Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Preserving the Adhesion of Catechol–Conjugated Hydrogels by Thiourea–Quinone Coupling

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Supporting Information

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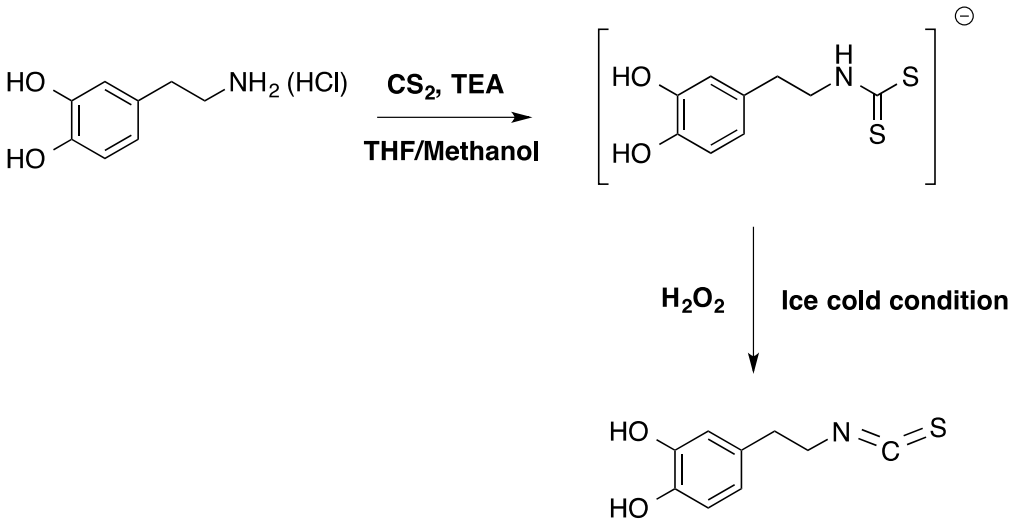
Materials

Dopamine hydrochloride, N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), 1-Hydroxybenzotriazole hydrate (HOBT), and methyl isothiocyanate were purchased from J&K Scientific. 4-armed poly (ethylene glycol)-NH_2 HCl was purchased from Jenkem Technology, USA. Gelatin (from bovine skin), triethylamine and other chemicals were purchased from Sigma-Aldrich unless otherwise specified.

Experimental Sections

Synthesis and Characterization of Dopamine-Isothiocyanate (Dopamine-ITC)

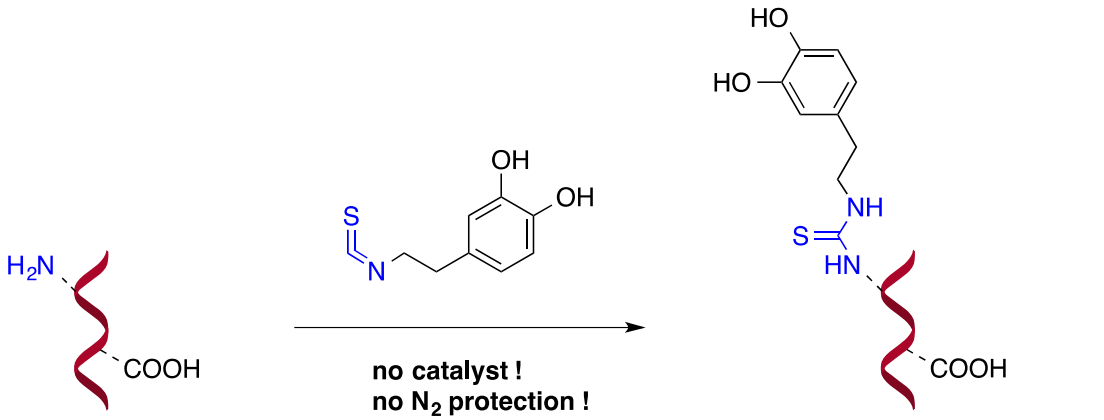


Dopamine Isothiocyanate

The dopamine-ITC was synthesized based on published procedures with slight modification.[1,2] Dopamine hydrochloride (6 g, 31.7 mmol) was dissolved in tetrahydrofuran (80 mL) with triethylamine (5.8 mL, 43 mmol), and methanol (82 mL) was slowly added to help dissolution under ice-cold condition and a nitrogen atmosphere. After forming a clear colorless solution, carbon disulfide (9.68 mL, 160.8 mmol) was added dropwise. A light yellow color developed. The reaction was left stirring for two hours at 5–10 °C. The mixture was then

warmed to room temperature while stirring for a further 10 to 12 hours. The reaction mixture was cooled again to 5 °C, and 30 % hydrogen peroxide (10.08 mL, 100.9 mmol) was slowly added under a nitrogen atmosphere. A brown-colored solution was developed with some yellow precipitations (sulfur). The mixture was neutralized with hydrochloric acid (37 %, 2 mL). The resulting mixture was filtered and concentrated under vacuum. The residue was filtered again and rinsed with DI water (50 mL, one time) and extracted with ethyl acetate (three times, 50 mL each). The ethyl acetate layers were combined, dried over $MgSO_4$ (s) and filtered. The crude product (yellow oil) was obtained by evaporation, which was further purified by a silica column chromatograph (300–400 mesh) by using a chloroform/ethyl acetate (3:1) mixture. The product was obtained as white powder. ^{1}H NMR spectrum of the compound is shown in Figure S1.

