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Supporting information

Thermoresponsive polymers with lower critical solution temperature: From fundamental aspects and measuring techniques to recommended turbidimetry conditions

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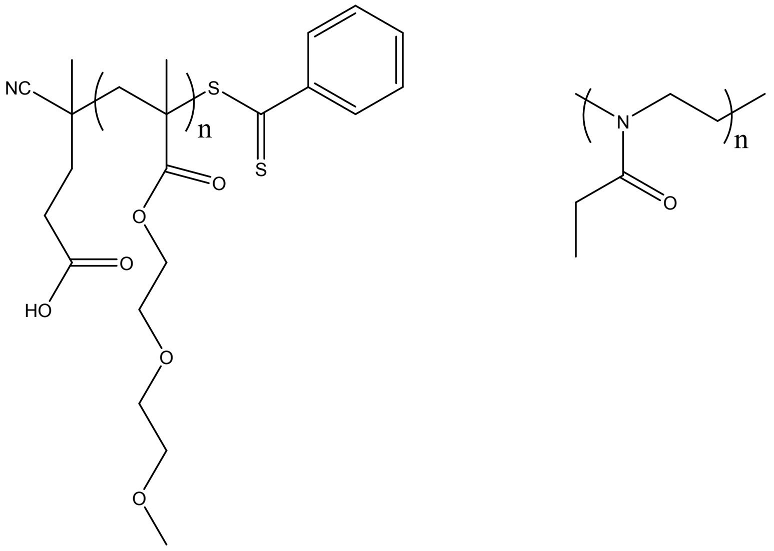
Experimental Section

All chemicals and solvents were commercially available and used as received unless otherwise stated. Dichloromethane, toluene, *N,N*-dimethylacetamide (DMAc), methanol, hexane are from Sigma Aldrich. Dichloromethane was distilled before use. Azobisisobutyronitrile (AIBN, 98%, Aldrich) was recrystallized from methanol (twice) and stored in the fridge. Poly(2-ethyl-2-oxazoline) (PEtOx, 50 kDa, Đ = 3-4, brand name Aquazol 50, see Scheme 1) was obtained from Polymer Chemistry Innovations Inc. Di(ethylene glycol) methyl ether methacrylate (Sigma Aldrich) was processed with neutral alumina column chromatography to remove the inhibitors, and were stored in fridge before use. 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid was purchased from Sigma Aldrich.

Size-exclusion chromatography (SEC) was performed on an Agilent 1260-series HPLC system equipped with a 1260 online degasser, a 1260 ISO-pump, a 1260 automatic liquid sampler, a thermostatted column compartment, a 1260 diode array detector (DAD) and a 1260 refractive index detector (RID). Analyses were performed on a PSS Gram30 column in series with a PSS Gram1000 column at 50 °C. DMAc containing 50 mM of LiCl was used as eluent at a flow rate of 0.6 mL/min. The SEC traces were analysed using the Agilent Chemstation software with the SEC add on. Molar masses and dispersity values were calculated against PMMA standards.

Turbidimetry measurements were performed on a Cary 300 Bio UV-Visible Spectrophotometer (Varian Inc.) equipped with a 12-Cell Holder and a Peltier Cary Temperature Controller (Varian Inc.). Two temperature sensors, probe and block, were equipped to follow/control the temperature of the sample and the sample holder, respectively. To perform the measurements, the samples in cuvettes were first cooled to a suitable temperature to fully dissolve the polymers, after which the sample was placed in the cell holder and heated to a certain temperature above the lower critical solution temperature. Plastic cuvettes (2.5 mL in volume, with round aperture and cap, made of polystyrene, Brand Gmbh + Co KG) made from polystyrene were used for the experiments unless otherwise specified. In addition, to evaluate the effect of the shape and material of cuvette on the measurement, a glass cuvette (12mm o.d. square glass cell with square aperture and cap., Malvern Instruments) and two types of quartz cuvettes (Hellma GmbH & Co. KG) were also used (see Figure S5 for the pictures).

Poly(di(ethylene glycol) methyl ether methacrylate (PmDEGMA, see Scheme 1) was prepared by reversible addition-fragmentation chain transfer (RAFT) polymerization. mDEGMA, AIBN and 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (as chain transfer agent, CTA) were first dissolved in toluene in a schlenk vial. The concentration of monomer was fixed at 2M, and the ratio of [mDEGMA]:[CTA]:[AIBN] was 70:1:0.1. After degassing the solution three times by freeze-vacuum-thaw cycles, the schlenk vial was filled with argon and immersed in a preheated oil bath at 70 °C while stirring. The polymerization was performed for six hours and stopped by immersing the schlenk vial into a dry ice/isopropanol bath. The resulting polymer was redissolved in dichloromethane and precipitated in hexane for three times to remove the impurities, and then dried under reduced pressure at 50 °C. Size exclusion chromatography was used to evaluate the number average molar mass (Mn) and dispersity (Đ) of the obtained polymer. Mn = 5.0 kDa, Đ = 1.21.



PDEGMA

PEtOx (Aquazol 50)

Scheme 1 Chemical structures for PDEGMA and PEtOx (Aquazol 50)

Results and discussions

The most important data obtained by turbidimetry are the transmittance (*%T*) or absorbance (*A*) *versus* temperature plots. However, serious deviations could be expected when plotting absorbance *versus* temperature because the results are strongly dependent on the sensitivity of the optical device used, in particular when the absorbance exceeds 1.5. This drawback of plotting absorbance data *versus* temperature can be easily overcome by converting it to transmittance (1). The resulting value is then plotted as $\%T$.

$$\%T = 10^{-A} \qquad (1)$$

For example, an absorbance value of 1.5 results in a transmittance value of 3.2%, and further increase in absorbance only gives marginal variations in the respective transmittance value. Hence, it is highly recommended to determine Tcp based on transmittance *versus* temperature plots rather than absorbance *versus* temperature plots. Therefore, all the following discussions are based on the transmittance *versus* temperature plots. The turbidimetry experiments were performed using a UV-vis spectrometer with Peltier temperature control as being used in most laboratories.

