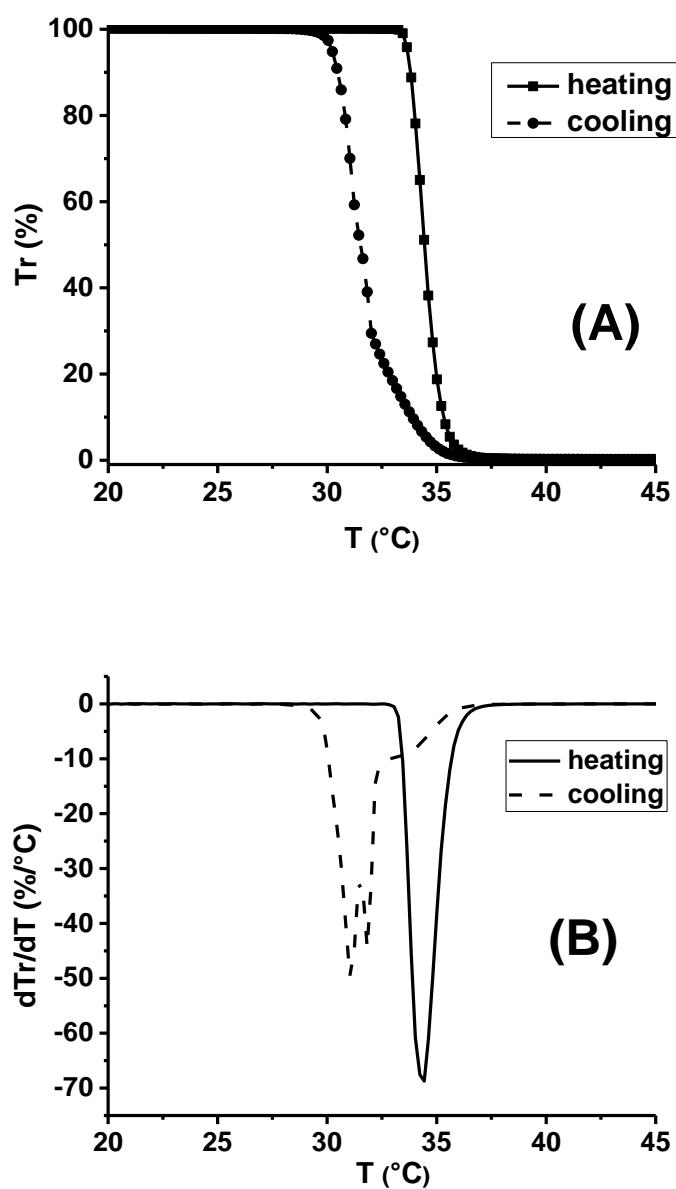


Figure 4. Transmittance-temperature curves (A) and their derivatives (B) during heating and cooling (conditions: 0.1 wt% PNIPAAm, 0.2 °C/min heating rate with 5 min equilibration, 488 nm wavelength).

As displayed in **Figure 4**, the phase transition observed during heating (demixing) is a reversible process with remarkable hysteresis. It has to be noted that this phenomenon of thermoresponsive polymers has not been investigated at all in most of the reported cases, i. e.

mainly results of the heating can be found in the literature, on the one hand. On the other hand, the existing sporadic reports do not deal with the effect or study only limited ranges^[41,42,48] of the conditions on the characteristics of the heating-cooling hysteresis for thermally responsive polymer solutions. We have not been able to identify any literature which describes detailed investigations of the heating-cooling hysteresis of such polymers by photometric cloud point determination. As **Figure 4** reveals, remixing (dissolution, clearing) of the aqueous PNIPAAm solution takes place at lower temperatures than the transmittance decrease during heating. As it can be seen in **Figures 4A** and **4B**, the remixing occurs in three stages, which is in good accordance with DSC results observed during cooling of PNIPAAm solutions.^[39,40,42] It has been found that the thermal demixing during heating usually leads to intrachain and interchain H-bonding, aggregation and even vitrification.^[40] As a consequence, breaking up the aggregates and H-bonds and finally hydration (dissolution) of the polymer chains will occur at different temperature regimes.^[42,43,45] These steps of remixing are better illustrated in **Figure 4B** showing that cooling first results in a slow transmittance increase followed by a faster phase, and finally full recovery of 100% transmittance is reached in a narrow temperature range. In this Figure, in line with literature results,^[39,43] the minimum at 31.8 °C during cooling is most likely due to the overlap between breaking up the aggregates and interchain/intrachain H-bonding, while the minimum (inflection point) at the lowest temperature at 31.1 °C is the real clearing temperature (T_{CL}) due to rehydration of the polymer chains. Measuring the transmittance versus temperature curves during heating and subsequent cooling for polymers with LCST (or cooling-heating with UCST) behavior and determining the cloud point (T_{CP}) and clearing point (T_{CL}) during heating and cooling, respectively, allow to quantify the hysteresis process by selecting the most characteristic features of this phenomenon. First, the difference between the cloud point (T_{CP}) and clearing point (T_{CL}) can be defined as the width of hysteresis (X_H):

$$X_H = T_{CP} - T_{CL} \quad (1)$$

In addition to the starting and ending transmittance values during a heating-cooling cycle, the extent of transmittance (or absorbance) recovery (Y_H), that is, the ratio of the differences between the starting and ending transmittance values is also an important data on the state of the dissolution (remixing) process, e. g. full dissolution or remaining aggregates etc. Thus, the extent of transmittance recovery (Y_H) can be calculated by the following equation:

$$Y_H(T_1, T_2) = (Tr_{C1} - Tr_{H2}) / (Tr_{H1} - Tr_{H2}) \quad (2)$$

where, $T_1, T_2, Tr_{C1}, Tr_{H1}$ and Tr_{H2} stand for the starting and ending temperatures of the investigated temperature range, the transmittance after cooling at T_1 , at the beginning of heating at T_1 and ending of heating (starting at cooling) at T_2 temperatures, respectively.