Synthesis and Characterization of Gelatin-Thiourea-Catechol (Gel-NCSN-cat)

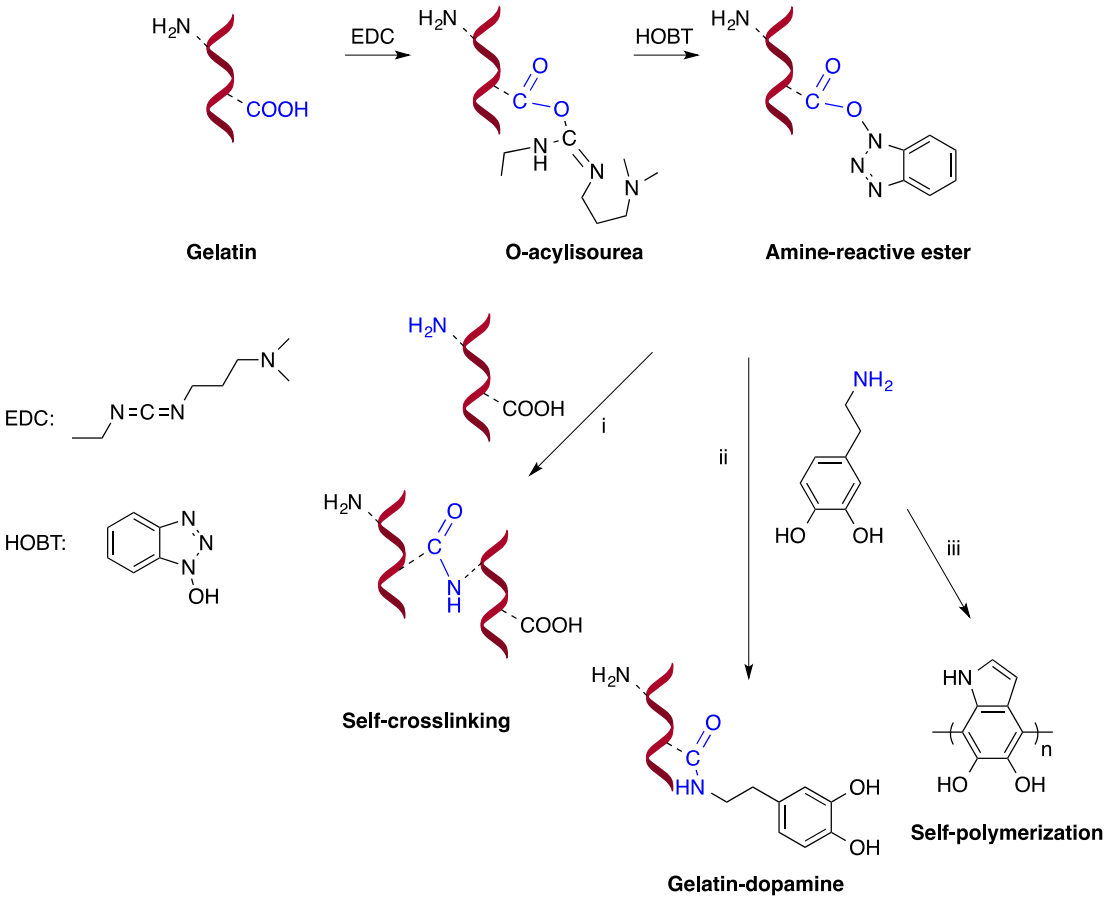


Gelatin **Gelatin-dopamineisothiocyanate**

Gelatin (2 g) was dissolved in a mixture of deionized water/dimethyl sulfoxide (1:1, 200 mL total) at 37 °C, and then the dopamine-ITC (320 mg, 1.64 mmol) was added to the mixture and left stirring overnight (~ 15–18 hours). The resultant polymer was purified by dialysis (MWCO = 6,000–8,000) in dimethyl sulfoxide for two days and in distilled water for four days at 37 °C, and then the purified solution was lyophilized to obtain the Gel-NCSN-cat. ^{1}H NMR

spectrum of the compound are provided in Figure S2a, and the content of dopamine-ITC as well as dopamine conjugated to the gelatin were determined by monitoring the absorbance at 280 nm by using UV-vis spectra (Figure S4a) based on a standard curve (Figure S4c and Figure S4e), obtained by using dopamine-ITC. The grafted amount of dopamine-ITC was 85.4 μmol per 1 g of Gel-NCSN-cat.

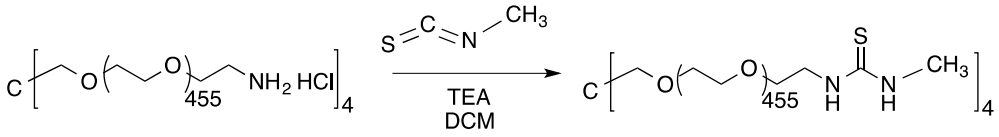
Synthesis and characterization of Gelatin-Catechol (Gel-cat)



Gel-cat was synthesized in accordance with a standard carbodiimide coupling protocol.[3] Gelatin (2 g) was dissolved in 200 mL (100 mL DI water and 100 mL DMSO as co-solvent) at 37 °C. EDC (307 mg, 1.6 mmol), HOBT (245 mg, 1.6 mmol), and dopamine hydrochloride (304 mg, 1.6 mmol) were added to the reaction mixture. The reaction was performed under nitrogen atmosphere (protect self-polymerization of dopamine) and left stirring overnight. The resultant polymer was purified by dialysis (MWCO = 6,000–8,000) in acidified

distilled water (pH < 2) for three days and in distilled water for another three days at 37 °C. The purified solution was lyophilized to obtain the Gel-cat. ^{1}H NMR spectrum of the compound are provided in Figure S2b, and the amount of dopamine conjugated to the gelatin was determined by monitoring the UV-vis absorbance at a wavelength of 280 nm (Figure S4b) based on a standard curve (Figure S4d and Figure S4f) obtained by using dopamine. The grafted amount of dopamine was 33.24 μmol per 1 g of Gel-cat.

