



Temperature responsive bio-compatible polymers based on poly(ethylene oxide) and poly(2-oxazoline)s

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ABSTRACT

This review covers the LCST behavior of two important polymer classes in aqueous solution, namely poly(2-oxazoline)s and systems whose thermo-responsiveness is based on their structural similarity to poly(ethylene oxide) (PEO). In order to elucidate the progress that has been made in the design of new thermo-responsive copolymers, experimental data that were obtained by different research groups are compared in detail. Copolymerization with hydrophilic or hydrophobic comonomers represents a suitable method to tune the coil to globule transition temperature of several homopolymers, and incorporation of other monomers provided further interesting features, such as pH responsiveness or sensing properties. In addition, living and controlled polymerization techniques enabled access to defined end groups and more advanced polymer architectures, such as graft copolymers or double responsive block copolymers. The effect of such structural variations on the temperature responsive behavior of the (co)polymers is discussed in detail.

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Nomenclature

AGET ATRP	Activator generated by electron transfer ATRP
ATRP	Atom transfer radical polymerization
BMDO	5,6-Benzo-2-methylene-1,3-dioxepane
BOx	2-Butenyl-2-oxazoline
BuMA	Butyl methacrylate
BuOx	2-Butyl-2-oxazoline
CROP	Cationic ring opening polymerization
DLS	Dynamic light scattering
DMAEMA	<i>N,N</i> -dimethyl aminoethyl methacrylate
DP	Degree of polymerization
DR	Disperse red
DSC	Differential scanning calorimetry
EGFP	Enhanced green fluorescent protein
EO	Ethylene oxide
FRP	Free radical polymerization
HEA	Hydroxy ethyl acrylate
HS-DSC	High sensitivity differential scanning calorimetry
LCSC	Lower critical solution concentration
LCST	Lower critical solution temperature
MAA	Methacrylic acid
MMA	Methyl methacrylate
NBA	<i>o</i> -Nitrobenzyl acrylate
NiPAm	<i>N</i> -iso-propylacrylamide
NMP	Nitroxide mediated polymerization
PBS	Phosphate buffered saline
PCL	Poly(ϵ -caprolactone)
PcPrOx	Poly(2-cyclo-2-propyl-2-oxazoline)
PTCO	Poly(1,3,6-trioxacyclooctane)
PDI	Polydispersity index
PDMAEMA	Poly(<i>N,N</i> -dimethyl aminoethyl methacrylate)
PDXL	Poly(1,3-dioxolane)
PEG	Poly(ethylene glycol)
PEO	Poly(ethylene oxide)
PEtOx	Poly(2-ethyl-2-oxazoline)

PhOx	2-Phenyl-2-oxazoline
PIC	Polyion complex
PLGA	Poly(lactide-co-glycolide)
PMeOx	Poly(2-methyl-2-oxazoline)
PMO	Poly(methylene oxide)
PNiPAm	Poly(<i>N</i> -iso-propylacrylamide)
PNonOx	Poly(2- <i>n</i> -nonyl-2-oxazoline)
PO	Propylene oxide
POx	Poly(2-oxazoline)
PPC	Pressure perturbation calorimetry
PPO	Poly(propylene oxide)
PPrOx	Poly(2-propyl-2-oxazoline)
PTCU	Poly(1,3,6,9-tetraoxacycloundecane)
RAFT	Reversible addition-fragmentation chain transfer
SLS	Static light scattering
T_{cp}	Cloud point temperature
TEM	Transmission electron microscopy
TMAEMA ⁺	Methacryl oxyethyl trimethylammonium chloride
UCST	Upper critical solution temperature
XRD	X-ray diffraction

1. Introduction

Polymers that respond with a property alteration towards environmental changes are often referred to as “stimuli-responsive”, “smart”, or “intelligent” materials. In cases where the external trigger is temperature, the polymer is said to exhibit thermo-responsive properties. An interesting feature that can be influenced by changes in temperature is the solubility of the polymer in aqueous systems. Besides individually dissolved polymer chains, thermo-responsive polymers can be designed to exist in various physical forms, such as hydrogels, functionalized surfaces, membranes, micelles and various types of

particles [1]. Thermo-responsive polymers that undergo a solubility transition in water have received major interest in recent decades based on their broad application potential in various interesting fields, such as protein chromatography [2], sensing devices [3], protein adsorption and tissue engineering [4], temperature triggered drug delivery and regenerative medicine [5–8]. Nowadays, living and controlled polymerization mechanisms are widely applied for the synthesis of thermo-responsive polymers providing a perfect tool for the construction of advanced polymer architectures and enabling the combination of thermo-responsiveness with a second response towards other environmental triggers, such as pH value, magnetic field or light, within one molecule [9].

Several polymers that respond with a solubility transition to temperature changes in aqueous solution are known from literature among whose the most well-known is poly(*N*-iso-propylacrylamide) (PNiPAm) since its lower critical solution temperature (LCST) in water, i.e. the temperature at which the polymer switches from hydrophilic to hydrophobic, is close to body temperature [10]. The progress in the design and application of new PNiPAm based thermo-responsive copolymers during the last years was already covered in a range of review articles [5,6,11]. On the other hand, besides polymers where the polyacrylamide structure is maintained, there are other interesting polymers that exhibit LCST behavior in water based on various structural motifs, such as methyl cellulose, polyethers, poly(2-oxazoline)s, poly(*N,N*-dimethyl aminoethyl methacrylate) (PDMAEMA), poly(vinyl caprolactone), certain polypeptides or poly(methyl vinyl ether) [7,12]. Apart from the latter polymer class, which was recently reviewed by Aoshima and Kanaoka [11] the thermo sensitive properties of these polymers were only briefly mentioned in recent reviews. In addition, Lutz discussed the synthesis [13] and application [14] of poly(oligo[ethylene oxide]methacrylate) based thermo-responsive materials in two recent highlights.

The scope of the current review is to provide an insight into the structural and environmental factors that can influence the thermo-responsive properties of certain biocompatible polymers. For this purpose, two specific polymer classes that are both interesting candidates for biomedical applications will be discussed [15]: poly(2-oxazoline)s (POx) and systems whose thermo-responsiveness is based on their structural similarity to poly(ethylene oxide) (PEO). Therefore, experimental data that were obtained by different groups will be compared in detail, although this is complicated by the different measurements conditions that were applied during the investigation of the LCST behavior. Nonetheless, as we will demonstrate, it is possible to draw conclusions about the influence of the polymer structure on its LCST behavior. Most of the polymers that will be discussed were synthesized by living or controlled polymerization techniques, but the applied synthesis methods will not be mentioned in detail since the focus of this review is on the detailed evaluation of the thermo-responsive properties of the synthesized materials.

2. Methods to investigate thermo-responsive polymers in solution

When a polymer is molecularly dissolved in a suitable solvent, it may become insoluble upon increase or decrease in temperature and, thus, precipitate from the solution. In other words, the binary polymer/solvent mixture undergoes a temperature induced phase separation from a one-phasic towards a bi-phasic system due to the existence of a miscibility gap in the phase diagram [16,17]. If elevation of temperature results in phase separation the systems exhibits lower critical solution temperature (LCST) behavior. To be more precise, the polymer does not simply precipitate from the solution, but two phases are formed in equilibrium, whereby one phase has a high polymer concentration and the other one has a low polymer concentration. As shown in Fig. 1, the LCST is defined as the temperature at the minimum of the binodal (or the coexistence curve) of the phase diagram. The corresponding concentration is the lower critical solution concentration (LCSC). The reverse case, where phase separation occurs upon decreasing temperature, is called upper critical solution temperature (UCST) behavior.

Due to the numerous possible applications in biomedical science there is an increasing interest in polymers that exhibit LCST behavior in water. Apparently, such a polymer behaves hydrophilic at low temperatures and turns hydrophobic at elevated temperature. Below the demixing temperature of the solution the polymer is capable to form hydrogen bonds with surrounding water molecules resulting in hydration. With increasing temperature those hydrogen bonds are weakened and it is more favorable for the water molecules to be expelled from the polymer structure into the bulk water. As a result, the polymer chains are (partially) de-hydrated and agglomerate. Since the phase separation is accompanied by conformational changes of the polymer, such an effect is often referred to as coil to globule transition of the responsive polymer (Fig. 2). From the thermodynamic point of view, hydrogen bonding between polymer chains and water molecules gives a favorable enthalpy contribution to the free energy of mixing whereas the binding of the water molecules to the polymer chain results in an enhanced ordering. Therefore, it contributes negatively (unfavorably) to the entropy of mixing. At higher temperatures, the entropy term $T\Delta S$ becomes predominant and the free energy of mixing gets positive, which is manifested in phase separation.

The easiest and most widely used method to obtain information about the coil to globule transition temperature of a polymer in solution is turbidimetry. A solution with a defined concentration of polymer is subjected to a variable temperature program, whereby the transmittance of light through the solution is constantly measured. As soon as the phase separation takes place, the transmittance will rapidly decrease due to agglomeration of the collapsed polymer globules forming aggregates that scatter the irradiated light. The temperature at which this happens is called the cloud point temperature (T_{cp}). If the investigated mixture is cooled down, the polymer re-dissolves and the transmittance increases again. In addition, dynamic light scattering (DLS) measurements are often applied in

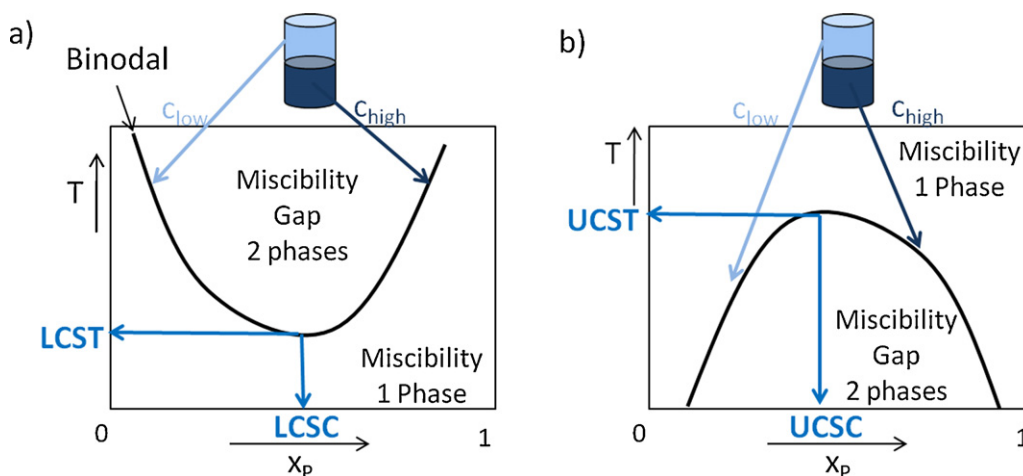


Fig. 1. Phase diagram for a binary polymer–solvent mixture exhibiting (a) LCST behavior and (b) UCST behavior.

order to gain information about the size and stability of the collapsed polymer globules. Since the coil to globule transition of a thermo-responsive polymer represents an endothermic process, it can also be followed by calorimetric methods, such as differential scanning calorimetry (DSC) and pressure perturbation calorimetry (PPC). In addition, (modulated) DSC allows the determination of the enthalpy of the transition (ΔH) and the glass transition temperature of the collapsed globules, whereas PPC can be used to determine the volume change $\Delta V/V$ during the phase transition.

In a range of applications, the thermo responsive polymer is exposed to a large amount of varying cosolutes in its aqueous solution. All those additives might alter the T_{cp} of the aqueous polymer solution. In the most simple case, one additive changes the binary system into a ternary system. Besides the effect of other solvents or surfactants, in particular the influence of salts has been investigated in detail for the polymers that are discussed within this review. In order

to understand the effect of salts on the solubility behavior of synthetic polymers it is helpful to apply the knowledge that has been achieved in protein chemistry: different salts increase or decrease the solubility of proteins since they induce changes in the secondary and tertiary structure. This observation is referred to as salting in or salting out effect, respectively. Salts that enhance the solubility (elevated T_{cp}) are called kosmotropes – they decrease the ordering of water. In contrast, salts that have a salting out effect (lowered T_{cp}) are chaotropes – they strengthen the hydrophobic interactions. Both, anions and cations exhibit such effects, but the influence of the anion is more pronounced. In the so-called Hofmeister series, anions and cations are ordered according to their ability to strengthen the hydrophobic interaction:

Anions : $\text{SO}_4^{2-} > \text{OAc}^- > \text{Cl}^- > \text{I}^- > \text{ClO}_4^- > \text{SCN}^-$

Cations : $\text{NH}_4^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+ > \text{Mg}^{2+} > \text{Ca}^{2+}$

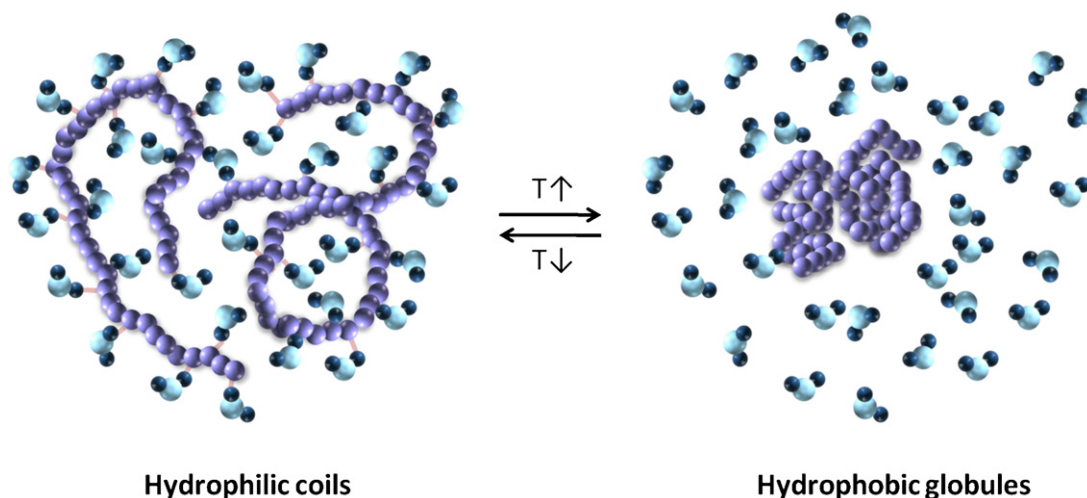


Fig. 2. Coil to globule transition of a thermo-responsive polymer in aqueous solution.

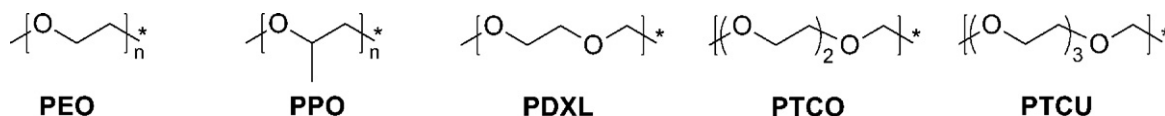


Fig. 3. Schematic representation of the structures of poly(ethylene oxide) (PEO), poly(propylene oxide) (PPO), poly(1,3-dioxolane) (PDXL), poly(1,3,6-trioxacyclooctane) (PTCO) and poly(1,3,6,9-tetraoxacycloundecane) (PTCU).

3. Linear polyethers

3.1. Poly(ethylene oxide)

Poly(ethylene oxide) (PEO, Fig. 3) is a hydrophilic polymer that retains its solubility in water upon heating at ambient pressure. However, increase of temperature above 100 °C results in phase separation and further heating solubilizes the PEO again. Thus, the phase diagram of the binary mixture PEO/water represents a “closed loop” coexistence type that shows both, an LCST as well as an UCST [18]. As shown in Fig. 4, increasing molar mass diminishes the solubility of the PEO in water resulting in lowered LCST but elevated UCST, as typical for Flory–Huggins Type

I behavior [19]. The observed cloud point temperatures were found to be independent of the pressure applied to the system, which could not be explained by simple van der Waals interactions between water and PEO. Instead, hydrogen bonds that are formed between the PEO chains and water are supposed to enhance the structuring of solvent molecules around the polymer coil [20]. This behavior is entropically unfavorable but the favorable enthalpy term is dominant at low temperatures resulting in miscibility. In contrast, the entropy contribution becomes predominant at higher temperatures causing a disruption of the water structure around the PEO and, therefore, a phase separation. The peculiar properties of the system PEO/water have attracted many theoretical scientists who tried to describe its phase behavior, mainly by modification of the Flory–Huggins theory [21–26]. Already in 1959 Bailey and Callard investigated the influence of inorganic salts as additives upon the phase transition temperature of PEO aqueous solutions ($c = 0.5$ wt%) and found salting out effects of both anions and cations to follow the Hofmeister series for proteins [27]. In addition, the salting out effects of sodium chloride and sodium propionate were further studied by Saeki and co-workers and the results could be related to the thermodynamic equation of state [28].