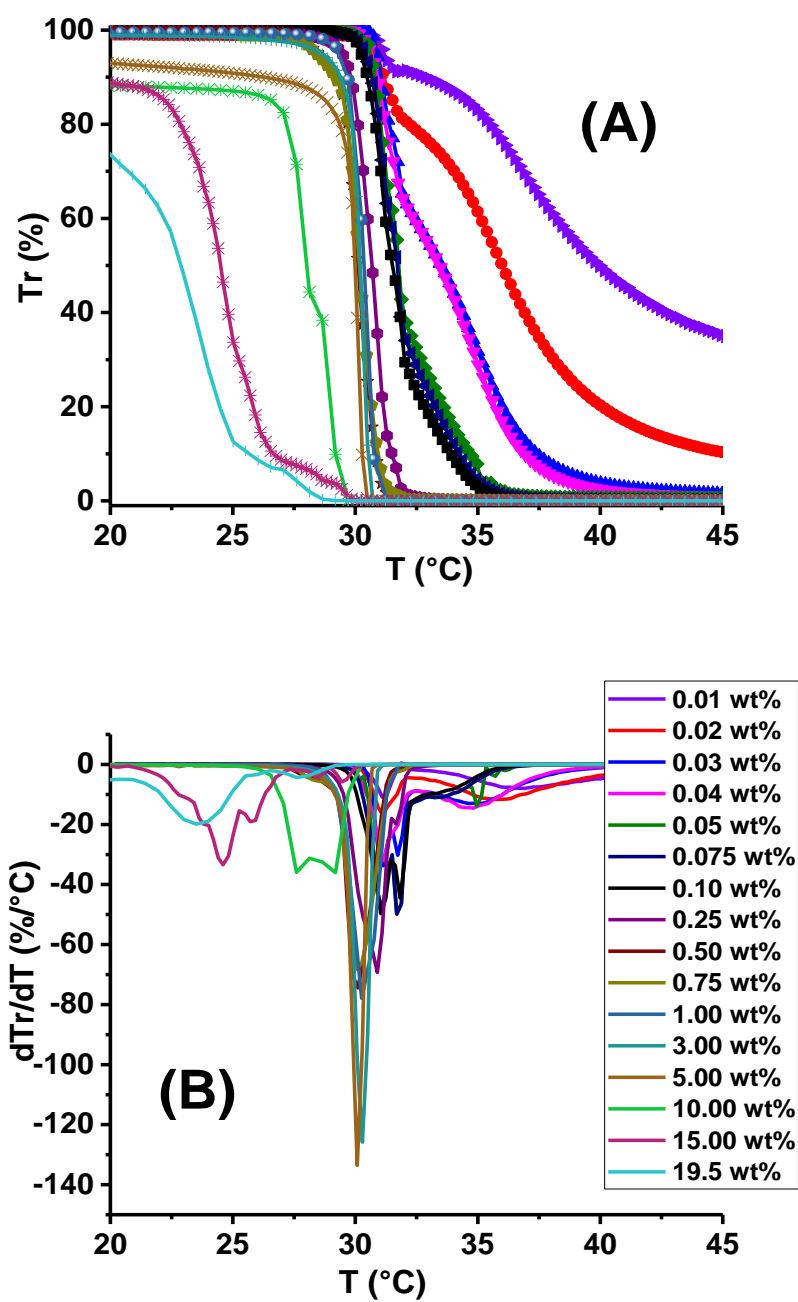


Figure 5. Transmittance as a function of temperature (A) and derivate curves of PNIPAAm solutions of different concentrations during cooling (conditions: 0.2 $^{\circ}C$ /min cooling rate with 5 min equilibration, 488 nm wavelength).