Synthesis and Characterization of 4-armed-PEG-NCSN-CH_3 (PEG-NCSN)



4-armed poly(ethylene glycol)-NH_2 HCl sat (200 mg, 0.01 mmol) was dissolved in anhydrous DCM (4 mL). Methyl isothiocyanate (88 mg, 1.2 mmol) and triethylamine (11 uL, 0.08 mmol) were added in the solution. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for at least three days. The mixture was poured into cold diethyl ether (50 mL) and the PEG product was obtained by centrifuge and vacuum dry. ^{1}H NMR and of the compound are shown in Figure S3, and the graft ratio of methyl isothiocyanate is 92.7 % which is determined by the integration of relevant peaks in the ^{1}H NMR spectrum.

Formation of Hydrogel (Gel-NCSN-cat and Gel-cat) at Different $NaIO_4$: Catechol Ratios

Hydrogels were formed by the crosslinking of catechol at different oxidant content. Firstly, the Gel-NCSN-cat and Gel-cat were dissolved in PBS, the concentration of Gel-NCSN-cat and Gel-cat were 40 mg/mL and 100 mg/mL, in order to set an equal molar amount of catechol. Then, the $NaIO_4$ was added and kept at a molar ratio of 0.5–6 relative to the catechol. The mixtures were immediately vortexed and the gelation time was determined when the polymer

solution ceased flowing after inverting the vials.[4,5]

Formation of Hydrogel (Gel-NCSN-cat and Gel-cat) at Different pH values

Hydrogels were formed by crosslinking catechol at different pH conditions. Firstly, the Gel-NCSN-cat and Gel-cat were dissolved in desired pH solutions (2, HCl; 4, MES; 6, PBS; 7.4, PBS; 8.5, PBS; 10, NaOH). The concentrations of Gel-NCSN-cat and Gel-cat were 40 mg/mL and 100 mg/mL in order to maintain an equal molar amount of catechol. The $NaIO_4$ was then added at a molar ratio of 1.0 relative to the catechol. The mixtures were immediately vortexed because of their short curing time, and the gelation time was determined when the polymer solution ceased flowing after inverting the vials.[4,5]

Formation of Hydrogel (Gel-cat with PEG-NCSN/PEG-NH_2)

Hydrogels were formed by the crosslinking of Gel-cat with 4-armed-PEG. 4% PEG-NCSN/PEG-NH_2 was added into the Gel-cat 10% solution at targeted pH values (pH = 2, pH = 4, and pH = 7.4) with $NaIO_4$: NCSN = 1:1. The mixture was immediately vortexed.

UV-vis Kinetic Studies

The investigations were carried out with a Gary 5000 UV-vis spectrophotometer (Agilent) consisting of a 1 cm light path quartz spectrophotometer cuvette. Briefly, Gel-cat (10%) + PEG-NCSN (4%); Gel-cat (10%) + PEG-NH_2 (4%); Gel-cat (10%) were dissolved in different pH solutions, respectively. $NaIO_4$ was added with 1.0 equivalent of catechol. Following the oxidant addition, the spectral changes were examined at one-minute scanning intervals (the scan ranged from 250 nm to 650 nm.)

Oscillatory Rheometry

The rheological properties of Gel-NCSN-cat and Gel-cat hydrogels were measured by using a rotating rheometer (Anton Paar MCR301) with a temperature controller. Frequency sweeps (10–0.01 Hz at 5% strain) were performed to determine the storage modulus (G') and the loss modulus (G'') with the temperature at 37 °C. Hydrogel discs (diameter = 25 mm, thickness = 0.125 mm) were formed by using parallel plates. Distilled water was applied around the edge of the hydrogel to prevent dehydration. All hydrogels were formed and incubated at 37 °C for about one hour to reach the equilibrium prior to testing.

For all measured hydrogels the measured storage modulus (G') values were independent of frequency, and they were greater than the loss moduli (G'') in all frequency ranges (10 Hz to 0.01 Hz) with the temperature at 37 °C, thereby indicating that the hydrogels were chemically crosslinked. Additional frequency sweeps both of the Gel-cat and the Gel-NCSN-cat in 24 hours were tested and they showed little difference compared to the one-hour results (Figure S5c), thereby indicating that the equilibrium points both of the Gel-cat and the Gel-NCSN-cat hydrogels were reached after one hour.

Lap-shear Adhesion Measurements

Gel-cat and PEG-NCSN/PEG-NH_2 were dissolved in desired pH solutions (pH = 2, pH = 7.4). The final concentration of Gel-cat and PEG was 100 mg/mL and 40 mg/mL, respectively. After adding sodium periodate ($NaIO_4$: NCSN = 1:1), the mixture was vortexed and added to one end of a piece of glass (2.6 cm × 7.6 cm). The adherents were formed by placing the second piece of glass over the first with a 2 cm overlap. The adherents were compressed with a 40 g weight for three minutes and further incubated in DI water at 37 °C for one hour. The samples were pulled to failure by using a universal testing machine with a speed of 1 mm/minute under ambient condition. The load and displacement were recorded.