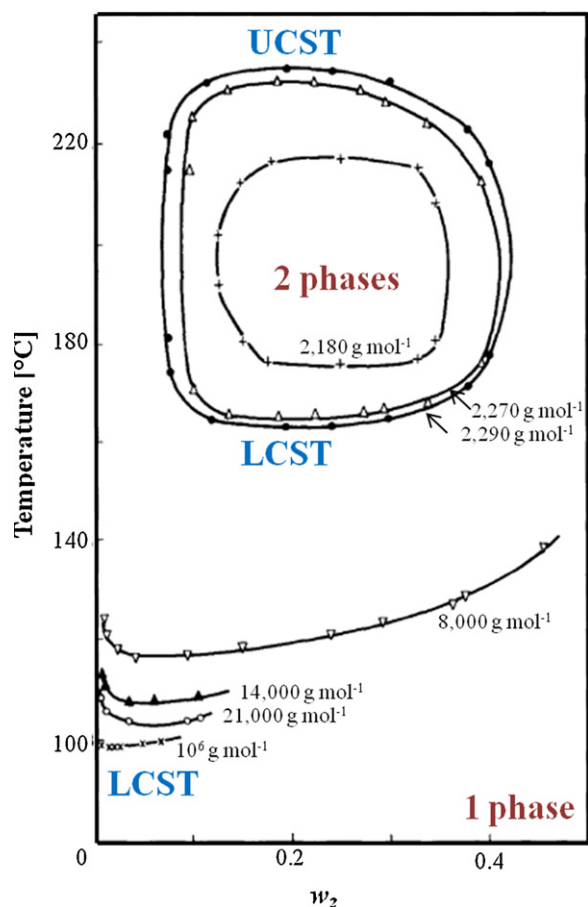


Fig. 4. Temperature/weight fraction phase diagram for the system PEO/water. Molar masses are provided as M_n . Reproduced from Saeki et al. [19] by permission of Elsevier Science Ltd., UK.

3.2. Poly(propylene oxide)

Although the LCST behavior of PEO in combination with its rather simple polymer structure is certainly of academic interest for the understanding of the LCST phenomenon, the high phase transition temperature is of little practical importance. However, when the hydrophobicity of the polyether is increased by addition of one carbon atom to the repeating unit, as in the case of poly(1,2-propylene oxide) (PPO), the phase transition temperature of the binary mixture with water decreases below the boiling point of the solvent under ambient pressure. Already in 1957, Malcolm and Rowlinson developed the phase diagram for this system and found a LCST of around 50 °C for PPO with a molar mass of $M_n = 400$ g mol^{−1} [18]. Saito determined the cloud point curves for PPO in the concentration range up to 80 mM [29]. The T_{cp} 's of aqueous solutions were found to decrease from around 38 °C to 20 °C and 17 °C with increasing molar masses of the PPO ($M_n = 1000$ g mol^{−1}, 2000 g mol^{−1} and 3000 g mol^{−1}, respectively). It should be noted that a large range of block copolymers of PEO and PPO are commercially available under the trade name “Pluronic” and have also been investigated with respect to their solubility behavior in aqueous systems, which will not be covered here.

3.3. Statistical copolymers of ethylene oxide and propylene oxide

Statistical copolymerization of EO with PO results in copolymers that exhibit LCST behavior in a temperature range in between the LCST of both homopolymers. This was already demonstrated in 1959 by Bailey and Callard who found a linear decrease of the cloud point temperatures of aqueous copolymer solutions ($c = 0.1$ and 1 wt%) with increasing content of PO in the statistical copolymer [27]. A further example is given by Tjerneld and co-workers who investigated a copolymer containing equal amounts of EO and PO with a molar mass of 4000 g mol^{-1} [30]. The experimentally determined cloud point curve revealed a minimum at 50°C and 10 – 20 wt% concentration. Based on this knowledge, the theoretical phase diagram was also constructed for degrees of polymerization from 39 to 156. The calculations using the Flory–Huggins theory provide hints towards a similar closed loop coexistence for the statistical copolymers as for PEO. With respect to applications of PEO–PPO copolymers for enzyme purification, Persson et al. determined the coexistence curves of three polymers with PO content of 50%, 70% and 80%, respectively [31]. The LCST was found to decrease with increasing content of hydrophobic PO from around 50 to 40 and 30°C , respectively. Copolymers with a lower content of PO from 12 to 26% but similar M_w of around $30,000 \text{ g mol}^{-1}$ were investigated by Louai et al. [32]. The LCSTs of their binary mixtures with water were determined as minima of the cloud point curves and were found to increase in a linear fashion from 74 to 87°C with increasing EO content of the copolymer. The phase diagrams were calculated in analogy to PEO. Determination of the heat of fusion of water and of the excess mixing volume revealed rather similar values as for PEO. This led to the conclusion that, despite the attachment of an additional methyl group at some repeating units, the structure of water around the copolymer is very similar to that in PEO solutions. Further experiments with these polymers were conducted in order to investigate the effect of various additives upon the phase transition temperature of the aqueous solutions [33]. The salting out effect of inorganic salts was shown to follow the Hofmeister series: $\text{KI} < \text{NaCl} < \text{Na}_2\text{CO}_3 < \text{Na}_3\text{PO}_4 < \text{Na}_2\text{B}_4\text{O}_7$. It is interesting to note that the more polar copolymers with higher EO content were affected in a stronger manner than the more hydrophobic ones, which is most likely a result of the enhanced dehydration of the more hydrophilic polymers in the presence of salts. In addition, also organic additives, such as carboxylic acids, alcohols, diols and amides, with varying hydrophilicity either increased or decreased the T_{CP} of the copolymer solutions ($c_{\text{polymer}} = 4 \text{ wt}\%$).

3.4. Other linear polyethers

Benkhira et al. performed comprehensive studies on the solubility behavior of linear polyethers that are composed of both EO and methylene oxide (MO) repeating units (Fig. 3). The cationic polymerization of cyclic monomers, 1,3-dioxolane, [34] 1,3,6-trioxacyclooctane and 1,3,6,9-tetraoxacycloundecane, [35] resulted in polyethers that contain varying molar fractions of EO and MO in an

alternating fashion. Since PMO is insoluble in water increasing EO content of the polymer led to an increased LCST, as shown by comparison of the phase diagrams, which were developed by turbidimetry (102°C for PTCU, 95°C for PTCO and 70°C for PDXL, respectively). In contrast to PTCU and PTCO, whose demixing curves had similar shapes as PEO, the solubility behavior of PDXL was more complex: in the concentration range between 20 and $40 \text{ wt}\%$ the binodal reached a plateau and strongly decreased at higher concentrations, where the demixing curve only represent a metastable state. When higher concentrated mixtures of PDXL and water were kept for several days, PDXL was found to crystallize even under ambient conditions. Analysis of the polymer series in aqueous solution by DSC revealed that PDXL forms a stable hydrate and that mainly the EO segments of the polymer chain were hydrated. In addition, the influence of anionic [36] and cationic surfactants [37] on the phase separation of polymer/water mixtures was well studied for the complete polyether series and compared to PEO.

Although the LCST behavior of PEO has been well investigated throughout the last decades, it is difficult to incorporate other functionalities to the polymer itself because of the rather complicated polymerization of the EO monomer, which is gaseous and highly toxic. Therefore, most of the studies relied on commercially available (co)polymers for their investigations. This drawback can be overcome by the end functionalization of short PEO chains with polymerizable units resulting in macromonomers. With this approach, the properties of PEO can be maintained and a straightforward access to a larger quantity of well-defined copolymers is provided using a variety of polymerization methods.

4. Systems containing oligomeric ethylene oxide side chains

By attachment of short hydrophilic PEO chains to various kinds of polymeric backbones, the advantages of PEO with respect to its biomedical applications can be maintained while the phase transition temperature is lowered due to the hydrophobicity of the polymer backbone. A suitable way for the synthesis of such comb shaped or “bottle brush” polymers is the macromonomer method where the short PEO chain is end functionalized with a moiety that can be polymerized in a subsequent step. Since the used abbreviations for the resulting polymers are not consistent in the literature, they will be systematically named during this review as illustrated in Fig. 4 (right). The degree of polymerization of the pendant EO side chains will be given by EO_n and the type of polymeric backbone will be abbreviated as follows: V for vinyl ether, St for styrene, A for acrylate, MA for methacrylate and LA for lactide. The end group of the pendant EO_n side chains will be indicated by: m for methyl, Et for ethyl and H for hydrogen.

4.1. Polymacromonomers with varying side chain length

Besides the nature of the used backbone, the thermoresponsive properties of the polymer will be strongly influenced by the length of the pendant PEO chains.

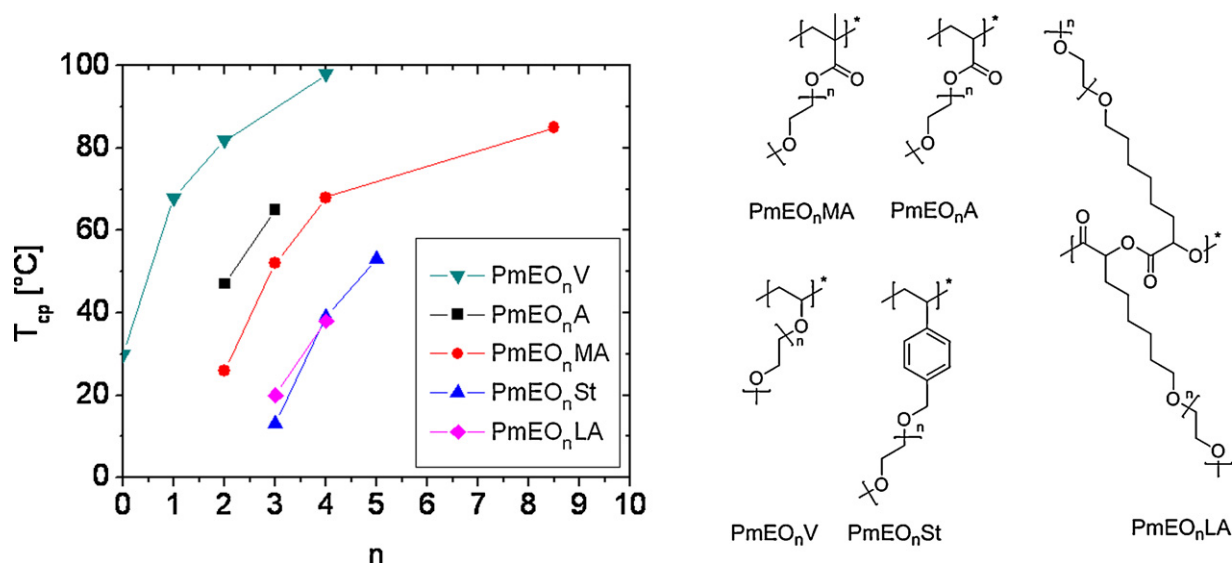


Fig. 5. Left: dependence of the cloud point temperatures of aqueous solutions of various PEO based polymacromonomers on the degree of polymerization of the pendant PEO chains (n). Right: schematic representation of the polymer structures. Data taken from Ref. [38] (▼), [39–41] (●), [42] (■), [43] (▲), [44] (◆). Measurement conditions are provided in the text. Lines are added to guide the eye.

Elongation of the PEO side chain length will increase the hydrophilicity of the polymer and, therefore, also the T_{cp} of its aqueous solution (Fig. 5, left). In addition, it can be clearly seen from Fig. 5 that increasing the hydrophobicity of the polymer backbone results in lower T_{cp} 's when the length of the pendant oligomeric EO chains is kept constant (the applied measurement conditions as well as the DP of the polymer backbone vary in parts within the literature data that are compared). The dependence of the T_{cp} 's upon the molar mass of the thermo-responsive polymers is summarized in Fig. 6 and will be discussed for each polymer class in the following section.

Already in the early 1990s, the living cationic polymerization of monodisperse oligo(ethylene oxide)vinyl ethers was applied to synthesize thermo-responsive polymers with short oligoEO side chains of varying length

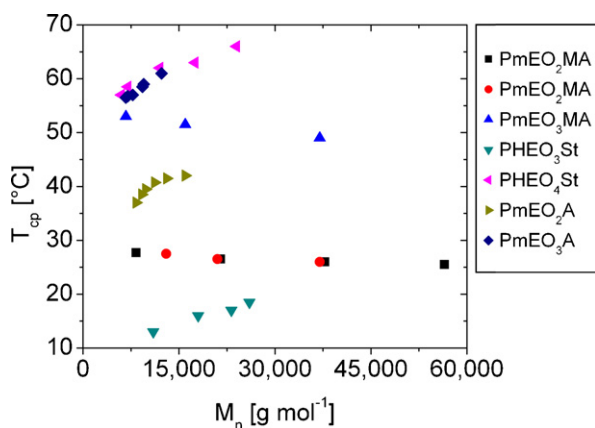


Fig. 6. Dependence of the cloud point temperatures of aqueous solutions of various PEO based polymacromonomers on the molar mass of the polymers. Data taken from Ref. [39] (●, ▲), [45] (■), [42] (▼, ◆, ▲, ◆).

by Kobayashi and co-workers (entrance ▼ in Fig. 5) [38]. For this polymer class, the polymer without PEO side chains, i.e. poly(methyl vinyl ether), already displays thermo-responsive properties around 30 °C. However, T_{cp} from turbidity measurements ($c = 1$ wt%) was found to be strongly increased to approximately 72 °C if one hydrophilic EO unit was present as side chain. Elongation of the side chain towards two and four EO units resulted in even higher T_{cp} 's around 83 °C and 98 °C, respectively. The dependence of the polymer molar mass ($M_n = 5000$ –70,000 g mol⁻¹) upon T_{cp} was investigated for PmEO₁V at varying concentrations (0.1, 1 and 10 wt%). Generally, increasing molar mass as well as concentration resulted in lower T_{cp} 's, and the effect was found to be much more pronounced at lower concentration regimes and for molar masses up to 20,000 g mol⁻¹ (decrease of about 5 °C). Many additional studies, including the development of phase diagrams [46], have been performed on this type of polymer class and are well summarized in a review that appeared in 2008 [11], to which the reader is referred for additional details.

The PEO macromonomer approach was extended by Ishizone and co-workers to methacrylate type polymers that were synthesized by anionic polymerization of the corresponding monodisperse mEO_nMA monomers (entrance ● in Fig. 5) [39]. PmEO₁MA was found to be insoluble in water, whereas elongation of the side chains towards 2 and 3 EO units enhanced the hydrophilicity of the polymers leading to polymers that exhibit LCST behavior in aqueous solution. Turbidity measurements of aqueous solutions ($c = 0.2$ wt%) revealed T_{cp} 's around 26 °C for PmEO₂MA and around 52 °C for PmEO₃MA, respectively. Increasing molar mass of the polymers (M_n range from 6700 g mol⁻¹ to 37,000 g mol⁻¹) resulted in a more sudden response towards temperature (represented by sharper transitions in the turbidity curves) and was found to decrease T_{cp} of

the aqueous solution by 5 °C (entrance ● and ▲ in Fig. 6). In addition, a small effect of the polymer tacticity upon the thermo-responsiveness was observed. ^1H NMR measurements in D_2O at elevated temperatures showed the complete disappearance of signals for PmEO_2MA above the cloud point of the solution whereas the longer side chains of PmEO_3MA remained mobile even upon precipitation of the polymer above T_{cp} . The corresponding PmEO_4MA , which was synthesized from a monodisperse macromonomer as well, was found to precipitate from its aqueous solution at 68 °C under similar measurement conditions reflecting a further increase of hydrophilicity by the longer EO_4 side chain [40].

PmEO_2MA as well as PmEO_3MA were also synthesized by ATRP by Yamamoto et al. who extended the investigated molar mass range of PmEO_2MA to $M_n = 60,000 \text{ g mol}^{-1}$ [45]. The T_{cp} values observed by turbidity measurements ($c = 3 \text{ mg mL}^{-1}$) are in excellent agreement with results obtained for the polymers that were synthesized by anionic polymerization as demonstrated in Fig. 6 (entrance ■). The polymer concentration was shown to have a stronger influence on the phase separation temperature for PmEO_3MA than for PmEO_2MA . In the investigated concentration range from 1 mg mL^{-1} to 10 mg mL^{-1} , T_{cp} decreased from 26 to 24 °C for PmEO_2MA and from 51 to 47 °C for PmEO_3MA upon increase of concentration. Next to turbidity measurements, the thermal properties of the aqueous polymer solutions were investigated by DLS revealing the formation of very large aggregates of $2.7 \mu\text{m}$ size upon precipitation at T_{cp} for PmEO_2MA . In addition, $\text{PmEO}_{\sim 4.5}\text{MA}$ was synthesized by RAFT polymerization of a commercially available (but not monodisperse) macromonomer [47]. Turbidity measurements in buffered solutions at pH 4, 7 and 10, respectively, and a polymer concentration of 5 mg mL^{-1} revealed a T_{cp} of 64 °C, which was unaffected by the pH of the solution.

An additional increase of the hydrophilicity of the PmEO_nMA polymers can be achieved when the EO chain is elongated to approximately, i.e. not monodisperse, 8 units as demonstrated by Laschewsky who applied RAFT polymerization of $\text{mEO}_{\sim 8}\text{MA}$ [41]. Turbidity measurements revealed a T_{cp} of 85 °C of a 1 wt% aqueous solution of a comb polymer with a DP of 16 in the polymer backbone. Further increase of the EO side chain length towards ~ 22 repeating units resulted in polymers that are water soluble in the entire temperature range of liquid water at ambient pressure [48].

