Tailoring Lifetimes and Properties of Carbodiimide-Fueled Covalently Crosslinked Polymer Networks

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Abstract

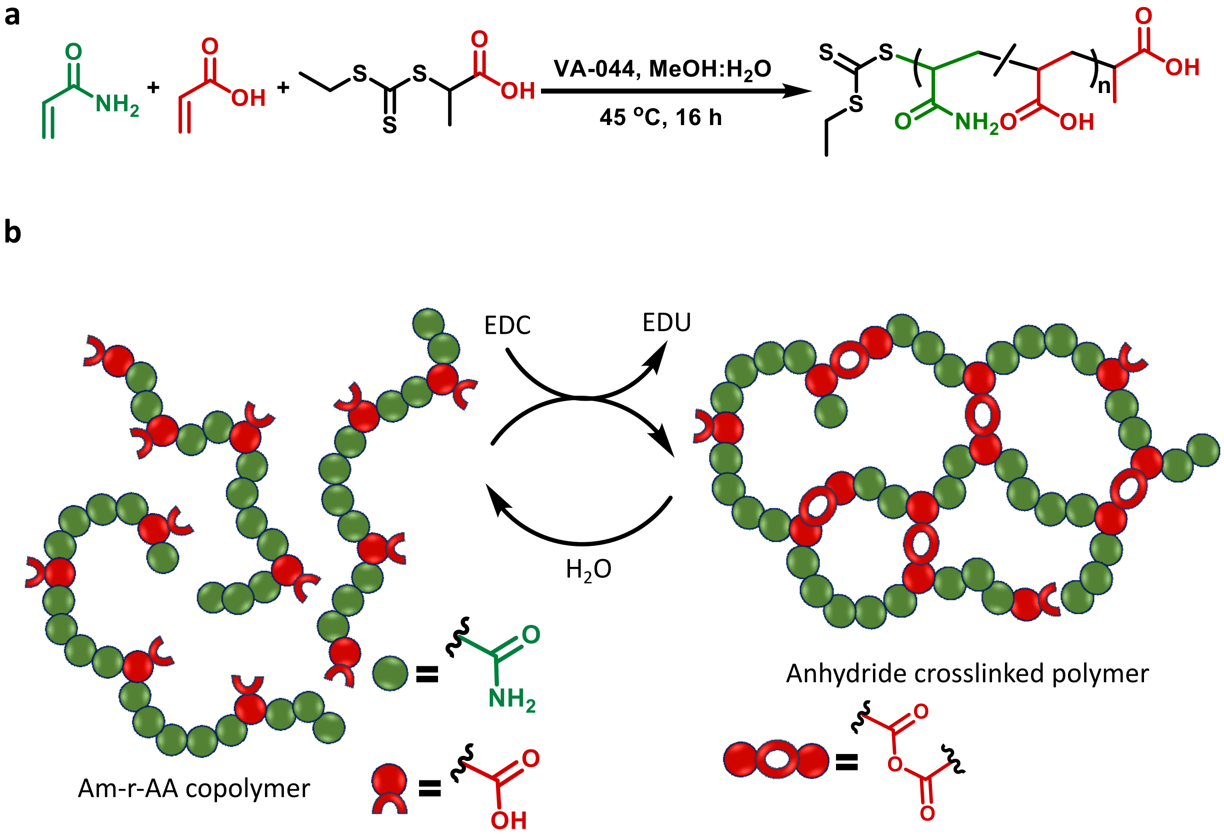
The mechanical properties of nonequilibrium polymer hydrogels obtained from the transient crosslinking of polymer chains by a chemical fuel were investigated. Aqueous polymers featuring pendant carboxylic acids were treated with a carbodiimide to give anhydride-crosslinked gels. The anhydrides spontaneously hydrolyze back to the polymer solution and the cycle can be repeated multiple times. Oscillatory rheology was employed to study the effects of temperature, fuel concentration, chain length, and polymer composition on the storage and loss moduli of the polymeric materials as well as the time taken for the polymers to undergo decrosslinking. Regardless of the temperature used, at constant carbodiimide concentration, degelation times are more sensitive to experimental temperature than are the peak storage moduli. Decrosslinking times decrease with increasing temperature. As carbodiimide concentration decreases there is a decrease in moduli and decrosslinking times. Within the scope of materials studied, the polymer structure was found to have relatively small impact on the transient properties of gel networks compared to the fuel concentration and temperature. These findings facilitate the design of tunable on demand networks and gels.

Key words: Chemical fuel, self-assembly, nonequilibrium, transient materials, polymeric hydrogels

Introduction

Dissipative self-assembly describes processes driven out of equilibrium by constant influx of energy (*e.g.,* fuel molecules or light). This is commonly observed in nature (*e.g.,* self-assembly of microtubules from tubulins[1]) but difficult to replicate artificially.[2] In biological systems, the energy provided by a chemical fuel facilitates spatiotemporal control over self-assembly, allowing the cell to perform complex functions like cell division and intracellular transport. Outside of biology, pioneering work by van Esch and Eelkema has inspired recent reports on chemically fueled assembly as a new approach towards complex self-assembled smart materials.[3–5] Chemically fueled assembly has now been exploited in, for example, supra-amphiphiles,[6,7] membrane transporters,[8,9] self-destructing gels,[3,4,10,11] supramolecular assemblies,[12–14] temporary inks,[15] and colloidal clusters.[16]

Boekhoven *et al.*[17–21] and our group[22,23] have employed the hydration of carbodiimides to fuel the formation of aqueous carboxylic anhydrides, which subsequently undergo hydrolysis back to the starting carboxylic acids giving overall formation of a transient bond. In this reaction cycle, the carbodiimide does not contribute to the structure of the transient state. This feature enables new forms of structural complexity;[24] for example, we showed that carbodiimides (specifically EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) can be used to crosslink polymer chains containing pendant carboxylic acids via formation of the corresponding anhydrides.[25] This gives transiently crosslinked polymer networks that upon anhydride hydrolysis return the starting polymer solution.



