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Chemically Fueled Covalent Crosslinking of Polymer Materials

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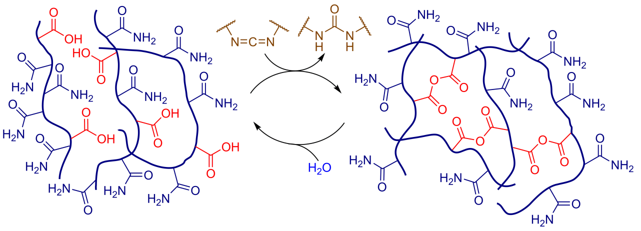
Transiently crosslinked dynamic polymer networks are developed, using carbodiimide hydration to link carboxylic acids as anhydrides. From aqueous polymer solutions, non-equilibrium hydrogels are transiently formed, which dissolve upon anhydride hydrolysis. The materials can be refueled using a subsequent injection of carbodiimide. The gels exhibit higher storage moduli compared to transient supramolecular gels as a result of their covalent crosslinks.

Chemically fueled, out-of-equilibrium processes are central to much of the rich behavior of biochemical systems.[1] Accordingly, dissipative processes are attracting increasing attention in the context of autonomous nonbiological self-assembly and related processes.[2, 3] Recent examples include pioneering studies on supramolecular gelation,[4, 5] supramolecular hosts,[6] molecular motors,[7] and aggregates,[8, 9] all of which exhibit temporally controlled behavior that is impossible at equilibrium (which, by definition, is unchanging). In many of these systems, the fuel is covalently bound to the substrate as an intermediate step in its decomposition, inducing transient change in the substrate's properties. Recently, the Boekhoven group[10-12] and our group[13] have investigated the hydration of carbodiimides as a fuel reaction for chemical assembly. When added to aqueous carboxylic acids, carbodiimides cause the rapid formation of carboxylic anhydrides, which subsequently hydrolyze back to the starting acids. An important feature of this process is that the transiently formed species do not incorporate any structural element of the fuel; thus, unlike many other systems, these carbodiimide-fueled processes can be used to create intramolecular bonds and couple complex species.

Here, we show that carbodiimides can be used to crosslink polymer chains with pendant acids via the formation of anhydrides, giving transient crosslinked polymer networks. Our design, shown in Scheme 1, is based on simple acrylamide (Am) and acrylic acid (AA) copolymers. The mechanical properties of the resulting materials have been characterized, showing gels that eventually decompose to liquids. As the gels are the result of covalent crosslinks, they are stronger than other currently reported chemically fueled systems based on non-covalent self-assembly. The polymers can undergo subsequent refueling cycles to regenerate the gel state.



Scheme 1: EDC fueled crosslinking of Am-AA co-polymers

We used reversible addition-fragmentation chain transfer (RAFT) polymerization[14] to make a short Am-containing oligomer with one carboxylic acid at the α-terminus (oligo(Am)COOH, Figure 1a), with a mean length of 7–8 units of Am as determined by ESI-MS (Figure S1). This material was used as a model to test for anhydride bond formation between polymer chains, ensuring, for example, that there is no interference from the 1° amides. The system was monitored with IR spectroscopy. Thus, the treatment of an aqueous solution of oligo(Am)COOH at 0 °C with ~0.7 eq of the common reagent EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) resulted in the appearance of an IR band at 1809 cm^{-1} that is characteristic of the symmetric carbonyl stretching mode of anhydrides (Figure 1, Figure S6), in good agreement with similar systems.[13] The asymmetric stretching mode, which is usually weaker, is presumably buried under bands assigned to the EDC and unreacted oligo(Am)COOH. In this system, which shows no

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Electronic Supplementary Information (ESI) available: [Experimental details, supplemental characterization of polymers, additional rheological and infrared data]. See DOI: 10.1039/x0xx00000x indication of gelation over the course of the experiment, the maximum concentration of anhydride is achieved after roughly 2 min, followed by slow decomposition via a first-order process with a rate coefficient of 1.8×10^{-4} s^{-1}. We conclude that anhydride formation and hydrolysis should be effective in Am/AA co-polymers.