Influence of the wavelength of incident light

For (binary) mixtures of thermoresponsive polymers with water that undergo an LCST phase separation upon heating, the homogeneous clear solution phase separates into a concentrated polymer phase, which is dispersed in the diluted polymer phase. The phase transition is usually accompanied by a transition from a clear solution to a cloudy solution due to the large difference in refractive index between the two phases, which can be followed by measuring the light transmittance of the solution at different temperatures. A low transmittance (usually 0%) is obtained for polymer solutions in the phase separated state due to the scattering of the incident light by the polymer globules while the one-phase system below Tcp scatter negligible light leading to almost 100% transmittance. As such, the phase separation is accompanied by a drop in transmittance.

According to the Rayleigh approximation (2), when a beam of unpolarized light of wavelength λ and intensity I_0 passes through a solution with small spheres of diameter d and refractive index n, the scattering of light (I) is given by:

$$I = I_0 \frac{1 + cos^2\theta}{2R^2}\left(\frac{2\pi}{\lambda}\right)^4\left(\frac{n^2 - 1}{n^2 + 2}\right)^2\left(\frac{d}{2}\right)^6 \qquad (2)$$

where R is the distance to the particle and θ is the scattering angle. The fact that $I \propto d^6$ explains the enhanced scattering of large particles, but I is also proportional to $1/\lambda^4$. Hence, the scattering intensity is increased when λ of the incident light is decreased, and smaller agglomerates should be detected more easily when a lower λ is applied during the turbidimetry measurement. As shown in Figure S1a, the decrease in transmittance in the respective turbidimetry plots for PEtOx slightly shifts to higher temperature with increasing incident light wavelength indicating the formation of smaller particles at the initial stage of phase separation followed by the formation of large particles with increasing temperature. This evolution of particle size with temperature might be related to the broad molar mass distribution of this PEtOx (Aquazol 50) sample leading to different transition temperature for different fractions of the sample and thus leading to an increase of polymer agglomerates with further increasing temperatures. In contrast, the phase transition of PmDEGMA as detected by turbidimetry exhibits less or no dependence on the utilized wavelength indicating the direct formation of large particles (Figure S1b). Note that the incomplete drop of transmittance is due to precipitation of the high concentrated polymer phase droplets indicating the importance of visual inspection of the vials when unexpected turbidimetry curves are obtained. Based on these results it is suggested to use an incident measurement wavelength of 600 nm, which is in the middle of the range of determined Tcp's for PEtOx, providing an average value for the broad molar mass distribution on the one hand while preventing interference of chromophoric groups that may absorb at 400 nm for other polymers on the other hand.[1]

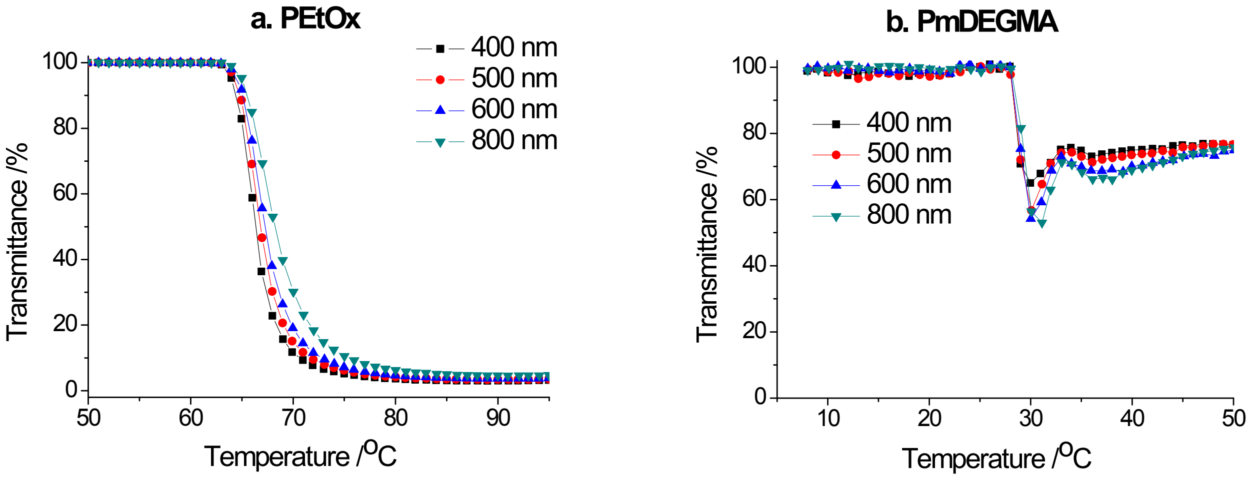


Figure S1 Transmittance *versus* temperature with different incident wavelengths of a) PEtOx (Aquazol 50) and b) PmDEGMA in water at 1 mg/mL, at a temperature ramp of 0.1 °C/min, without stirring.