The utilization of monodisperse macromonomers based on acrylates for NMP yielded the corresponding PmEO_nA with 2 as well as 3 repeating units of EO as side chains, as was demonstrated by the Zhao group (entrance ■ in Fig. 5) [42]. Turbidity measurements of aqueous polymer solutions were carried out in a concentration range from 0.05 wt% to 5 wt% and revealed T_{cp} 's from 35 °C to 47 °C for PmEO_2A and from 57 °C to 65 °C for PmEO_3A , respectively. The cloud point curves of both polymers were found to decrease steeply at lower concentrations (up to 1 wt%) whereas they became rather flat at higher polymer concentrations. ^1H NMR spectroscopy in D_2O revealed line broadening of the peaks above the cloud point, but the signals were still visible indicating a high hydrophilicity

of the polymers even after dehydration. In contrast to the PmEO_nMA discussed above, an increase of the molar mass of the PmEO_nA resulted in elevated T_{cp} 's (by around 5 °C) in the M_n range from 8000 g mol^{-1} to $16,000 \text{ g mol}^{-1}$, which was attributed to the relatively large hydrophobic polymer end group resulting from NMP synthesis that has a larger impact on the solubility of shorter polymer chains (entrance ► and ◆ in Fig. 6).

A similar study was carried out applying NMP of monomers that are based on styrene comprising monodisperse oligomeric EO units (entrance ▲ in Fig. 5) [43]. The aqueous solutions ($c = 0.5 \text{ wt\%}$) of the resulting PmEO_nSt containing 3, 4 and 5 repeating units of EO as side chains displayed T_{cp} 's of 13 °C, 39 °C and 53 °C, respectively. For PmEO_3St , the aromatic signals of the polymer backbone completely disappeared from the ^1H NMR spectrum in D_2O as soon as the cloud point of the polymer solution was reached while the PEO signals remained visible, although broadened. However, the increasing hydrophilicity of the structures obtained with higher EO content (PmEO_4St and PmEO_5St) was reflected in the fact that even the signals deriving from the highly hydrophobic PS backbone remained visible above T_{cp} .

The use of this strategy was also applied in order to synthesize thermo-responsive polyglycolides (entrance ◆ in Fig. 5) from the corresponding EO containing cyclic dilactides [44]. Due to the relatively long and, thus, hydrophobic alkyl chains that were used to connect the oligomeric EO units to the lactide, the polymers containing one as well as two EO units were found to be insoluble in water. However, turbidity measurements of aqueous solutions at polymer concentrations of 15 wt% revealed cloud point temperatures at 20 °C and 38 °C for PmEO_3LA and PmEO_4LA , respectively. The polymer signals in ^1H NMR spectra in D_2O were broadened above the cloud point, and DLS measurements at 3 mg mL^{-1} confirmed the agglomeration of the polymer chains into large aggregates above the cloud point.

4.2. Effect of side chain end groups

All values discussed in the previous section are based on polymer systems with methyl groups at the end of the pendant PEO chains. However, since a comb polymer contains as many of these end groups as side chains, they have a large impact on the solubility properties of the entire polymer. In addition, those end groups point towards the outside of the macromolecular “bottle brush” structure in solution as demonstrated in Fig. 7. This further enhances their effect on the phase transition temperature since the surrounding water molecules will strongly interact with these end groups upon hydration of the polymer.

This effect has already been observed by Aoshima et al. in the 1990s for their vinyl ether based poly-macromonomers [38]. Replacement of the methyl moieties at the end of the oligomeric EO side chains with ethyl groups resulted in T_{cp} 's that were decreased by 40–50 °C (entrance ▲ and ▼ in Fig. 7). Similar results were found for the methacrylate based systems of Ishizone et al. although the T_{cp} 's were only decreased by around 20–25 °C when compared to the corresponding values from polymers carrying methyl end groups (entrance ■ and ● in Fig. 7) [40].

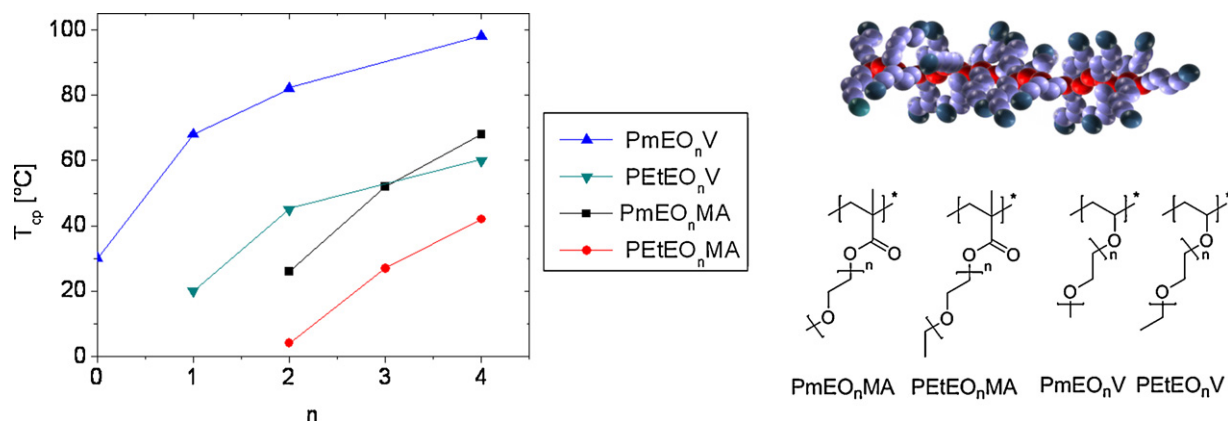


Fig. 7. Effect of the hydrophilicity of the side chain end groups on the cloud point temperatures of aqueous solutions of various PEO based polymers and schematic representation of the polymer structures. Data taken from Ref. [38] (\blacktriangle , \blacktriangledown), [39] (\blacksquare), [40] (\bullet). Lines are added to guide the eye.

PETEO₃MA was also synthesized via RAFT polymerization, and the T_{cp} 's of buffered polymer solutions ($c = 5 \text{ mg mL}^{-1}$) at pH 4, 7 and 10 are in agreement with values obtained for the anionically synthesized polymers [48]. A similar influence can be observed when PETEO₂A, which has recently been obtained by NMP [49], is compared to PmEO₂A [42]: the T_{cp} of a 0.5 wt% aqueous solution of PETEO₂A was found to be 13 °C, which is 15 °C lower than the T_{cp} of PmEO₂A at the same concentration. In addition, the T_{cp} of PETEO₂A solutions was slightly increased to 16 °C when the polymer was synthesized by FRP, which is a result of the large hydrophobic backbone end group derived from NMP synthesis.

On the other hand, replacement of the side chain end groups by a proton results in hydroxyl end groups that significantly enhance the hydrophilicity of the macromolecules so that the corresponding PHEO_nMA and PHEO_nA are water soluble in the entire temperature range of liquid water at ambient pressure [39]. In agreement to that the T_{cp} of an aqueous solution of PmEO₄St is increased from 39 °C to 64 °C for PHEO₄St [42]. Since this type of polymers was synthesized by NMP, the large hydrophobic end group of the polymer backbone caused an increase in T_{cp} with increasing molar mass (entrance \blacktriangledown and \blacktriangleleft in Fig. 6).

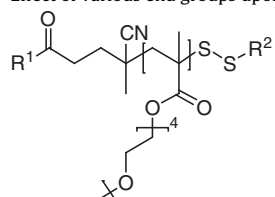
4.3. Effect of backbone end group

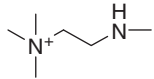
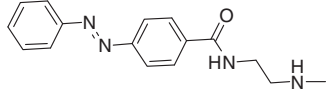
The application of living and controlled polymerization techniques during the synthesis of thermo-responsive polymers provides the opportunity to obtain polymeric structures with well-defined end groups. The fact that some of those end groups can be used for further modification of the obtained polymer with hydrophilic or hydrophobic moieties enables the investigation of their effect upon the LCST behavior. Since there are only two end groups at the polymer backbone (at the α and at the ω chain end) it seems to be quite obvious that the impact of the backbone end groups on the solubility behavior of the entire macromolecule will be much less pronounced than the effect of the side chain end groups. However, the effect of end

groups will be more significant for polymers with lower molar masses [50].

Theato and co-workers recently applied the RAFT polymerization technique to obtain PmEO₄MA from a commercially available methacrylate monomer having approximately 4 repeating units of EO [51]. The obtained polymer ($DP \sim 10$) was subsequently modified at both α as well as ω end groups resulting in a library of PmEO₄MA having the same DP but a large variety of end groups (see Table 1). T_{cp} of the aqueous polymer solutions ($c = 10 \text{ mg mL}^{-1}$) having the same α end group was found to decrease when the hydrophobicity of the ω end group was increased following the order: $-\text{CH}_3$, $-\text{C}_{16}\text{H}_{33}$, $-\text{CH}_2\text{CH}_2\text{C}_6\text{F}_{13}$. When $-\text{CH}_3$ was kept constant as ω end group, the same trend could be followed when the hydrophobicity of the α end group was increased in the following order: ammonium, PEG, C_3H_7 , $\text{C}_{16}\text{H}_{33}$, $\text{C}_8\text{F}_{17}\text{CH}_2$, $(\text{C}_{18}\text{H}_{37})_2$. However, by introduction of two very hydrophobic moieties at both α as well as ω end group deviations from this series could be observed, which was attributed to the formation of micellar structures below the cloud point as could be confirmed by DLS measurements. Aromatic end groups, such as $\text{C}_6\text{F}_5\text{O}-$, $-\text{SCSPH}$ and an azo dye, were found to decrease T_{cp} more than long aliphatic chains, which was proposed to be a result of the rigidity of those structures making them even more difficult to hydrate by the solvent molecules. In particular, the azo dye (last entrance in Table 1) represents a very interesting end functionality since irradiation with UV light induces a *cis-trans* isomerization of the azo moiety that is accompanied by an increased dipole moment and, thus, elevated T_{cp} (by 2–11 °C, $c = 10 \text{ mg mL}^{-1}$) [50]. This UV triggered solubility change was found to be fully reversible after re-isomerization of the azo functionality.

Soeriyadi et al. functionalized the vinylic polymer end group of PmEO₂MA, which was synthesized via catalytic chain transfer polymerization, with 2-mercaptoethanol, 1-dodecanethiol and benzyl mercaptan and found a similar influence of the hydrophobicity of the polymer end group [52].

Table 1Effect of various end groups upon the cloud point temperature of aqueous solutions of PmEO₄MA [51].


R ¹	R ²	T _{cp} [°C] c = 1 wt%
C ₆ F ₅ O–	–SCSPh	42.8
CH ₃ (OCH ₂ CH ₂) ₁₁ NH–	–CH ₃	62.7
	–C ₁₆ H ₃₃	62.4
	–CH ₂ CH ₂ C ₆ F ₁₃	62.1
C ₃ H ₇ NH–	–CH ₃	58.3
	–C ₁₆ H ₃₃	50.1
	–CH ₂ CH ₂ C ₆ F ₁₃	49.7
C ₁₆ H ₃₃ NH–	–CH ₃	53.6
	–C ₁₆ H ₃₃	45.8
	–CH ₂ CH ₂ C ₆ F ₁₃	44.7
C ₈ F ₁₇ CH ₂ NH–	–CH ₃	50.9
	–C ₁₆ H ₃₃	51.7
	–CH ₂ CH ₂ C ₆ F ₁₃	50.3
(C ₁₈ H ₃₇) ₂ NH–	–CH ₃	48.9
	–CH ₂ CH ₂ C ₆ F ₁₃	48.9
	–CH ₃	66.3
	–C ₁₆ H ₃₃	62.5
	–CH ₂ CH ₂ C ₆ F ₁₃	62.1
	–CH ₃	49.1

4.4. Block copolymers composed of two thermo-responsive parts

In cases where both blocks of a block copolymer consist of thermo-responsive polymers, the aqueous solution might display a single cloud point at an intermediate temperature, or both blocks might collapse independently from one another. Both behaviors have been observed for polymers based on EO macromonomers and will be discussed in the following section.

Ishizone and co-workers reported that the aqueous solution of a block copolymer of PmEO₂MA and PmEO₃MA displays a single cloud point at 39 °C, which is in between the *T_{cp}*'s of both independent blocks (at 26 °C and 52 °C, respectively) [39]. Yamamoto et al. also observed single cloud points of 0.1 wt% aqueous solutions of two PmEO₂MA-*b*-PmEO₃MA block copolymers that were synthesized by ATRP [45]. Although both *T_{cp}*'s were in between the cloud point temperatures of the homopolymers, the exact value for the block copolymer containing a longer PmEO₃MA block was found to be higher than the expected value for a statistical copolymer of both monomers. This deviation is most likely caused by the formation of micelles that consist of the collapsed PmEO₂MA block in the core and still water soluble PmEO₃MA in the shell below *T_{cp}*. Indeed, DLS confirmed the presence of aggregates that were about 100 nm in size already before *T_{cp}* (which was determined by turbidimetry) was reached. Further increase in temperature resulted in the formation of larger aggregates

(>1 μm) above the cloud point indicative of a full collapse of the block copolymer.

Similar observations were made by Zhao et al. for block copolymers of PmEO_nSt having EO side chains of varying DP from 3 to 5 [43]. The aqueous solutions of 5 block copolymers (PmEO₃St-*b*-PmEO₄St, PmEO₃St-*b*-PmEO₅St, PmEO₄St-*b*-PmEO₅St) displayed phase transition temperatures that coincide with values that were calculated from the *T_{cp}*'s of the corresponding homopolymers using a formula that takes into account the degree of polymerization (DP) of each block:

$$T_{cp} = \frac{DP_1}{DP_1 + DP_2} \times T_{cp,1} + \frac{DP_2}{DP_1 + DP_2} \times T_{cp,2} \quad (1)$$

In contrast, aqueous solutions of various mixtures of the homopolymers showed phase separation at the *T_{cp}*'s of the homopolymer with the lowest phase transition temperature.

Alexander and co-workers prepared hybrid block copolymers that consist of a statistical copolymer of EtEO₃MA with mEO_{~8.5}MA as first block and of PmEO_{~8.5}MA homopolymers as second shorter block using AGET ATRP [53]. Aqueous solutions of the polymers (*c* = 1.5 mg mL^{−1}) revealed *T_{cp}*'s that were increased by 4–7 °C when compared to those of the first block. The difference was found to be enlarged upon increase of the block length of the second, more hydrophilic block due to the formation of micelle-like assemblies of 40–100 nm radii upon collapse of the first thermo-responsive block, as could be

confirmed by DLS, TEM as well as fluorescent probing with pyrene. The addition of sodium sulfate as kosmotrope to those systems resulted in changes of the micellar structure leading to a sharp burst release of carboxyfluorescein that was encapsulated into the core of the micelles.

On the other hand, block copolymers where the cloud points of the individual blocks, PmEO_3A ($T_{\text{cp}} = 62^\circ\text{C}$) and PmEO_3St ($T_{\text{cp}} = 13^\circ\text{C}$), are further apart underwent a series of phase transitions in water, as demonstrated in Fig. 8 [54]. Heating of a 1 wt% aqueous solution of $\text{PmEO}_3\text{A}-b\text{-PmEO}_3\text{St}$ led first to the collapse of the more hydrophobic PmEO_3St block at transition temperatures that were up to 20°C higher when compared to the homopolymer PmEO_3St . This first phase transition temperature was found to be decreased with increasing length of the PmEO_3St block and, thus, came closer to the T_{cp} of the PmEO_3St homopolymer. Upon further increase in temperature to around $25\text{--}39^\circ\text{C}$, the turbid solutions became clear, and micelles consisting of the collapsed PmEO_3St block in the core and the still water soluble PmEO_3A block in the shell were formed. The hydrodynamic diameter of these spherical micelles with narrow size distributions was increased from 27 nm to 58 nm with increasing length of the PmEO_3St block, as could be shown by DLS measurements. Further increase in temperature towards $45\text{--}55^\circ\text{C}$ resulted in a collapse of the PmEO_3A blocks and, therefore, the formation of large aggregates. This second cloud point was lowered compared to the PmEO_3A homopolymer and, again, increasing length of the PmEO_3St block resulted in a shift to lower temperatures. This fact can nicely be explained by the larger hydrophobicity of the longer, collapsed PmEO_3St block. The independent collapse of both blocks could also be followed by ^1H NMR spectroscopy in D_2O revealing a broadening of the aromatic signals of the PmEO_3St around the first transition temperature and a broadening of the peak derived from the methyl side chain end group of PmEO_3A at the second cloud point.

4.5. Statistical copolymers of monomers with different length of oligomeric EO

The homopolymers described above already cover a wide temperature range in their responsive behavior. However, sometimes the monomers have to be synthesized since not all of them are commercially available. In addition, specific monomers result in polymers with a defined T_{cp} and intermediate temperatures are not accessible using homopolymers. An easy and straightforward method to more accurately tune the phase transition temperature of PmEO_nMA is therefore the statistical copolymerization of two monomers with different EO chain length. The resulting copolymers will display thermo-responsive properties in a temperature interval ranging in between the T_{cp} 's of both homopolymers. Most studies involve the copolymerization of mEO_2MA with monomers having a longer EO chain so that the rather low phase transition temperature of PmEO_2MA is increased. The increased PEO chain length of the second monomer enhances the hydrophilicity of the copolymer, which is reflected by the steeper decrease of the T_{cp} values with increasing mEO_2MA content, as displayed in Fig. 9.