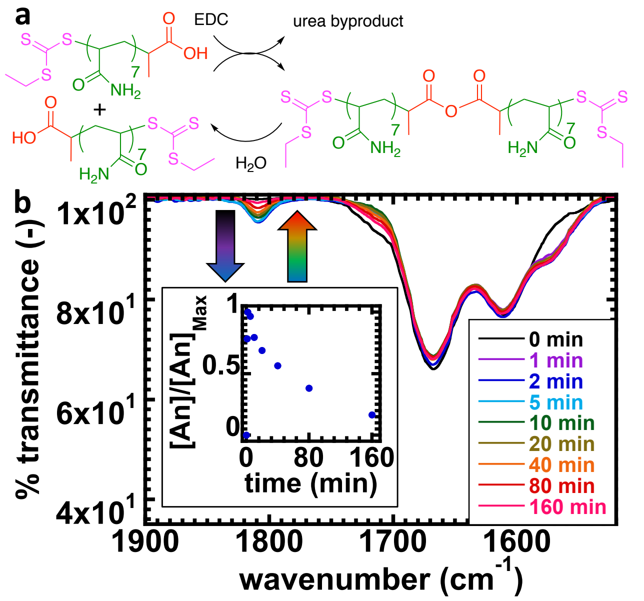

Figure 1. (a) Anhydride bond formation in oligo(Am)COOH. (b) IR spectra of oligo(Am)COOH collected after treatment with EDC (0.6 mL, 0.5 g/mL oligo(Am)COOH, ~0.7 eq EDC, 0 °C); inset shows variation of relative anhydride concentration over time.

We then explored dynamic crosslinking in polymer systems. Two copolymers of the same length (~100 units) with different ratios of Am to AA were synthesized by RAFT polymerization,[14] one with the molar ratio CTA:Am:AA of 1:85:15 (poly(Am85-r-AA15), Figure 2a) and one of 1:65:35 (poly(Am65-r-AA35)). These were characterized by MALDI-TOF mass spectrometry (Figures S2-3). On treatment with EDC, aqueous solutions of the polymers exhibited transient gelation according to inverted vial tests (Figure 2). The addition of 0.5 eq of EDC (with respect to the net number of acid groups) at 4 °C gave the rapid formation of a gel, which then liquified over time. The complete de-crosslinking time was sensitive to the proportion of acid groups in the polymers: 9.5 h for poly(Am65-r-AA35) but only 3.5 h for poly(Am85-r-AA15). Taken together, these results suggest that gelation results from the formation of temporary anhydride crosslinks, with increased opportunities for crosslinks in the polymer poly(Am65-r-AA35) with a greater proportion of carboxylic acid groups.

Rheological studies were performed on the transient gels to quantify the changes in material properties. The time evolution of the crosslinking and hydrolysis processes for the poly(Am85-r-AA15) and poly(Am65-r-AA35) systems were monitored via the storage (*G′*) and loss moduli (*G″*) at 4 °C (Figure 3). Polymer solutions were injected onto the rheometer followed by EDC solutions; the motion of the rheometer was used to mix these solutions. Immediately after injection of the EDC, for both polymers there is a significant increase in *G′*, consistent with the formation of anhydride crosslinks. Importantly, in both systems the *G′* exceeds *G″* for several hours, indicating a solid or gel-like material. Control experiments showed time-independent rheological properties of the polymer solution in the absence of EDC (Figure S6); no meaningful rheological data could be obtained for the EDC solution alone.

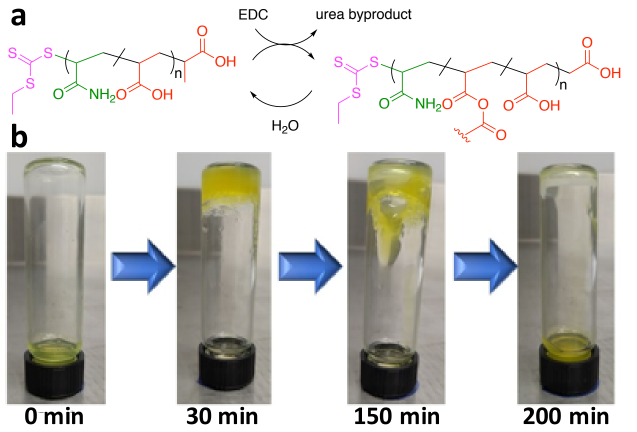

Figure 2. a) Representation of the cross-linking between polymer chains due to EDC fueled anhydride formation and decomposition in water. b) Inverted vial tests for poly(Am85-r-AA15) upon treatment with EDC (0.8 mL, 0.5 g/mL polymer, 0.5 eq EDC).

