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Efficient Formation of Multicompartment Hydrogels by Stepwise Self-Assembly of Thermoresponsive ABC Triblock Terpolymers

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Supporting Information

ABSTRACT: The gelation behavior of a poly(ethylene-*alt*-propylene)-*b*-poly(ethylene oxide)-*b*-poly(*N*-isopropylacrylamide) (PON) triblock terpolymer and a poly(*N*-isopropylacrylamide)-*b*-poly(ethylene oxide)-*b*-poly(*N*-isopropylacrylamide) (NON) triblock copolymer was studied by rheology over the concentration range 1−5 wt %. In comparison to the NON copolymer, gelation of the PON terpolymer was achieved at a much lower concentration, with a much sharper sol−gel transition. This is due to a stepwise gelation of PON terpolymers involving micellization at room temperature and gelation at elevated temperatures. The separation of micellization and gelation leads to the formation of a two-compartment network as observed by cryoTEM. The results highlight the intricate and tunable nanostructures and new properties accessible from ABC terpolymer hydrogels.

Hydrogels are water-swollen polymeric networks with broadly tunable characteristics that enable wide utility in, for example, coating, cosmetic, drug delivery, tissue engineering, and sensing applications.[1,2] Both chemical hydrogels, formed by covalent cross-linking of hydrophilic polymers, and physical hydrogels comprising block copolymers or other self-associating polymers held together by hydrophobic, hydrogen bonding, or ionic interactions, are of interest.[1] Reversible physical hydrogels from block polymers are particularly appealing, as they can exhibit a sol−gel transition in response to external stimuli, and have potential for site-specific drug-delivery applications.[3,4] In addition, mechanical properties and mesh size can be readily tuned by changing copolymer concentration, composition and molar mass.

Hydrogel formation by ABA triblock copolymers containing hydrophilic midblocks and hydrophobic end blocks has been extensively studied (Figure 1a).[5−12] However, in general, the gelation of such systems is inefficient, in the sense that minimum gelation concentrations often exceed 10 wt % polymer, and that the sol−gel transition is relatively broad for thermoreversible systems. At least two underlying factors contribute to this. First, there are three possible conformations of the midblocks in ABA hydrogels: (i) loops, when both end blocks belong to the same microdomain; (ii) bridges, when the end blocks connect two different microdomains; (iii) dangling ends, when one end block is unassociated with any microdomain.[13] Both looped chains and dangling ends are network defects, whereas only bridges contribute to the network elasticity; it is difficult to achieve a majority of bridges in ABA systems. At low concentrations, ABA copolymers form discrete, flowerlike micelles as the looping conformation is preferred.[14] As the concentration increases, a fraction of the midblocks will form bridges between different micellar cores, resulting in the formation of an elastic network. Secondly, for thermoreversible systems, the association of A blocks is rather haphazard, leading to a disorganized gelation process. It is therefore of interest to consider ABC triblock terpolymers, in which A and C are both hydrophobic, but mutually immiscible. In principle, this architecture should completely suppress looping.[15] Furthermore, by making the C block association reversible, it is possible to produce hydrogels in a stepwise manner, by first forming micelles with an A core, then gels by subsequent C block association (Figure 1b). We demonstrate here that this strategy produces a very sharp gelation transition, and at a much lower polymer concentration, than an equivalent CBC triblock.

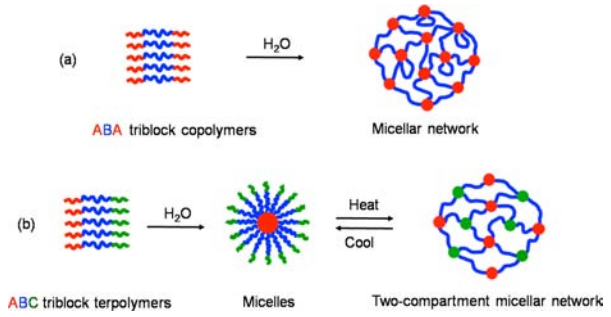

Figure 1. Schematic illustration of gelation of (a) ABA and (b) thermoresponsive ABC polymers.