Successive adhesive strength measurements were conducted (five times) to

investigate the reversible adhesion properties of the hydrogels. The adherents after lap-shear test were resembled and incubated in DI water for three hours. Then the lap-shear strength was measured in the same manner.[6]

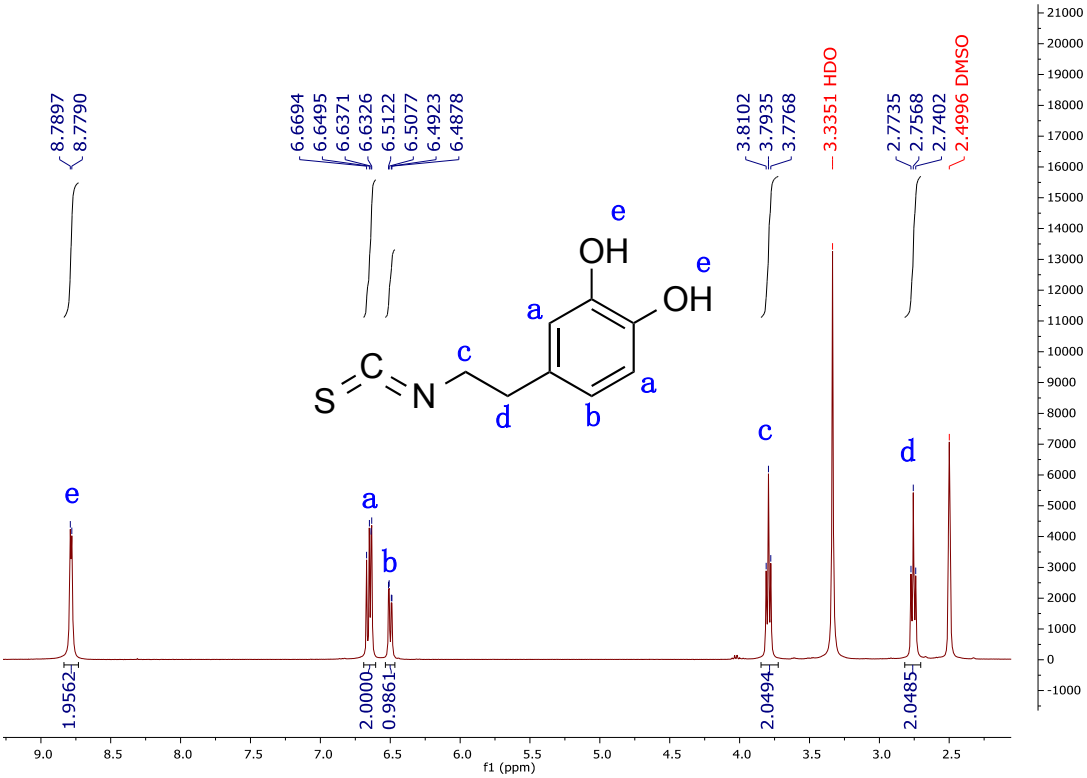
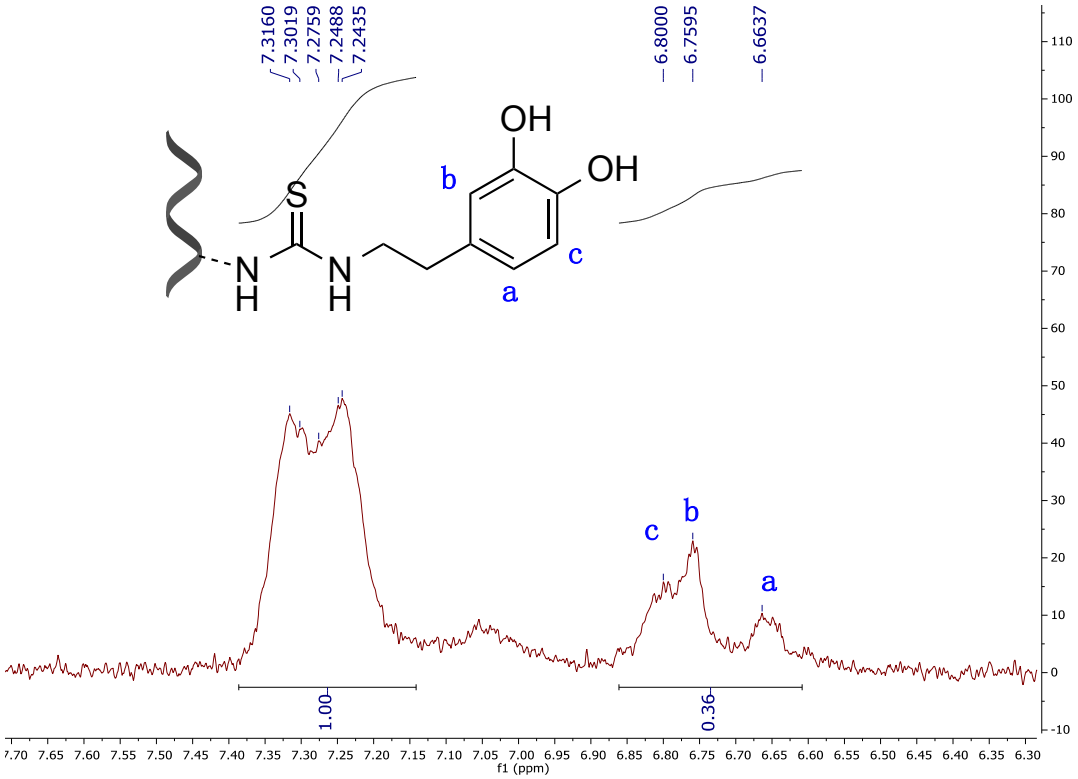