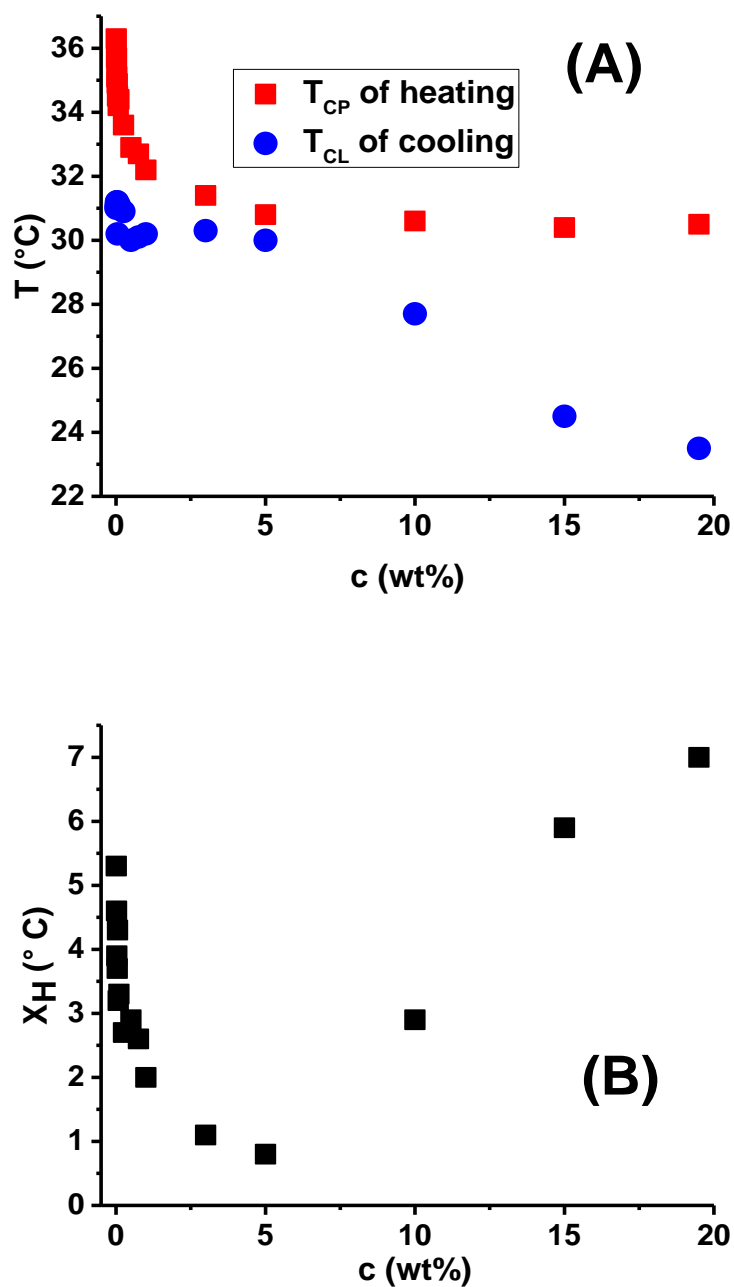


Figure 6. Cloud point (T_{CP}) and clearing point (T_{CL}) during heating and cooling (A), respectively, and the width of hysteresis (X_H) (B) as a function of PNIPAAm concentration (conditions: 0.2 $^{\circ}C/min$ heating rate with 5 min equilibration, 488 nm wavelength).

Figure 5 indicates that similar to the effect of concentration on the transmittance change during heating, the polymer concentration has significant effect on the transmittance-

temperature relationship during cooling as well. The influence of concentration on the hysteresis process is summarized in **Figures 6 and 7**. These Figures show the cloud points of the heating (T_{CP}) and clearing point of the cooling (T_{CL}) cycles, the width of the heating-cooling hysteresis (X_H), the starting and ending transmittance values and the extent of the transmittance recovery (Y_H) of the heating-cooling cycles as a function of polymer concentration observed in the 20-45 °C range. In **Figure 6A**, the cloud point (T_{CP}) of heating and the clearing point (T_{CL}) of cooling as a function of polymer concentration are depicted. The strong effect of concentration on both T_{CP} and T_{CL} can clearly be observed in this plot. Both parameters decrease with increasing concentration, and their difference is also concentration dependent. As displayed in **Figure 6A**, the T_{CP} decreases sharply during heating at low concentrations, i.e. from 36.4 °C to 34.5 °C with 0.01 wt% and 0.1 wt% concentrations, respectively. Further decrease of T_{CP} occurs to 32.2 °C and further to 30.4 °C at 1 wt% and at higher than 10 wt% concentrations, respectively. In the range of ~10-20 wt% concentration, the T_{CP} values become nearly constant, i. e. independent of concentration. In accordance with a recent survey^[5] on the phase diagrams of aqueous solutions of PNIPAAm, these findings indicate that the frequently referred LCST of about 32 °C for PNIPAAm is rather a rough estimate than the real value of LCST of PNIPAAm, on the one hand. On the other hand, our results also reflect the strong dependence of the single-point T_{CP} determination on concentration, which is usually claimed erroneously as the LCST. The T_{CL} as a function of concentration obtained during cooling in **Figure 6A** clearly shows that the clearing point during the cooling cycle also remarkably depends on polymer concentration. The T_{CL} of cooling lies between 30-32.7 °C up to 5 wt% concentration, and significantly decreases above 10 wt% to 24.5 °C and 23.5 °C at 15 wt% and 19.5 wt% concentrations, respectively, resulting in relatively large differences between T_{CP} and T_{CL} . This is better illustrated in **Figure 6B** exhibiting the width of hysteresis (X_H) as a function of concentration. As shown in this Figure, strikingly the X_H versus concentration plot result in a minimum

curve. The width of hysteresis decreases from 5.3 to 2 °C by changing the polymer concentration from 0.01 wt% to 1 wt%. Then X_H decreases to a minimum value of 0.8 °C in the range of about 5 wt%. Increasing the concentration to 15 wt% and 19.5 wt% results in large differences between the T_{CP} and T_{CL} values of heating and cooling, i.e. in X_H values of 5.9 °C and 6.9 °C for PNIPAAm solutions with 15 wt% and 19.5 wt% concentrations, respectively. These findings indicate that the difference between the heating and cooling cycles, and thus the characteristic temperatures of demixing (T_{CP}) and remixing (T_{CL}) can be fine tuned in a certain range by selecting the right concentration. Figure 6B also reveals that the same X_H value can be reached at both relatively low and high concentrations as well. In other words, for the same effect in respect to the width of hysteresis, polymers with lower or higher viscosities might be selected depending on application requirements.