Kitano et al. used small amounts (3–10%) of $\text{mEO}_{\sim 8.5}\text{MA}$ and $\text{mEO}_{\sim 22}\text{MA}$ as comonomers during free radical copolymerization of mEO_2MA in order to investigate the effect of added macrocycles that might be able to incorporate a few longer pendant PEO chains [55]. Turbidity measurements of 0.1 wt% aqueous solutions revealed increased T_{cp} 's with increasing comonomer content as well as increasing length of the oligomeric EO chain of the comonomer (entrance ■ and ● in Fig. 9). Addition of SCX6, a macrocycle that contains six anionically charged sulfonate groups, was found to raise the phase transition temperature by up to 12°C due to the fact that the pendant PEO chains were enclosed into the macrocycle obstructing the coil to globule transition of the polymer. β -Cyclodextrin had a similar effect, although much less pronounced, whereas hydrogen bonding of neighbouring hydroxyl groups of α -cyclodextrin caused a slight decrease of T_{cp} .

A study that covered a wider range of copolymer compositions was carried out by Lutz and Hoth who copolymerized mEO_2MA with $\text{mEO}_{\sim 8.5}\text{MA}$ using ATRP [56]. Turbidity measurements ($c = 3\text{ mg mL}^{-1}$) in H_2O revealed a linear increase of T_{cp} with increasing $\text{mEO}_{\sim 8.5}\text{MA}$ content for polymers that contained up to 30% $\text{mEO}_{\sim 8.5}\text{MA}$ so that the phase transition temperature could be precisely tuned between 28°C and 59°C (entrance ▲ in Fig. 9). The polymer concentration was found to have only a small effect since T_{cp} only varied by “a few degrees” in a concentration range from 1 mg mL^{-1} to 20 mg mL^{-1} . Subsequently, the copolymer containing 5% $\text{mEO}_{\sim 8.5}\text{MA}$ was further investigated due to the fact that it exhibited LCST behavior at a similar temperature as PNIPAm [59]. The turbidity curve of the aqueous solution was found to be sharper with less hysteresis between heating and cooling compared to PNIPAm. Addition of sodium chloride ($c \leq 0.2\text{ mol L}^{-1}$) caused a similar small salting out effect of about 2°C for both polymers. In addition, the dependence of T_{cp} of the polymer solutions on the concentration was found to be very similar, decreasing by $\sim 3^\circ\text{C}$ for concentration between 1 mg mL^{-1} and 10 mg mL^{-1} . The effect of the degree of polymerization upon T_{cp} was found to be even smaller for the PmEO_nMA copolymer than for PNIPAm when varying the DP from 25 to 100. DLS experiments of 1 mg mL^{-1} aqueous solutions were applied for the PmEO_nMA copolymers containing 10% and 20% $\text{mEO}_{\sim 8.5}\text{MA}$, respectively [60]. Below the cloud point of the solution, both polymers showed the coexistence of particles having a hydrodynamic radius of around 4 nm, which most likely represent individually solvated polymer coils, with larger particles of about 150 nm size, which contain less than 0.01% of the polymer chains and were attributed to loose aggregates. Increase of temperature resulted in a slight shrinkage of the particles, which was attributed to the partial loss of hydration water, before the individual polymer chains formed larger aggregates of 300 nm and 200 nm upon their coil to globule transition at T_{cp} . The reason for the fast reversibility of the phase transition was proposed to be the fact that the polymer aggregates are only held together by weak van der Waals interactions instead of intramolecular hydrogen bonds as being the case for PNIPAm. With respect to further use of this type of polymer in biological systems, the biodegradability of the polymer was enhanced by copolymerization

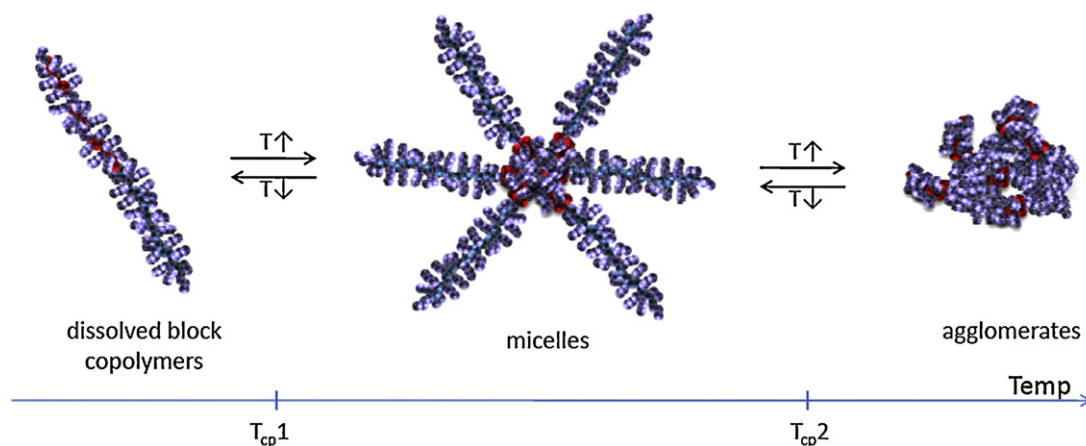


Fig. 8. Conformational changes of a copolymer consisting of two thermo-responsive parts in aqueous solution upon temperature variation.

with BMDO [57]. Since BMDO was incorporated mainly via a ring opening mechanism, the resulting ester functionalities inside the methacrylate backbone could either be cleaved enzymatically or under basic conditions. As displayed in Fig. 9 (entrance ▼) inclusion of the hydrophobic BMDO resulted in a slight decrease of the observed T_{cp} values ($c = 3 \text{ mg mL}^{-1}$) when compared to the copolymer without BMDO.

Copolymerization of mEO_2MA with a monomer containing a shorter oligomeric EO chain, $\text{mEO}_{\sim 4.5}\text{MA}$, results in decreased hydrophilicity of the copolymer at the same mEO_2MA content as depicted in Fig. 9 (entrance ♦) [58]. Nevertheless, turbidity measurements at a polymer concentration of 3 mg mL^{-1} revealed that T_{cp} can be tuned in a similar way in between the cloud point temperatures of PmEO_2MA and $\text{PmEO}_{\sim 4.5}\text{MA}$ at 28°C and 65°C , respectively. PBS was shown to have a small salting out effect since the observed T_{cp} 's were $3\text{--}4^\circ\text{C}$ lower than in water. In addition, DLS measurements revealed the presence of free hydrated polymer chains with a hydrodynamic

diameter of 3.5 nm below the cloud point and the formation of mesoglobules of sizes around 250 nm upon the temperature induced collapse of the polymer. A comparison between ATRP and free radical polymerization as synthesis techniques revealed a T_{cp} that was lowered by 2°C for the conventionally synthesized copolymer, which may be a result of its higher molar mass.

Yamamoto et al. applied ATRP in order to obtain well-defined copolymers of mEO_2MA with mEO_3MA [45]. Investigation of the thermal properties of their aqueous solutions ($c = 0.1 \text{ wt\%}$) by turbidity measurements showed a linear increase of T_{cp} with increasing content of mEO_3MA from 26°C to 53°C . As demonstrated in Fig. 9 (entrance ►), the shorter oligomeric EO chain of mEO_3MA results in a decreased hydrophilicity of the statistical copolymers and, thus, lower cloud points when compared to the copolymers described above.

Besides the copolymerization of mEO_2MA , also the copolymerization of other oligoEO based monomers with one another was used in order to finetune the LCST of

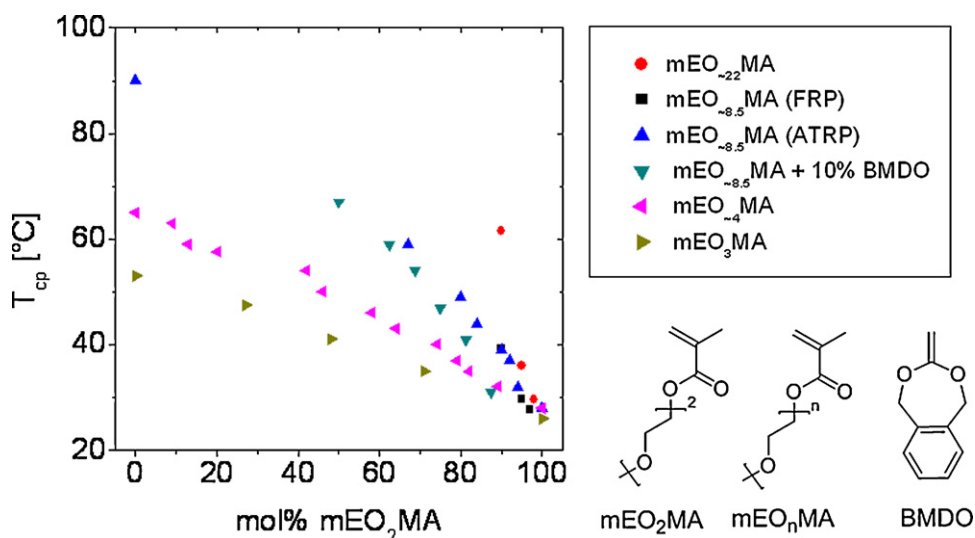


Fig. 9. Cloud point temperatures observed for aqueous solutions of statistical copolymers of mEO_2MA with other monomers containing oligomeric EO chains (mEO_nMA). Data taken from Ref. [55] (■, ●), [56] (▲), [57] (▼), [58] (◆), [45] (►).

the corresponding homopolymers. The AGET ATR copolymerization of EtEO₃MA with mEO_{~8.5}MA revealed a series of statistical copolymers with mEO_{~8.5}MA content of up to 20 mol% whose aqueous solutions ($c = 1.5 \text{ mg mL}^{-1}$) displayed thermo-responsive behavior in a temperature range from 24 °C to 42.5 °C [53]. The T_{cp} 's measured by turbidimetry increased linearly with the content of the more hydrophilic mEO_{~8.5}MA in the polymer. In addition, the effect of various salt additives on the demixing temperature of aqueous polymer solutions was investigated for salt concentrations up to 0.5 mol L^{-1} . The observed effects for both sodium as well as potassium salts followed the Hofmeister series of anions: $\text{SCN}^- > \text{ClO}_4^- > \text{I}^- > \text{Cl}^- > \text{OAc}^- > \text{SO}_4^{2-}$, the first 3 (kosmotropes) causing a salting-in effect and the latter 3 (chaotropes) causing a salting-out effect. The additives altered the T_{cp} of PEO_{*n*}MA solution (33 °C) over a broad temperature range from 3 °C for Na₂SO₄ to 46 °C for NaSCN, causing stronger effects than for aqueous solutions of PNIPAm, which might be due to the highly ordered water structure around PEO or crownether type interactions between PEO and the cations.

The approach has been extended to acrylate based monomers, mEO₂A and mEO_{~8.5}A, which were copolymerized using ATRP by Laschewsky and co-workers [61]. Aqueous solutions ($c = 3 \text{ mg mL}^{-1}$) of the obtained statistical copolymers containing up to 30 mol% mEO_{~8.5}A exhibited phase transitions in a temperature range from 9 °C to 50 °C, as was followed by turbidity measurements. Also in this case increasing the mole fraction of the more hydrophilic mEO_{~8.5}A was found to increase the hydrophilicity of the copolymer resulting in elevated T_{cp} of the aqueous solution. Sodium chloride was shown to cause a salting out effect of up to ~ 4 °C for salt concentrations of up to 12 g L^{-1} . The molar mass of the copolymer had only a very small effect on its coil to globule transition since T_{cp} 's only deviated by 1 °C in the M_n region of 10,000–30,000 g mol^{-1} . Recently, Cornelissen and co-workers reported copolymers of mEO₁A and mEO₂A with an equal content of both monomers that were prepared by ATRP [62]. Turbidimetry in PBS ($c = 0.5 \text{ wt\%}$) revealed T_{cp} 's between 25 °C and 35 °C that decreased with increasing molar mass of the polymer ($M_n = 9000\text{--}30,000 \text{ g mol}^{-1}$). Increasing concentration of polymer and added amount of NaCl resulted in decreased T_{cp} 's. Coupling of the thermo-responsive polymers to enhanced green fluorescent protein (EGFP) induced the formation of micellar structures above the coil to globule transition temperature of the thermo-responsive copolymer, as shown by DLS as well as TEM measurements. In addition, random copolymers of mEO₁A and HEO₁A with varying composition were synthesized by RAFT polymerization [63] as well as NMP [64]. The T_{cp} of aqueous polymer solutions ($c = 0.5 \text{ wt\%}$) could be tuned from 2 °C to 60 °C and was found to increase with increasing content of the more hydrophilic HEO₁A monomer. Increasing the molar mass resulted in lower T_{cp} values, whereas removal of one benzylic end group of the polymer significantly increased the coil to globule transition temperature of the polymers. Hoogenboom, Keul and Moeller recently reported the copolymerization of EtEO₂A with HEO₁A by FRP as well as NMP [49]. The T_{cp} 's of aqueous polymer solutions ($c = 0.5 \text{ wt\%}$) were found to increase

from 13 °C for PEtEO₂A to 48 °C for a copolymer with 75% HEO₁A content with increasing mole fraction of the more hydrophilic HEO₁A in the copolymer, as determined by turbidimetry. Comparison of the polymers that were synthesized by NMP and FRP revealed 3 °C higher T_{cp} values for the free radically polymerized copolymers, which was ascribed to the hydrophobicity of the nitroxide end group of the polymers obtained by NMP. Increasing amount of HEO₁A in the copolymer resulted in a larger heating cooling hysteresis, whereas the PDI of the polymers had no significant effect on the phase transition.

4.6. Copolymers with other monomers

Besides the copolymerization of oligoEO based monomers with one another, these monomers may also be copolymerized with other monomers that are able to provide additional interesting features to the resulting thermo-responsive copolymer. Various groups combined PEO with the most well-known LCST polymer, PNIPAm, either by grafting PEO onto PNIPAm [65], by copolymerization of mEO_{*n*}MA [66] or HEO_{*n*}MA [67] with the NiPAm monomer, or by terpolymerization with dodecyl methacrylate [68]. In these works EO_{*n*}MA is mainly regarded as comonomer in order to investigate its effect on the phase transition temperature of PNIPAm since increasing length of the PEO chain as well as increasing mole fraction of EO_{*n*}MA in the copolymers resulted in an increased T_{cp} of the aqueous PNIPAm solutions due to the hydrophilicity of the pendant PEO chains. In contrast, also a wide range of other monomers has been copolymerized with EO_{*n*}MA in order to investigate their effect upon the phase transition temperatures of PEO_{*n*}MA aqueous solutions (Fig. 10).

4.6.1. Copolymers with hydrophobic monomers

Ali and Stover applied ATRP for the synthesis of PHEO_{~7.3}MA [69]. Due to the hydrophilicity of the hydroxyl end groups of the pendant side chains, the PHEO_{~7.3}MA homopolymers were water soluble in the investigated temperature range up to 80 °C. However, copolymerization with MMA rendered them more hydrophobic so that the resulting copolymers displayed LCST behavior in aqueous solutions of 1 wt% concentration. Turbidity measurements revealed increasing T_{cp} from 43 °C to 56 °C with increasing mole fraction of PHEO_{~7.3}MA in the copolymers (24–40%). Vincent and co-workers reported a range of copolymers of mEO_{~5.5}MA with BuMA that were synthesized by free radical polymerization to show LCST behavior in 1 wt% aqueous solution at temperatures ranging from 45.8 °C to 53.6 °C, but their research was focused on the investigation of the formation of micellar structures at room temperature rather than on LCST phenomena [70].

4.6.2. Copolymers with ionizable monomers

4.6.2.1. Cationically charged comonomers. As already discussed in Section 4.5, it is possible to tune the phase separation temperature of aqueous solutions of copolymers that are composed of two different monomers (whose homopolymers also display LCST behavior) between the

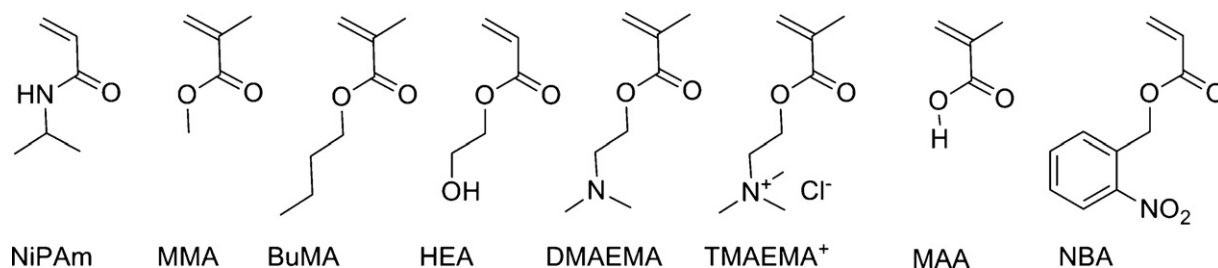


Fig. 10. Schematic representation of monomers that have been applied for copolymerization with EO_nMA or EO_nA.

