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Thermo-responsive self-immolative nanoassemblies: Direct and indirect triggering

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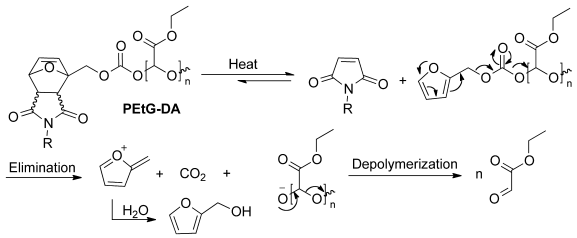
A thermo-responsive end-cap based on a retro-Diels-Alder and subsequent furan elimination reaction was developed. It was used to cap poly(ethyl glyoxylate), allowing end-to-end depolymerization upon thermal triggering. Using block copolymers, thermo-responsive micelles and vesicles were prepared and shown to disassemble upon heating. Thermal degradation could also be triggered indirectly by magnetic field hyperthermia after incorporation of iron oxide nanoparticles into the assemblies.

Nanoassemblies are of great interest due to their ability to mimic biological nanostructures and their capacity to perform diverse functions such as controlled release[1], catalysis,[2] and templating of inorganic structures.[3] Stimuli-responsive macromolecular assemblies have attracted significant attention,[4] due to their ability to undergo morphological or functional changes in response to stimuli. Assemblies responsive to stimuli such as light,[5] heat,[6] and pH[7] have been reported. Heat is particularly attractive as it can be easily applied either directly with good spatiotemporal control, or indirectly through photothermal[8] or magnetothermal effects[9]. Many examples of thermo-responsive polymer assemblies employing polymers such as poly(*N*-isopropylacrylamide) that undergo solubility changes in response to temperature have been reported.[10] There are very few examples involving thermally-initiated bond cleavage.[11]

Over the last decade, a new class of stimuli-responsive polymers, often termed self-immolative polymers (SIPs), has been developed. These polymers can be triggered to degrade from end-to-end upon the cleavage of a stimulus-responsive end-cap from the terminus.[12] The propagating depolymerization mechanism amplifies the stimulus-mediated event. Various backbones including polycarbamates,[13] poly(benzyl ether)s,[14] and polyaldehydes[15-17] have been reported. A single SIP backbone can respond to different signals by simply changing the end-cap.[18] SIPs responsive to stimuli including light,[19] oxidizing or reducing agents,[20] and enzymes[13] have been reported. Assemblies such as vesicles[19] and micelles[21] have been prepared from SIPs and shown to undergo disintegration and payload release in response to stimuli.



Scheme 1. Proposed end-cap cleavage and depolymerization mechanism of PEtG end-capped with a DA adduct (**PEtG-DA**).

Thus far, one thermo-responsive SIP end-cap was reported.[22] This 1,2-oxazine end-cap underwent cycloreversion to an unstable carbamoylnitroso intermediate that hydrolyzed and decarboxylated to initiate depolymerization. However, the end-capping was challenging, as it required generation of the unstable nitroso species to perform the cycloaddition. Furthermore, the end-cap cleavage and depolymerization were very slow, occurring over tens of days. Here we report that simple Diels-Alder (DA) adducts of furan and maleimides can serve as thermo-responsive end-caps. Upon heating, a retro-Diels Alder reaction occurs, revealing a furan.[23] Based on the known instability of similarly substituted furan derivatives,[24] it is proposed that the released furfuryl carbonate undergoes an elimination reaction to release an uncapped poly(ethyl glyoxylate) (PEtG) SIP for depolymerization (Scheme 1). Exploiting the easy synthetic modification of this end-cap, amphiphilic block copolymers can be prepared, and self-assembled into thermo-responsive vesicles and micelles. Furthermore, indirect triggering of SIP nanoas-

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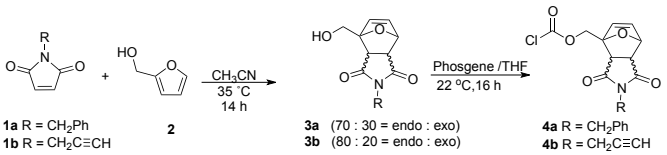
semblies is demonstrated for the first time using the magneto-thermal effect with iron oxide nanoparticles (IONPs) under an alternating magnetic field.