Scheme 1. (a) Synthesis of typical Am-r-AA co-polymer using RAFT polymerization and (b) out-of-equilibrium dynamic covalent crosslinking of Am-r-AA co-polymer network using EDC (R = Et, R' = $(CH_2)_3N^+(H)(CH_3)_2$) as a chemical fuel.

Recently, Wang and co-workers also reported similar out-of-equilibrium anhydride-crosslinked polymer hydrogels generated by EDC, which was used for the controlled formation and isolation of pharmaceutical crystals.[26] In their case, they copolymerized acrylic acid (AA) with N-isopropylacrylamide (NIPAm) to form poly(NIPAm-co-AA) as hydrogel precursor. Structure-property effects in this study revealed that increased EDC fuel concentration increases the lifetime and viscoelasticity of polymer hydrogels. A denser crosslinked network was observed with more EDC, up to a point: further increase in EDC had no impact on the maximum storage modulus (G'_{max}) owing to the finite number of carboxylic groups on the copolymer. These initial reports demonstrate the concept of transient polymer crosslinking. However, little is known about how these systems respond to fundamental changes in conditions and polymer microstructure.

Here, we present new insights into how fuel concentration, experimental temperature, and polymer architecture influence the mechanical properties of transient polymer hydrogels. Reversible addition-fragmentation chain transfer (RAFT) polymerization[27] was used to synthesize simple acrylamide (Am) and AA random copolymers, denoted as poly(Am_x-r-AA_y) for polymers with x units of Am and y units of AA. These polymers serve as precursors to transient hydrogels and are quite simple to synthesize. In addition, their architecture allows for easy modulation of their underlying structures. Polymers with variable chain lengths and potential crosslink densities illustrate how structural factors impact the properties of transiently crosslinked polymers. This work provides the basis to better understand how materials featuring temporal changes in mechanical properties can be designed and tuned on demand.

Results and Discussion

RAFT polymerization was used to synthesize four well-defined Am and AA co-polymers of different chain lengths (~100 and 200 units long) with different ratios of Am to AA (65:35 and 85:15), as described in the Supplemental Information. 2-(((Ethylthio)carbonothioyl)thio)-propanoic acid (PAETC) was employed as the chain transfer agent (CTA). The polymers under study thus had CTA:Am:AA molar ratios of 1:65:35 (poly(Am_{65}-r-AA_{35})), 1:130:70 (poly(Am_{130}-r-AA_{70})), 1:85:15 (poly(Am_{85}-r-AA_{15})), and 1:170:30 (poly(Am_{170}-r-AA_{30})). MALDI-TOF mass spectrometry was used to characterize all polymers (Figure S1-S4). They all had dispersities (Đ) of ~1.02-1.03.

In our previous work, we conducted a model study using a short Am-based oligomer containing one carboxylic acid group at the α-terminus with mean length of 7-8 units of Am. This model material tested the formation of anhydride bonds between polymer chains and showed that there is no interference from 1° amides leading to possible formation of permanent imide crosslinks.[25] To further confirm the formation of transient anhydrides, 0.6 mL a 40 wt% aqueous solution of poly(Am_{130}-r-AA_{70}) was treated with 0.2 mL of 1.5 M EDC and monitored using Attenuated Total Reflection Infrared Spectroscopy (ATR IR). An IR band at 1809 cm^{-1}, assigned to the symmetric carbonyl stretching mode of anhydrides, appeared within 30 seconds after EDC addition. A decrease in the anhydride peak was observed over time denoting polymer decrosslinking through hydrolysis (Figure 1). After 65 minutes, the crosslinked polymer had dissolved to form a dilute aqueous polymer solution.

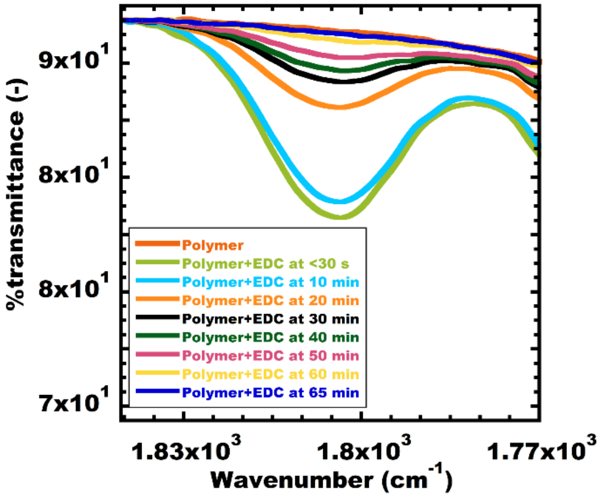


Figure 1. ATR-IR-monitored treatment of poly(Am_{130}-r-AA_{70}) (2.61 mmol of AA) with EDC (1.31 mmol) showing anhydride formation and the time dependence of anhydride hydrolysis after EDC addition to polymer.