Influence of heating ramp and polymer concentration

The turbidimetry plots for polymer solutions at different concentrations during heating at 0.1, 0.5, 1 and 5 °C/min are plotted in Figure S2. The phase transition temperature for both of the two polymers shifts to higher temperatures with faster heating, which is due to the relatively slower response of precipitation of polymer chains compared to the heating rate and/or a lag time in between heating of the sample holder and the solution. When looking into the details of the two plots, a different dependence on the heating ramp could be observed, i.e. PmDEGMA is more sensitive to the heating ramp than PEtOx indicating a slower collapse, i.e. dehydration, of the PmDEGMA chains compared to PEtOx. As such, variation of heating rates provides further information on the phase transition kinetics. Note that the transmittance goes up when heating beyond the Tcp for PmDEGMA heated at 0.1 °C/min, which can be ascribed to the macroscopic precipitation of the polymer sample in the long heating process revealing an important drawback of too slow heating/cooling rates. As such, it is evident that 0.1 °C/min is too slow for the measurement while 5 °C/min is too fast resulting in a large overestimation of Tcp. Therefore, it is recommended to use a 0.5 °C/min heating rate for Tcp determination for an accurate Tcp determination, which will be further supported when comparing different cuvettes and temperature control modes, vide infra. It should be noted that it may be interesting to also determine the Tcp with 1.0 °C/min to assess the kinetics of the phase transition.

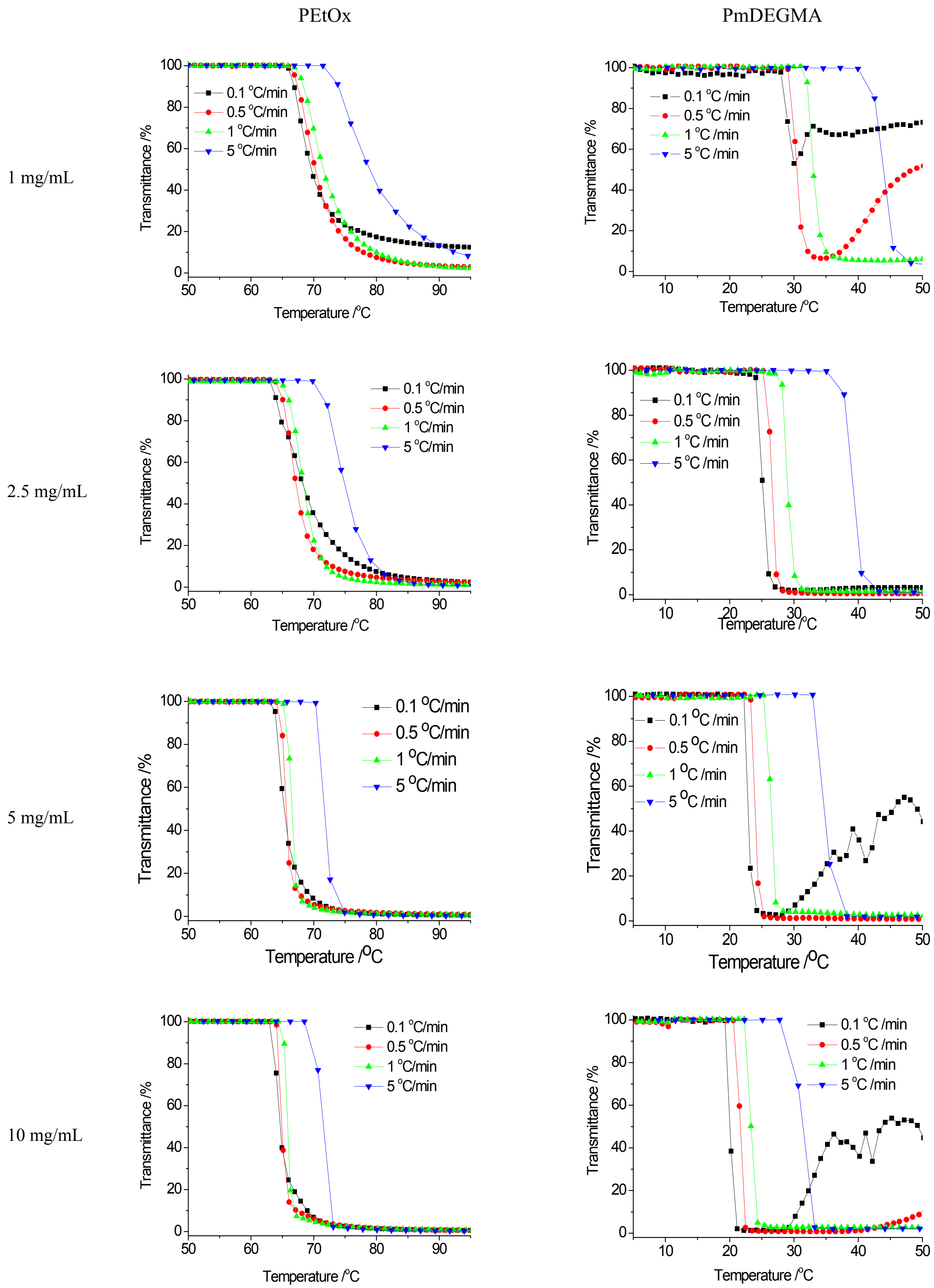


Figure S2. Transmittance *versus* temperature plots recorded with different temperature ramps of PEtOx (Aquazol 50) and PmDEGMA in water at 1, 2.5, 5 and 10 mg/mL, respectively, with stirring and incident wavelength of 600 nm.