The starting transmittance at 20 °C for the heating, the ending/starting transmittance for heating/cooling at 45 °C and the recovered transmittance after cooling at 20 °C is plotted as a function of concentration in **Figure 7A**. The data in this Figure indicate that the starting transmittance at 20 °C, i. e. at lower than the cloud point temperature, is lower than 100% and decreases to the range of 85-90% for polymer solutions with higher than 10 wt% concentrations even after long dissolution times. As shown in **Figure 7B**, the extent of transmittance recovery (Y_H) also depends on polymer concentration. It is near to one up to 15 wt% but drops to 0.83 for polymer solution with 19.5 wt% concentration. This result confirms that the cloud point determination and hysteresis investigation by UV-vis spectrophotometry under carefully selected conditions lead to well-reproducible starting and ending transmittance values in a broad range of concentrations, and this technique is a valuable tool to investigate the fine details of the hysteresis process as well.

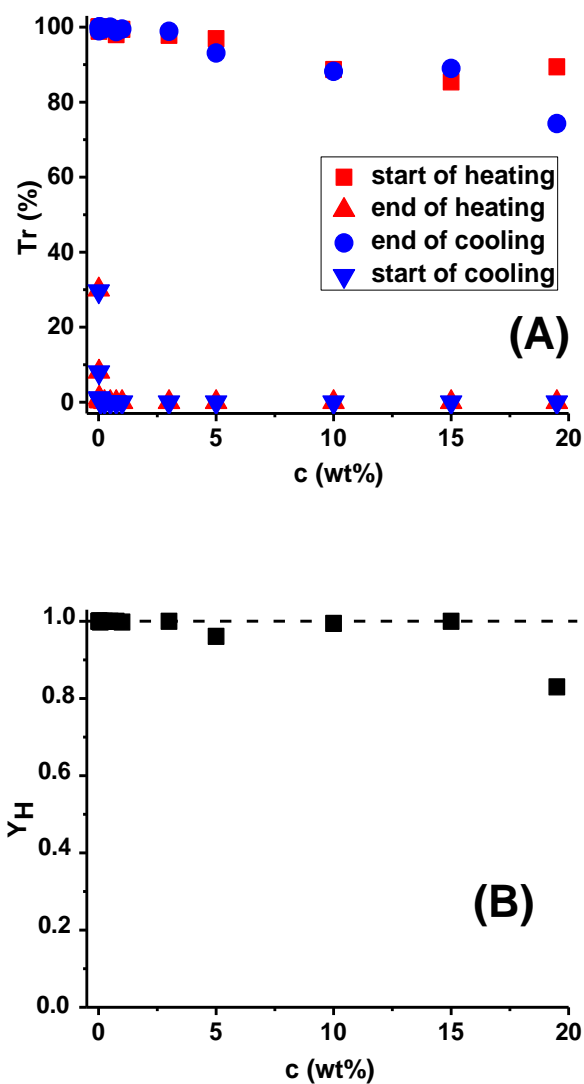


Figure 7. The starting and ending transmittances (A) and the extent of heating-cooling transmittance recovery (Y_H) (B) as a function of polymer concentration (conditions: 0.2 °C/min heating rate with 5 min equilibration, 488 nm wavelength).

The results on the strong effect of concentration on the cloud point (T_{CP}) and clearing point (T_{CL}) of solutions of the investigated thermoresponsive polymer as shown in **Figures 3-7** clearly indicate that a practically feasible polymer concentration should be selected for comparative cloud point determinations and for the characteristics of the heating-cooling

hysteresis as well. As already mentioned above, using 0.1 wt% polymer solution for obtaining widely comparable phase transition data is proposed.

As shown in **Tables S1** and **S2**, a large variety of wavelengths have been used to determine LCST by UV-vis spectroscopy (turbidimetry) or light scattering, ranging from 380 to 800 nm. Due to the dependence of the intensity of light scattering, and thus the transmittance of polymer solutions on wavelength, the effect of this parameter on the transmittance-temperature curves during heating and cooling was also examined. **Figure 8** shows the Tr-T curves during heating and cooling and the T_{CP} and T_{CL} values of a PNIPAAm solution at various wavelength of the incident light. As shown in **Figures 8A** and **8B**, the transmittance-temperature curves are shifted to higher temperature values with increasing the wavelength. This is in line with expectations due to the well-known relationship of the intensity of scattered light with λ^{-4} (where λ is the wavelength of the incident light).^[65] This means that higher relative transmittance is expected at higher wavelength for polymer solutions containing scattering polymer aggregates. Indeed, higher transmittance values of the polymer solution in the range of thermal phase transitions during both heating and cooling are obtained at the same temperatures by using higher wavelengths as displayed in **Figure 8A** and **8B**. Interestingly, while the cloud point slightly increases with increasing wavelength (1 °C between 400 and 800 nm), the clearing point of the cooling cycle is independent of the wavelength (**Figure 8C**). In order to obtain comparable results with light scattering measurements, it is proposed to select 488 nm for the wavelength for cloud point determinations, because this is the wavelength of the laser of a broad variety of light scattering apparatuses used for studying polymer solutions.