Rheological time sweep experiments were carried out to study the mechanical properties of the transiently formed gels by closely monitoring the changes in storage (G’) and loss (G”) moduli, and thus the crosslinking and decrosslinking times of gels, under different experimental conditions. The experiments were performed by injecting a polymer solution onto the static Peltier plate of the rheometer followed by immediate injection of the EDC solution into the polymer solution. Mixing of polymer and EDC solutions was achieved by the motion between a 20 mm parallel plate geometry and the static lower plate (Figure S6). Figure 2 shows typical time sweep data for each polymer system under similar conditions.

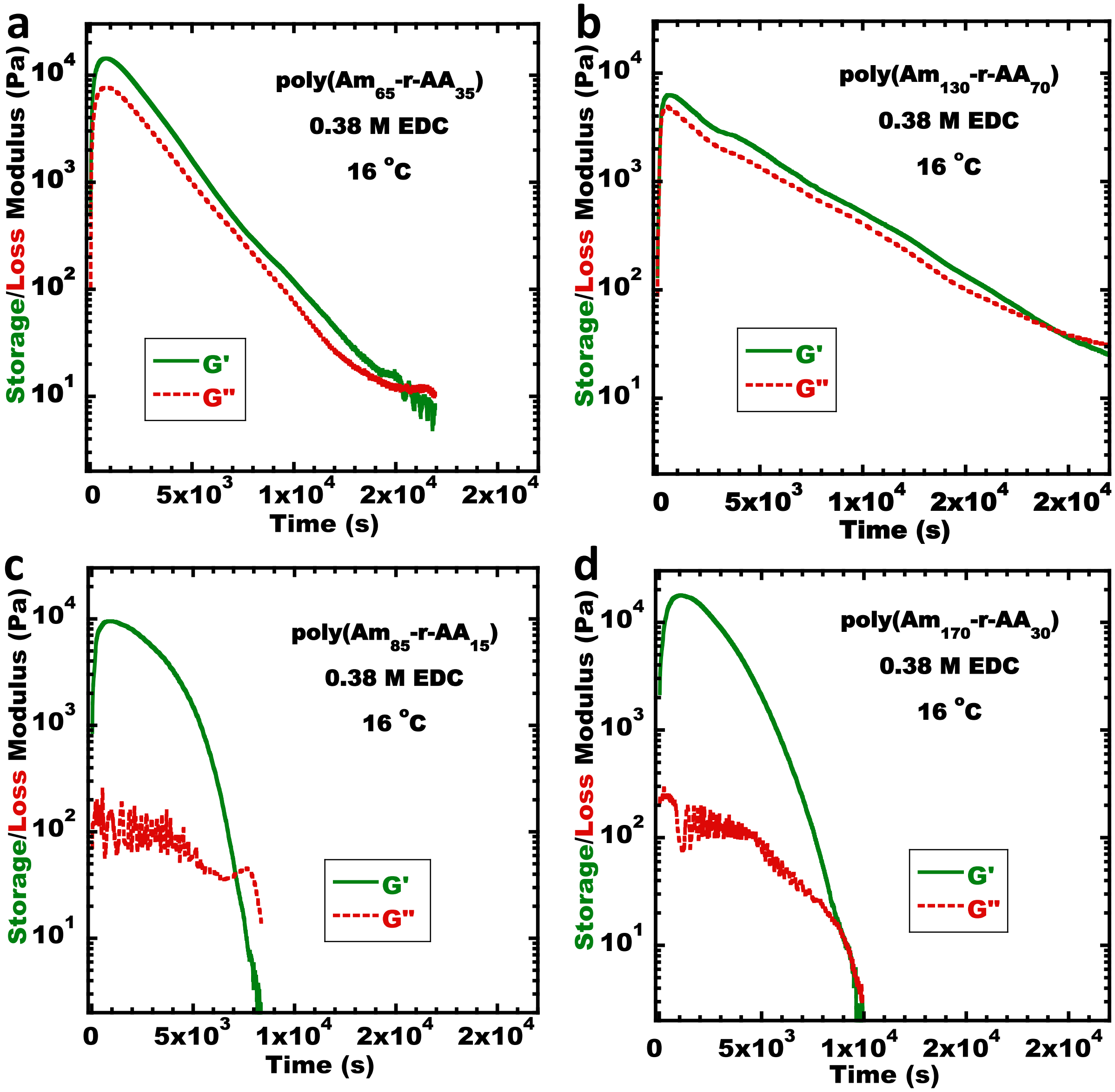


Figure 2. Typical rheology time sweeps at 16 °C using 0.38 M EDC for (a) poly(Am$_{65}$-r-AA$_{35}$), (b) poly(Am$_{130}$-r-AA$_{70}$), (c) poly(Am$_{85}$-r-AA$_{15}$), and (d) poly(Am$_{170}$-r-AA$_{30}$).