Figure S1. ^{1}H NMR spectra of dopamine-ITC in DMSO-d_6.

a



b

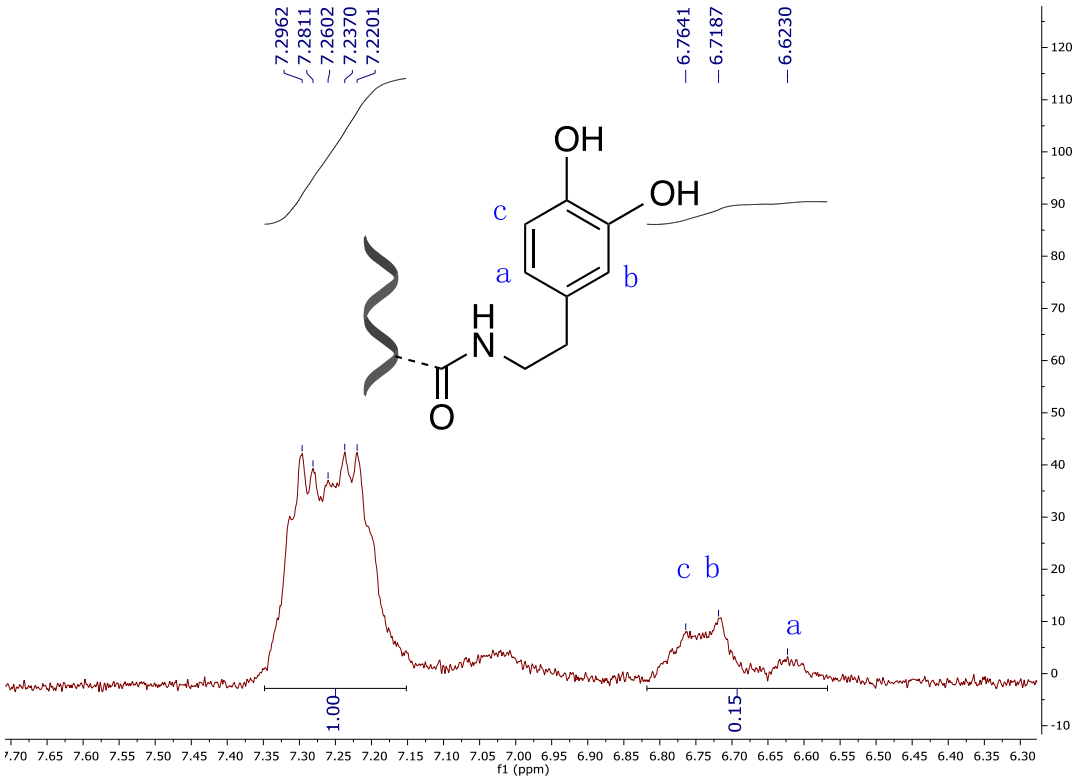


Figure S2. ^{1}H NMR spectra of (a) Gelatin-Thiourea-Catechol (Gel-NCSN-cat), and (b) Gelatin-Catechol (Gel-cat) in D_2O.

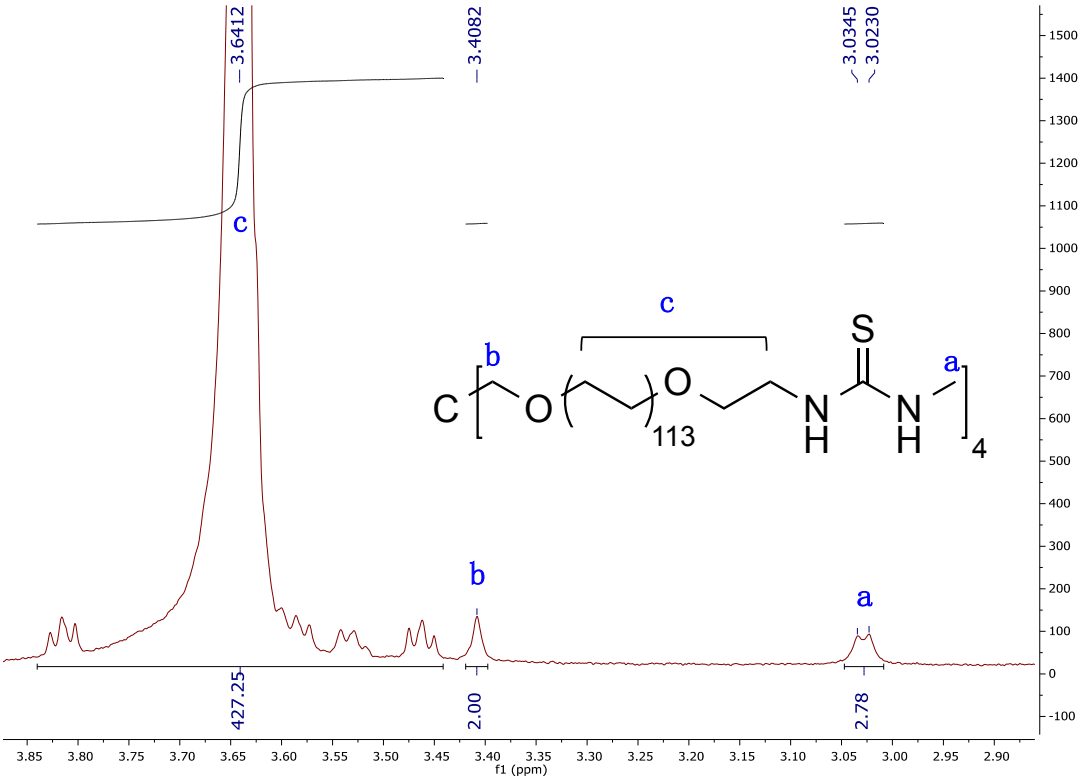


Figure S3. ^{1}H NMR spectra of thiourea-functionalized-4-armed poly(ethylene glycol) crosslinkers (PEG-NCSN) in $CDCl_3$.