T_{cp} 's of the homopolymers. This approach has been followed by Fournier et al. who prepared a series of gradient copolymers of DMAEMA with mEO_{~4.5}MA using the RAFT polymerization technique ($r_{DMAEMA} = 0.93$; $r_{mEO_{~4.5}MA} = 0.66$) [47]. Turbidity measurements of aqueous solutions ($c = 5 \text{ mg mL}^{-1}$) at pH 4 only revealed LCST behavior for a copolymer with a mEO_{~4.5}MA content of 90% as well as the PmEO_{~4.5}MA homopolymer due to the fact that the amine function of DMAEMA was protonated and, therefore, charged resulting in a high hydrophilicity. However, at pH 7 the DMAEMA moieties were only partially protonated and the T_{cp} of the aqueous solutions was found to increase linearly from 47 °C (PDMAEMA) to 71 °C (PmEO_{~4.5}MA) with the mole fraction of mEO_{~4.5}MA in the copolymer. Under basic conditions at pH 10, all amine functions existed in an unprotonated state resulting in a decrease of the T_{cp} values by ~10 °C. A similar study was performed by Yamamoto et al. who applied turbidity measurements of aqueous solutions ($c = 0.3 \text{ wt\%}$) of copolymers of mEO₂MA with DMAEMA that were synthesized by ATRP [71]. For this copolymer system, the coil to globule transition temperature of PDMAEMA is higher than that of PmEO₂MA and, thus increasing content of DMAEMA (up to 26 mol%) was found to increase the T_{cp} of the aqueous copolymer solution. This effect was more pronounced at pH 7 ($T_{cp} = 22.5\text{--}48^\circ\text{C}$) than at pH 9 ($T_{cp} = 22.5\text{--}29^\circ\text{C}$) since the amino functions of the DMAEMA are partially protonated at pH 7 resulting in a further increase of hydrophilicity.

Tenhu and co-workers investigated the formation of complexes between two oppositely charged polyions, namely PEO-*b*-poly(sodium methacrylate) as anion and poly(methacryl oxyethyl trimethylammonium chloride) (PTMAEMA⁺) as polycation [72]. Turbidity measurements as well as DLS revealed increasing T_{cp} (5–55 °C) of the aqueous solutions of the polymer complexes ($c = 0.25\text{--}1 \text{ mg mL}^{-1}$) with increasing content of sodium chloride ($c = 0.5\text{--}0.6 \text{ mol L}^{-1}$) and sodium nitrate ($c = 0.5\text{--}0.6 \text{ mol L}^{-1}$). When a statistical copolymer of TMAEMA⁺ with mEO_{~4.5}MA was used as polycation, the hydrophilicity of the resulting complex was increased resulting in higher T_{cp} than for aqueous solutions of complexes without grafted oligoEO.

4.6.2.2. Anionically charged comonomers. The copolymerization of mEO_nMA with MAA results in polymers whose solubility in water is not only dependent on the temperature but also on the pH of the solution since MAA as Brönsted acid will be deprotonated under basic conditions.

Under acidic conditions, the protonated MAA moieties will serve as hydrogen bond donors and can “occupy” hydrogen bond accepting sites of the oligoEO chains. These competing interactions result in the formation of less hydrogen bonds with surrounding water molecules that keep the polymer in solution and, thus, LCST transitions at lower temperatures, as illustrated in Fig. 11. In addition, the deprotonated ionic form is much more hydrophilic compared to the protonated form.

Jones et al. varied the comonomer ratio between 0 and 100% during the free radical copolymerization of mEO_{~4.5}MA and MAA (Fig. 11) [73]. The aqueous solutions ($c = 3 \text{ wt\%}$) of the resulting copolymers containing up to 5% mEO_{~4.5}MA displayed T_{cp} 's that were decreasing from 68 °C to 12 °C with increasing content of mEO_{~4.5}MA. Polymers that contained 6–24% mEO_{~4.5}MA were found to be insoluble in water due to the formation of hydrogen bonds between the protonated MAA moieties with ether functions of the oligoEO side chains. Further increase of the mole fraction of mEO_{~4.5}MA rendered them water soluble again, and the T_{cp} was found to increase with the mEO_{~4.5}MA content up to 61 °C for PmEO_{~4.5}MA. A similar behavior could be observed for copolymers of MAA with longer oligoEO side chains, mEO_{~9}MA and mEO_{~22.7}MA, but the phase transition temperatures were elevated at constant ether to acid ratios due to the increasing hydrophilicity of the longer oligomeric EO chains. In addition, the demixing temperatures of aqueous solutions were found to be increased by 15 °C with increasing pH value from 2 to 5 because of a partial deprotonation of the MAA moieties. Increasing polymer concentration in a range from 0.5 to 3 wt% was found to lower the T_{cp} by 6 °C, which was partially attributed to a decreased pH value of the more concentrated solutions. The addition of urea (0.8 mol L^{-1}), which disrupts the intramolecular hydrogen bonding, resulted in T_{cp} 's that were elevated by 4 °C. Introduction of more hydrophobic comonomers, such as BuMA or lauryl methacrylate, to the copolymers resulted in an almost linear decrease of the T_{cp} values with increasing content of hydrophobic comonomer (0.8 °C per mol%). Surprisingly, copolymers with more hydrophilic glycidyl methacrylate showed a similar trend, although less pronounced (0.5 °C per mol%) presumably also due to intramolecular hydrogen bonding. Schubert and co-workers performed a similar study applying copolymers of MAA with PmEO_{~8.5}MA and PmEO_{~22}MA, respectively, that were synthesized by RAFT polymerization [48]. Turbidity measurements ($c = 5 \text{ mg mL}^{-1}$) at pH 2 and pH 4 revealed T_{cp} 's that were

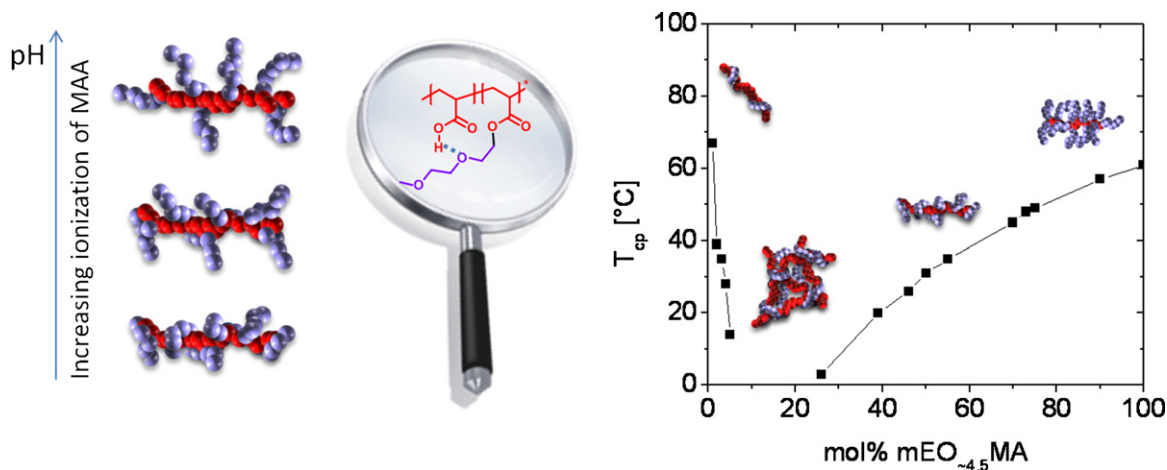


Fig. 11. Influence of the ionization of MAA (left) and the copolymer composition (right) upon intramolecular hydrogen bonding in copolymers of MAA and EO_nMA.

Reproduced from Jones et al. [73] by permission of John Wiley & Sons Ltd., UK.

increasing with the mEO_nMA content of the copolymers from 30 to 90 °C for P(mEO_{~8.5}MA-*stat*-MAA) and from 38 to 95 °C for P(mEO₂₂MA-*stat*-MAA), respectively. Polymers with mole fractions of mEO₂₂MA larger than 40% were found to be water soluble up to 100 °C. Increase of the pH value to pH 7 and pH 10 resulted in deprotonation of the MAA moieties, which rendered the polymers more hydrophilic and, thus, water soluble.

Instead of direct copolymerization with MAA, similar copolymers were obtained by hydrolysis of statistical copolymers of mEO₂MA and *tert*-butyl methacrylate that were synthesized by ATRP [71]. The thermal behavior of the copolymer solutions was affected in a similar fashion as described above. As long as the MAA moieties of the copolymer were protonated in de-ionized water, increasing the mole fraction of MAA up to 28% resulted in decreased T_{cp} 's (27–15 °C). Partial deprotonation resulted in the opposite effect, as demonstrated by turbidity measurements at pH 7 and 9. The tremendous influence of MAA was revealed during investigation of the solubility properties of a terpolymer that consisted of 89% mEO₂MA, 6% MAA and 5% DMAEMA. Its aqueous solution exhibited a T_{cp} of 34 °C at pH 4 and of 40 °C at pH 7, whereas it was water soluble up to 80 °C at pH 9. The lowered T_{cp} under acidic conditions hints towards the fact that the hydrogen bonding, which is caused by the protonation of the MAA, dominates over the increased hydrophilicity of the protonated DMAEMA moieties.

Jiang and Zhao investigated in detail the effect of pH upon the phase transition temperatures of aqueous solutions ($c = 0.2$ wt%) of a mEO₂MA based copolymer containing 13 mol% MAA [74]. The T_{cp} 's were in the range of 24–60 °C and increased with increasing pH value from 4 to 6.8. A block copolymer with linear PEO was found to form micelles that consisted of the collapsed thermo-responsive block in the core and hydrophilic PEO in the shell when the aqueous solution was heated above its cloud point. These micelles could be disrupted by increase of the pH value and reformed either upon further elevation of the temperature

or by decrease of the pH value, as was confirmed by DLS as well as fluorescence spectroscopy and is illustrated in Fig. 12.

In addition, a similar system that undergoes reversible micellization at two different temperatures and is triggered by UV irradiation was developed by Zhao and co-workers [75]. A statistical copolymer of mEO₃A with 13 mol% of *o*-nitrobenzyl acrylate (NBA) exhibited a T_{cp} in 0.2 wt% aqueous solution at 18.5 °C, which is lowered by 17.5 °C when compared to PmEO₃A due to the hydrophobicity of NBA. Upon irradiation with UV light, *o*-nitrosobenzaldehyde is cleaved and an acrylic acid moiety is formed. The aqueous solution of the resulting copolymer displayed a higher T_{cp} at 30 °C due to the absence of the hydrophobic aromatic moieties. Utilization of a PEO macroinitiator for the synthesis of the mEO₃A based polymer resulted in a block copolymer that undergoes reversible formation of micelles above the T_{cp} of the thermo-responsive block, as demonstrated by DLS and fluorescence spectroscopy. Cleavage of the NBA moiety caused disruption of the micelles since the resulting acid containing PmEO₃A block was still below its phase transition temperature. Upon increase of temperature towards 36 °C, the coil to globule transition of this newly formed thermo-responsive block was reached and the micelles were re-formed.

4.6.3. Copolymers with dye containing monomers

Pietsch et al. developed a dual polymeric sensor for temperature and pH value based on the LCST behavior of PmEO₂MA in water [76]. After detailed kinetic studies on the copolymerization of mEO₂MA and mEO_{~22}MA [77], RAFT polymerization was applied for the copolymerization of mEO₂MA with 5 mol% of a monomer that is based on a solvatochromic azo dye, disperse red 1. Below the T_{cp} of an aqueous solution (pH 1, $c = 1$ mg mL⁻¹) of the copolymer at 17 °C, the dye is in contact with water and, thus, in a polar environment. Upon precipitation of the PmEO₂MA, the hydrating water molecules are expelled from the polymer

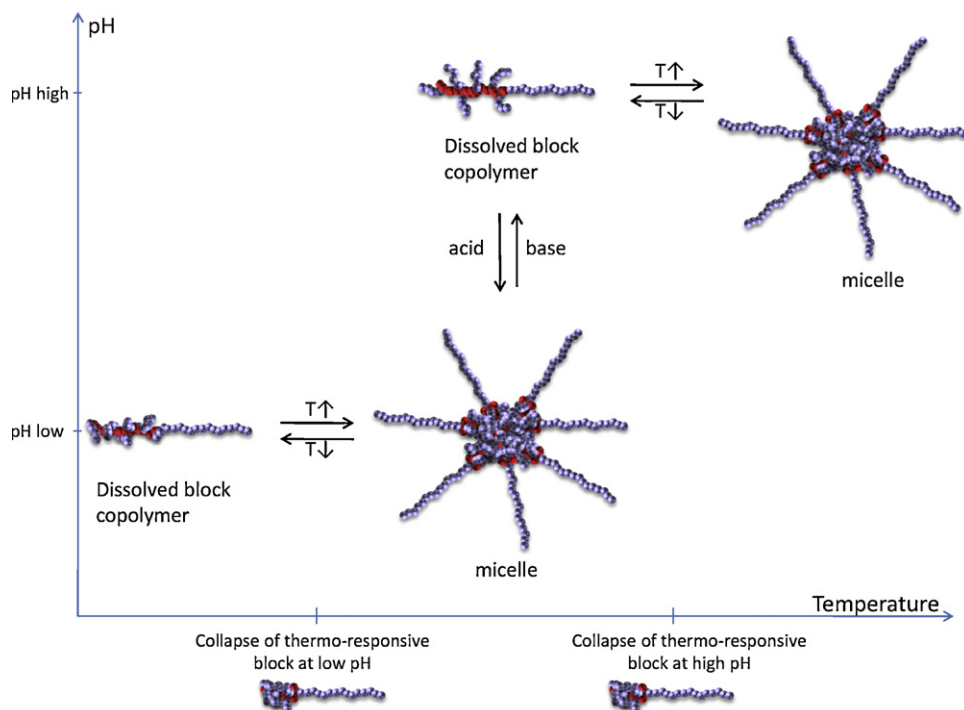


Fig. 12. Reversible formation of micellar structures from pH and temperature responsive block copolymers.

and the azo dye is directly protonated in its nonpolar environment, which induces a color change (Fig. 13). Since the protonation of DR1 is not possible under basic conditions, this color change could not be observed at $\text{pH} > 7$, as quantified by UV/vis spectroscopy of the aqueous solutions in a temperature range around the cloud point. Unfortunately, the high hydrophilicity, even above its coil to globule transition at 92°C , of a tercopolymer that contained 45% mEO_2MA , 50% $\text{mEO}_{\sim 22}\text{MA}$ and 5% DR1 prevented the extension of this interesting concept towards an elevated temperature range.

A similar sensor that is based on the fluorescence of pyrene was realized by RAFT copolymerization of mEO_2MA with 5 mol% pyrene-1-ylmethyl methacrylate [78]. Below the T_{cp} of the aqueous polymer solution at 18°C the

hydrophilic polymeric structure facilitates the formation of excimers between the pyrene moieties. Upon the coil to globule transition of the thermo-responsive polymer, the hydrophobic surrounding of the pyrene decreased the excimer formation, which was manifested in a decrease of the excimer fluorescence band between 11°C and 21°C . DLS measurements at varying temperatures confirmed the formation of particles with sizes about 180 nm in this temperature range.

4.7. Grafted structures

The possibility to use functional initiators for the ATRP of mEO_nMA has been applied for the synthesis of comb and graft copolymers that contain PmEO_nMA as side chains

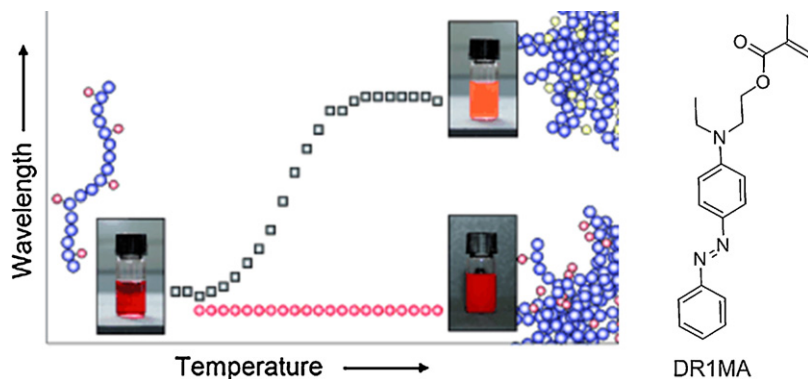


Fig. 13. Color change of an aqueous solution containing a PmEO_2MA based temperature sensor upon coil to globule transition of the polymeric sensor. Reproduced from Pietsch et al. [76] by permission of John Wiley & Sons Ltd., UK.