For each system, the addition of the EDC solution leads to a significant increase in G’ in the first few minutes followed by a more gradual decrease. The rapid rises in both G’ and G” in Figure 2a-d and larger value of G’ indicates that elastic response is prevalent, and a transient gel is formed. This is consistent with transient covalent crosslinking of diacids using EDC via anhydride formation.[25,26] An eventual crossover between G’ and G” was observed in all time sweep data indicating that G” becomes dominant after the EDC fuel is consumed and the transient gels return to aqueous polymer solutions. The results indicate formation of temporary anhydride crosslinks and 1-ethyl-3-(3-dimethylaminopropyl)urea (EDU) followed by eventual hydrolysis of anhydrides to the starting acids (Scheme 1). A model study was conducted to investigate the formation of *N*-acylureas as possible byproducts,[28] where propionic acid was employed as a carboxylic acid source and EDC as a chemical fuel (Scheme S1). ^{1}H and ^{13}C NMR after 3 hours of reaction time gave no evidence of N-acylurea, instead showed that the starting acids and EDU were obtained (Figures S31-32).

Fuel concentration and mechanical properties of transient gels

As shown in Figure 2, the number of acid groups in the polymers has relatively little effect on the peak G’ but does influence the times taken for the gels to undergo complete decrosslinking. Poly(Am_{65}-r-AA_{35}) and poly(Am_{130}-r-AA_{70}) both displayed higher crossover times compared to poly(Am_{85}-r-AA_{15}) and poly(Am_{170}-r-AA_{30}). Slightly lower crossover time values in poly(Am_{85}-r-AA_{15}) compared to the longer chain length variant poly(Am_{170}-r-AA_{30}) indicates that longer chains length does not have significant effect on the gel lifetime. This is also consistent with poly(Am_{130}-r-AA_{70}) having slightly higher crossover time values compared to poly(Am_{65}-r-AA_{35}), especially at 0.38 M EDC fuel concentration. Thus, the ratio of Am to AA has a significant effect on the polymer lifetime but not chain length.

The EDC concentration affects both the maximum G’ and crossover time, as shown in Figure 3. For example, the crossover time of poly(Am_{65}-r-AA_{35}) increased from ~9.0×10^{3} s to ~1.5×10^{4} s by increasing the EDC concentration from 0.12 M to 0.38 M. Similar trends were observed for poly(Am_{85}-r-rAA_{15}), poly(Am_{130}-r-rAA_{70}), and poly(Am_{170}-r-rAA_{30}) (Figure 3). This implies that larger proportions of acid groups and increased EDC concentrations result in more anhydride crosslinks, causing the progressive increase in G'_{max} shown for all polymer hydrogels in Figure 3a. This aligns with the recent literature report[26] that increasing EDC fuel leads to crosslinking of more AA and therefore increased crosslink density.

We conclude that increasing fuel concentration increases the opportunity for crosslinks in polymers with greater carboxylic acid group proportions. Note that since rheology can only measure elastically effective linkers, these data indicate that higher densities of anhydrides lead to increases in the elastically effective crosslink density. In contrast, lower carboxylic acid densities can potentially lead to more loops or other defects.[29]

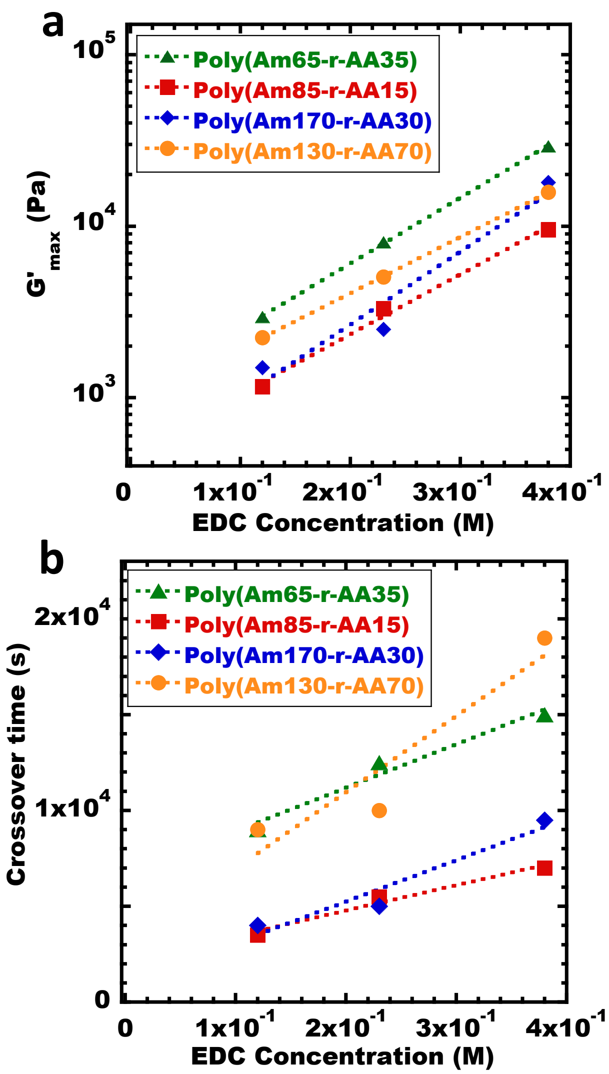


Figure 3. Dependence of (a) maximum storage modulus and (b) crossover time on EDC concentration varying from 0.12 M, 0.23 M, and 0.38 M at 16 °C.