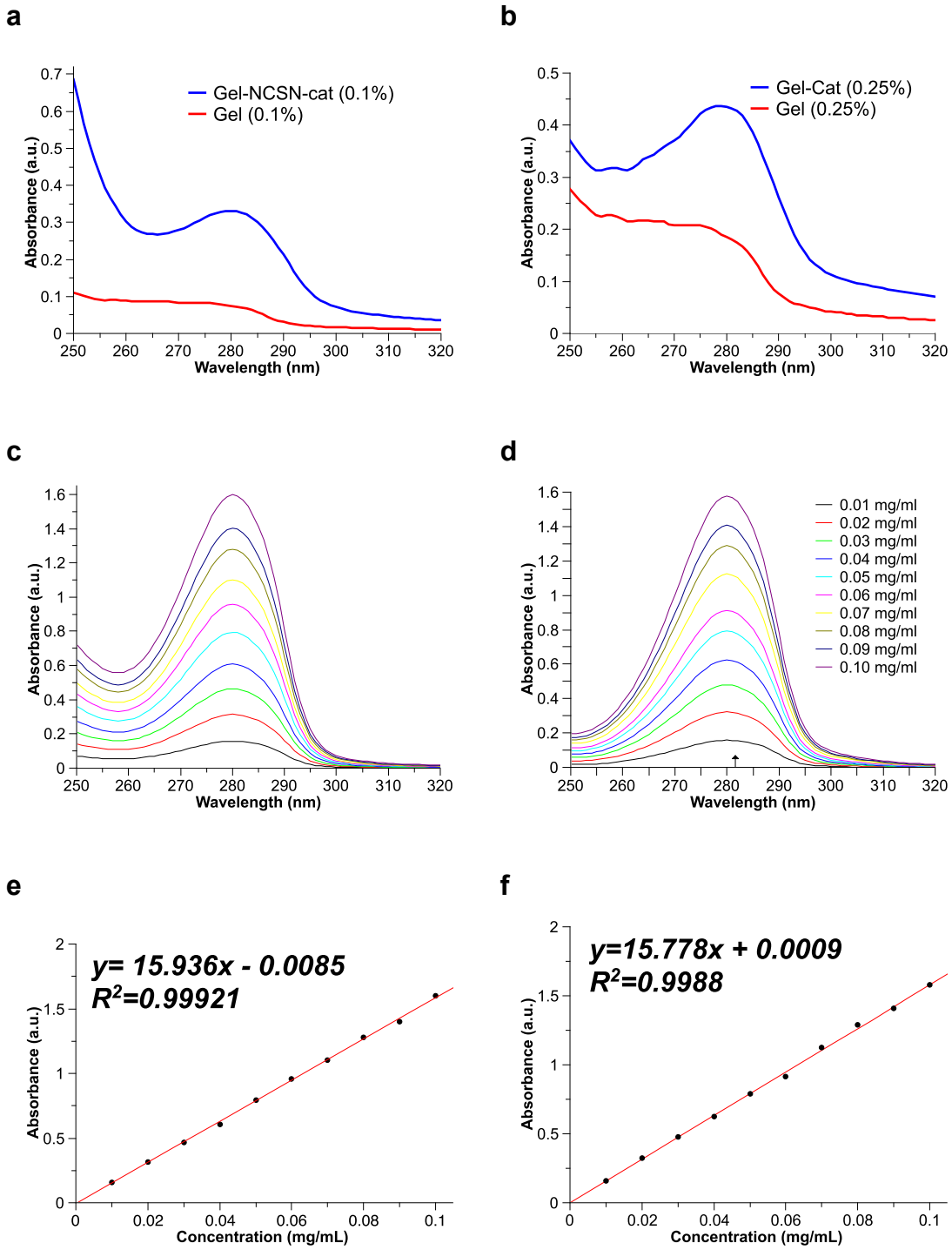


Figure S4. Catechol content determination of catechol-conjugated gelatin polymers by UV-vis measurements. (a) UV-vis spectra for Gel-NCSN-cat and gelatin (w/w = 0.1%). (b) UV-vis spectra for Gel-cat and gelatin (w/w = 0.25%). (c) Dopamine-ITC UV-vis standard curve. (d) Dopamine UV-vis standard curve. (e) Dopamine-ITC UV-vis standard curve equation. (f). Dopamine UV-vis standard curve equation.