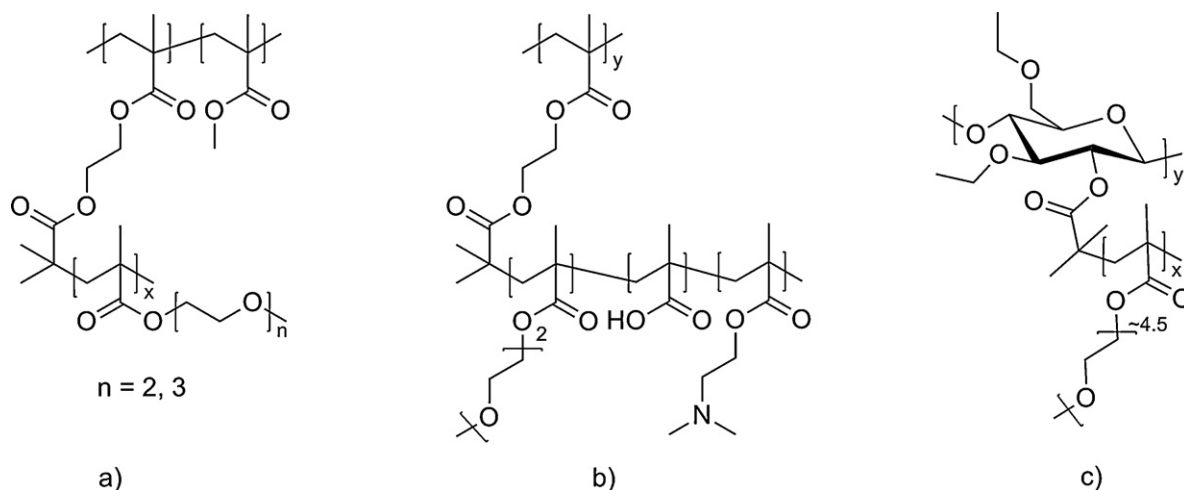


Fig. 14. Schematic representation of grafted structures with PmEO_nMA side chains.

via the “grafting from” approach. As depicted in Fig. 14, methacrylate [45,71] as well as ethyl cellulose [79] based polymeric backbones were functionalized with an ATRP initiator that was subsequently used to graft the PmEO_nMA side chains from these polymeric backbones.

Matyjaszewski and co-workers prepared a series of comb polymers with PmEO₂MA side chains of varying DP of 25, 50 and 115 from the same polymethacrylate backbone (Fig. 14a) [80]. The aqueous solutions of the comb polymers ($c = 0.3$ wt%) were investigated via turbidity measurements and revealed similar T_{cp} values of 22 °C during the heating run, which is slightly lower compared to linear PmEO₂MA. The heating-cooling hysteresis of the turbidity curves was significantly smaller for the polymers with longer side chains hinting towards a delayed collapse of the PmEO₂MA parts close to the polymeric backbone due to steric hindrance. The grafting density was shown to have no effect on the phase separation temperature of aqueous polymer solutions since copolymers with varying amounts of MMA (24–70 mol%) in the polymer backbone exhibited similar T_{cp} in water. However, DLS revealed increasing agglomerate sizes of the precipitated collapsed polymer globules above T_{cp} with increasing grafting density. When the side chains of the polymeric structure consisted of statistical copolymers of mEO₂MA and mEO₃MA, the T_{cp} of the aqueous polymer solutions was found to increase with the content of the more hydrophilic mEO₃MA in a similar fashion to their linear analogues. Again, the demixing temperature of a solution of comb shaped PmEO₃MA of 45 °C was found to be lowered by 6 °C when compared to linear PmEO₃MA. Comb polymers that contained side chains consisting of block copolymers with PmEO₂MA in the inner sphere and PmEO₃MA on the outside exhibited thermo-responsive behavior at 50 °C, which is higher than expected for a statistical copolymer of the same composition, because the polymer chains were still held in solution by the more hydrophilic PmEO₃MA parts. DLS measurements indeed revealed a shrinking of the dissolved macromolecules at temperatures lower than T_{cp} hinting towards a collapse of the inner parts (PmEO₂MA) of the

polymer structure. In contrast, such comb polymers were found to aggregate at 26 °C when the more hydrophobic PmEO₂MA was located in the shell of the structure. In this case, DLS revealed a shrinking of the polymer globules at temperatures higher than T_{cp} due to the coil to globule transition of the PmEO₃MA segments at elevated temperatures.

In addition, the composition of the side chains was further varied by copolymerization with pH responsive monomers, such as MAA and DMAEMA (Fig. 14b) [71]. In general, the thermo-responsive properties of the PmEO₂MA were affected in a similar fashion as the linear analogues (see Section 4.6), but some additional effects of the comb structure will be discussed here. The influence of the intramolecular hydrogen bonding that is present in the MAA containing copolymers was found to be enhanced when compared to linear copolymers of PmEO₂MA and MAA due to the proximity of hydrogen bond accepting ether moieties and hydrogen bond donating protonated MAA moieties at pH 7 in the dense bottle brush like structure. On the other hand, T_{cp} 's of aqueous solutions of comb like copolymers of mEO₂MA and DMAEMA showed the same trend as their linear analogues at pH 7, but the copolymer composition did not affect the phase transition temperature at pH 9. In addition, the thermo-responsiveness of a comb polymer, whose side chains represented a terpolymer of mEO₂MA, MAA and DMAEMA, was less affected by changes in pH than its linear analog. These observations were proposed to be a result of the additional electrostatic association of the side chains in the complex structure of the comb polymer that made its thermo-responsive behavior less sensitive to composition.

Liu and co-workers grafted PmEO_{~4.5}MA from a functionalized ethyl cellulose backbone in grafting densities of 2% and 20% (Fig. 14c) [79]. Turbidity measurements of aqueous graft copolymer solutions ($c = 1$ mg mL⁻¹) revealed T_{cp} 's of around 64 and 67 °C for 20% and 2% grafting density, respectively. TEM as well as DLS investigations showed the presence of micellar structures with the hydrophilic PmEO_{~4.5}MA as shell already below the cloud point (which explains the rather small difference of

the observed T_{cp} 's). These micelles (100 nm and 65 nm) further agglomerated upon coil to globule transition of the PmEO_nMA segments and formed particles of slightly larger size (175 and 120 nm, respectively).

4.8. Towards bio-medical applications

The excellent thermo-responsive properties gave rise to many initial studies aiming for applications of in particular PmEO_nMA based polymers that were recently reviewed in a highlight by Lutz [14] and, thus, are only briefly summarized here.

PmEO_nMA based polymers have been applied for the fabrication of functionalized surfaces: functionalization of silica monoliths resulted in suitable column materials for the separation of steroids and proteins [81]. Functionalized gold or glass surfaces allowed switching of cell adhesion: above the phase transition temperature of the polymer in PBS at 35 °C, the surface behaved hydrophobic and could be used to grow L929 mouse fibroblast [82,83] and MCF-7 breast cancer cells [84]. Afterwards, the cell sheet could be easily removed from the surface by decreasing the temperature to 25 °C since the surface behaved hydrophilic below the coil to globule transition temperature of the copolymer. Very recently, this approach could even be applied to mouse embryonic stem cells [85]. Attachment of a bactericidal protein mangainin-I to the thermo responsive surface resulted in a coating that is bactericidal below and cell-repellent above the temperature at which the PmEO_nMA collapses [86,87].

In addition, PmEO_nMA based polymers have been applied for the preparation of “smart” particulate systems: grafting to magnetite nanoparticles resulted in promising MRI contrast agents [88], while PLGA [89] and PCL [90] microparticles were coated with PmEO_nMA to form thermo-responsive dispersions that may be applied to support cell growth. Nanoparticles were prepared from PLGA block copolymers with PmEO_nMA coating for potential bio-medical applications [91]. Other approaches involve the application of mEO_nMA copolymers with DMAEMA as nonviral gene transfection agents [92], the complex formation of P(DMAEMA-*b*-mEO₂MA) with coxsackieviral B2 virus by electrostatic interaction [93] or the synthesis of protein hybrids [94,95] and polymer conjugates with other biologically active molecules, such as biotin and thyroxine [96,97].

With respect to future biological application of PmEO_nMA's their LCST behavior was also studied in a range of buffers that are typically applied for biomedical studies, cell culture media [98] as well as bovine serum [99]. The fact that T_{cp} differed by up to 5 °C in some of these media compared to pure water stresses the necessity to tailor the composition of the thermo-responsive polymer specifically for the environmental conditions of the targeted application.

5. Poly(2-oxazoline)s

Various 2-oxazoline-based monomers can be polymerized via a living cationic ring-opening polymerization (CROP) mechanism (Fig. 15). Due to the livingness of the polymerization, the use of functional electrophilic

initiating as well as nucleophilic end-capping agents enables the synthesis of both α - and ω -end-functionalized polymers. The nature of the substituent R^1 in 2-position of the oxazoline monomer determines the hydrophilicity of the resulting poly(2-oxazoline) (POx): poly(2-methyl-2-oxazoline) (PMeOx) is water soluble in the entire temperature range of liquid water under atmospheric pressure, poly(2-ethyl-2-oxazoline) (PEtOx) and poly(2-propyl-2-oxazoline) (PPrOx) exhibit LCST behavior in aqueous solution whereas longer substituents result in water insoluble hydrophobic polymers.

Several studies on the investigations of the biocompatibility of POx in comparison to the widely applied PEO have been carried out revealing similar protein repellent properties of PMeOx functionalized surfaces [100,101] and rapid blood clearance rates of ¹¹¹In and ¹²⁵I radioactively labeled PMeOx and PEtOx when injected to mice without significant accumulation in the body [102,103]. PEtOx and PMeOx grafted liposomes showed similar enhanced blood circulation times and tissue distribution as the corresponding PEO conjugates [104,105]. In addition, cytotoxicity studies of PEtOx [106] and a wide range of amphiphilic di- and tri-block copolymers (comprising PMeOx, PEtOx, PPrOx and poly(2-butyl-2-oxazoline) (PBuOx) [107] revealed a fast endocytosis of especially more hydrophilic polymers without affecting the viability or activity of the cells. These encouraging findings already led to initial application studies of POx for drug delivery purposes: PMeOx-*b*-PBuOx block copolymer micelles have been successfully applied as carrier systems for the delivery of the anticancer drug Paclitaxel into mice [108]. In addition, PEtOx conjugates with enzymes (i.e. trypsin, RNase, uricase, catalase), insulin and the anticancer drug Ara-C revealed similar enzyme activity and cytotoxicity as PEO conjugates [109,110]. Despite these very promising studies demonstrating the biocompatibility of POx, there are almost no reports regarding biomedical applications of thermoresponsive POx yet.

5.1. Homopolymers

Lin et al. were the first who reported the LCST behavior of three high molar mass PEtOx homopolymers in 1988 [111]. The performed cloud point measurements revealed T_{cp} 's in the range of 61–69 °C for concentrations of 0.5–20 wt% PEtOx in aqueous solution. The cloud point curves showed minima at a concentration of about 2–3 wt% and the corresponding temperatures were 61 °C, 63 °C and 63.5 °C for PEtOx having a molar mass of 20 kg mol⁻¹, 50 kg mol⁻¹ and 500 kg mol⁻¹, respectively. NaCl induced a salting out-effect, whereas addition of tetrabutyl ammonium bromide resulted in a salting-in effect. In addition, the T_{cp} 's were found to increase by the addition of dioxane as co-solvent to the aqueous solutions. The investigation of the molar mass dependence of the thermo-responsiveness was extended to lower molar mass PEtOx by Du Prez and co-workers [112] showing that the LCST behavior of PEtOx ceases to exist in the range of 0–100 °C for PEtOx with molar masses lower than $M_n = 10,000$ g mol⁻¹ (which corresponds to a DP of approximately 100, entrance Fig. 16). The authors also proposed the PEtOx/water binary

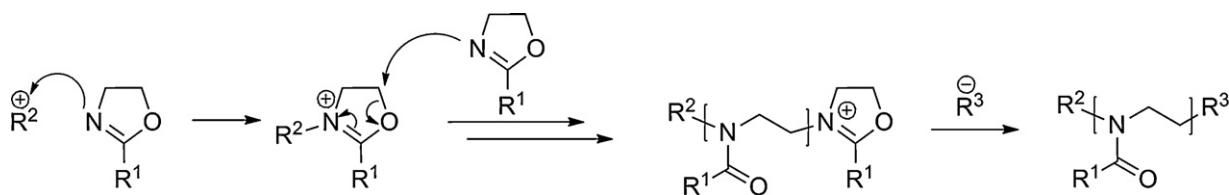


Fig. 15. Schematic representation of the CROP of 2-oxazolines.

mixture to follow Flory–Huggins Type I behavior due to the lowered T_{cp} for higher molar mass polymers. Similar results were found by Hoogenboom and Schubert who investigated PEtOx with DP from 50 to 500 using turbidity measurements in 0.5 wt% aqueous solutions (entrance ▼ in Fig. 16) [113].

Recently, three investigations describing the thermo-responsive behavior of star shaped PEtOx were published. In accordance to linear PEtOx, an increasing molar mass of star shaped PEtOx with a hyperbranched polyglycerol core resulted in lowered T_{cp} 's from 75 to 62 °C ($c = 5 \text{ mg mL}^{-1}$), which is lowered by 7–10 °C compared to the T_{cp} of aqueous solutions of linear PEtOx of similar molar mass [114]. On the other hand, aqueous solutions of star shaped PEtOx with poly(propylene imine) dendrimers as core revealed T_{cp} 's of around 90 °C ($c = 5 \text{ mg mL}^{-1}$) that were not dependent on the molar mass of the polymer and elevated compared to linear PEtOx [115]. In addition, star shaped PEtOx, which was prepared by cross-linking of micelles with a poly(2-(3-butynyl)-2-oxazoline) core, showed T_{cp} 's in the range of 60–64 °C [116] in aqueous media at varying pH values ($c = 0.1 \text{ wt\%}$). pH sensitivity was introduced by functionalization of the core with amino- and carboxylic acid moieties, respectively, resulting in elevated T_{cp} when the pH sensitive groups were in their charged state (i.e. at

pH = 3 for the amine containing star and at pH = 11 for the acid containing star). The phase transition temperature of the acid containing star could be shifted from 48 to 82 °C by addition of 0.1 M Na_2SO_4 or NaSCN as salts from the Hofmeister series to the solution.

Next to the simple observation of T_{cp} by turbidity measurements, detailed thermodynamic investigations of the demixing process in aqueous solutions have been carried out already in the 1990s using light scattering experiments as well as osmometry [120,121]. The LCST behavior of high molar mass PEtOx with broad PDI value ($M_w = 116 \text{ kg mol}^{-1}$, $\text{PDI} = 2.4$) was reflected in a decrease of the second virial coefficient with increasing temperature, and the θ temperature (at which the solution displays the properties of an ideal solution) was determined to be 56 °C [120]. Since the second virial coefficients describe the “non-ideality” of the mixture, excess thermodynamic dilution functions, such as chemical potential $\Delta\mu_1^E$, enthalpy Δh_1^E and entropy Δs_1^E of mixing could be derived. At 30 °C, $\Delta\mu_1^E$ is negative due to the fact that it is dominated by the negative enthalpy term Δh_1^E , whereas at 60 °C, $\Delta\mu_1^E$ is positive due to the fact that it is dominated by the unfavorable entropy term $T\Delta s_1^E$. As a result, the overall chemical potential $\Delta\mu_1 = \Delta\mu_1^{\text{id}} + \Delta\mu_1^E$ becomes positive at 60 °C resulting in demixing. In other words, the phase separation at elevated temperatures is a result of the increased entropy of the mixture when water molecules are released from interactions with the polymer. The fact that hydrogen bonding of water molecules with the carbonyl oxygen of the PEtOx amide structure contributes to those polymer–water interactions was shown by a shift of the carbonyl vibration of PEtOx to lower wavenumbers in D_2O when compared to acetonitrile as a non hydrogen-bonding solvent. In addition, the free energy of mixing as a function of the concentration was calculated for several temperatures. The cloud point and the coexistence curve were determined up to a PEtOx concentration of 0.2 g mL^{-1} revealing a critical concentration of 0.109 g mL^{-1} and a critical temperature of 65.3 °C from the minimum of the coexistence curve, which was found to be inconsistent with the minimum of the cloud point curve (ca. 63 °C and 0.025 g mL^{-1}). This large deviation may result from the polydispersity of the utilized PEtOx. DLS as well as SLS were performed at several concentrations and temperatures revealing long-range concentration fluctuations that reflect partial organization even below the LCST. The approach to determine thermodynamic values from SLS measurements below the LCST was further followed by the characterization of more well-defined PEtOx with different molar masses ($\text{PDI} = 1.3$, $M_w = 20, 30, 60 \text{ kg mol}^{-1}$, respectively) [121]. Second virial coefficients as well as Δh_1^E and Δs_1^E were found to decrease with increasing molar mass

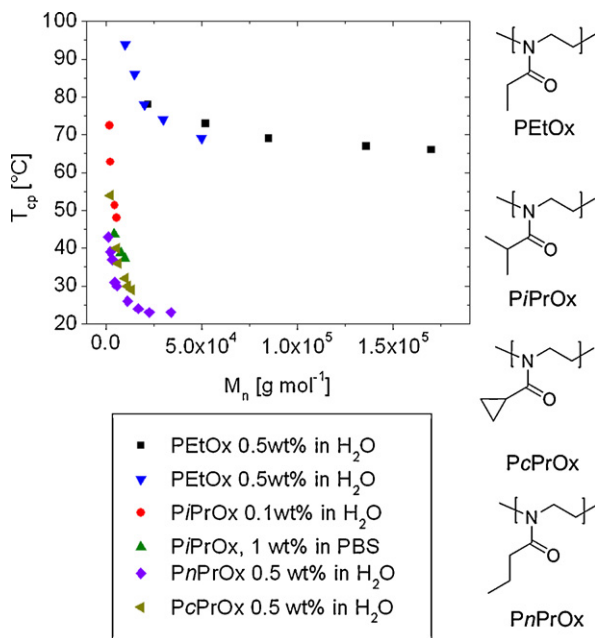


Fig. 16. Cloud point temperatures observed for aqueous solutions of PEtOx, PiPrOx and PnPrOx with varying molar mass. Data taken from Ref. [112] (■), [113] (▼, ◆), [117] (●), [118] (▲), [119] (◇).

of the polymer, which was suggested to be a result of the increasing availability of the carbonyl groups in the PEtOx chain to form hydrogen bonds with water with increasing molar mass.