Temperature and mechanical properties of transient gels

Understanding the temperature dependence of the mechanical properties of hydrogels is relevant to their eventual applications.[30,31] Rheological time sweep experiments were performed as described above for poly(Am$_{65}$-r-AA$_{35}$) (Figures S9-S10), poly(Am$_{130}$-r-AA$_{70}$) (Figures S13-S14), poly(Am$_{85}$-r-AA$_{15}$) (Figures S17-18), and poly(Am$_{170}$-r-AA$_{30}$) (Figures S21-22) at 10, 16, and 22 °C. These distinct temperatures were chosen to mimic useful conditions near ambient temperature. Higher temperatures led to the potential for water evaporation, complicating the analysis of time sweep rheology. Simplified plots generated from rheological data gave the dependence of G'_{max} on temperature for all four polymers (Figure 4a) under otherwise similar experimental conditions. These plots suggest that increasing experimental temperature has negligible impact on G'_{max} in the temperature range studied, although we note that there is significant scatter in the plots.

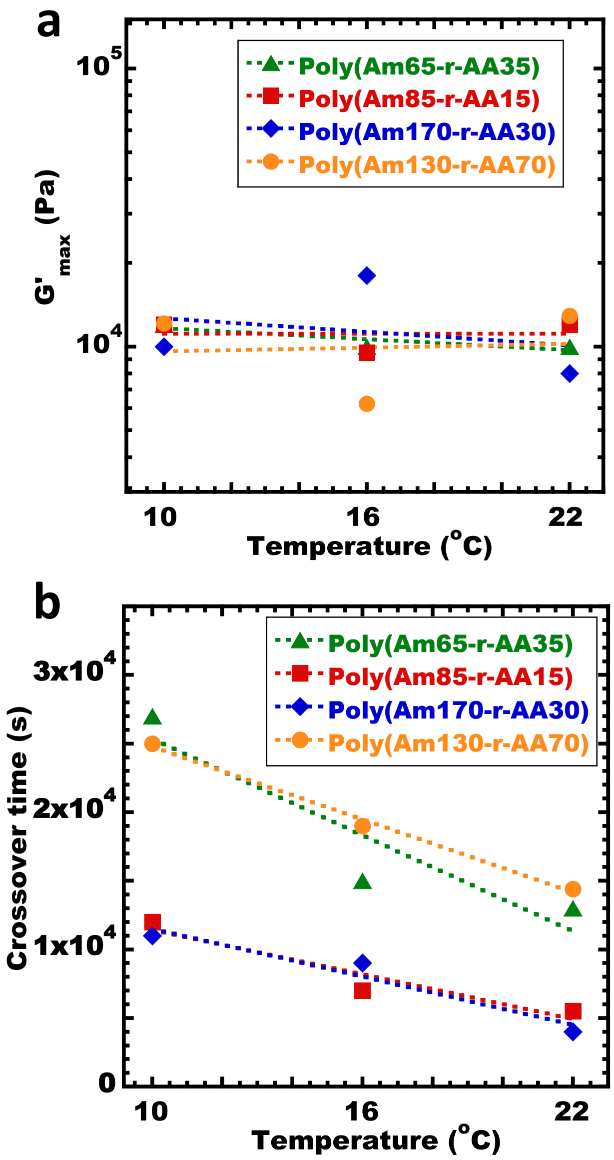


Figure 4. Effect of varied rheological experimental temperature from 10 °C, 16 °C, and 22 °C at 0.38 M EDC on (a) maximum storage modulus and (b) crossover time.

Figure 4b shows the dependence of crossover time on temperature. As expected, higher temperatures led to faster degelation times and shorter gel lifetimes. In particular, poly(Am$_{85}$-r-AA$_{15}$) and poly(Am$_{170}$-r-AA$_{30}$) had the fastest gelation and degelation profiles for all three temperatures. This is due to the smaller AA group proportion in poly(Am$_{85}$-r-AA$_{15}$) and poly(Am_{170}-r-AA_{30}) compared to poly(Am_{65}-r-AA_{35}) and poly(Am_{130}-r-AA_{70}). Hence, degelation time is more sensitive to experimental temperature than the G'$_{max}$ of transient gels. Overall, these findings suggest that, under otherwise same experimental conditions, the maximum number of anhydride crosslinks is similar with changing temperature (within the uncertainty in Figure 4a) but the gelation and especially hydrolysis kinetics may be impacted.

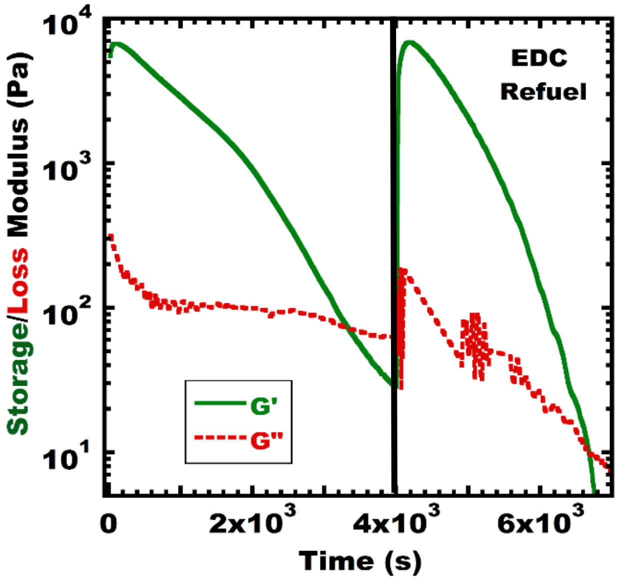


Figure 5. Rheology time sweep data at 22 °C for poly(AM_{65}-r-AA_{35}) at 10 Hz using 0.38 M EDC and refueling using 0.38 M EDC.