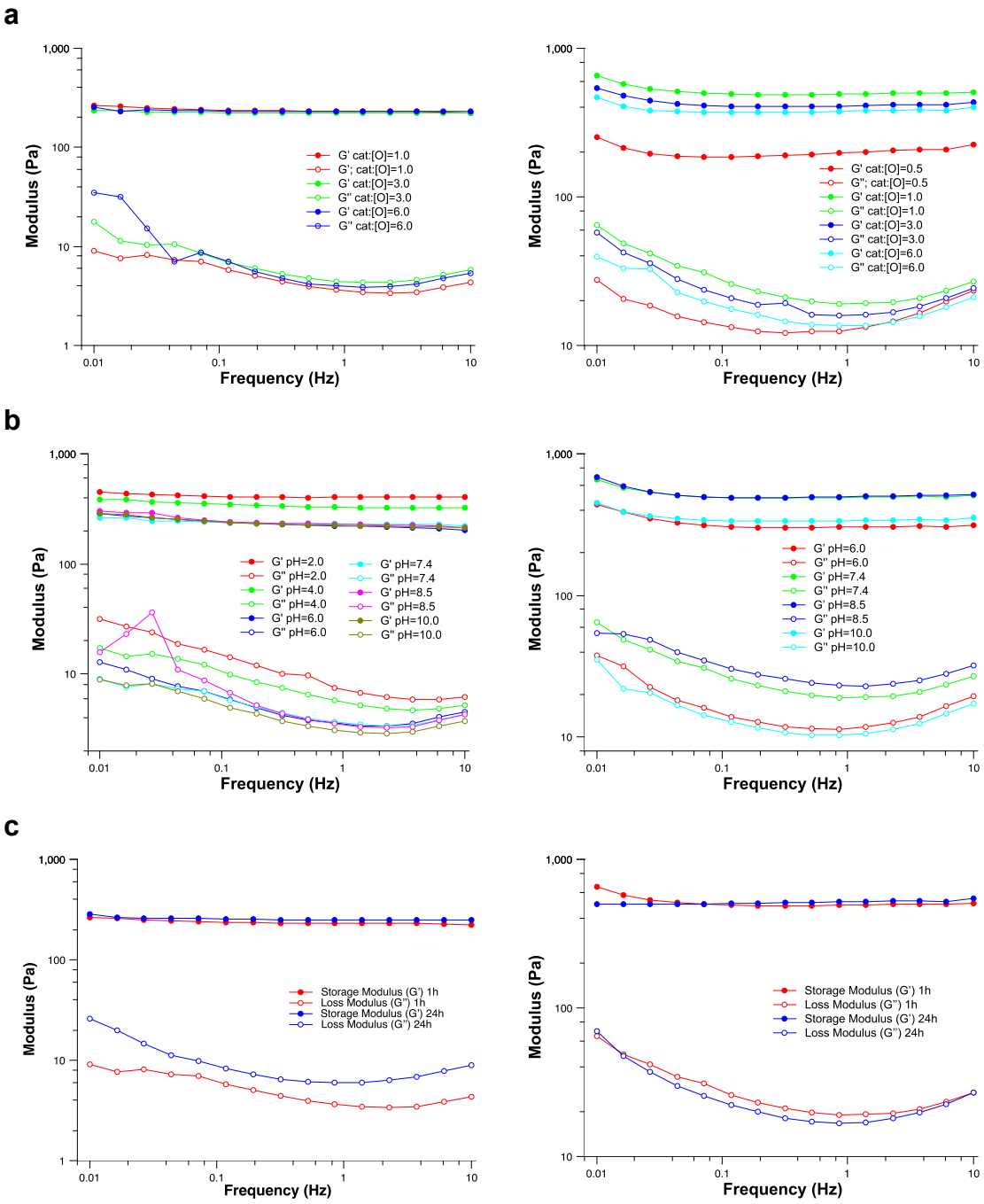


Figure S5. Rheology analysis (frequency sweeps) of (a) Gel-NCSN-cat (left) and Gel-cat (right) hydrogels formed in different pH levels. (b) Gel-NCSN-cat (left) and Gel-cat (right) hydrogels formed in a different oxidant content and (c) Gel-NCSN-cat (left) and Gel-cat (right) hydrogels formed in one hour and 24 hours in a condition of pH = 7.4, $NaIO_4$:cat = 1:1.