In 1992, Uyama and Kobayashi reported that PiPrOx ($M_n = 16,700 \text{ g mol}^{-1}$, $\text{PDI} = 1.13$) exhibits LCST behavior in the range of the human body temperature using turbidity measurements of aqueous solutions. The T_{cp} was found to decrease from 39°C to 35°C with increasing polymer concentration (0.1–1 wt%). A salting-out effect of NaCl was reported whereas the addition of ionic surfactants such as sodium dodecylsulfate and dodecyltrimethylammonium chloride led to an increase of T_{cp} [122]. A detailed investigation of the LCST behavior of four PiPrOx with very low PDI values of 1.03–1.05 was performed by Winnik and co-workers applying not only turbidity measurements, but also high sensitivity DSC (HS-DSC) as well as PPC [117]. T_{cp} as well as T_{max} and T_{onset} of the endothermic peaks from DSC of aqueous solutions of PiPrOx ($c = 1 \text{ mg mL}^{-1}$) were found to decrease from 73 to 48°C with increasing molar mass ($\text{DP} = 17\text{--}50$, entrance ● in Fig. 16). Both techniques verified the salting-out effect of NaCl and showed lowered phase transition temperatures in D_2O compared to H_2O due to the difference in “hydrogen”-bond strength in both solvents. Increasing concentration ($0.5\text{--}5 \text{ mg mL}^{-1}$) resulted in decreased T_{cp} 's ($56\text{--}49^\circ\text{C}$) for PiPrOx with a DP of 41, as could be verified by HS-DSC, too. Additionally, the enthalpy of transition ΔH could be obtained from the latter technique. Interestingly, ΔH increased almost linearly with the molar mass of the polymer, which might hint towards an increasing availability of the polymer's carbonyl groups to form hydrogen bonds with water. This explanation was confirmed by PPC measurements that revealed a strong increase of the volume change $\Delta V/V$ during the phase transition with increasing molar mass. $\Delta V/V$ was also found to be increased with increasing NaCl content of the solutions as well as in D_2O when compared to H_2O . Subsequently, Park et al. found that the concentration dependence of T_{cp} becomes less pronounced with increasing molar mass of quite similar PiPrOx ($M_n = 3600\text{--}9700 \text{ g mol}^{-1}$, entrance ▲ in Fig. 16) [118]. The LCST behavior of four PiPrOx samples with M_n from 3000 to $13,000 \text{ g mol}^{-1}$ was recently investigated in detail by Van Mele and co-workers who applied modulated DSC in order to establish the phase diagrams [123]. Since both LCST as well as LCSC were decreasing with increasing molar mass of PiPrOx (33.8°C at 29.8 wt\% for $M_n = 3000 \text{ g mol}^{-1}$ and 26.2°C at 19.8 wt\% for $M_n = 13,000 \text{ g mol}^{-1}$) the binary system PiPrOx/water was shown to follow Type I Flory–Huggins miscibility behavior. The reversible enthalpy of demixing ΔH_{dm}^{rev} decreased with the molar mass of the polymer but remained almost constant up to concentrations of 20 wt\% . At higher concentrations, ΔH_{dm}^{rev} decreased because too few water molecules were available to build up the full hydration shell around the polymer coils. In addition, the (de)mixing kinetics during the phase transition could be followed by quasi-isothermal modulated DSC measurements showing that the de-mixing is a faster process than the re-mixing. The equilibrium was reached in all cases after 180 min , which is much faster than for PNIPAm, since the molecular structure of PiPrOx without hydrogen bond donating

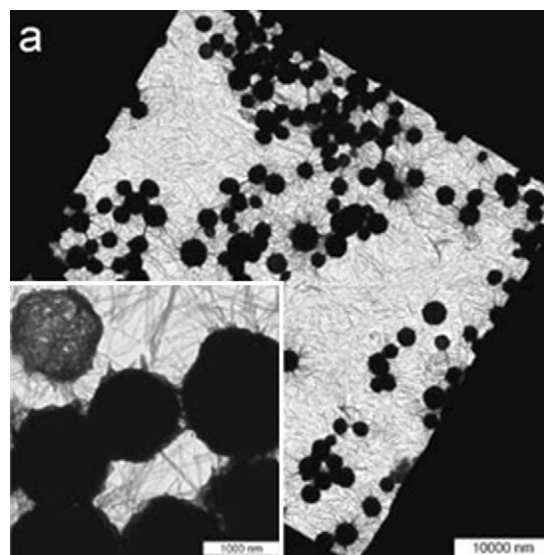


Fig. 17. Transmission electron micrographs of coagulate particles. Reproduced from Meyer et al. [124] by permission of The Royal Society of Chemistry.

moieties only requires the disruption of polymer/water hydrogen bonds, and vitrification effects are excluded due to the fact that the T_g of the precipitated polymer phase is well below the de-mixing temperature.

All the cited references have described the phase transition to occur abruptly and reversibly with small heating-cooling hysteresis of the determined values. However, Schlaad discovered that PiPrOx carrying either one or two positively charged amino-functions at the end of the chain form so-called “cotton balls” when kept in aqueous dispersion far above the cloud point (65°C for 24 h) [124]. These coagulates are built from fibrillar aggregates that are formed by crystallization of PiPrOx (see Fig. 17) [125]. Most likely, non-specific hydrophobic interaction draws the *i*-propyl groups together resulting in interaction and alignment of the amide-dipoles of the, thus, stretched polymer backbone. Interestingly, the LCST transition is fully reversible during continuous heating-cooling cycles while crystallization occurs upon prolonged heating above the phase transition temperature. This behavior indicates that the initial liquid–liquid phase transition is entropy driven while upon isothermal treatment above the T_{cp} , the concentrated phase undergoes a second irreversible crystallization induced phase transition.

After the LCST behavior of PEtOx and PiPrOx was already reported almost 20 years ago, it is astonishing that it was only recently reported that PnPrOx also exhibits LCST behavior by Park and Kataoka [126]. Due to fact that the linear *n*-propyl group reduces the area that is accessible for water molecules, T_{cp} was found to be almost 15°C lower than for a comparable PiPrOx solution (23.8°C for PnPrOx and 38.7°C for PiPrOx, in 1 wt\% PBS solution). Subsequently, Hoogenboom and Schubert investigated the effect of the molar mass of PnPrOx ($\text{DP} = 10\text{--}300$) upon the cloud points of aqueous solutions using turbidity measurements ($c = 0.5 \text{ wt\%}$) [113]. The T_{cp} 's were found to decrease from 43 to 24°C with increasing molar mass of the

polymer (entrance ♦ in Fig. 16). In 2011, the propyl substituent of PPrOx was further varied towards a cyclic structure. The resulting aqueous PcPrOx (DP = 20–112) solutions ($c = 0.1$ – 3 wt%) exhibited thermo-responsive properties at intermediate temperatures when compared to PnPrOx and PiPrOx (entrance ♦ in Fig. 16) [119]. In addition, the polymer was found to be amorphous and the LCST behavior of the system PcPrOx/water was investigated by means of viscosimetry revealing increased viscosities shortly below T_{cp} due to deterioration of the solvent quality and more favorable interactions between the polymer chains. DLS measurements showed the formation of large aggregates upon coil to globule transition of the polymer.

The effect of various salts on the cloud point of aqueous solutions of PEtOx, PiPrOx and PnPrOx was recently investigated by Hoogenboom and Schubert using turbidity measurements in 0.5 wt% solutions with a systematic variation of the salt concentration (0 – 0.5 mol L⁻¹) [127]. The effects of the investigated additives were found to follow the Hofmeister series of anions in an analog fashion as for PEtEO₃MA (compare Section 4.5). Also for POx the effects were more pronounced for the more hydrophilic polymers (PEtOx > PiPrOx > PnPrOx) covering a temperature range of 90 °C, whereas the polymer architecture seemed to cause no significant effects as was shown by comparison of turbidity measurements of linear PEtOx and a PEtOx comb polymer.

5.2. Effect of the polymer end group and block copolymers

The livingness of the CROP of 2-oxazolines offers the possibility to functionalize both α - as well as ω -chain ends of the POx with end groups of varying hydrophilicity and the straightforward synthesis of block copolymers. The use of a functional initiator will attach the desired end group to the “beginning” of the polymer chain, whereas variation of the polymer terminal end can either be achieved by the direct attack of nucleophiles on the cationic poly-oxazolinium species or by subsequent modification of terminal –OH groups. Schematic representations of the end groups that will be discussed in the following part are depicted in Table 2.

It is quite obvious that the impact of the end groups on the LCST behavior of the polymer will be stronger for polymers with lower molar mass than for those with higher molar mass. In this context, a 3,3-diethoxy-propyl moiety at the α -chain end of PiPrOx ($M_n = 9600$ g mol⁻¹, corresponding to DP = 85) did not affect the thermo-responsive properties of the PiPrOx when compared to the most frequently used –CH₃ end group of a polymer with similar molar mass [118]. In contrast to that, the attachment of a hydrophobic acrylate function at the terminal end of a PiPrOx with a DP of 40 resulted in a T_{cp} that is decreased by 5 °C when compared to –OH terminated PiPrOx ($c = 1$ – 10 g L⁻¹) [128]. Besides this, the transmittance curves were found to be much broader, and the salting-out effect of NaCl ($c = 0$ – 0.15 mol L⁻¹) was enhanced for the acrylate oligomer solutions due to the strong hydrating contribution of the –OH terminal group. In addition, polyion complex (PIC) micelles that contain two

oppositely charged poly(amino acids) as the inner core and PiPrOx as outer shells revealed T_{cp} 's that remained remarkably unaffected by changes in concentration or NaCl content but were significantly lowered when compared to the free outer PiPrOx blocks [128]. This could be either attributed to a high local concentration of PiPrOx in the shell of the PIC micelles or to the fact that the hydrophobic methyl groups at the α chain ends point towards the outside of the micelles.

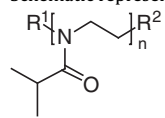
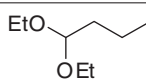
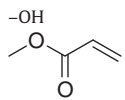
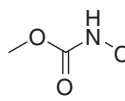
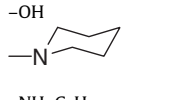
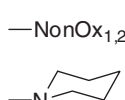
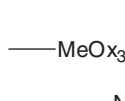
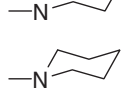
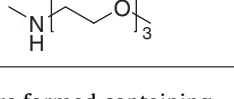
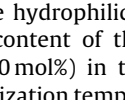
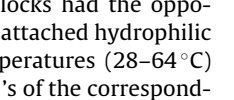
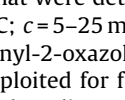
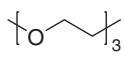
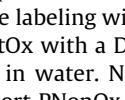
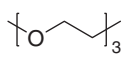
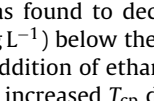
A very detailed investigation on the effect of octadecyl α - and ω -end groups on the phase transition of PiPrOx (DP = 57, 85, 111) solutions was carried out by Obeid et al. applying turbidity as well as HS-DSC and PPC measurements in dilute concentration regimes ($c = 0.1$ – 10 mg mL⁻¹) [129]. As expected, the hydrophobic end group(s) had the largest influence on the solubility behavior of the shortest PiPrOx: T_{cp} of a 1 mg mL⁻¹ solution was decreased by 12 °C and 16 °C for PiPrOx of DP 57 with one and two hydrophobic end groups, respectively. HS-DSC and PPC revealed the existence of two separate transitions during heating of the aqueous solutions that contain the hydrophobically modified PiPrOx in micellar structures. The transition at lower temperatures could be correlated to the cloud point of the solution and was assigned to changes of the packing of the C₁₈ chains in the micellar core since it was hardly affected by the polymer concentration. On the other hand, the second endothermic peak in the DSC and PPC traces followed the characteristics of the collapse of the PiPrOx chains, such as dependence on concentration of the polymer, increasing ΔH_{tr} values with increasing molar mass, and a large contribution to the volume change due to release of bound water.

An investigation of the effect of end groups with varying hydrophilicity on the LCST behavior of PiPrOx oligomers with a DP of 25 was recently published by Huber et al. [130]. Introduction of a hydrophobic nonyl-substituent at one chain end resulted in a T_{cp} in 2 wt% aqueous solution that was decreased by 19 °C. In contrast, the T_{cp} of a solution of PiPrOx bearing nonyl substituents at both α - and ω -chain end was only decreased by 15 °C, which was attributed to possible formation of micelles. The comparison with turbidity results obtained from PiPrOx having short second PNonOx blocks (1 and 2 repeating units) showed that the influence of the nonyl substituent was even enlarged when connected as side chain to the polymer. Similar modification of the system with short hydrophilic PMeOx blocks at the chain ends of the PiPrOx resulted in an increased T_{cp} (by 6 °C) for the MeOx₃-*b*-iPrOx₂₅-*b*-MeOx₃ triblock copolymer. The more hydrophilic piperazyl end group slightly increased the T_{cp} values with respect to the less hydrophilic piperidyl end group. Surprisingly, the functionalization of either one or both chain ends of the PiPrOx with triethyleneglycol moieties was found to decrease the T_{cp} of the investigated system by 5 °C, although PEG is often regarded as a strongly hydrophilic polymer.

Hruby et al. investigated the micellization of PMeOx-*b*-(PiPrOx-*stat*-BuOx)-*b*-PMeOx triblock copolymers using SLS as well as DLS measurements at a polymer concentration of 0.5 mg mL⁻¹ [131]. Above the coil to globule transition temperature of the central thermo-sensitive PiPrOx based block incorporating some BuOx units to

Table 2

Schematic representations of end-group modified PiPrOx discussed in this section.

	R^1	R^2	n	Ref.
			85	[118]
CH ₃ –			40	[128]
C ₁₈ H ₃₇ –			57, 85, 111	[129]
C ₁₈ H ₃₇ –			57, 85, 111	[129]
C ₉ H ₁₉ –			25	[130]
C ₉ H ₁₉ –			25	[130]
CH ₃ –			23, 24	[130]
CH ₃ –MeOx ₃ –			25	[130]
CH ₃ –MeOx ₃ –			25	[130]
CH ₃ –			25	[130]
			25	[130]
			25	[130]

lower the T_{cp} , micellar structures were formed containing the PiPrOx block in the core and the hydrophilic PMeOx blocks in the shell. An increasing content of the more hydrophobic BuOx monomer (10–20 mol%) in the middle block resulted in decreased micellization temperatures whereas elongation of the PMeOx blocks had the opposite effect. However, as a result of the attached hydrophilic PMeOx blocks, all micellization temperatures (28–64 °C) were significantly higher than the T_{cp} 's of the corresponding P(iPrOx-*stat*-BuOx) copolymers that were determined by turbidity measurements (17–28 °C; c = 5–25 mg mL^{−1}). In addition, incorporation of 2-butenyl-2-oxazoline into the thermo-responsive block was exploited for functionalization of the micellar core with a phenolic moiety that was subsequently used for radioactive labeling with ¹²⁵I.

It was already mentioned that PEtOx with a DP below 100 does not exhibit LCST behavior in water. Nevertheless, the high hydrophobicity of a short PNonOx block of an EtOx₉₀-*b*-NonOx₁₀ copolymer was found to decrease the T_{cp} of an aqueous solution (c = 5 g L^{−1}) below the boiling point of water (to 69 °C) [132]. Addition of ethanol as co-solvent (up to 12 wt%) resulted in increased T_{cp} due to better solvation of the EtOx block of the copolymer.

5.3. Random and gradient copolymers

The copolymerization of EtOx, *n*PrOx and *i*PrOx either with each other or with of a range of different 2-oxazoline monomers offers the opportunity of varying the specific LCST behavior of PEtOx, P*n*PrOx and PiPrOx to further broaden the accessible temperature range. Depending on the copolymerization conditions and the reactivity of the comonomer, the resulting copolymer will be either of random or gradient composition, which has to be taken into account during interpretation of the resulting solubility behavior. In general, incorporation of monomers containing a hydrophobic substituent (such as butyl-, nonyl- or phenyl-) in 2-position of the oxazoline results in a decreased cloud point of the aqueous polymer solution. On the other hand, more hydrophilic co-monomers will increase the phase separation temperature.

