

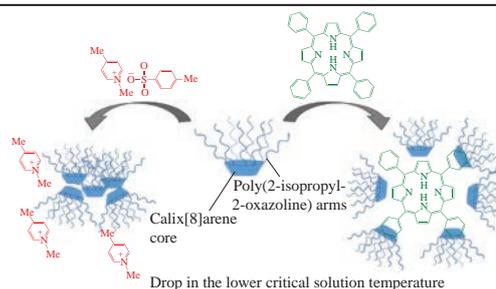
## Influence of hydrophilic and hydrophobic low-molecular-weight additives on the thermoresponsiveness of star-shaped poly-2-isopropyl-2-oxazoline in solution

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**Both hydrophilic *N*-methylpyridinium *p*-toluenesulfonate and hydrophobic 5,10,15,20-tetraphenylporphyrin additives decrease the phase transition temperature of thermo-responsive poly-2-isopropyl-2-oxazoline star-shaped polymer in aqueous solutions.**



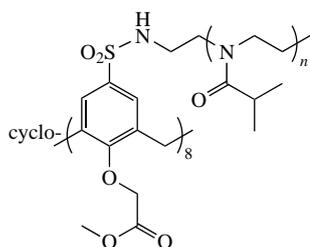
**Keywords:** thermoresponsive polymers, light scattering, polyalkyloxazolines, aggregation, low critical solution temperature.

Thermoresponsive polyoxazolines are known as promising materials for drug delivery due to their ability to form intra- and intermolecular hydrogen bonds resulting in compaction and aggregation of single polymer chains.<sup>1,2</sup> From the influence of physiological media on the hydrogen bond formation and related properties of thermoresponsive polymers, including polyoxazolines, it has been demonstrated that even small NaCl concentration can dramatically affect their low critical solution temperature (LCST).<sup>3,4</sup> In this work, we have investigated star-shaped poly-2-isopropyl-2-oxazoline (polyPr<sup>i</sup>Ox) in aqueous solution in the presence of *N*-methylpyridinium *p*-toluenesulfonate (N-PTS). The latter compound can be considered as a model for cetylpyridinium chloride known for its antimicrobial and antifungal effects. On the other hand, N-PTS influences the LCST of thermoresponsive polymers, because both *N*-methylpyridinium cation and tosylate anion affect the hydrogen bond network of water, though in the opposite ways. Namely, tosylate belongs to the chaotropic anions class,<sup>5,6</sup> which typically breaks the hydrogen bond network and thus increases the polymer solubility.<sup>7</sup> Contrary to that, alkylpyridinium represents a chaotropic cation<sup>5,8</sup> weakly bound to water, which therefore promotes salting out effect and lowers the phase separation temperature. We also have explored the influence of

hydrophobic 5,10,15,20-tetraphenylporphyrin (TPhP), known as photosensitizer in photodynamic cancer therapy,<sup>9</sup> on polyPr<sup>i</sup>Ox as a potential polymeric carrier. The obtained results on the role of both model drugs in the thermoresponsive behavior of polyPr<sup>i</sup>Ox in water have been compared with the data collected in the absence of any additives as well as in physiological media.<sup>4</sup>

Star-shaped polyPr<sup>i</sup>Ox bearing a calix[8]arene core and eight arms grafted at the upper rim was synthesized and characterized<sup>10</sup> by weight-average molecular weight  $M_w = 16300$  Da, molar mass dispersity index  $M_w/M_n = 1.37$  and polymerization degree of the arms equal to 16. PolyPr<sup>i</sup>Ox solutions in water at the ratio of one N-PTS molecule per one oxazoline monomer unit were investigated using static and dynamic light scattering as well as turbidimetry at the polymer concentration  $c = 0.0002\text{--}0.0050$  g ml<sup>-1</sup>. Besides, the solutions of lower N-PTS content, namely (i) one molecule per one polyPr<sup>i</sup>Ox macromolecule and (ii) one molecule of N-PTS per one polyPr<sup>i</sup>Ox arm, were prepared (for details, see Online Supplementary Materials). Temperature responsiveness of the polyPr<sup>i</sup>Ox–TPhP complex was measured spectrophotometrically<sup>†</sup> in one cm layer at a heating rate of 0.5 °C min<sup>-1</sup> and  $\lambda = 714$  nm (see Online Supplementary Materials).