In prior work on ABC triblock terpolymers,[13,16−21] Taribagil et al. studied 1,2-(poly)butadiene-*b*-poly(ethylene oxide)-*b*-poly (perfluoropropylene oxide) (PB-PEO-PFPO) hydrogels with two highly immiscible end blocks and revealed a bicontinuous structure composed of PFPO disks distributed within a hydrophobic PB sheet covered by hydrophilic PEO chains. The formation of a bicontinuous structure instead of a two-compartment micellar network was attributed to the exceptionally high interfacial energy (i.e., incompatibility) between PFPO and water.[17] Reinicke and co-workers prepared pH and thermoresponsive hydrogels from poly(2-vinylpyridine)-*b*-poly(ethylene oxide)-*b*-poly(glycidyl methyl ether-*co*-

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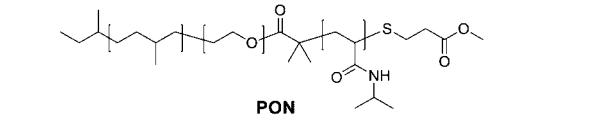
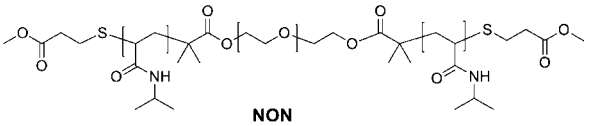
ethyl glycidyl ether) (P2VP-PEO-P(GME-*co*-EGE)) block terpolymers at high polymer concentration (18 wt %), but gelation of the corresponding ABA copolymers was not studied.[18] Armes et al. reported the mechanical response of thermoresponsive hydrogels from poly(propylene oxide)-*b*-poly(2-methacryloyloxyethyl phosphorylcholine)-*b*-poly(*N*-isopropylacrylamide) (PPO-PMPC-PNIPAm) and PNIPAm-PMPC-PNIPAm,[9,20] but remarkably they found that the gelation efficiency and mechanical properties of PNIPAm-PMPC-PNIPAm hydrogels were superior to PPO-PMPC-PNIPAm hydrogels, in conflict with our working hypothesis. One possible explanation is the availability of rapid exchange of PPO chains between micelles.[21,22] Conversely, Shen et al. demonstrated that looping was suppressed in hydrogels formed from a triblock protein with dissimilar end domains, leading to better mechanical properties.[23] Thus, whether ABC triblock terpolymers are beneficial for hydrogel formation in comparison to ABA triblock copolymers remains an open question.





A poly(ethylene-*alt*-propylene)-*b*-poly(ethylene oxide)-*b*-poly(*N*-isopropylacrylamide) (PON) triblock was prepared using a combination of anionic and reversible addition−fragmentation chain transfer (RAFT) polymerizations,[24] and an analogous NON copolymer was synthesized by RAFT polymerization from a α,ω-dihydroxy-PEO precursor (M_n = 20 kg mol^{-1}) following a reported procedure.[25,26] The product of each reaction step was confirmed by ^{1}H NMR spectroscopy and characterized by size exclusion chromatography (Figure S1). Samples investigated in this work are listed in Table 1

Table 1. Molecular Parameters of PON and NON polymers

sample[a]	N_P[b]	N_O[b]	N_N[b]	f_P[c]	f_O[c]	f_N[c]	$Đ$[d]
PON(3-25-10)	45	565	89	0.11	0.63	0.26	1.05
NON(10-25-10)	–	454	91	–	0.49	0.51	1.05

[a]The numbers in the parentheses correspond to the molar masses of P(PEP), O(PEO), and N(PNIPAm), respectively, in kg mol^{-1} as determined by ^{1}H NMR spectroscopy. [b]Number average degree of polymerization as determined by ^{1}H NMR spectroscopy. [c]The volume fraction was calculated using the molecular weight and the RT densities: ρ(PEP) = 0.856 g/cm^3, ρ(PEO) = 1.12 g/cm^3, and ρ(PNIPAm) = 1.07 g/cm^3. [d]The dispersity was measured by SEC with THF/*N*,*N*,*N*′,*N*′-tetramethylethylenediamine as the eluent.

along with the molecular characteristics. We previously reported the micellization behavior of PON triblock terpolymers in water at low concentrations (0.5 and 0.05 wt %).[24] The terpolymers formed well-defined micelles with hydrophobic PEP cores surrounded by hydrophilic PEO-PNIPAm coronae at low temperatures, and these micelles associated to form larger aggregated structures upon heating above the lower critical solution temperature (LCST) of the PNIPAm block. At higher concentrations, we expect the formation of PEP micelles with PEO-PNIPAm coronae at low temperatures and a two-compartment network with exclusively bridging conformations for the PEO midblocks upon heating (Figure 1b). On the basis of the aggregation number and hydrodynamic radius of dilute PON(3-25-10) micelles described previously,[24] we estimate a "critical micelle overlap" concentration of about 2 wt %, consistent with a very efficient use of bridging PNIPAm blocks. Note that these PEP blocks are sufficiently hydrophobic that no changes in micelle aggregation number are expected with increasing temperature.[27,28]

A 5 wt % sample of PON(3-25-10) is a free-flowing transparent liquid at room temperature, and becomes a free-standing opaque hydrogel when heated to 50 °C (Figure S2). Repeated heating and cooling experiments indicate that the sol−gel transition is completely thermoreversible. Dynamic shear measurements were performed on a 5 wt % PON(3-25-10) sample over the temperature range 25−55 °C. Representative data at 25, 42, and 45 °C are shown in Figure S3. At 25 °C, the storage modulus (G') is smaller than the loss modulus (G''), and follows typical terminal rheological behavior for a viscoelastic fluid. At an intermediate temperature of 42 °C, G' is almost equal to G'' and show similar power law dependences on ω: $G' \approx G'' \sim \omega^{0.5}$. This temperature is identified as the critical gelation temperature (T_{gel}).[29] At 45 °C, $G' > G''$ at all frequencies and is nearly frequency independent, indicating solid-like behavior.

