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Influence of molecular architecture on behavior of thermoresponsive poly-2-ethyl-2-oxazine in saline media

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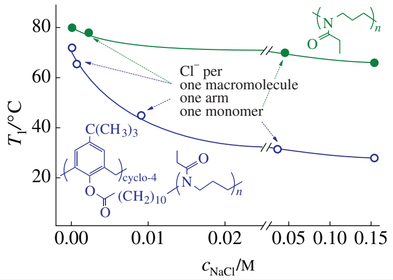
Linear and calix[4]arene based star-shaped poly-2-ethyl-2-oxazines have been explored in saline solutions at NaCl concentration of 0.0050 g cm^{-3}. The calix[4]arene core promotes an aggregation in solutions of the star-shaped polymer and a decrease in its phase separation temperature. Poly-2-ethyl-2-oxazine is more sensitive to the presence of NaCl in solution than the known poly-2-isopropyl-2-oxazolines.



Keywords: star-shaped polymer, thermoresponsive polymer, poly-2-alkyl-2-oxazine, solution properties, saline solution.

Thermoresponsive polymers have a high potential for applications in medicine.[1] Poly-2-alkyl-2-oxazines (pAlkOz) as polymers of 2-alkyl-4,5-dihydro-1,3-oxazines represent promising biocompatible materials for targeted drug delivery due to their ability to form intra- and intermolecular hydrogen bonds leading to self-organization and aggregation of polymer chains, similar to that found for poly-2-alkyl-2-oxazolines (pAlkOx).[2] For the pAlkOz polymers, a specific loading of hydrophobic drugs compared with the pAlkOx ones has been found.[3] Biomedical applications of thermoresponsive polymers stimulate investigations of ionic effects in their solutions[4] to explore the interaction mechanism of ions with macromolecules in relation to their lower critical solution temperature (LCST). It has been established[5] that an understanding of the Hofmeister salt effects on the LCST behavior, namely the arrangement of ions in the order of their influence on the solvent properties, particularly on the dissolving ability and salting out, requires an investigation of the polymer architecture as well as the structure of its functional groups. In this work, we have analyzed the molecular layout effect on the properties of thermoresponsive polymers in solutions in the presence of salt. Linear poly-2-ethyl-2-oxazine (pEtOz) and star-shaped four-arm poly-2-ethyl-2-oxazine (C4A–pEtOz) with calix[4]arene (C4A) core (Scheme S1, Online Supplementary Materials) were investigated in pure aqueous and saline solutions. It is known, that calixarene core ensures an effective complexation with low molecular weight compounds,[6] while chloride ions represent weakly hydrated ones and thus contribute to salting out and a decrease in the phase separation temperature.[7]

Star-shaped C4A–pEtOz has been already synthesized and characterized[8] with M_w = 7100 g mol^{-1} and hydrodynamic radius of the macromolecule R_h = 3.0 nm. Linear pEtOz was obtained according to the method described for EtOz copolymers[9] (for details, see Online Supplementary Materials) with M_w = 2200 g mol^{-1} by GPC and R_h = 2.3 nm by static light scattering.

The behavior of C4A–pEtOz and pEtOz in water–salt solutions was explored at polymer concentration c = 0.0050 g cm^{-3}. The salt concentrations were selected as one NaCl formula unit per one macromolecule, per one arm of the polymer star and per one monomer unit. Besides, physiological saline (0.9 wt% NaCl or 0.154 mol dm^{-3}) and pure aqueous solutions were investigated. Thus, NaCl concentrations employed were 0.00070, 0.0091, 0.0364 and 0.154 mol dm^{-3} for C4A–pEtOz as well as 0, 0.0023, 0.0455 and 0.154 mol dm^{-3} for pEtOz. The values and temperature dependence for light scattering intensity I, optical transmittance I^*, hydrodynamic radii R_h of the scattering objects and their contribution S_i to the total light scattering intensity were determined according to the known procedure[10] (for details, see Online Supplementary Materials).

For pEtOz water–salt solutions at 21 °C, the distribution of light scattering intensity I *vs.* hydrodynamic radii R_h of the scattering objects was unimodal (Figure S4, Online Supplementary Materials). The observed particle radius R_{fast} was almost constant with variation of the salt concentration (Figure 1) and its average value coincided with the hydrodynamic size R_h of the polymer molecules, *i.e.*, the pEtOz solutions were molecularly dispersed. Alternatively, the

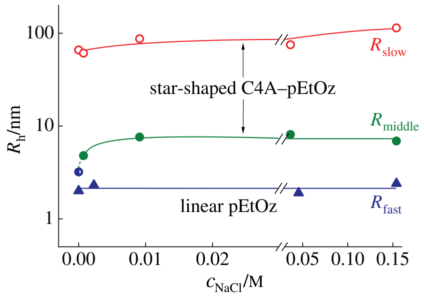


Figure 1 Hydrodynamic radii R_{fast} (blue triangles), R_{middle} (olive circles) and R_{slow} (red open circles) *vs.* NaCl concentration for solutions of pEtOz and C4A–pEtOz at 21 °C.

distribution for solutions of star-shaped C4A–pEtOz was bimodal at 21 and 33 °C (Figure S5). The reason for aggregation of pAlkOz and pAlkOx polymers with calixarene cores is their hydrophobic interactions.[8,11] Even with a minimum salt content of one NaCl formula unit per one polymer molecule, isolated macromolecules of the star-shaped polymer could not be observed and only small aggregates with a middle mode radius R_{middle} appeared in the solution. The aggregate size increased with an elevation of the salt concentration (see Figure 1). Thus, the presence of NaCl promotes the aggregation in C4A–pEtOz solutions.