The concentration dependence of the turbidimetry plots is depicted in Figure S3. In general, the two polymers exhibit similar concentration dependence, i.e. the phase transition shifts to lower temperatures with higher concentrations, which is also widely reported in the literature and can be ascribed to enhanced polymer-polymer interactions at higher concentrations.[2-4] Furthermore, at very dilute conditions, this higher observed Tcp is also related to the turbidimetry technique that only allows the detection of relatively large phase separated droplets of which less are present at the initial stages of phase separation at lower concentrations. Besides, the phase transition occurs over a broader temperature window in the diluted polymer solutions, in particular for PEtOx indicating a more gradual collapse and coagulation of the polymer chains instead of a fast simultaneous process. The slower transition for PEtOx can be ascribed to the high dispersity of the polymer resulting in non-identical phase transition temperatures for different molar mass fractions of the polymer sample. With samples at high concentration, sharp transitions from 100% transmittance to 0% transmittance are obtained. This is due to the high mass of polymer present in the solution, which quickly leads to the formation of large concentrated phase droplets and, therefore, produces high turbidity. Based on the data shown in Figure S3 it is recommended to use 5-10 mg/mL (0.5-1 wt%) polymer solutions for the determination of Tcp by turbidimetry, depending on availability of the material.

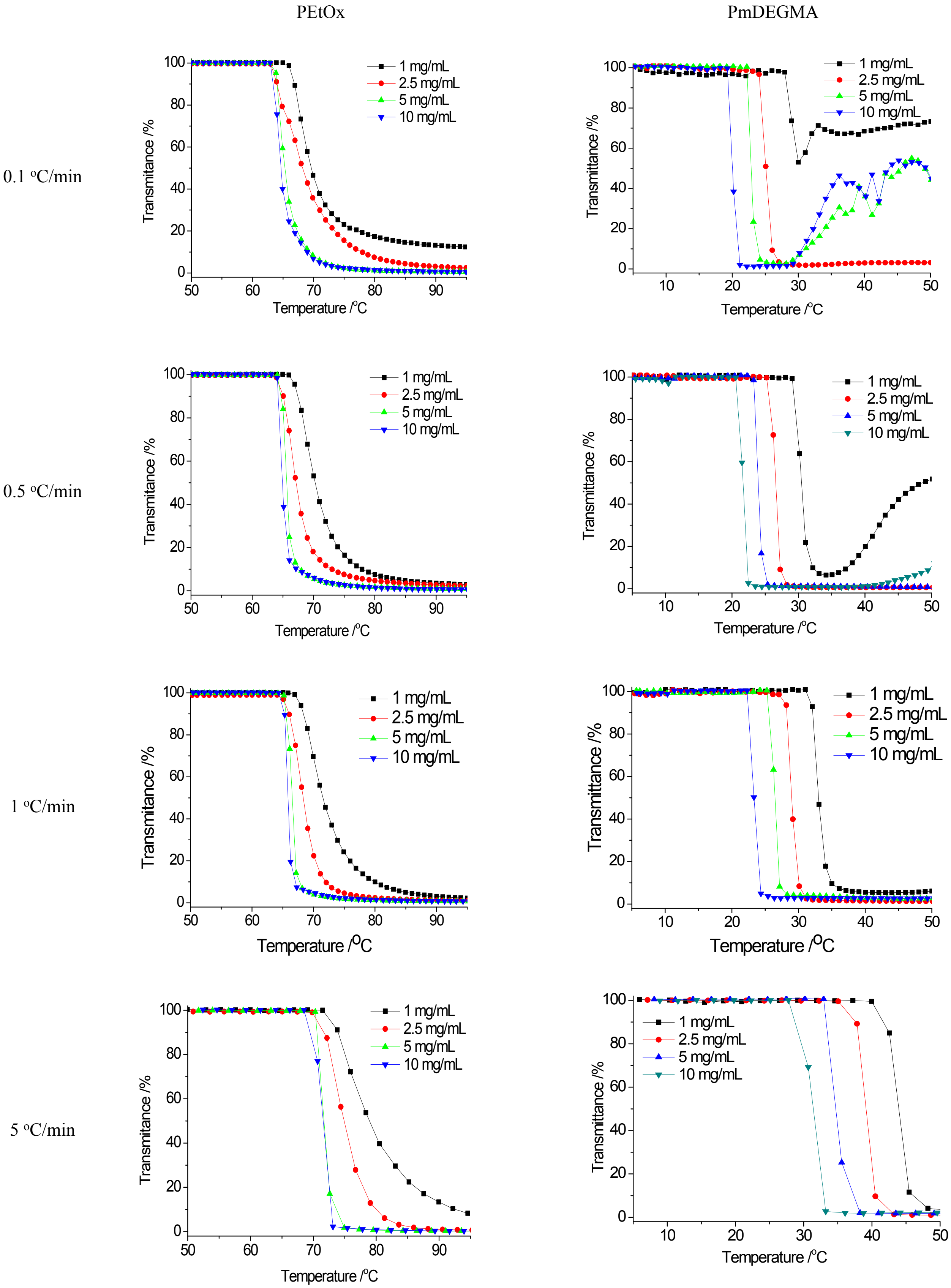


Figure S3. Transmittance *versus* temperature in different concentrations for PEtOx (Aquazol 50) and PmDEGMA in water at temperature ramp at 0.1, 0.5, 1 and 5 °C/min, respectively, with stirring and incident wavelength of 600 nm.