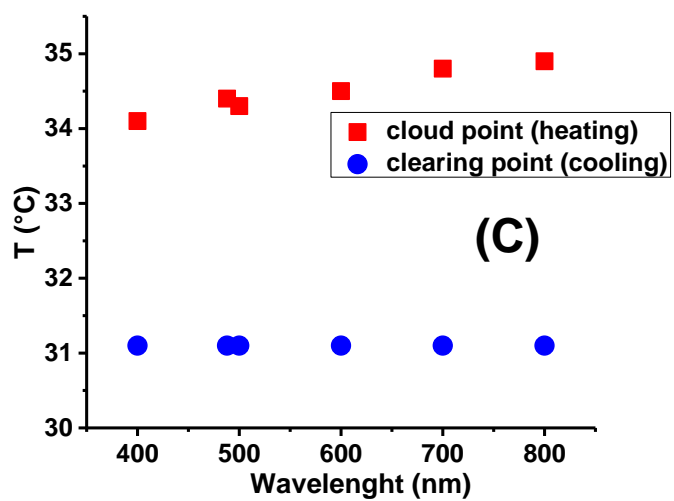
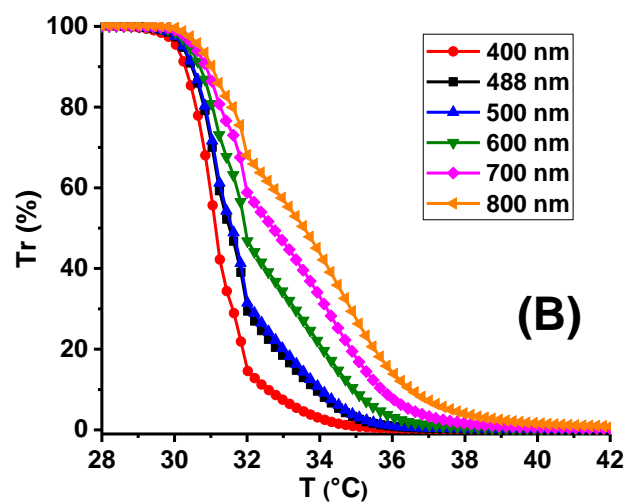
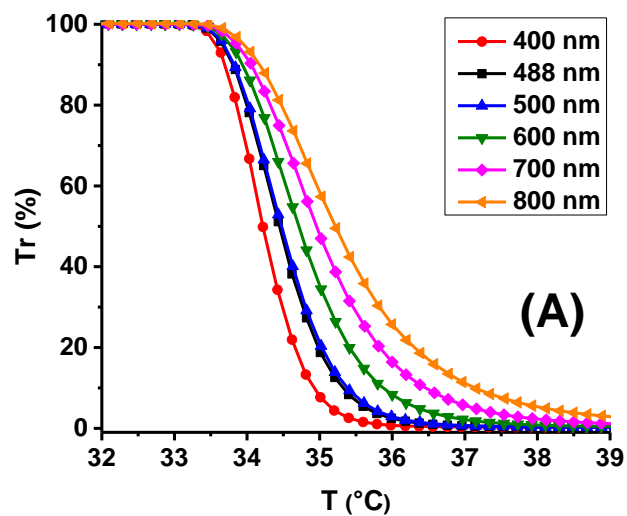


Figure 8. Transmittance-temperature curves during heating (A) and cooling (B) and the cloud points and clearing points (C) of a PNIPAAm solution at different wavelengths (conditions: 0.1 wt% PNIPAAm, 0.2 °C/min heating rate with 5 min equilibration).

The effect of the number of heating-cooling cycles on T_{CP} and T_{CL} was also investigated by us. As displayed in **Figure 9**, both values are independent of the number of cycles. On the one hand, this finding indicates the reversibility of the thermoresponsive behavior of PNIPAAm solutions for a large number of cycles, which might be of significant importance for a variety of application purposes. On the other hand, this result allows us to conclude that the cloud point and clearing point determination with this polymer is independent of the number of heating-cooling cycles. However, it is proposed to carefully investigate the reversibility and reproducibility of the thermoresponsive behavior for sufficiently large number of cycles in the course of investigations with such polymer systems.