To test the proposed concept, we capped PEtG with the DA adduct and evaluated its thermo-responsiveness. First, *N*-benzylmaleimide (**1a**) was reacted with furfuryl alcohol (**2**) in a DA reaction to obtain **3a** as a 70:30 mixture of *endo*:*exo* diastereomers (Scheme 2). **3a** was then activated as the chloroformate **4a**. Polymerization of ethyl glyoxylate was conducted at -20 °C,[15] and the resulting PEtG was end-capped *in situ* by reaction with **4a** to afford **PEtG-DA-Bn** (Scheme 3). The number average molecular weight (M_n) was 33 kg/mol and the dispersity (*Đ*) was 1.8 based on size exclusion chromatography (SEC). Successful end-capping was confirmed by thermogravimetric analysis as the capped polymer was stable to > 165 °C in the solid state, whereas uncapped polymer degraded below 100 °C [15] (Fig. S28, Table S1).

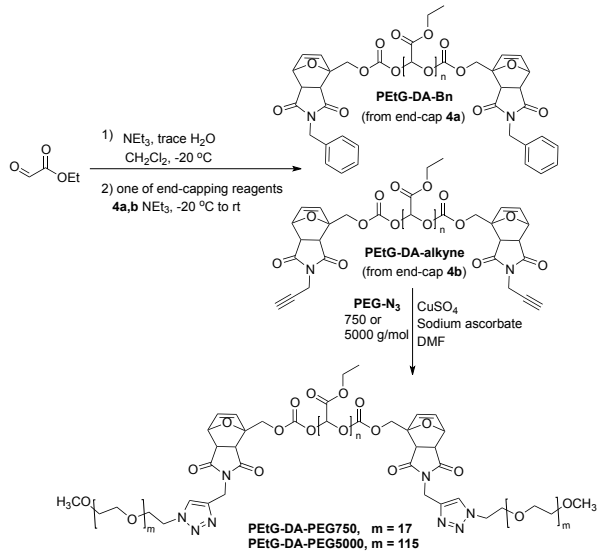
To test the thermo-responsiveness of **PEtG-DA-Bn**, the polymer was dissolved in 9:1 CD_3CN:D_2O and incubated at different temperatures. A non-responsive PEtG end-capped by benzyl chloroformate (**PEtG-control**, Scheme S1)[25] was also examined. The depolymerization was monitored by ^{1}H NMR spectroscopy. Initially, the spectrum of **PEtG-DA-Bn** consisted of broad peaks attributable to the polymer (Fig. 1). When the polymer was heated, a sharp peak at 5.1 ppm corresponding to the depolymerization product ethyl glyoxylate hydrate (EtGH) emerged. The extent of depolymerization was quantified based on the integrations of polymer and EtGH peaks. Over 24 h, 85% depolymerization occurred at 75 °C, 53 % at 60 °C, and 8% at 40 °C (Figs. 2a, S22). In contrast, **PEtG-DA-Bn** at 22 °C and **PEtG-control** at 75 °C underwent less than 10% depolymerization even after 4 days (Figs. 2a, S21, S23). Overall, the rate of end-cap cleavage and depolymerization was much faster for **PEtG-DA-Bn** than that of the previously reported oxazine system,[22] which required several days, resulting in substantial background degradation of the controls. Thus, DA adducts can provide rapid and selective thermo-responsive degradation.

To prepare thermally-responsive assemblies from amphiphilic block copolymers, end-cap **4b** with a propargyl group was synthesized (Scheme 2). **4b** was installed on PEtG to afford **PEtG-DA-alkyne** with an M_n of 63 kg/mol and a *Đ* of 2.0 (Scheme 3). This polymer underwent depolymerization at rates very similar to those of **PEtG-DA-Bn** (Figs. 2a, S24-25). It was then coupled with two different lengths of azide-functionalized poly(ethylene glycol) (PEG-N_3; 750 and 5000 g/mol) to give copolymers **PEtG-DA-PEG750** and **PEtG-DA-PEG5000**. The success of the couplings was confirmed by SEC (Figs. S30-31) and ^{1}H NMR and ^{13}C NMR spectroscopy (Figs. S15-18).