Determination of the Tcp value from turbidimetry plots

The Tcp represents the phase transition temperature of the mixture of the thermoresponsive polymer with water at the respective concentration. However, different researchers define the position of Tcp on the transmittance *versus* temperature curve in different ways. Even with similar data collected by a UV–Vis spectrometer with the same experimental settings, four different types of definition for Tcp's are used by different researchers as listed by Chytrý and Ulbrich.[5] The lack of unity in the definitions makes it difficult to compare the data reported by different researchers.[6] Besides, Tcp's obtained by some of the definitions are strongly dependent on the sensitivity of the optical device used and the presence of insoluble particles in the solution.[5]

As discussed previously, two types of plots could be used to define the Tcp, i.e. absorbance *versus* temperature and transmittance *versus* temperature.[7] However, as explained transmittance *versus* temperature is more accurate and only these will be further discussed here. Based on the transmittance *versus* temperature plot, the Tcp is usually defined as the temperature of a certain stage of the drop in transmittance. The onset of decrease,[8] 10, 50 or 80% of transmittance have been reported as definition to determine Tcp. For thermoresponsive polymers with sharp transition from clear to cloudy, e.g. PmDEGMA, these definitions provide, however, only minor deviations in Tcp. However, for solutions of thermoresponsive polymers with broad transitions ranges, the transmittance decreases more gradually from 100 to 0%, which leads to a large deviation in Tcp depending on the used definition (Table S1). This is of particular importance in case of broad UCST type transitions, or for broad LCST type transitions that either result from a low polymer concentration or from a high polydispersity of polymer samples with strong influence of the molar mass on the Tcp, such as PEtOx (Aquazol 50, *vide supra*). An ideal definition of the cloud point should, on the one hand show no dependence on the sensitivity of the device or experience of experimentalist, while on the other hand it should be sensitive to the parameters used, e.g. concentration and heating rate. Based on the survey on the reported results and our own experience, it is proposed to use 50% of transmittance as definition for Tcp in the future to make it easy to compare data from different reports. For broad polymer phase transitions, it may be valuable to also report the 80% values as a measure for the broadness of the phase transition.

Table S 1 Tcps of PEtOx (Aquazol 50) and PDEGMA at different concentrations based on different definitions at a heating rate of 5 °C/min

Stage of transmittance	Concentration of PEtOx (Aquazol 50)				Concentration of PDEGMA			
	1 mg/mL	2.5 mg/mL	5 mg/mL	10 mg/mL	1 mg/mL	2.5 mg/mL	5 mg/mL	10 mg/mL
Onset of decrease	65.1	64.1	64.2	64.3	40.0	35.1	32.9	27.8
80 %	69.2	66.7	65.9	65.5	44.1	39.2	34.3	29.3
50 %	71.5	68.3	66.6	65.9	43.9	39.1	34.7	31.4
10 %	80.0	72.0	67.8	67.1	46.0	40.6	37.4	33.0

Influence of stirring

The influence of stirring was evaluated with PEtOx (Aquazol 50) at various concentrations and heating ramps. As shown in Figure S4b, a clear difference of Tcp detected with and without stirring is found, which is most pronounced with heating ramps of 0.1 and 5 °C/min. The Tcp appears at relatively higher temperatures in absence of stirring with too fast heating (5 °C/min) due to inefficient thermal conductivity. In other words, it takes longer to heat the entire solution without stirring, which leads to an increased lag time and an overestimation of Tcp. In contrast, turbidimetry plots of Tcp for polymer solutions measured at 0.1 °C/min exhibit complex behavior due to the slow temperature change. Visual inspection of the cuvettes revealed that large polymer aggregates are formed during the measurement and stick on the wall of cuvettes due to the long incubation time of the mixture above its phase transition temperature. This precipitation can also be noticed from the transmittance *versus* temperature plots for solutions with high polymer concentration, which shows a shift of Tcp to higher temperatures at the second heating cycle as the apparent polymer concentration has decreased (Figure S4-a3 and S4-a4). And hence, both methods (with and without stirring) lead to inaccurate results making turbidimetry an inappropriate method in in this case. However, when using the optimal concentration of 5 to 10 mg/mL with 1 °C/min heating rate, there is no significant change in Tcp when the two methods are compared.