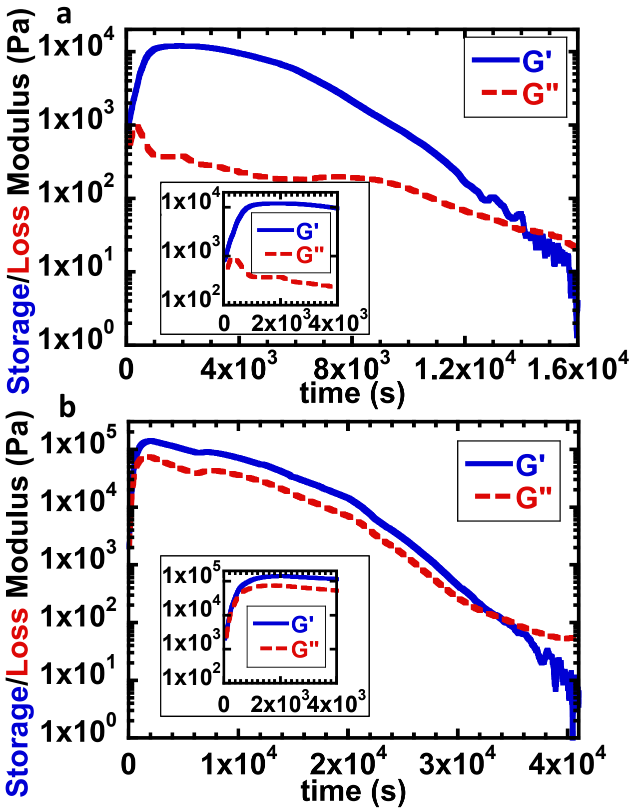

Figure 3: Rheology time sweeps of polymer solutions at 4 °C a) Time sweep at 4 °C of *G′* and *G″* for poly(Am85-r-AA15) at 10 Hz. b) Time sweep at 4 °C for the poly(Am65-r-AA35) at 10Hz. Insets show the first 4000 s of each system.

The profiles in Figure 3 have similar trends in the storage and loss moduli but different maxima and timescales due to the various ratios of Am to AA in each polymer chain. Initially, *G′* and *G″* in both cases rise quickly (insets in Figure 3). This feature suggests that the elastic response prevails and the gel is rapidly formed, corresponding with the principle that EDC reacts with pendant carboxylic acids generating anhydride crosslinks. Subsequently, the storage moduli reach their maximum values, indicating that the polymers reach a maximum crosslink density. After around 2000 s, the storage moduli continuously decrease as the anhydrides are hydrolyzed. The relatively flat, slightly curved regions in these plots following the maxima suggests that the EDC continues to be consumed over the course of approximately 1×10^{4} s and 2×10^{4} s for poly(Am85-r-AA15) and poly(Am65-r-AA35), respectively, with anhydride crosslink regeneration competing with hydrolysis. The steeper linear decay of log(*G′*) with time at the ends of the plots, after all the EDC has been consumed, indicates that the anhydrides undergo first-order decomposition. Assuming the storage modulus is proportional to the crosslink density, we estimate rate coefficients of $2.4\times10^{-4}\ s^{-1}$ for the poly(Am65-r-AA35) and $6.0\times10^{-4}\ s^{-1}$ for the poly(Am85-r-AA15) systems. These rate coefficients of hydrolysis are in the same order of magnitude as the hydrolysis rate for dimers of oligo(Am)COOH under similar conditions, indicating a good match between the chemistry in the model experiment and polymer crosslinking systems.

Eventually, in both systems there is a crossover point between *G′* and *G″*, beyond which the loss modulus *G″* exceeds the storage modulus *G′*. In this region, the viscosity dominates, suggesting that essentially all of the anhydride crosslinks have been hydrolyzed, giving a dilute polymer solution. We take the crossover time as the point of degelation. From Figure 3a and 3b, poly(Am65-r-AA35) needs 32,000–35,000 s (9–10 h) to decrosslink, whereas the poly(Am85-r-AA15) requires just 12,000–15,000 s (3–4 h) to decrosslink, in excellent agreement with the inverted vial tests. The frequency of oscillation of the rheometer was verified to have no impact on the hydrolysis time frame (Figure S7). As anticipated, higher temperatures, such as room temperature, led to faster gelation and hydrolysis kinetics, with the poly(Am85-r-AA15) system showing essentially complete degelation in 55 min at room temperature, compared to over 150 min at 4 °C. (Figure S8).

These results are consistent with the intuitive picture that increasing acrylic acid content in the polymer should produce more anhydride bonds, forming a network with a much tighter structure. As the crosslink density increases, it takes more time to break all the crosslinks needed to create a free flowing polymer solution. We also note that the poly(Am65-r-AA35) system has a higher peak storage modulus than the poly(Am85-r-AA15) system, again presumably because it achieves a higher crosslink density. Interestingly, the loss modulus of the poly(Am65-r-AA35) is also higher than the poly(Am85-r-AA15), which could be due to degenerate exchange of anhydrides and free carboxylic acid groups,[15] which would be facilitated at higher AA loadings.