Assemblies were prepared by nanoprecipitation involving the addition of H_2O into THF for **PEtG-DA-PEG750** and DMSO into H_2O for **PEtG-DA-PEG5000**. Based on dynamic light scattering (DLS), the Z-average diameters of the assemblies were 480 ± 80 nm for **PEtG-DA-PEG750** and 87 ± 3 nm for **PEtG-DA-PEG5000** (Figs. S33-34). Transmission electron microscopy (TEM) showed that **PEtG-DA-PEG750** formed vesicles, while **PEtG-DA-PEG5000** formed solid spherical nanoparticles (Fig. 3).



Scheme 2. Synthesis of end-caps **4a** and **4b**.



Scheme 3. Synthesis of end-capped PEtG and its block copolymers.

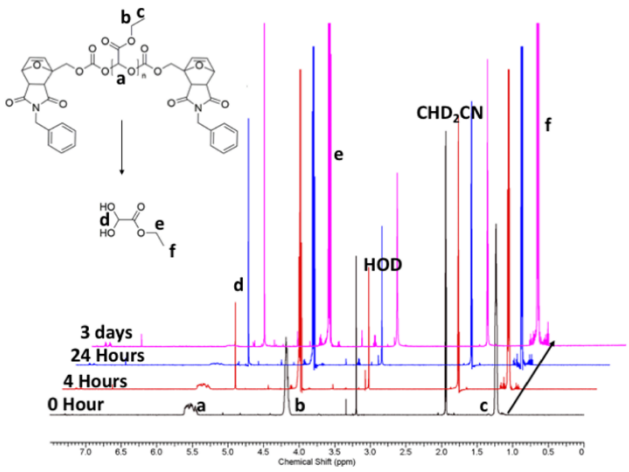


Fig. 1. ^{1}H NMR spectra of **PEtG-DA-Bn** incubated in 9:1 CD_3CN:D_2O at 75 °C. Spectra are offset to allow the progression over time to be clearly observed.

After incubation of the assemblies at 75 °C for 16 h, no remaining assemblies were detected by TEM (Fig. S37). This suggests that depolymerization of the PEtG resulted in disassembly. This disassembly was further probed using DLS by fixing the detector attenuation factor and recording the mean count rate (CR), which is proportional to the number and molar mass of the scattering species. Both the thermo-responsive vesicles and micelles prepared from **PEtG-DA-PEG750** and **PEtG-DA-PEG5000** showed an 80% decrease in CR when incubated at 75 °C for 10 h (Fig. 2b). In contrast, when these systems were incubated at 22 °C less than a 20% change in CR was observed. To ensure

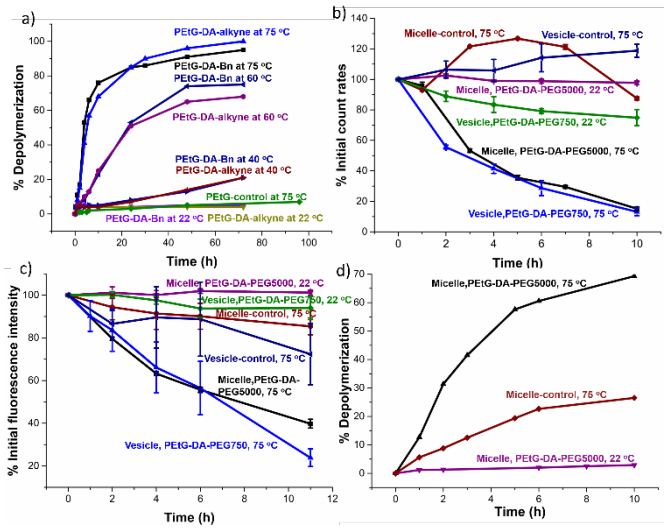


Fig. 2. a) Depolymerization of polymers in 9:1 CD_3CN:D_2O monitored by NMR spectroscopy; Assembly degradation in pH 7.4 phosphate buffer monitored by b) DLS count rate changes, c) Nile red fluorescence changes, and d) NMR spectroscopy.