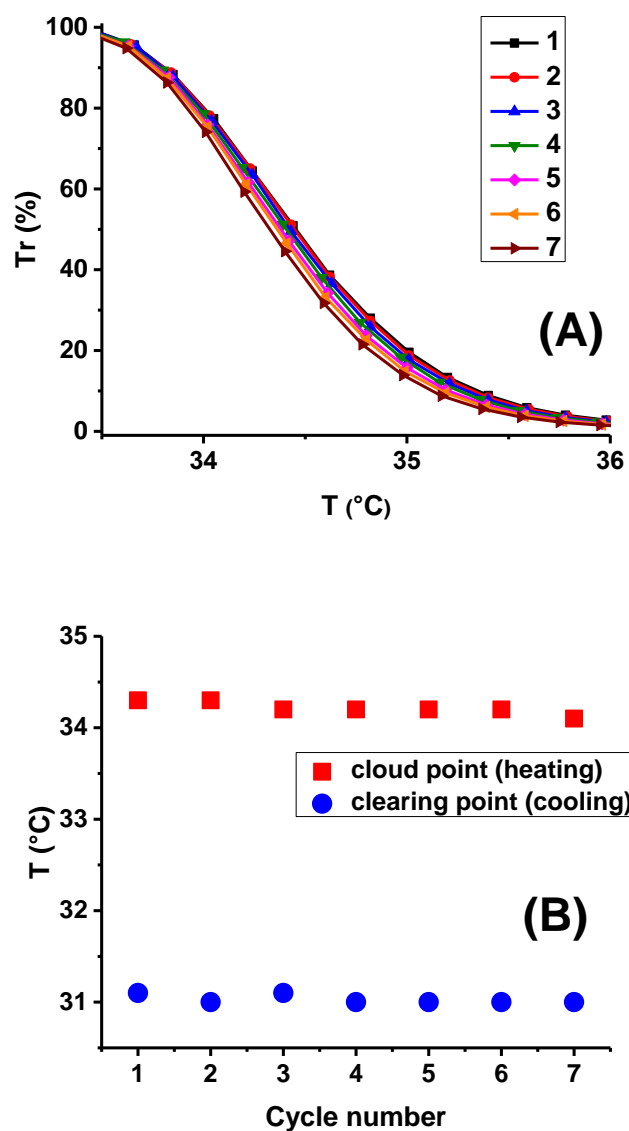


Figure 9. Transmittance-temperature curves (A) and cloud point and clearing point (B) of a PNIPAAm solution with different cycle numbers (conditions: 0.1 wt% PNIPAAm, 0.2 °C/min heating rate with 5 min equilibration, 488 nm wavelength).

4. Conclusions

Systematic investigations have been carried out by us to reveal the effect of experimental conditions on the transmittance change for finding optimal conditions for cloud point (T_{CP}) determination and on the heating-cooling hysteresis of aqueous solutions of a typical

thermally responsive polymer, poly(N-isopropylacrylamide) (PNIPAAm). The detailed assessment of the literature indicates that a broad variety of individual conditions are applied for determining the cloud point by single-point turbidity measurements or the critical solution temperature (CST) by other techniques, which is inappropriately identified as the LCST in the vast majority of literature. The single-point methods apply one selected set of conditions in terms of the evaluation method of the transmittance(absorbance)-temperature curves, the heating-cooling rates, polymer concentrations, wavelength of the incident light and heating-cooling cycle numbers. Strikingly, this latter process, that is, its reversibility and reproducibility, resulting in hysteresis has not been explored yet in details by applying broad range of experimental conditions. It has been found by us that all investigated parameters, with the exception of cycle number for one selected condition set, has significant influence on the cloud point (T_{CP}) and clearing temperature (T_{CL}) during heating and cooling, respectively. The data obtained during detailed exploration of the heating-cooling cycles led to results which show that there is significant concentration dependent hysteresis occurs in the case of the investigated polymer solutions. Determination of T_{CP} and T_{CL} allowed to define the width of hysteresis (X_H) as the difference between these two values: $X_H = T_{CP} - T_{CH}$. Surprisingly, X_H decreases with increasing concentration up to ~5 wt% having a minimum value of 0.8 °C, then increases to 6.9 °C at 19.5 wt% concentration. In contrast to this finding, the extent of transmittance recovery (Y_H), which is a measure of the transmittance change between the starting and ending transmittances, is constant with a value near to one up 15 wt% concentration, then decreases to 0.83 for the solution of 19.5 wt%. These results offer valuable information for application possibilities in which thermoresponsive polymers with many cycles should be used. It was found by us that repeating the heating-cooling cycles up to 8 times do not influence the transmittance-temperature curves, that is, the T_{CP} and T_{CL} , either in the heating or the cooling cycles.

Based on our findings on the effect of all these parameters on the shape and transition width of the transmittance-temperature curves, T_{CP} , T_{CL} and on the literature survey (see also the broadly scattering data in **Tables S1** and **S2**), we propose to use the following conditions for obtaining reliable single-point cloud point determination for thermally responsive polymers with LCST- or UCST-type behavior in order to obtain results comparable all over the world:

1. Using 0.2 °C/min heating rate with 5 minutes of thermal equilibration before increasing (decreasing) the temperature with 0.2 °C increment again is proposed.
2. The inflection point of the transmittance(absorbance)-temperature curves should be determined and referred to as cloud point (T_{CP}) and clearing temperature (T_{CL}).
3. Polymer solution with 0.1 wt% concentration is suggested to be used for the cloud point determination by turbidimetry or critical solution temperature (CST) measurements by other techniques.
4. The wavelength of 488 nm of the UV-vis spectrophotometer is proposed to be selected for measuring transmittance (or absorbance) as a function of temperature in order to obtain comparable results with that of light scattering experiments carried out by equipments having laser source with this wavelength.
5. Heating-cooling cycles should be investigated for all cases, and the width of hysteresis (X_H) and the extent of transmittance (absorbance) recovery (Y_H) are suggested to be determined and evaluated which may provide fundamental results on the demixing/remixing processes and application possibilities.

In order to design complex macromolecular assemblies with components of thermoresponsive polymers, revealing the fundamental characteristics, especially the heating-cooling (hysteresis) behavior, is essential for obtaining polymer structures with well-defined properties for applications ranging from sensors, biomaterials to unique nanostructured materials. Therefore, the conditions for obtaining the basic features of such polymer

architectures useful for macromolecular designing requires well-defined (standardized) technical (experimental) conditions as well in order to create a library of comparable scientific and technical data for such purposes. The above listed dataset of conditions for determining the most important characteristics of thermoresponsive polymers is expected to result in such a library of data for thermoresponsive smart polymers.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author (Tables S1 and S2, reference list for tables).