The solution behavior of star-shaped polyPr<sup>i</sup>Ox at one molecule of N-PTS per one oxazoline monomer unit was similar to that in the absence of N-PTS,<sup>4</sup> with trimodal distribution of light scattering intensity vs. hydrodynamic radius and close values of hydrodynamic radii at room temperature [see Figure S1(a) in Online Supplementary Materials]. As for the solution in pure water, the three particle



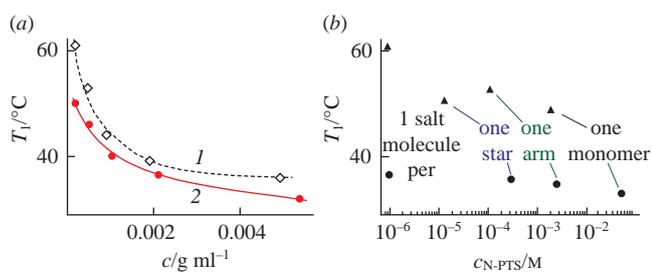
Star-shaped poly-2-isopropyl-2-oxazoline (polyPr<sup>i</sup>Ox)

<sup>†</sup> The UV-VIS spectra were recorded using a LOMO Fotonika SF-256 spectrophotometer (Russia) and turbidimetry experiments were carried out on a modified Specord UV-VIS apparatus (Carl Zeiss Jena, Germany).

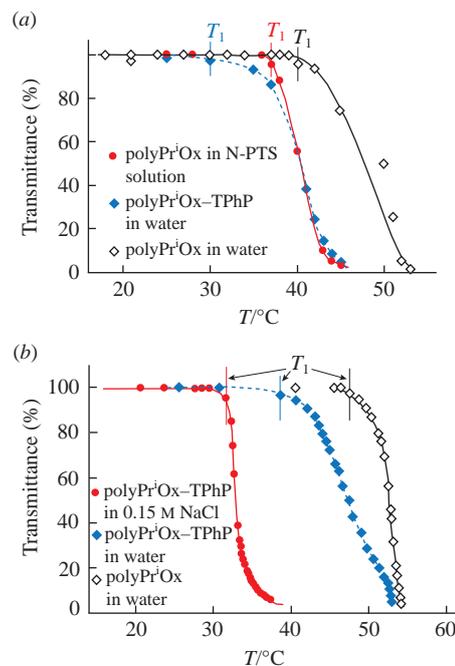
types represented single macromolecules as well as middle-size and large-size aggregates, which had formed due to hydrophobic interaction of the star macromolecule cores. However, a contribution of large aggregates to light scattering was higher for the N-PTS solution compared with water, which indicated more intensive aggregation in the presence of N-PTS as well as its salting out effect.

Despite moderate difference in aggregation in the presence of N-PTS, it caused significant decrease in the phase separation temperature  $T_1$  [Figure 1(a)], determined as the temperature of onset of the drop in transparency [Figure 2(a)], compared with water at the same polymer concentration. This discrepancy in the  $T_1$  values between the N-PTS solutions and water increased from 4 to 11 °C with dilution. This pronounced influence of N-PTS on the polyPr<sup>i</sup>Ox solution confirmed its salting out effect with an increase in surface tension at the hydrophobic-aqueous interface and polarization of water molecules involved in hydrogen bonding, followed by the polymer dehydration, and thus indicated the salt behavior according to Hoffmeister series.<sup>7</sup> Taking into account the opposite effects of chaotropic tosylate anion and chaotropic *N*-methylpyridinium cation on the shift of the phase separation temperature, one can suppose that the anion does not influence the  $T_1$  values, while the effect of the cation is stronger. The decrease in  $T_1$  for the star-shaped polyPr<sup>i</sup>Ox solutions was observed as well in physiological saline,<sup>4</sup> where the temperature drop was more pronounced due to higher content of chloride anion, namely *ca.* four anions per one monomer unit. The influence of N-PTS on the phase separation at polymer concentrations 0.0002 and 0.005 g ml<sup>-1</sup> was analyzed [Figure 1(b)]. For both concentration values, the N-PTS salting out effect was evident at the lowest salt content, namely one N-PTS molecule per one macromolecule. The observed flattening of both curves [Figure 1(a)] indicates the LCST approximation, *i.e.* the existence of minimum in miscibility diagram, which is almost reached for the polyPr<sup>i</sup>Ox solution in water.