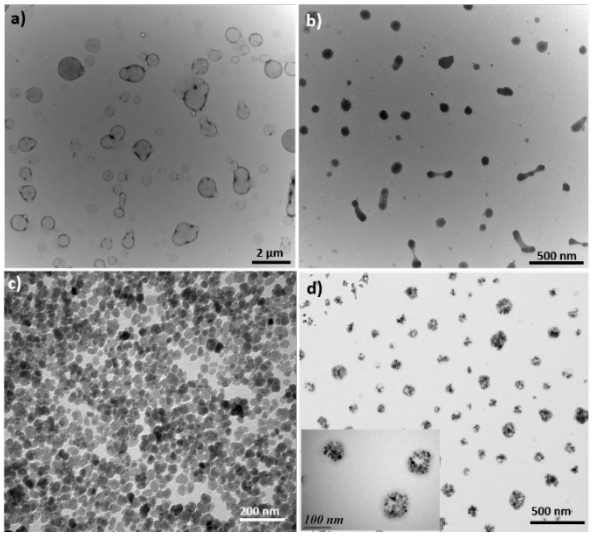


Fig. 3. TEM images of a) **PEtG-DA-PEG750** vesicles, b) **PEtG-DA-PEG5000** micelles, c) unloaded IONPs, d) IONP-loaded **PEtG-DA-PEG5000** micelles.

that the degradation at 75 °C did not arise from non-specific thermal bond cleavage, we prepared additional vesicle and micelle controls (**Vesicle-control** and **Micelle-control**) from previously reported PEtG-PEG block copolymers containing a photocleavable nitrobenzyl end-cap/linker, that should not be thermo-responsive (Scheme S1).[15] For both of these systems, the CR initially increased, which could be attributed to some aggregation, but it remained within 20% of its initial value.

The release of cargo from the vesicles and micelles was also explored. Nile red is a hydrophobic dye with strong fluorescence emission in a hydrophobic environment, but greatly reduced fluorescence due to quenching in hydrophilic environments such as water. Nile red was incorporated into the responsive vesicles and micelles as well as their corresponding controls. When the thermo-responsive assemblies were incubated at 75 °C, the fluorescence intensity of Nile red decreased by 60-70% over 11 h (Fig. 2c), consistent with its release into the aqueous environment. However, for either the same assemblies incubated at 22 °C or the non-thermo-responsive controls incubated at 75 °C, only ~10-20% intensity decrease was observed after 11 h.

To confirm that breakdown of the assemblies was induced by the depolymerization of the PEtG blocks, the depolymerisation of **PEtG-DA-PEG5000** micelles was studied by ^{1}H NMR spectroscopy. In this case, the assemblies were prepared by nanoprecipitation of the polymer in DMSO-d_6 into pH 7.4 phosphate buffered D_2O (DMSO-d_6: D_2O = 1:5). Only the peak corresponding to the PEG block was observed initially, consistent with self-assembly of PEtG at the particle cores (Fig. S26). However, after 1 h at 75 °C, peaks corresponding to EtGH appeared, confirming depolymerization. As shown in Fig. 2d, ~65% depolymerization had occurred over 10 h at 75 °C, whereas less than 5% depolymerisation occurred at 22 °C. For **Micelle-control**, at 75 °C, there was only about 20% depolymerization, which could be induced by nonspecific hydrolysis of the carbonate group of the end-cap. Combined, these data support that the thermo-responsive assemblies can degrade in response to external heat, and that this is due to the thermally responsive end-cap.

For some applications, direct bulk heating to trigger depolymerisation can be a viable process. However, in other cases, it would be necessary to apply a more selective and localized heating. Therefore, we also explored the incorporation of IONPs into the micelle core and the use of magnetic field hyperthermia (MFH) to obtain localized heating around the IONPs. This magnetothermal effect has previously been shown to enable a similar cleavage of bonds at the IONP surface.[26]

Hydrophobic IONPs were synthesized via the "polyol" process (11.2 ± 1.9 nm diameter, Fig. 3c),[8] and then coated with Beycostat NE surfactant to be incorporated into the micelle cores via nanoprecipitation of co-assembled IONPs and **PEtG-DA-PEG5000** from a THF solution into water.[27] The pure micelle solution was transparent and colorless. When the IONPs were introduced, the colloidal suspension became brown and darkened as the concentration of iron increased (Fig. S39). However, even at 35 mass % of IONP relative to polymer, the suspension was still transparent, confirming the IONPs were well dispersed and not precipitating. TEM showed that the IONPs were aggregated in spherical shapes with dimensions of ~100 nm (Fig. 3d).