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Keywords: Lower Critical Solution Temperature (LCST), cloud point, poly(N-isopropylacrylamide), thermal phase transition, hysteresis

References

- [1] A. Constantinou, T. Georgiou, *Eur. Polym. J.* **2016**, *78*, 366-375.
- [2] V. Rosa, P. Woisel, R. Hoogenboom, *Materials Today* **2016**, *19*, 44-55.
- [3] M. Tibbitt, J. Dahlman, R. Langer, *J. Am. Chem. Soc.* **2016**, *138*, 704-717.
- [4] Q. Zhang, R. Hoogenboom, *Prog. Polym. Sci.* **2015**, *48*, 122-142.
- [5] A. Halperin, M. Kröger, F. Winnik, *Angew. Chem. Int. Ed.* **2015**, *54*, 15342-15367.
- [6] P. Schattling, D. Jochum, P. Theato, *Polym. Chem.* **2014**, *5*, 25-36.

- [7] M. Gibson, R. Reilly, *Chem. Soc. Rev.* **2013**, *42*, 7204-7213.
- [8] V. Aseyev, H. Tenhu, F. Winnik, *Adv. Polym. Sci.* **2011**, *242*, 29-89.
- [9] M. Ward, T. Georgiou, Thermoresponsive polymers for biomedical application. *Polymers* **2011**, *3*, 1215-1242.
- [10] I. Dimitrov, B. Trzebicka, A. Müller, A. Dworak, C. Tsvetanov, *Prog. Polym. Sci.* **2007**, *32*, 1275-1343.
- [11] S. Aoshima, S. Kanaoka, *Adv. Polym. Sci.* **2008**, *210*, 169-208.
- [12] E. Manek, B. Berke, N. Miklósi, M. Sabján, A. Domján, T. Fukuda, O. Czakkel, K. László, *eXPRESS Polym. Lett.* **2016**, *8*, 710-720.
- [13] E. Manek, A. Domján, J. Madarász, K. László, *Eur. Polym. J.* **2015**, *68*, 657-664.
- [14] S. J. Sigg, F. Seidi, K. Renggli, T. B. Silva, G. Kali, N. Bruns, *Macromol. Rapid Commun.* **2011**, *32*, 1710-1715.
- [15] H. Zhou, Y. Lu, M. Zhang, G. Guerin, I. Manners, M. Winnik, *Macromolecules* **2013**, *49*, 4265-4276.
- [16] Y. Zhang, J. Cai, C. Li, J. Wei, Z. Liu, W. Xue, *J. Mater. Chem. B.* **2016**, *4*, 3733-3749.
- [17] J. Zhou, T. He, J. Wang, *Enzym. Microbiol. Technol.* **2016**, *87-88*, 47-51.
- [18] W. Huang, J. Yang, Y. Xia, X. Wang, X. Xue, H. Yang, G. Wang, B. Jiang, F. Li, S. Komarneni, *Polymers* **2016**, *8*, 183.
- [19] G. Wang, P. Wu, *Langmuir* **2016**, *32*, 3728-3736.
- [20] Y. Wu, H. Yang, Y. Lin, Z. Zheng, X. Ding, *Mater. Lett.* **2016**, *169*, 218-222.
- [21] W. Zheng, L. Chen, G. Yang, B. Sun, X. Wang, B. Jiang, G. Yin, L. Zhang, X. Li, M. Liu, G. Chen, H. Yang, *J. Am. Chem. Soc.* **2016**, *138*, 4927-4937.
- [22] X. Li, Y. F. Gao, M. J. Serpe, *Macromol. Rapid Commun.* **2016**, *36*, 1382-1392.
- [23] N. Xue, X.-P. Qiu, Y. Chen, T. Satoh, T. Kakuchi, F. M. Winnik, *J. Polym. Sci., Part B: Polym. Phys.* **2016**, *54*, 2059-2068.

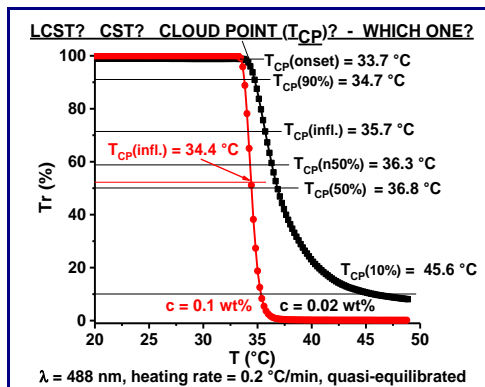
- [24] M. Heskins, J. Guillet, *J. Macromol. Sci. Chem.* **1968**, *2*, 1441-1455.
- [25] C. Wu, S. Zhou, *Macromolecules* **1995**, *28*, 5388-5390.
- [26] C. Wu, S. Zhou, *Macromolecules* **1995**, *28*, 8381-8387.
- [27] C. Wu, S. Zhou, *Macromolecules* **1997**, *30*, 574-576.
- [28] X. Zhang, B. H. Tan, C. B. He, *Macromol. Rapid Commun.* **2013**, *34*, 1761-1766.
- [29] Y. Chen, N. Xiao, M. Fukuoka, K. Yoshida, Q. Duan, T. Kakuchi, *Polym. Chem.* **2015**, *6*, 3608-3616.
- [30] Y. Shangguan, D. Guo, H. Feng, Y. Li, X. Gong, Q. Chen, B. Zheng, C. Wu, *Macromolecules* **2014**, *47*, 2496-2502.
- [31] Y. Xia, N. Burke, H. Stöver, *Macromolecules* **2006**, *39*, 2275-2283.
- [32] A. Simula, V. Nikolaou, A. Anastasaki, F. Alsubaie, G. Nurumbetov, P. Wilson, K. Kempe, D. Haddleton, *Polym. Chem.* **2015**, *6*, 2226-2233.
- [33] M. Constantin, M. Cristea, P. Ascenzi, G. Fundueanu, *eXPRESS Polym. Lett.* **2011**, *5*, 839-848.
- [34] N. SzeJeong, M. Hasan, D. Phillips, Y. Saaka, R. O'Reilly, M. Gibson, *Polym. Chem.* **2012**, *3*, 794-799.
- [35] D. Phillips, M. Wilde, F. Greco, Gibson, *Biomacromolecules* **2015**, *16*, 3256-3264.
- [36] T. Congdon, P. Shawa, M. Gibson, *Polym. Chem.* **2015**, *6*, 4749-4757.
- [37] T. Swift, J. Lapworth, K. Swindells, L. Swanson, S. Rimmer, *RCS. Adv.* **2016**, *6*, 71345-71350.
- [38] X. Wang, X. Qiu, C. Wu, *Macromolecules* **1998**, *31*, 2972-2976.
- [39] H. Cheng, L. Shen, C. Wu, *Macromolecules* **2006**, *39*, 2325-2329.
- [40] K. Durme, G. Assche, B. Mele, *Macromolecules* **2004**, *37*, 9596-9605.
- [41] Y. Ding, G. Zhang, *Macromolecules* **2006**, *39*, 9654-9657.
- [42] Y. Ding, X. Ye, G. Zhang, *Macromolecules* **2005**, *38*, 904-908.