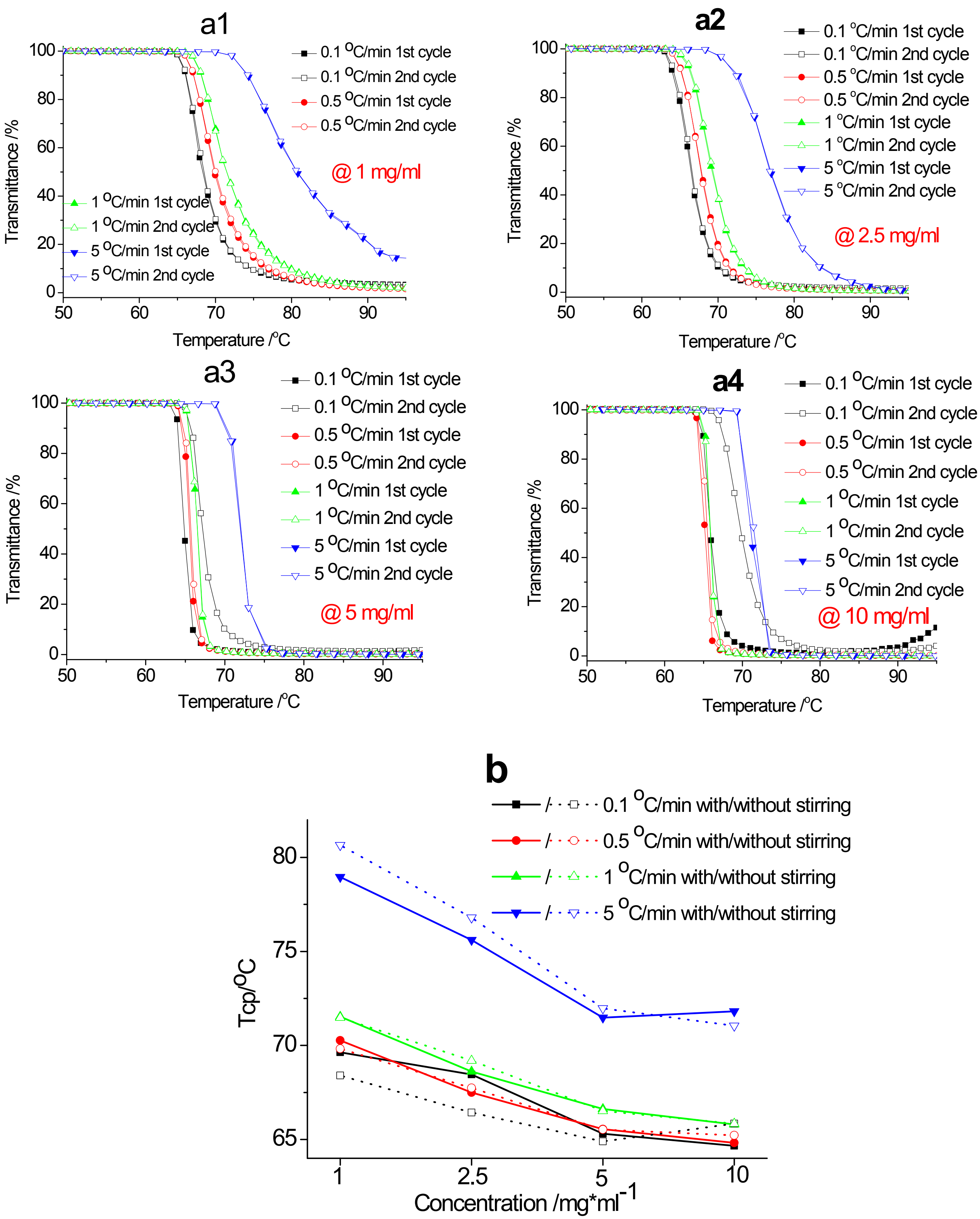
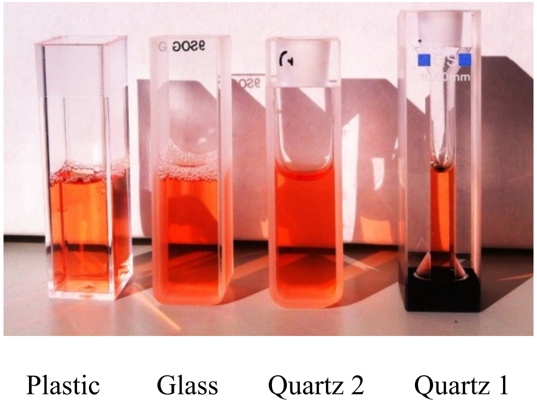


Figure S4. a) Transmittance *versus* temperature plots with different temperature ramps of PEtOx (Aquazol 50) in water of 1, 2.5, 5 and 10 mg/mL, respectively; data were collected during the first and second heating ramps (cooling plots not shown) without stirring, incident wavelength was 600 nm, and b) dependence of Tcp of PEtOx (Aquazol 50) on the concentration determined with and without stirring. The transmittance *versus* temperature plots with stirring are not shown as the first and second heating plots match well. Lines are added to guide the eye.

Influence of cuvette and temperature sensor

The reliability of the temperature control is essential for the accuracy of the turbidimetry measurements. Both heating and cooling of the samples were performed and followed to obtain the cloud point temperature upon heating (Tcph) and clearance point temperature (Tcpc) upon cooling, respectively. Besides, several other parameters can also influence the temperature monitoring, and hence the accuracy of the Tcp determination by turbidimetry measurement performed in a UV-Vis spectrometer. For instance, the monitoring of temperature can be done with a sensor inside or outside of the cuvette, corresponding to the probe or block mode in the UV-Vis spectrometer. Furthermore, the type of cuvette influences heat transport from the block to the solution.

The Tcp for PEtOx (Aquazol 50) and PmDEGMA were determined in different cuvettes (Figure S5) with a temperature probe inside the cuvette or in the measuring block. As expected, when the temperature probe is present in the cuvette, the Tcp determination is most accurate and least dependent on the type of cuvette since it monitors directly the temperature of polymer solution. The Tcp determined in quartz cuvette 1 (Figure S5), however, deviates a lot from the others, which could be ascribed to the inefficient stirring of the polymer solution in the long and narrow chamber as well as the large amount of insulating quartz. For Tcp determination with temperature control of the cuvette holder (block mode) as is mostly done in practice, deviations in Tcp are always present due to the temperature difference between the inside and the outside of the cuvettes. For different temperature ramps with block probe, a medium heating/cooling rate at 0.5 °C/min provides the best results as indicated by the lowest deviation from the probe mode. It is logical that slow changing of temperature allows a more accurate determination of Tcp since it enables more efficient thermal transfer through the cuvette. The temperature ramp at 0.1 °C/min, however, exhibits higher deviation than that with 0.5 °C/min and a reversed hysteresis. This unexpected phenomenon can be ascribed to the formation of large globules and sticking of polymer globules on the wall of cuvette during the long measurement time as also discussed above. This assumption was confirmed by visual inspection of the cuvette during the measurement. The Tcp obtained at 1 °C/min revealed the highest deviation due to the relatively fast temperature change. The deviation of the Tcp for different polymer samples also varies depending on the temperature. For PmDEGMA, the Tcp values obtained by block mode are higher or lower than that obtained by probe mode depending on the temperature program, which is mainly due to the lag of the temperature inside the cuvettes. In contrast, the Tcp for PEtOx (Aquazol 50) detected by block mode are always higher than the results from probe mode.