*i*PrOx has been copolymerized with EtOx [133], *n*PrOx [126,134], *n*BuOx [134] and *n*NonOx [134] resulting in gradient copolymers with *i*PrOx-rich chain ends due to the lower reactivity of *i*PrOx. The range of cloud point temperatures that could be observed by turbidity measurements is illustrated in Fig. 18. Park et al. showed a linear increase

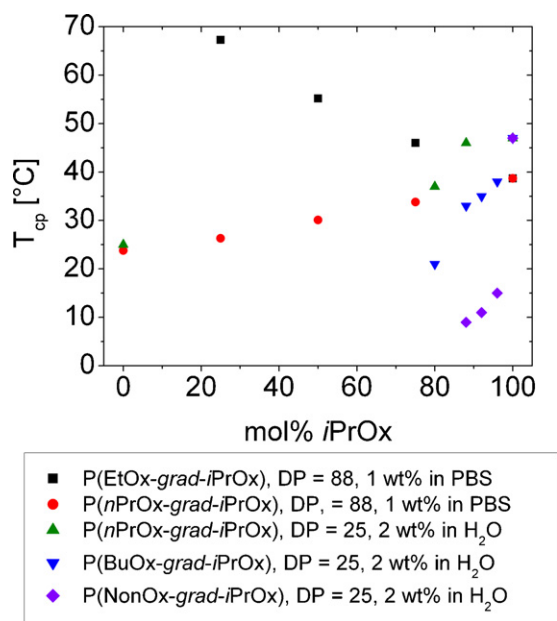


Fig. 18. Cloud point temperatures observed for aqueous solutions of gradient copolymers with varying *i*PrOx content. Data taken from Ref. [133] (■), [126] (●), [134] (▲, ▼, ◆).

of T_{cp} with increasing EtOx content of the copolymers (entrance ■ in Fig. 18, DP=88, turbidity measurements were performed in 1 wt% PBS solutions) [133] whereas increasing content of *n*PrOx resulted in the opposite effect (entrance ● in Fig. 18) [126]. Huber et al. were able to further decrease the phase transition temperature using more hydrophobic comonomers, such as BuOx (entrance ▼ in Fig. 18) and 2-nonyl-2-oxazoline (NonOx, entrance ◆ in Fig. 18), in a similar study with gradient copolymers of DP=25 (turbidity measurements were performed in 2 wt% aqueous solutions) [134]. However, the direct comparison of both series is complicated by several factors, such as differences in measurement conditions and molar masses as well as the different end-groups of the polymers (–OH and –piperidyl), which might affect the solution properties.

The solubility properties of *Pn*PrOx could be tuned in a similar fashion by copolymerization of *n*PrOx with EtOx that resulted in random copolymers although the reported reactivity ratios of both monomers slightly differ when the polymerization is carried out at 42 °C [126] and at 140 °C [113]. Park et al. observed a linear increase of T_{cp} with increasing content of EtOx for copolymers with a DP of 88 until an EtOx content of 68% followed by a steeper increase for polymers with higher EtOx content [126]. This behavior was further elucidated using high-throughput methods by Hoogenboom and Schubert who investigated a library of random copolymers with varying EtOx content as well as varying molar mass (compare Fig. 16) enabling them to establish a predictive model for this polymer type [113]. Both increasing molar mass and increasing *n*PrOx content resulted in lowered cloud points of the polymer solutions (see Fig. 19).

In addition, several copolymers of EtOx with less polar PhOx (20 and 30%) and NonOx (10 and 20%) as comonomers

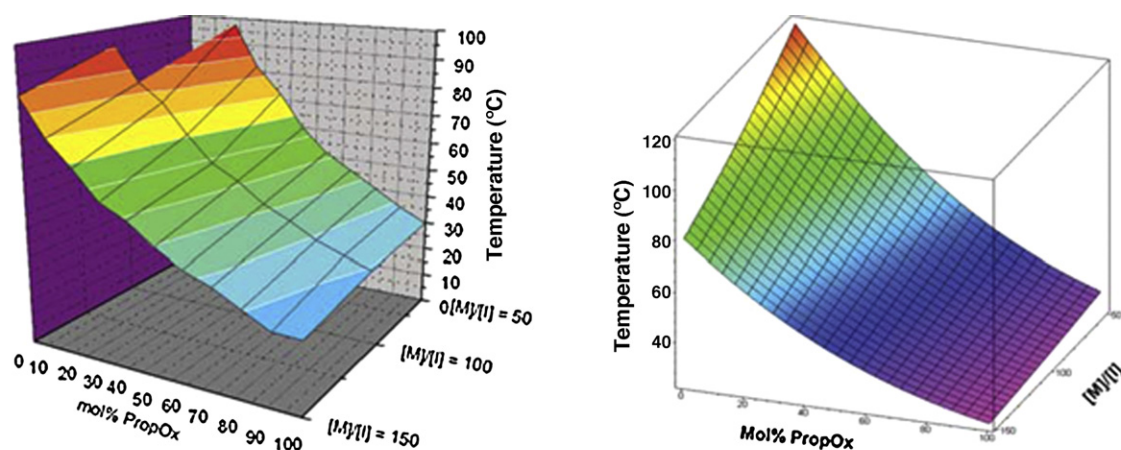
were found to exhibit LCST behavior in water as well as in ethanol/water mixtures [132,135]. The T_{cp} was found to increase linearly from 47 to 65 °C for *PEtOx*₈₀-*grad*-*PhOx*₂₀ and from 54 to 73 °C for *PEtOx*₇₀-*grad*-*PhOx*₃₀ with increasing ethanol content (0–20%) of the solvent mixture due to better solubilization of the *PEtOx* [135]. Surprisingly, this effect could not be observed in similar studies with *PEtOx*₉₀-*r*-*NonOx*₁₀, which shows LCST behavior in the range of 20–30 °C in ethanol/water mixtures of 0–25% [132]. This fact was attributed to hydrophobic interaction of the polymer with ethanol molecules.

5.4. Post polymerization modification

Next to copolymerization of *i*PrOx with other oxazoline-based monomers, post-polymerization modification reactions offer the opportunity not only to “tune” the transition temperature but also to introduce functionalities that would disturb the cationic polymerization process. As a prerequisite to enable subsequent post polymerization modification by thiol-ene “click” chemistry, Schlaad and co-workers recently reported the copolymerization of *i*PrOx with 3-butenyl-2-oxazoline (BOx) [136]. The determination of the apparent rate constants for both monomers led to the conclusion that the synthesized copolymers (DP=52–248, 0–32 mol% BOx content) have a random monomer distribution. Turbidity measurements showed that T_{cp} in 0.1 wt% aqueous NaCl solution ($c=0.15 \text{ mol L}^{-1}$) decreased linearly from 46 to 23 °C with increasing BOx content of the copolymer. “Thio-click” modification (Fig. 20, left) of a copolymer containing 6 mol% BOx resulted in decreased T_{cp} ’s when hydrophobic per-acetylated thio-glucose and 1-octanethiol were reacted, whereas the utilization of hydrophilic 2-mercaptoethanol and 1-thioglycerol increased the T_{cp} ’s of the polymer solution. Introduction of 3-mercaptopropionic acid led to slightly decreased T_{cp} under acidic and elevated T_{cp} under basic measurement conditions. T_{cp} was found to strongly and linearly decrease with increasing content of protected sugar until the copolymers with more than 24 mol% were not water soluble anymore. On the other hand, deprotection of the glucose resulted in higher hydrophilicity and, thus, T_{cp} increased in a linear fashion with increasing sugar content. The copolymer with 32 mol% of deprotected glucose did not even display LCST behavior below 100 °C anymore. In contrast, a longer and, thus, more flexible spacer between glucose and the polymeric backbone (Fig. 20, right) facilitated hydrogen bonding between the hydroxyl groups of the sugar and the carbonyl moieties of the POx amide structure resulting in decreased T_{cp} ’s of the aqueous glycopolymer solutions ($c=4 \text{ mg mL}^{-1}$) in comparison to *PEtOx* homopolymer [137]. In addition, more advanced thermo-responsive glycopolymeric POx structures were recently reported by Schubert et al. who applied a combination of thiol-ene chemistry and a sugar oxazoline monomer for the synthesis and additionally investigated the lectin binding of the polymer [138].

5.5. Comb and graft copolymers

The application of a wide range of available functional initiators or end-capping agents for the CROP of



$$\ln(\text{LCST}) = a \cdot n\text{PropOx}^3 + b \cdot n\text{PropOx}^2 + c \cdot n\text{PropOx} + d \cdot \text{MI}^2 + e \cdot \text{MI} + f \cdot n\text{PropOx}^2 \cdot \text{MI} + g$$

Fig. 19. Left: cloud points as function of both composition and molar mass for EtOx-*n*PropOx statistical copolymers in water (5 mg mL⁻¹). Right: predictive model for the cloud points.

Reproduced from Hoogenboom et al. [113] by permission of The Royal Society of Chemistry.

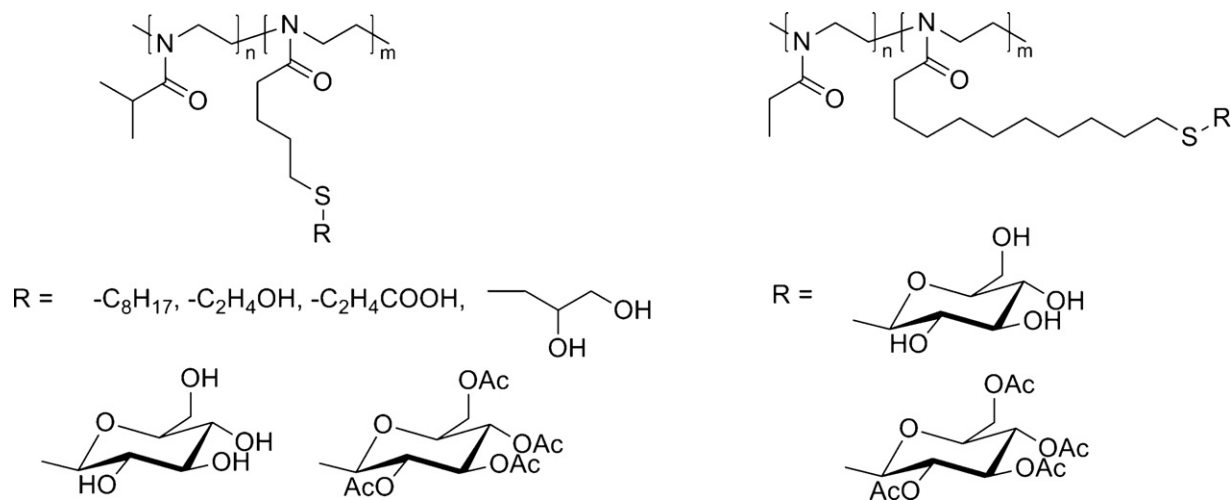


Fig. 20. Schematic representation of POx copolymers modified by "thio-click" reaction.

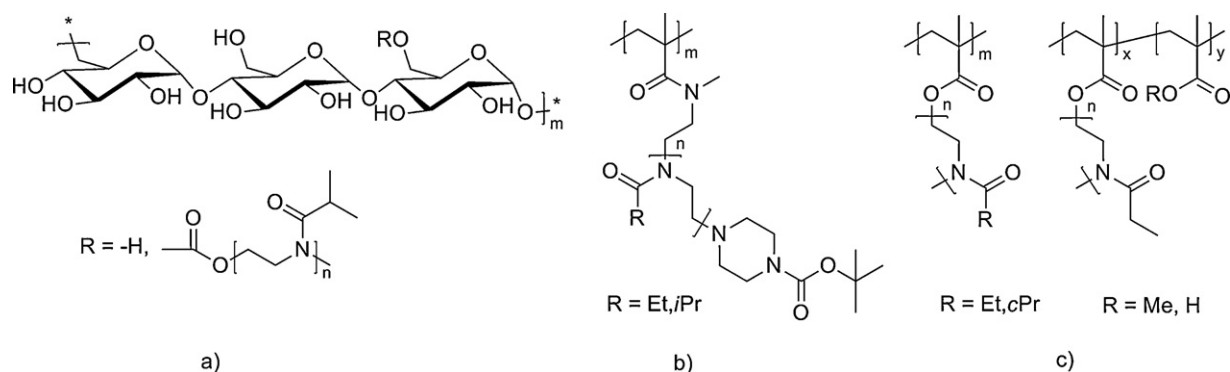


Fig. 21. Schematic representation of the structure of POx based comb and graft copolymers.

2-oxazolines enables the straightforward synthesis of comb and graft copolymers containing thermo-responsive POx as side chains of several polymeric backbones, such as PS, poly(methacrylates), poly(methacrylamides) or pullulan. David et al. as well as Rueda et al. were the first to report on graft copolymers with LCST behavior containing PEtOx and PMeOx as hydrophilic side chains of a PNiPAm backbone using either the macromonomer [139] or the grafting-from method [140]. However, the observed phase transitions were based on the thermo-responsiveness of PNiPAm, which was shown to be shifted to higher temperatures due to the hydrophilicity of PMeOx and PEtOx with respect to PNiPAm. In contrast to that, Morimoto et al. investigated a graft copolymer that displayed thermo-responsive properties based on PiPrOx [141]. Hydroxy-terminated PiPrOx (DP = 29 and 65) was activated using carbonyldiimidazole and subsequently grafted onto pullulan resulting in graft polymers that bear one side chain per 100 glucose backbone units (Fig. 21a). HS-DSC revealed endothermic peaks at higher T_m but with lowered ΔH than for the precursor side chains, which could be explained by the presence of hydrogen bonds that are formed between the amide functions of the PiPrOx side chains and –OH groups of the pullulan backbone. The phase transitions of the graft copolymer with shorter side chains were found to be reversible, whereas stable ring-like structures with diameters of 750–1200 nm were formed upon heating the polymer with longer side chains (as could be shown by TEM). XRD measurements revealed that the formation of such stable particles resulted from crystallization of the PiPrOx side chains. The restricted mobility of the side chains at temperatures above 50 °C was also demonstrated by broadening of the PiPrOx signals in the ^1H NMR spectrum in D_2O .

Comb polymers containing exclusively PiPrOx and PEtOx side chains (DP = 20) were prepared by grafting the POx chains from polycationic macroinitiator salts (Fig. 21b) [142]. Turbidity measurements in 1 wt% aqueous solution revealed lower T_{cp} 's for the comb polymers (53 °C for the PEtOx and 27 °C for the PiPrOx based polymers) with respect to linear PEtOx and PiPrOx, which was attributed to the effect of the hydrophobic Boc-end groups of the side chains pointing towards the outside of the polymer structure. Indeed, removal of the Boc-protection groups resulted in a T_{cp} that was increased by 23 °C. Quite similar PEtOx based comb and graft copolymers were reported by Hoogenboom and Schubert utilizing RAFT to polymerize OEtOx- and OPrOx- methacrylate macromonomers with a DP of 3–22 (Fig. 21c) [119,143]. Turbidity measurements in 5 mg mL^{-1} aqueous solutions of a series of comb polymers revealed T_{cp} 's in the range from 66 to 84 °C that were dependent on the side chain length as well as on the backbone length of the comb polymer and lowered when compared to linear POx. Copolymerization with MMA resulted in graft copolymers whose T_{cp} 's in aqueous solution decreased from 85 to 40 °C with increasing content of hydrophobic MMA (45–80%) [143]. This approach was further extended by copolymerization of the macromonomer with MAA resulting in graft copolymers that were not only temperature, but also pH-responsive [144]. The variation in the backbone length by utilization of

the macromonomer method could be overcome by grafting the living PEtOx chains directly onto a PMAA backbone. The resulting graft copolymers with grafting densities below 60% were soluble in the entire investigated temperature range in buffered solutions at pH 10, whereas the polymer solutions with higher grafting densities showed T_{cp} 's above 85 °C due to the high hydrophilicity of the deprotonated MAA moieties. Under acidic conditions at pH 4, hydrogen bonding between the protonated MAA groups of the polymer backbone and amide moieties of the PEtOx side chains averted the solubilization of copolymers with less than 35% grafting. The copolymers with higher grafting density showed T_{cp} 's that were increasing from 14 to 70 °C with increasing content of PEtOx side chains (up to 92%). In addition, ^1H NMR investigations in D_2O revealed the disappearance of backbone signals due to line broadening upon precipitation of the polymer at the cloud point, whereas the end groups of the polymer side chains remained mobile.

6. Conclusion

The temperature at which the coil to globule transition of a thermo-responsive polymer in water occurs strongly depends on its hydrophilic–hydrophobic balance. Increasing hydrophilicity of the polymer structure results in increasing cloud point temperature. For all of the reviewed polymer structures, PEO based polymers as well as POx, statistical copolymerization with either hydrophilic or hydrophobic comonomers has been successfully applied in order to fine tune the LCST behavior of the thermo-responsive homopolymers. In particular controlled radical polymerization techniques have been used to incorporate functional moieties into PEO based polymers, which provide further interesting features, such as pH responsiveness or sensing properties. On the other hand, functional groups that are not tolerated by the living CROP mechanism, such as carboxylic acids, or glucose, were introduced by post polymerization modification of POx based copolymers. Functional initiators and end capping agents have also proven to be useful to tailor the LCST behavior. Although hydrophobic end groups decrease the T_{cp} , possible micellization of longer aliphatic end groups further affects the thermal properties in the aqueous system. In addition, several systems based on block copolymers that undergo reversible micellization upon changes in temperature have been designed. The opportunities that are provided by living and controlled polymerizations have also been used in order to synthesize more complex comb and graft polymer structures that exhibit thermo-responsive properties.

Modern polymer synthesis offers the possibility not only to tune the LCST behavior of a polymer but also to introduce multiple functional moieties. There is a large demand for intelligent targeted drug delivery systems as well as for thermo-responsive surfaces. PEO has already been widely applied as carrier molecule in biomedical applications for years and also POx show potential for application in this field due to the fact that they exhibit stealth behavior in the body [145]. Therefore, we believe that future research will combine the biological compatibility of PEO and POx based polymers with the knowledge that has been obtained in application of PNiPAm as

thermo-responsive polymer in biomedical applications. In fact, some of the discussed polymers have already been applied for tissue engineering and the coupling of biologically relevant moieties, such as sugars or proteins, to obtain functional smart polymers in an emerging field of science.

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