- [43] K. Jain, R. Vedarajan, M. Watanabe, M. Ishikiriya, N. Matsumi, N. Tumble Polym. Chem. **2015**, *6*, 6819-6825.
- [44] A. Constantinou, T. Georgiou, *Polym. Chem.* **2016**, *7*, 2045-2056.
- [45] A. Milewska, J. Szlodoszki, R. Rebelo, *J. Polym. Sci., Part B: Polym. Phys.* **2003**, *41*, 1219-1233.
- [46] B. Sun, Y. Lin, P. Wu, H. Siesler, *Macromolecules* **2008**, *41*, 1512-1520.
- [47] L. Makinen, D. Varadharajan, H. Tenhu, S. Hietala, *Macromolecules* **2016**, *49*, 986-993.
- [48] M. Füllbrandt, R. Klitzing, A. Schönhals, *Soft Matter* **2012**, *8*, 12116-12123.
- [49] X. Zheng, Z. Tong, X. Xie, F. Teng, *Polym. J.* **1998**, *30*, 284-288.
- [50] F. Afroze, E. Nies, H. Berghmans *J. Mol. Struct.* **2000**, *554*, 55-68.
- [51] R. Pamies, K. Zhu, A. Kjoniksen, B. Nystrom, *Polym. Bull.* **2009**, *62*, 487-502.
- [52] L. Blackman, D. Wright, M. Robin, M. Gibson, R. O'Reilly, *ACS Macro. Lett.* **2015**, *4*, 1210-1214.
- [53] S. Furyk, Y. Zhang, D. Ortiz-Acosta, P. Cremer, D. Bergbreiter, *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 1492-1501.
- [54] Z. Tong, Z. Zeng, X. Zheng, *Macromolecules* **1999**, *32*, 4488-4490.
- [55] S. Fujishige, K. Kubota, J. Ando, *J. Phys. Chem.* **1989**, *93*, 3311-3313.
- [56] Y. Ding, X. Ye, G. Zhang, *Macromolecules* **2005**, *38*, 904-908.
- [57] K. Nishi, T. Hiroi, K. Hashimoto, K. Fujii, Y. Han, T. Kim, Y. Katsumoto, M. Shibayama, *Macromolecules* **2013**, *46*, 6625-6633.
- [58] Y. Chen, N. Xiao, M. Fukuoka, K. Yoshida, Q. Duan, T. Satoh, T. Kakuchi, *Polym. Chem.*, **2015**, *6*, 3608-3616.
- [59] Z. Song, K. Wang, C. Gao, S. Wang, W. Zhang, *Macromolecules* **2016**, *49*, 162-171.
- [60] C. Boutris, E. Chatzi, E. C. Kiparissides, *Polymer* **1997**, *38*, 2567-2570.

- [61] H. Lai, P. Wu, *Polymer* **2010**, *51*, 1404-1412.
- [62] Y. Yan, L. Huang, Q. Zhang, H. Zhou, *J. Appl. Polym. Sci.* **2015**, *132*, 41669-41677.
- [63] X. Ye, Y. Ding, J. Li, *J. Polym. Sci., Part B: Polym. Phys.* **2010**, *48*, 1388-1393.
- [64] D. Bergberiter, H. Fu, *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 186-193.
- [65] I. Teraoka, *Polymer solutions: an introduction to physical properties*, Wiley-Interscience, New York, 2002.

The table of contents entry

Systematic investigations on the thermal phase transition and heating-cooling hysteresis of poly(N-isopropylacrylamide) by turbidimetry shows strong condition dependence. These results, in comparison with literature, indicate the need for standardization of cloud point (TCP) determination, and a proposal is made. The width of hysteresis and extent of transmittance recovery are defined, and unusual concentration dependence of these fundamental parameters is reported for the first time.



ToC figure