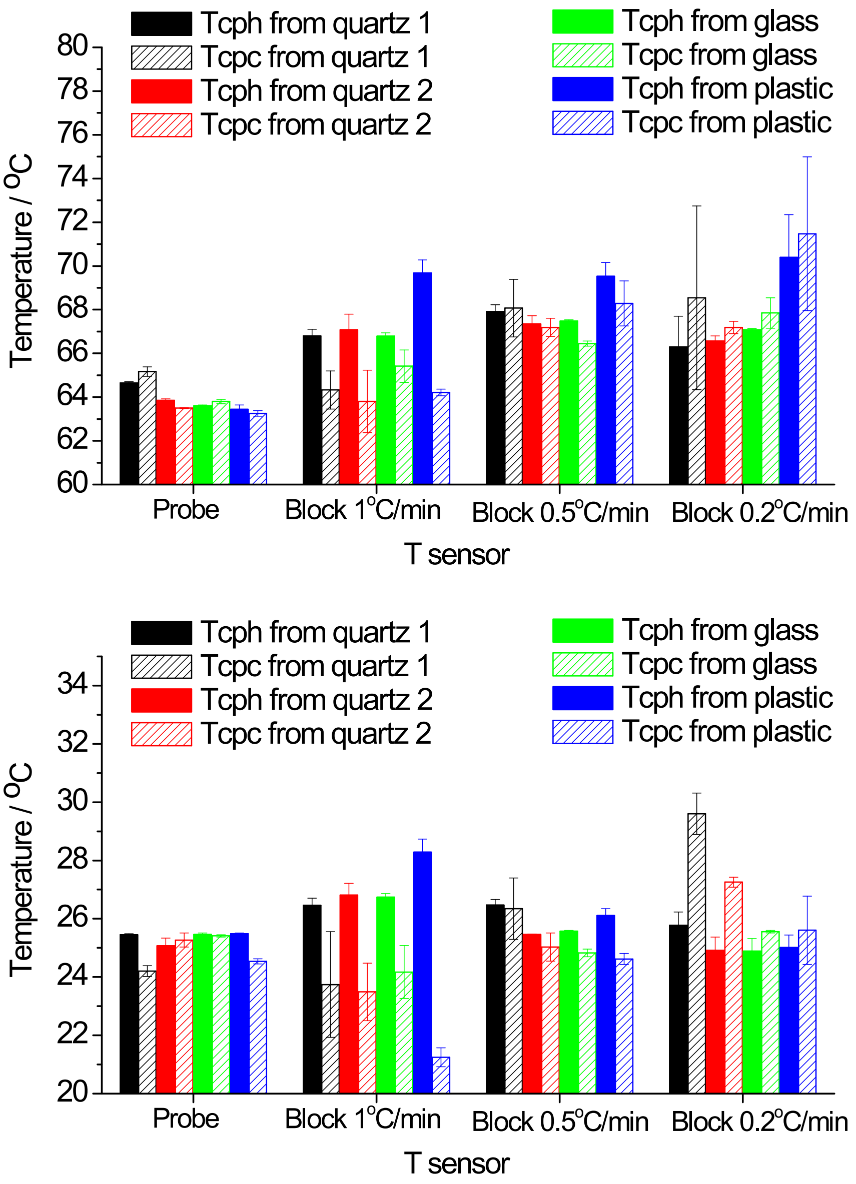


Figure S5 Top) Different cuvettes used in the determination of turbidimetry on a UV-Vis spectrometer, Nile red solution was filled in the cuvette allowing a clear visual of the shape, from left to right: plastic (polystyrene), glass, quartz 2 and quartz 1 cuvettes, and Bottom) dependence of Tcph and Tcpc for PEtOx (Aquazol 50) and PmDEGMA on the temperature sensor (probe and block) and ramp (1, 0.5 and 0.2 °C/min) with stirring, wavelength of incident light was 600 nm.

Although the best results are obtained at a temperature ramp of 0.5 °C/min, a heating ramp of 1 °C/min has been employed for most of the publications. Hence, it would be valuable to relate the values obtained in block mode to the values obtained at probe mode as calibration. An easy and accurate way for the calibration is to relate the actual temperature of the solution (temperature detected by probe sensor) to the block temperature. Therefore, the temperatures detected by block and probe sensors in pure water and different cuvettes were recorded with a 1 °C/min temperature program controlled by the block T-sensor. Figure S6 shows the plot of probe temperature *versus* block temperature during heating and cooling in all cuvettes. For the plastic cuvettes, the probe temperature inside the cuvette firstly exhibited an initial slow heating stage, after which a kind of equilibration was reached as indicated by the linear dependence of probe temperature on block temperature, whereby the probe temperature is lower than the block temperature. Hence, a linear fit of block temperature *versus* probe temperature was performed as calibration for the block temperature. The fitted intercept and slope values for different cuvettes during heating or cooling are listed in Table S2. The adjusted determination coefficients (R square higher than 0.9999) obtained by linear fits indicate the strong and predictable relationship between the temperatures detected by the two temperature sensors.

To assess the proposed calibration and the parameters obtained, the Tcp_H and Tcp_C for PEtOx (Aquazol 50) and PmDEGMA obtained by block mode with a ramp of 1 °C/min are recalibrated and listed in Table S3. The recalibrated Tcp values are highly comparable with the Tcp obtained by probe mode with an error lower than 0.5 °C for most of the cases. Exceptions are found for quartz cuvette 1 with errors higher than 1 °C most likely due to inefficient stirring.