Impact of polymer chain length and composition on repetitive addition of chemical fuel

A key feature of chemically fueled systems is that refueling should regenerate the transient species, although the production of byproduct(s), and dilution, can negatively impact successive cycles. All four polymers were subjected to repeated doses of EDC. Rheological profiles for repeated crosslinking of poly(Am_{170}-r-AA_{30}) are shown in Figure 5a. After the first anhydride crosslinking, a subsequent addition of EDC to the decrosslinked polymer led to the formation of new anhydride crosslinks. To verify if using comparable EDC concentration during a refuel will give similar G'_{max} in both the first and second cycles, we ran rheological time sweeps of poly(Am_{65}-r-AA_{35}) first using 0.38 M EDC and a refuel experiment using the same final EDC concentration (Figure 5b). Using similar EDC concentration in both cycle 1 and 2 resulted in a similar G'_{max} which is in excellent agreement with the recent findings of Wang et al.[26] In contrast, when using 0.38 M EDC for the first fueling and the same number of moles of EDC, but at a lower concentration of 0.30 M for the second fueling , a decline in G'_{max} was observed in the second (refueled) cycle as seen in Figure S26. This is likely due to overall lower EDC concentration and further dilution of the polymer solution in the refueled system.

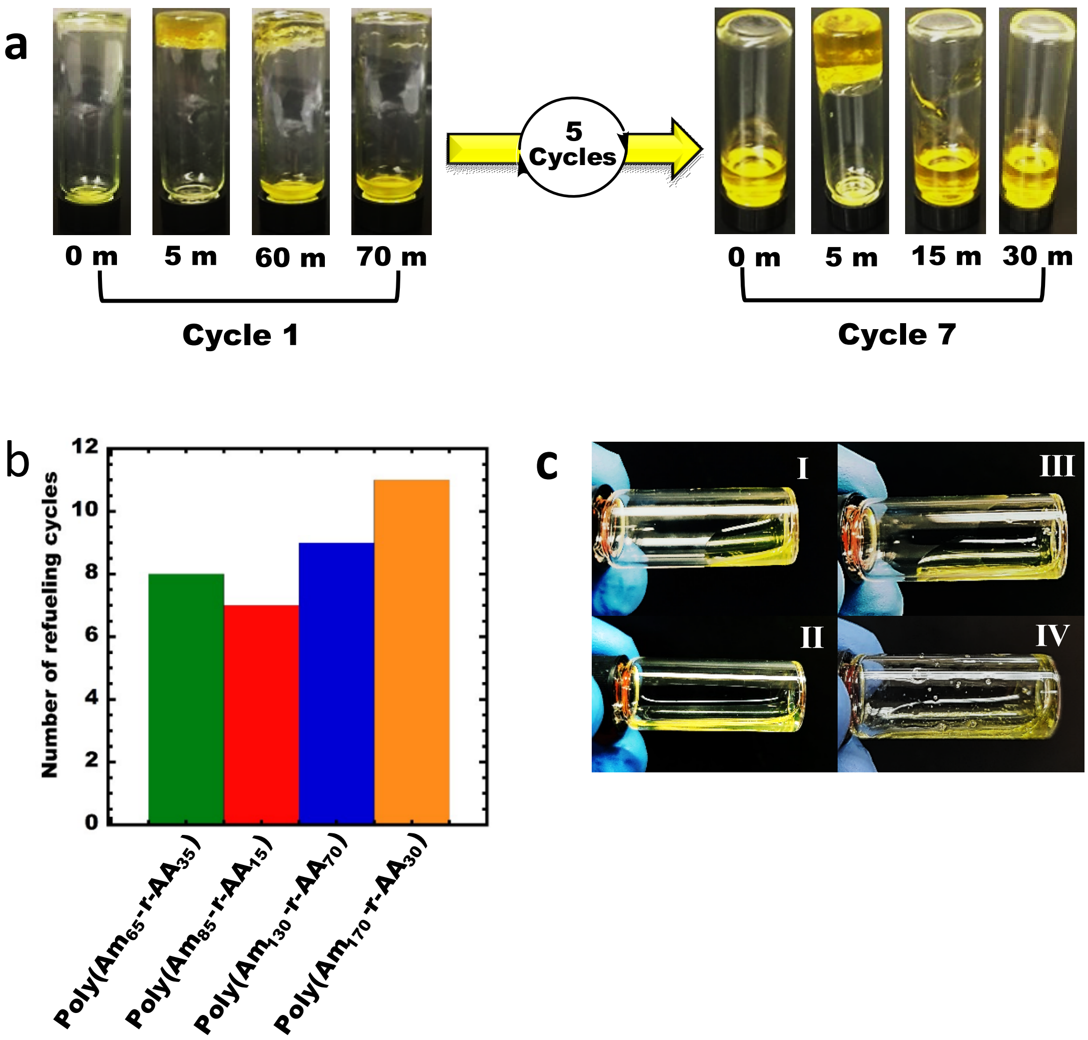


Figure 6. (a) Typical inverted vial test for poly(Am$_{85}$-r-AA$_{15}$) upon treatment with EDC seven times and (b) number of refuels for all four polymer systems upon treatment with EDC at room temperature (c) Inverted vial test of poly(Am$_{65}$-r-AA$_{35}$) showing (i) polymer solution (ii) polymer solution after addition of stoichiometric EDU amount (iii) polymer solution before addition of EDU (iv) polymer solution after addition of excess EDU

Inverted vial tests were used to quantify the possible number of refueling cycles these systems can undergo before crosslinking is inhibited by EDU build-up and polymer dilution (Figure 6a-b). A control experiment where EDU was employed as a crosslinking fuel instead of

EDC was carried out at room temperature using an inverted vial test (Figure 6c). Adding stoichiometric amount of EDU solution to poly(Am_{65}-r-AA_{35}) resulted in a free-flowing solution as shown in Figure 6c(ii) and addition of excess EDU to a fresh solution of poly(Am_{65}-r-AA_{35}) nonetheless gave yet another free-flowing solution as shown in Figure 6c(iv). This confirms that EDU does not contribute to the formation of crosslinks in these systems.