For the porphyrin derivative, it was found that grinding of polyPr<sup>i</sup>Ox with TPhP led to their physicochemical interaction and formation of a stable host–guest water-soluble inclusion complex. The calculated concentration of polyPr<sup>i</sup>Ox in the complex solution was  $1.2 \times 10^{-4}$  M (0.002 g ml<sup>-1</sup>) and the one of TPhP was  $2 \times 10^{-5}$  M, which corresponded to a 6:1 molar ratio of polyPr<sup>i</sup>Ox to TPhP. This high ratio can originate from (i) the ability of the large porphyrin guest molecule to form complexes with the calixarene core in a ratio distinct from 1:1 and (ii) possible large amount of free polymer molecules in the solution. The former reason seems to be more probable, because the light scattering data reveal an absence of single macromolecules or small aggregates in the presence of TPhP [Figure S1(b)].



**Figure 1** (a) Phase separation temperature  $T_1$  vs. concentration of polyPr<sup>i</sup>Ox solution: (1) in pure water<sup>4</sup> and (2) in aqueous N-PTS at one molecule of N-PTS per one monomer unit of the polymer. (b)  $T_1$  values for solutions at polyPr<sup>i</sup>Ox concentration of 0.0002 (indicated as triangles) and 0.005 g ml<sup>-1</sup> (indicated as circles) for the ratios of one N-PTS molecule per one star-shaped macromolecule, one arm and one monomer unit.

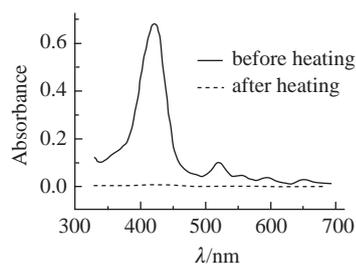


**Figure 2** Turbidimetry curves for (a) 0.002 g ml<sup>-1</sup> polyPr<sup>i</sup>Ox solutions with N-PTS at one its molecule per one monomer unit of the polymer and with TPhP (as polyPr<sup>i</sup>Ox-TPhP complex), compared with pure water, the measurements were carried out at each temperature after an equilibrium state had been achieved; (b) polyPr<sup>i</sup>Ox-TPhP complex in NaCl solution and in water compared with polyPr<sup>i</sup>Ox in water at a heating rate of 0.5 °C min<sup>-1</sup>.

Temperature dependence of optical transmittance for the polyPr<sup>i</sup>Ox-TPhP complex solution is demonstrated in Figure 2(b). Above 42 °C the polymer undergoes a phase transition, which results in destruction of the complex and precipitation of TPhP as fine suspended particles. After cooling and filtration through a 0.2 µm filter, the UV-VIS spectrum of the filtrate manifested almost complete absence of TPhP (Figure 3).

Comparative transmittance measurements for solutions of polyPr<sup>i</sup>Ox and polyPr<sup>i</sup>Ox-TPhP complex in pure water and isotonic saline revealed, that the complex formation significantly decreased the phase transition temperature  $T_1$ . Note, that the medium exhibited a significant effect on the entire phase transition process [Figure 2(b)].

In summary, it has been demonstrated, that hydrophobic TPhP can be dissolved in water *via* the complex formation with star-shaped polyPr<sup>i</sup>Ox accompanied by a decrease in the polymer LCST. Hydrophilic N-PTS also lowers the polyPr<sup>i</sup>Ox LCST due to salting out effect. At the same polymer concentration, the phase separation temperature decreases in order N-PTS → TPhP → 0.15 M NaCl as compared with water. Thus, the development of drug carriers based on thermoresponsive polymers should include consideration of polymer behavior in the presence of the drug.



**Figure 3** UV-VIS spectra of polyPr<sup>i</sup>Ox-TPhP complex solution before and after heating to the phase transition temperature followed by filtration.

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#### Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.07.033.

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