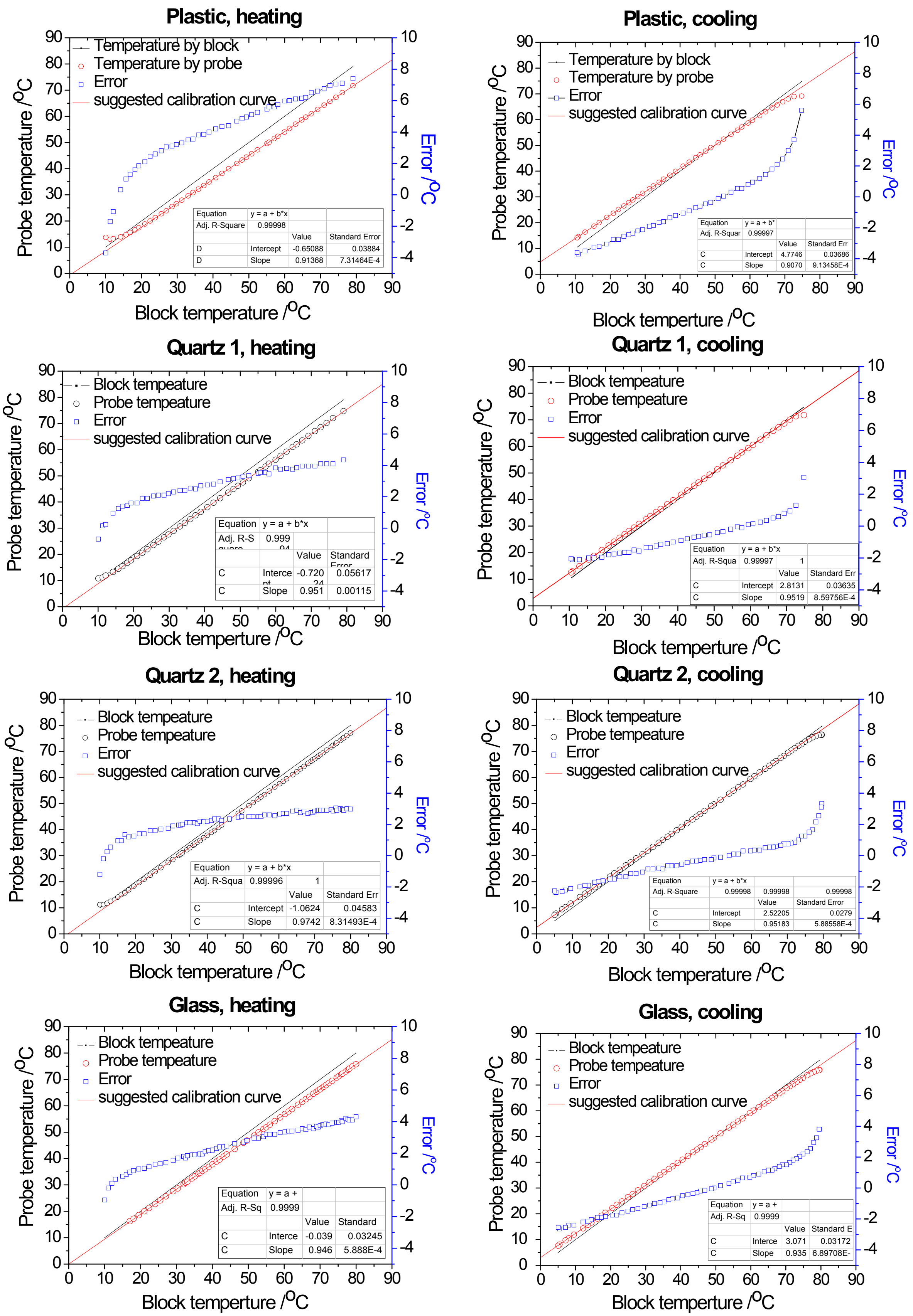


Figure S6. Suggested calibration curves for different types of plastic (polystyrene), quartz and glass cuvettes during heating and cooling. Red circle represent the probe temperature *versus* block temperature; the error represented as open squares was calculated by probe temperature - block temperature.

Table S 2 Calibration parameters for different cuvettes via heating and cooling

Cuvette/temperature program [a]	Intercept	Slope	R square
P/H	-0.65	0.91	0.9999
P/C	4.77	0.91	0.9999
Q1/H	-0.72	0.95	0.9999
Q1/C	2.81	0.95	0.9999
Q2/H	-1.06	0.97	0.9999
Q2/C	2.52	0.95	0.9999
G/H	-0.04	0.95	0.9999
G/C	3.07	0.94	0.9999

[a] Q, G and P represent quartz, glass and plastic cuvette, respectively; while H and C represent heating and cooling, respectively.

Table S 3 Calibrated phase transition temperature and error for different cuvettes

Cuvette/temperature program[a]	Tcph or Tcpc for PmDEGMA /°C				Tcph or Tcpc for PEtOx /°C			
	Block	Probe	Calibrated	Error[b]	Block	Probe	Calibrated	Error[b]
Q1/H	26.46	25.45	24.41	1.04	66.80	64.65	62.74	1.91
Q1/C	23.74	24.21	25.36	1.15	64.33	65.17	63.92	1.25
Q2/H	26.81	25.07	24.94	0.13	67.08	63.85	64.00	0.15
Q2/C	23.49	25.27	24.83	0.44	63.80	63.5	63.13	0.37
G/H	26.75	25.46	25.37	0.09	66.79	63.61	63.41	0.20
G/C	24.17	25.41	25.78	0.37	65.42	63.80	64.56	0.76
P/H	28.28	25.49	25.18	0.31	69.68	63.44	63.01	0.43
P/C	21.25	24.54	24.05	0.49	64.21	63.25	63.01	0.24

[a]Q, G and P represent quartz, glass and plastic cuvette, respectively; while H and C represent heating and cooling, respectively. [b]The error is absolute value of the difference between probe and block temperature.

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