As shown in Figure 4, crosslinking can be repeated by subsequent addition of additional EDC. In this refueling experiment with poly(Am85-r-AA15), after the crossover point (3.5 h) an additional 0.5 eq of EDC was injected on the top of reactant residue. The subsequent increase and decrease of the *G′* and *G″* demonstrates that multiple gelations and hydrolysis processes can be carried out in these systems. In the second cycle, the peak storage modulus is lower and there is a much shorter flattened period immediately following the maximum, suggesting that the fueling by EDC becomes less efficient and/or the structure of the network is disrupted. In this specific system, this likely results from dilution and the buildup of the urea byproduct. The overall rate of anhydride hydrolysis is, however, qualitatively similar in both cycles. Repeated inverted vial tests on poly(Am85-r-AA15) system were also performed to determine when the dilution and the buildup of the urea could inhibit the gelation. We then surprisingly found out eight cycles of EDC fuelled gelation followed by anhydride hydrolysis could go be performed, as shown in Figure S9.

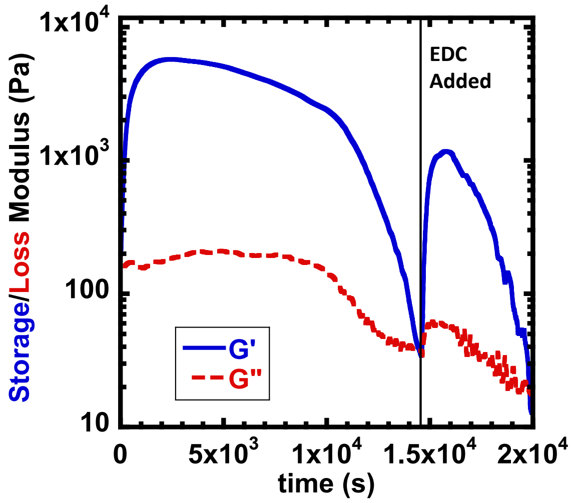

Figure 4. Time sweep at 4 °C for the poly(AM85-r-AA15) at 10 Hz with repetitive addition of EDC.

The mechanical properties of hydrogels are often tested between 10–80 °C, especially relevant for biological applications.[16, 17] The experiments described above were carried out at 4 °C for experimental convenience, although our system does undergo transient gelation at ambient temperature. We note that this system exhibits significantly higher maximum storage and loss moduli than many other reported dynamic hydrogels at 4 °C,[18-20] indicating that the mechanical properties achieved here are application-relevant. Potential uses of non-equilibrium[21, 22] hydrogel materials are envisioned where transient strength, stiffness or elasticity are needed.[23, 24] For instance, if a material is only to be temporarily subject to high-stress environments (e.g., impact absorption), it could be beneficial to have it controllably become stiffer or stronger before returning to a more flexible state. Similarly, temporary stiffness has applications in biomedical settings, where a high stiffness is useful during surgery but not longer-term.[24]

Previously reported chemically fueled, transient hydrogel systems function on the principle that the fuel activates the precursor molecules toward non-covalent interactions. This activation can be achieved, for example, by quenching electrostatic repulsion[5, 10] or by appending peptides that promote self-assembly.[25] In these cases, after the self-assembled gel is formed, the constituent molecules decompose to thermodynamic products (either starting materials or something new), resulting in the dissolution of the gel network. Transient gels based on temporary changes in pH have also been reported. For example, biocatalytically controlled acidity via the hydrolysis of urea has been shown to control the lifetime of a peptide gelator,[26] and the oxidation of sucrose has been used to form a peptide hydrogel via the production of carbonic acid.[27] Disulfide reduction has been used to decompose a transient hydrogel assembled following protonation of the precursors.[28] Unlike these other systems, our approach forms a covalent polymer network, rather than supramolecular assembly. Consequently, the hydrogels are much stiffer, with maximum storage moduli (*G′*) at least an order of magnitude higher than those in other systems.[5, 10, 25, 26, 28] This strategy is only possible because the carbodiimide is used exclusively as an energy source for transient covalent bond formation, and not for structural modification of the activated state.[13]

In addition, advances in RAFT polymerization,[14] enable the polymer architecture and functionality to be easily tuned. This implies that targeted transient mechanical properties could be easily and predictably achieved by varying the underlying structure of the polymer. Simply varying the pendant carboxylic acid density when comparing the poly(Am65-r-AA35) and poly(Am85-r-AA15) materials led to a predictable increase in the storage modulus with higher crosslink density. Future work will explore the impact of reaction conditions on the transient mechanical properties of these materials.

In conclusion, chemically fueled and transiently crosslinked polymer materials were synthesized based on the conversion of carboxylic acids to anhydrides. The covalent crosslinking was fueled using EDC, with return to the original solution by hydrolysis of the transient anhydrides. Small molecule, macroscopic and rheological properties of this reaction were investigated, showing that this is a powerful platform for materials which need temporary changes in mechanical properties.

Conflicts of interest

There are no conflicts to declare.

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