The thermoreversible nature of this sol−gel transition was verified using dynamic temperature sweep measurements (Figure 2a), in which G' and G'' were measured as a function of temperature during a ramp from 25 to 55 °C at a heating rate of 1 °C/min. At low temperature, the values of both G' and G'' are low, and $G' < G''$, indicating a free-flowing sol state. On increasing temperature, the magnitude of both G' and G'' increase abruptly and then G' reaches a plateau. As the increase in G' is more significant than G'', G' becomes much larger than G'' at higher temperatures indicating the solid-like behavior. The crossover of G' and G'', identified as T_{gel}, is 42 °C, consistent with the results in the dynamic frequency sweep measurements. The remarkably sharp gelation transition is unusual for flexible coil block polymers at such low concentrations.

We compared the gelation properties of PON(3-25-10) with NON(10-20-10) by examining the temperature dependence of G' and G'' at 5 and 2 wt % polymer (Figure 2). PON gelation is very sharp, within 5 °C, at both concentrations, whereas the NON copolymer shows a very gradual and broad transition at the concentration of 5 wt % and no gelation at the concentration of 2 wt %. Such a broad sol−gel transition has been observed in other thermoresponsive ABA hydrogels.[11] The critical gelation concentration can be obtained from dynamic temperature sweep measurements at different concentrations. Figures S4 and S5 show the temperature dependence of G' and G'' obtained for aqueous solutions of PON(3-25-10) and NON(10-20-10) at varying polymer concentrations. The PON terpolymer shows temperature induced gelation behavior at 1, 2, and 5 wt %, while the NON copolymer does not form a well-defined hydrogel until a concentration of 10 wt %, indicating the critical gelation concentration of PON terpolymer is much smaller than that of the NON copolymer.

On the basis of these data, we posit that the PON terpolymer undergoes a two-step gelation mechanism, involving the initial formation of micelles with PEP cores at room temperature and gelation due to the PNIPAm block aggregation at elevated

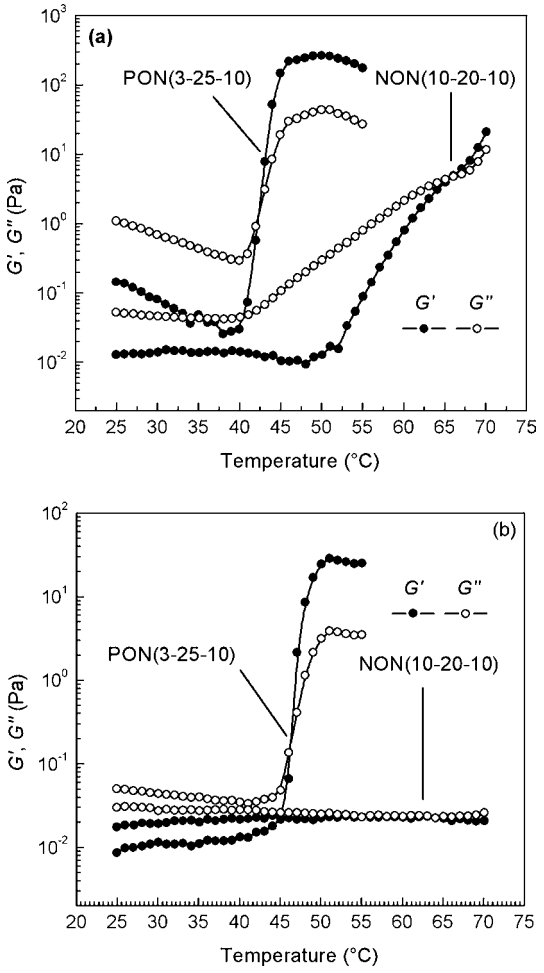


Figure 2. Temperature-dependent dynamic shear moduli (G' and G'') for (a) 5 wt % PON(3-25-10) and NON(10-20-10) and (b) 2 wt % PON(3-25-10) and NON(10-20-10) solutions at a frequency ω = 10 rad/s and heating rate of 1 °C/min.