After heating the water–salt solutions the phase separation was observed. The temperature of its onset T_1 was determined as the beginning of a rapid increase in the light scattering intensity I and the decrease in optical transmittance I^* (Figure S6). Temperature T_2 of the end of the phase separation was measured only for the star polymer, because for the linear one it was above 80 °C and could not be obtained reliably due to slow equilibration.

At temperature below 60 °C, the solutions of linear pEtOz remained molecularly dispersed. At 60 °C, aggregates with a hydrodynamic radius R_{slow} appeared in the solutions (Figure 2). For aqueous solutions of the polymers investigated, so-called dehydration process occurs at elevated temperature, which consists in destruction of polymer–water hydrogen bonds with formation of polymer–polymer ones.[12] It can be assumed that the dehydration of poly-2-ethyl-2-oxazine begins at temperatures slightly lower than 60 °C, and at this temperature the fraction of dehydrated units becomes sufficiently high for aggregation. It is known that the dehydration of poly-2-ethyl-2-oxazoline begins at ~54 °C.[13] The hydrodynamic radii R_{slow} of the aggregates and their contribution to total light scattering increased with heating. The radius R_{fast} did not depend on temperature and above the T_1 value isolated macromolecules were not observed by dynamic light scattering, since they were aggregated.

In the same way, the radius R_{middle} of smaller aggregates in C4A–pEtOz solutions did not change with temperature (see Figure 2). The radius of large aggregates changed slightly at moderate temperature and then rapidly increased reaching a maximum value at T_2. This caused an increase in the contribution S_i of these particles to the light scattering intensity.

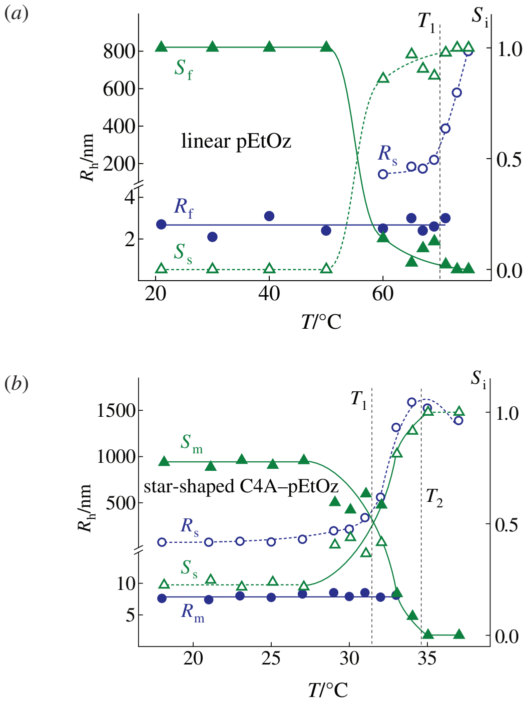


Figure 2 Hydrodynamic radii R_h of scattering species and their contribution S_i to the total intensity of light scattering for solutions of (*a*) linear pEtOz at c_{NaCl} = 0.0455 M and (*b*) star-shaped C4A–pEtOz at c_{NaCl} = 0.0364 M.

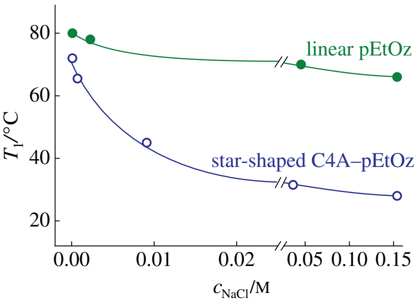


Figure 3 Temperature T_1 of the phase separation onset *vs.* NaCl concentration for investigated solutions.

For both polymers investigated, the temperatures of the phase separation onset T_1 decreased with raising c_{NaCl} (Figure 3). However, there was a significant difference in the behavior of linear and star-shaped polymers. For pEtOz, the temperature T_1 decreased by 14 °C in saline media compared with pure water, while for the star-shaped polymer this difference was three times larger.

Thus, the presence of salt affects the behavior of the linear pEtOz and star-shaped C4A–pEtOz polymers in different ways. Probably, the C4A core plays an important role including the interaction of the core with NaCl, an increase in hydrophobicity of C4A–pEtOz molecules and lowering the phase separation temperature. A comparison of the obtained results with those for star-shaped poly-2-isopropyl-2-oxazoline (pPr^{i}Ox)[14] as a structural isomer of pEtOz allows us to conclude that pEtOz is more sensitive to the presence of chloride ions. Indeed, the difference in T_1 between saline and pure water media for star-shaped pPr^{i}Ox is ~10 °C, *i.e.*, less than for linear pEtOz. Moreover, for pPr^{i}Ox one or more chloride ions per monomer unit are necessary for noticeable changes in self-aggregation, while the addition of one NaCl formula unit to one C4A–pEtOz star changes significantly the solution behavior on heating. The results obtained lead to a suggestion that due to the C4A core star-shaped pAlkOz polymers are more promising for medical applications compared with their linear counterparts and taking into account their biocompatibility, low toxicity, resistance to enzymes and an improved binding of water-insoluble drugs compared with the pAlkOx polymers.[3] Due to the presence of an additional methylene moiety in the monomer unit, the phase separation temperature of pAlkOz is somewhat lower than that of pAlkOx and closer to the temperature of a human body.

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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.2020.11.038.

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