Samples with the highest IONP content of 35 mass%, theoretically able to produce the largest increases of temperature, were studied. **Micelle-control** was also loaded with IONPs. The samples were first heated to 72 °C and equilibrated for 1 h. The Z-average particle diameter and scattering CR were measured using an *in situ* MFH-DLS setup as previously reported (Fig. S40).[9] No changes in CR or diameter were observed during the initial 1 h, suggesting that the composite structure may stabilize the assemblies. Then, magnetic field oscillations at maximum amplitude of 10.2 kA m^{-1} and 755 kHz were applied. Heat generated by the IONP-loaded micelles led to only a slight increase in temperature of ~2 °C for the bulk suspension. Nevertheless, the MFH had a rapid effect on the magnetic micelles, leading to an increase in diameter and large decrease in the CR. Normally,

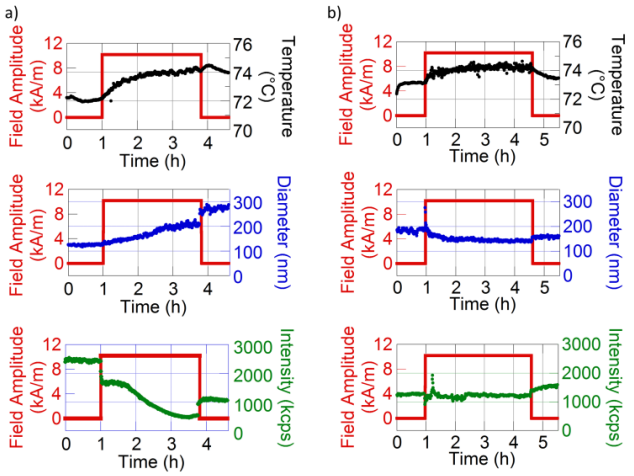


Fig. 4. Bulk temperature, particle diameter, and count rate measured before, during, and after magnetic hyperthermia using an *in situ* DLS for 35 mass% IONP-loaded a) **PEtG-DA-PEG5000** micelles and b) **Micelle-control**.

an increase in diameter would be expected to lead to an increase in CR. However, based on the experiments carried out on non-magnetic micelles, we hypothesize that upon application of MFH, the polymer degraded, leading to an overall reduction of the concentration of scattering species and reduced CR. The resulting unstabilized hydrophobic IONPs then aggregated, resulting in an increased diameter. In the case of the control IONP-loaded **Micelle-control**, a similar elevation of temperature was recorded, but diameters and intensities remained relatively constant. The experiment was also conducted at lower initial temperatures (53 °C) for the IONP-loaded thermo-responsive micelles but no significant changes were observed by DLS over the same time period (Fig. S41). Thus, while elevated initial temperatures were required for the MFH effect, these experiments demonstrate the ability to indirectly and selectively trigger the disassembly of the thermo-responsive micelles. A complementary small angle neutron scattering (SANS) study confirmed the thermo-induced degradation of the assemblies, both for pure and IONP-loaded **PEtG-DA-PEG5000** micelles heated at 80 °C for 30 min (Fig. S42).

In conclusion, we showed that a simple DA adduct of a furan and maleimide could serve as a new thermo-responsive end-cap for SIPs. It was readily functionalized to enable conjugation of PEG, forming block copolymers. Thermo-responsive PEtG-PEO copolymers with different PEG weight fractions were prepared and self-assembled to form micelles or vesicles. These nanoassemblies were triggered to disassemble upon heating as demonstrated by TEM, DLS, release of nile red, and NMR spectroscopy. Furthermore, MFH was used as an indirect stimulus to trigger the degradation of IONP-loaded micelles. Future work will involve the tuning of the structures of the end-caps and the assemblies to enable them to respond to heat stimuli both directly and indirectly at lower temperatures.

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