All four polymers undergo multiple refuel cycles. For example, poly(Am_{130}-r-AA_{70}) endures one more cycle of EDC refuel (Figure S27) than poly(Am_{65}-r-AA_{35}). The disparity in number of refueling cycles between poly(Am_{130}-r-AA_{70}) and poly(Am_{170}-r-AA_{30}) is likely due to more acid groups in the former making it more resilient to multiple refuel cycles. Therefore, more acids lead to more crosslinks and consequently more robust gelation. However, more crosslinks imply higher build-up of EDU byproduct, the effect of which on these systems is not currently well-understood. It is important to note that EDU is always present after transient gels are hydrolyzed back to starting acids. These effects are in fact not observed since multiple refuels with EDC solution resulted in formation of transiently crosslinked gels and a subsequent complete degelation leading to free polymer solution as highlighted in Figure 6a-b. Interestingly, poly(Am_{85}-r-AA_{15}) can be crosslinked via EDC refueling for a total of seven cycles (Figure 6a) and the longer chain length variant poly(Am_{170}-r-AA_{30}) can undergo eleven refuel cycles (Figure S28). This difference suggests that longer polymer chain length allows for higher crosslinking opportunities and consequently greater resilience in the system.

Furthermore, to probe the possible contribution of intra-chain crosslinking to trends observed, a time sweep was performed on diluted poly(Am_{65}-r-AA_{35}) solution where the polymer was dissolved in more water by a ratio of 1:3 (polymer to water) thus creating higher opportunity for intra-chain crosslinking on treatment with EDC solution. It was observed that the G'$_{max}$ obtained from crosslinking a dilute poly(Am$_{65}$-r-AA$_{35}$) solution was lower (Figure S23) compared to the same material with polymer to water ratio of 2:3 (Figure S10). While this finding logically confirms that intra-chain crosslinking is a possible contributor to the gelation observed, it is also plausible to add that dilution plays a major role in the mechanical properties of these systems. Additionally, after every refuel cycle, a slight decrease in pH was observed (Table S1) and this is because crosslinking experiments were performed using pure water. Since a buffer solution was not used, pH of a completely recovered polymer solution is lower compared to the previous polymer solution prior to refuel. This gives better insight into why shorter gel lifetime is observed after several refuel cycles. It is noteworthy that EDU is a very weak acid and is contributing to the trend observed in Table S1 since total amount of EDU increases after every refuel cycle.

Overall Trends

The overall trends in these materials and their sensitivity to external parameters is summarized below. Within the studied conditions, the most important parameter that determines the properties of these transiently crosslinked networks is the concentration of EDC, closely followed by the temperature. Higher EDC concentrations allow the formation of more anhydride crosslinks, leading to higher storage moduli at the peak and networks with longer lifetimes, as defined by the time needed to cross from a viscoelastic solid (G'>G") to a rheological liquid (G">G'). Similarly, temperature has a significant impact on the lifetimes of the gels, but a relatively small impact on G'$_{max}$.

These data suggest that the peak storage modulus is primarily dictated by the EDC concentration and number of potential anhydrides, not the rate of anhydride bond formation. However, the transient network lifetime is not only dictated by the number of crosslinks but also the anhydride hydrolysis rate. Higher EDC loading gives a higher density of anhydride crosslinks, extending the time needed to hydrolyze enough linkers to form a liquid. Lower temperatures decrease the hydrolysis rate, extending the lifetime of the network. The time from the peak of the storage modulus (t_{peak}) to the delegation or crossover time for the network (t_{cross}) has the system dominated by anhydride hydrolysis. Therefore, this time should be inversely proportional to the hydrolysis rate coefficient.

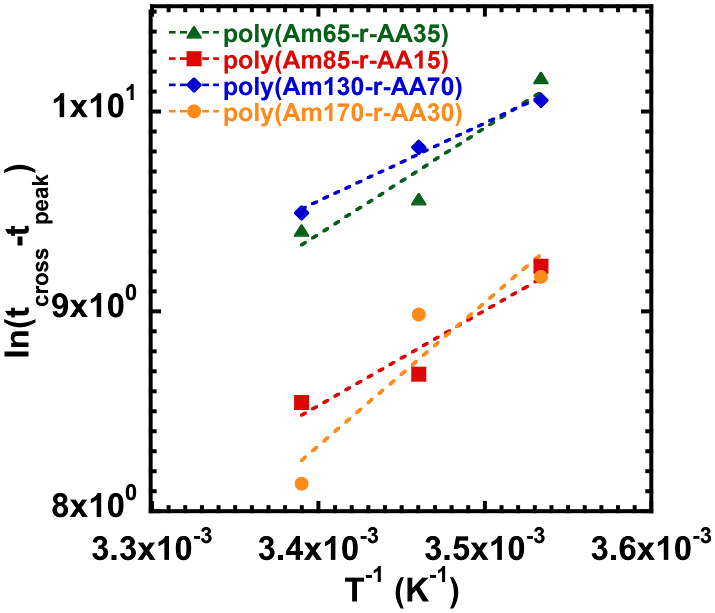


Figure 7. Arrhenius analysis of t_{cross} -t_{peak} against 1/T.