temperatures. The gelation of NON arises solely from the hydrophobic association of PNIPAm end blocks above the LCST; micellization and gelation occur simultaneously. We propose that the separation of micellization and gelation in the PON hydrogels leads to the formation of a two-compartment network with exclusively bridged conformations for the PEO midblocks, while both looping and bridging conformations are possible for the NON hydrogels. With more bridging chains in PON hydrogels, gelation can be achieved at a lower concentration. Furthermore, the presence of the PEP-core micelles serves to distribute the PNIPAm end blocks predominantly in the intermicellar regions, thereby "pre-concentrating" the cross-linking moieties. In such a case, large-scale reorganization of the preformed micellar solution is no longer required, and the sol−gel transition of PON terpolymers is very sharp.

To investigate whether the two-compartment micellar network illustrated in Figure 1b is a realistic description of this system, we utilized cryogenic transmission electron microscopy (cryoTEM), as shown in Figure 3 for a 1 wt % PON solution. In Figure 3a, the sample was vitrified after annealing at 25 °C, that is, below the gel temperature. The PEP micellar cores with liquid-like arrangement are clearly visible. The same solution gave the image shown in Figure 3b after

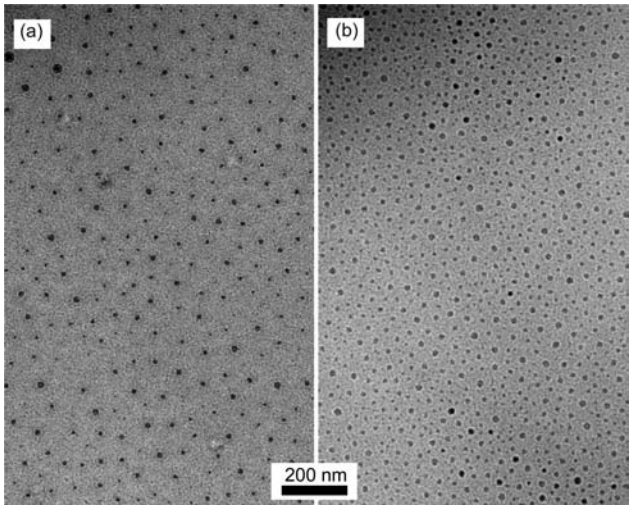


Figure 3. CryoTEM images of 1 wt % PON(3-25-10) samples prepared at (a) 25 °C and (b) 50 °C.

annealing at 50 °C, in the gel state. A 3- to 4-fold increase in the number of micellar cores is clearly evident, thereby providing the first direct evidence for the structure posited in Figure 1b. It is not possible to quantify precisely the increase in the number of micelles on gelation, but if one assumes roughly comparable micellar core radii, the ratio of PNIPAm to PEP volume fractions suggests that there should be 2−3 times more PNIPAm cores, consistent with observation. Similarly, although the cryoTEM images cannot directly confirm the absence of looping conformations, they do provide strong evidence for the overwhelming predominance of bridges. Corresponding cryoTEM images of 5 wt % NON(10-20-10) solutions prepared at 25 and 60 °C show no evidence of aggregates at low temperature, and inhomogenously distributed aggregates of micelles at high temperature (Figure S6), consistent with our interpretation. The gel modulus could, in principle, provide insight into the number of elastically effective network strands, but the low polymer concentration means that there is insufficient material to permeate the entire sample volume at the preferred midblock extension, leading to large scale heterogeneity. This heterogeneity decreases with increasing polymer concentration, as shown in Figure S2. Nevertheless, the remarkably low gelation concentration and sharp gelation transition confirm that the thermosensitive ABC terpolymer approach can provide significantly more effective gelation than an equivalent ABA copolymer.

In conclusion, we prepared thermoresponsive ABC hydrogels from PON triblock terpolymers. The terpolymers form micelles in water at low temperatures with hydrophobic PEP cores surrounded by hydrophilic PEO-PNIPAm coronae. These micelles associate to form a hydrogel upon heating above the LCST of PNIPAm. The separation of micellization and gelation leads to the formation of a two-compartment network with a very high fraction of bridging conformations for the PEO midblocks. Therefore, gelation can be achieved at a much lower concentration, with a much sharper sol−gel transition, as compared to NON copolymer hydrogels. The detailed gel structure for this PON terpolymer and others with different PEO and PNIPAm block lengths is currently under investigation using rheology and small-angle neutron scattering.

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■ ASSOCIATED CONTENT

Supporting Information

Experimental section; SEC trace of NON copolymers; photographs of PON solutions at room temperature and elevated temperatures; dynamic frequency sweep of PON solutions; dynamic temperature sweep of PON and NON solutions; cryoTEM of NON solutions. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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