Figure 7 shows Arrhenius analysis of t_{cross} -t_{peak} against 1/T. The estimated activation energies from the Arrhenius analysis are 44 kJ/mol for poly(Am_{65}-r-AA_{35}), 33 kJ/mol for poly(Am_{130}-r-AA_{70}), 35 kJ/mol for poly(Am_{85}-r-AA_{15}), and 60 kJ/mol for poly(Am_{130}-r-AA_{70}), giving a mean activation energy of 43 kJ/mol with a standard error of 7 kJ/mol. This sits comfortably within the measured activation energy of aliphatic anhydride hydrolysis; for instance, acetic anhydride has reported activation energies in the range of 40-50 kJ/mol.[32–34]

Interestingly, the polymer structure has a relatively smaller impact on the transient properties of the network. The data suggest that temperature and EDC fuel concentration play the primary role in modulating the lifetime and peak strength properties of the gels, implying that future predictive design and synthesis of fueled polymeric systems will largely depend on fuel concentration and temperature. However, the polymer composition does have a measurable effect, especially on the lifetime of the network, with systems having a higher potential crosslink density, with more AA, having generally longer lifetimes. The higher AA content has a smaller impact on the peak storage modulus at a constant EDC loading, although some increase is observed even at the same EDC concentration with more AA along the backbone. This is most likely due to the higher content of potential crosslinks from AA favoring elastically effective linkers; at lower AA loadings processes such as direct hydrolysis of the EDC-carboxylic acid intermediates or elastically ineffective loop formation that does not contribute to gelation will be more prevalent. Note that substantially higher storage moduli were possible with higher AA loadings in earlier work,[25] when even higher EDC concentrations were used. The effect of chain length on material properties was relatively small, within the scope of the materials studied. The overall trend observed specifically for the effect of EDC concentration on the mechanical properties of the transient gels is in excellent agreement with a recent report by Mathers et al.,[35] who reported the dependence of spinodal decomposition of poly(norbornene dicarboxylic acid) on EDC fuel concentration and pH.

Additionally, RAFT polymerization was used to synthesize poly($DMAm_{70}$-r-AA_{30}) where N,N-dimethylacrylamide (DMAm) was used in lieu of Am. A dispersity Đ of 1.20 (Figure S5) was obtained after characterization using size exclusion chromatography. Figure S24 shows the rheological time sweep of the poly($DMAm_{70}$-r-AA_{30}) material. The general trends and features of the DMAm-based material's rheological response to fueling was similar to that of the Am material. DMAm is not able to react with anhydrides in a permanent manner. Figure S25 further shows an EDC-refueled rheological time sweep experiment for the poly($DMAm_{70}$-r-AA_{30}) material. After the first injection of the EDC solution the gel completely hydrolyzes back to the starting acids. The resulting polymer solution was again refueled using a comparable EDC concentration to the first injection and the gel again hydrolyzes over time, similar to the Am-based networks. We found that poly($DMAm_{70}$-r-AA_{30}) could undergo up to seven refuel cycles in an inverted vial test (Figure S29), confirming the absence of permanent crosslinks. The similar responses of the DMAm and Am based networks, as well as the IR spectra, confirms that the dominant role of EDC is to generate anhydrides rather than imides,[26] without the formation of permanent crosslinks.

Conclusion and Outlook

RAFT polymerization was used to synthesize four random copolymers of Am and AA with different chemical compositions and chain lengths. The functionality and architecture of copolymers could be modulated due to the robustness of RAFT polymerization.[27] Hydrophilic poly(Am_{65}-r-AA_{35}), poly(Am_{130}-r-AA_{70}), poly(Am_{85}-r-AA_{15}), and poly(Am_{170}-r-AA_{30}) were prepared using large proportions of water-soluble acrylamide (Am) monomer. EDC solutions were employed as chemical fuels to covalently crosslink polymer solutions via formation of transient carboxylic acid anhydrides. Rheological experiments were used to characterize the mechanical properties of the resulting gels. The impact of fuel concentration, temperature, polymer chain length and composition on transient mechanical properties were investigated based on the conversion of pendant carboxylic acids to anhydrides and subsequent hydrolysis to starting carboxylic acids. Varying EDC concentration indicated an increase in peak G’ and crossover points as fuel concentration increases. Interestingly, higher temperatures decreased the crossover points but did not significantly impact the peak G’ within the scope of experimental conditions covered in this investigation. This discovery is especially relevant for applications where gelation time can be modulated by varying either fuel concentration or polymer composition. Future endeavors will focus on decoupling the specific effects of how EDU build-up and polymer dilution in refueled systems affect gelation and decrosslinking.

Conflict of Interest

The authors declare no competing interest.

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Supporting Information Available

Supporting information includes: Experimental methods and details of characterization techniques, characterization data of polymers, supporting rheological characterization data of polymers with different EDC concentrations and temperatures, pH measurement upon multiple refueling, images of inverted vial tests for refueling, NMR data for small molecule studies of model compounds.

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