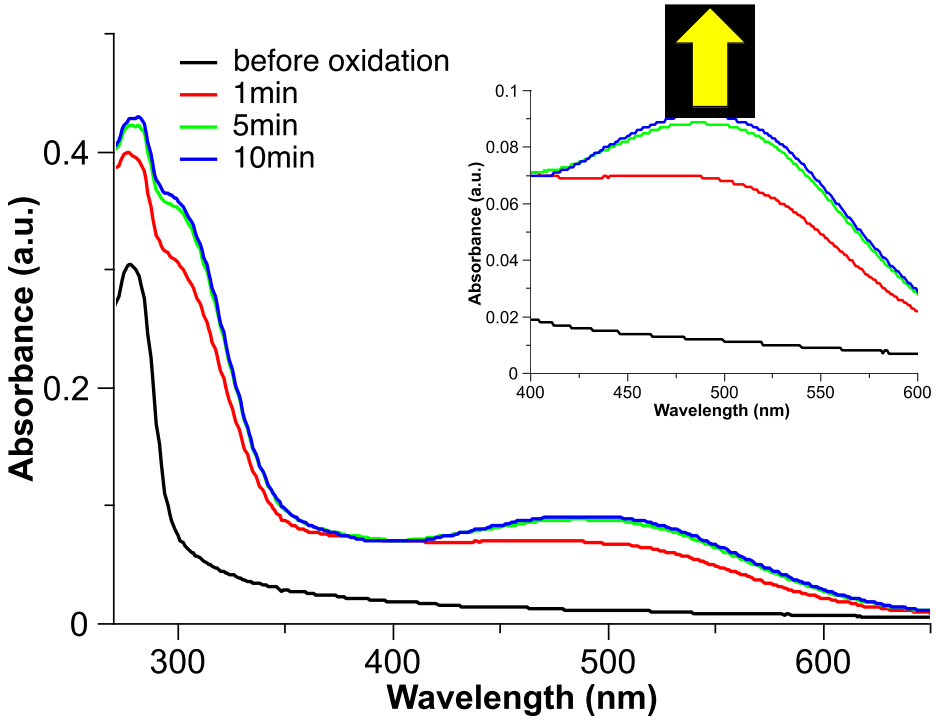
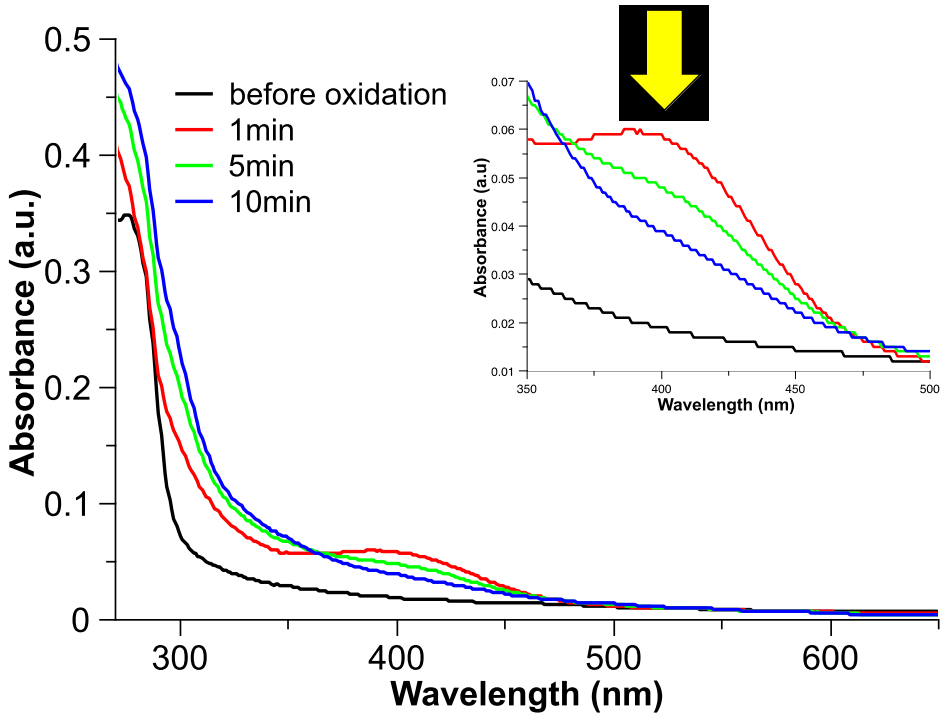


Figure S6. Evolution of the UV-vis spectrum with time for solutions containing: Gel-cat, and $NaIO_4$ at pH =7.4.The dicatechol peaks (281 nm and 484 nm) are more prominent due to the faster reaction at this pH.

a



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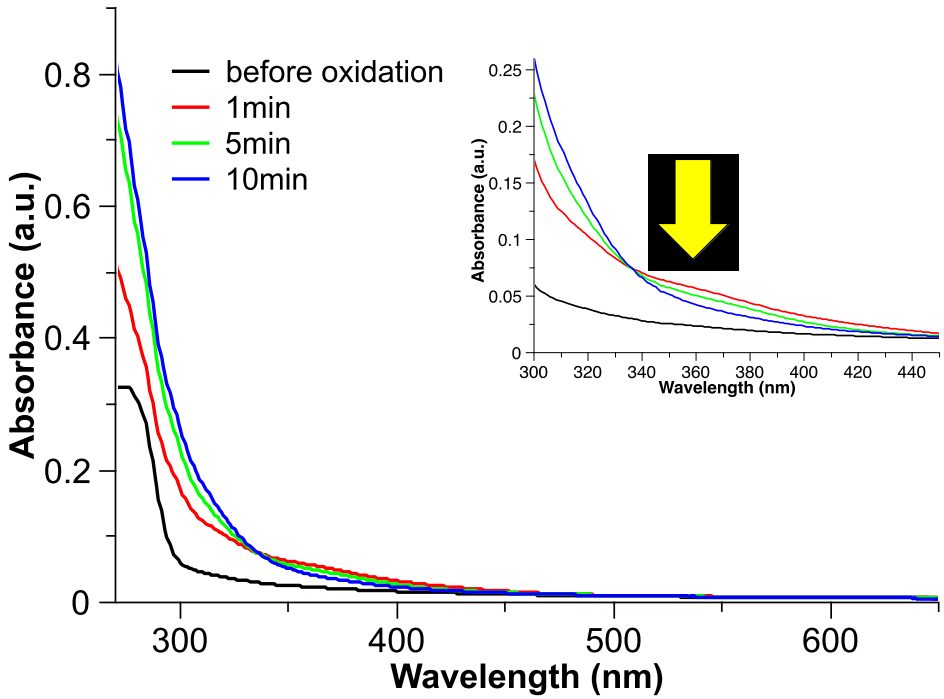

Figure S7. Evolution of the UV-vis spectrum with time for solutions containing. (a) Gel-cat, PEG-NCSN and $NaIO_4$ at pH = 4 ($NaIO_4$:cat = 1:1), and (b) Gel-cat, PEG-NCSN and $NaIO_4$ at pH = 2 ($NaIO_4$:cat = 1:1). Under more acidic conditions, the rapid consumption of quinone by the reducing thiourea becomes more significant.

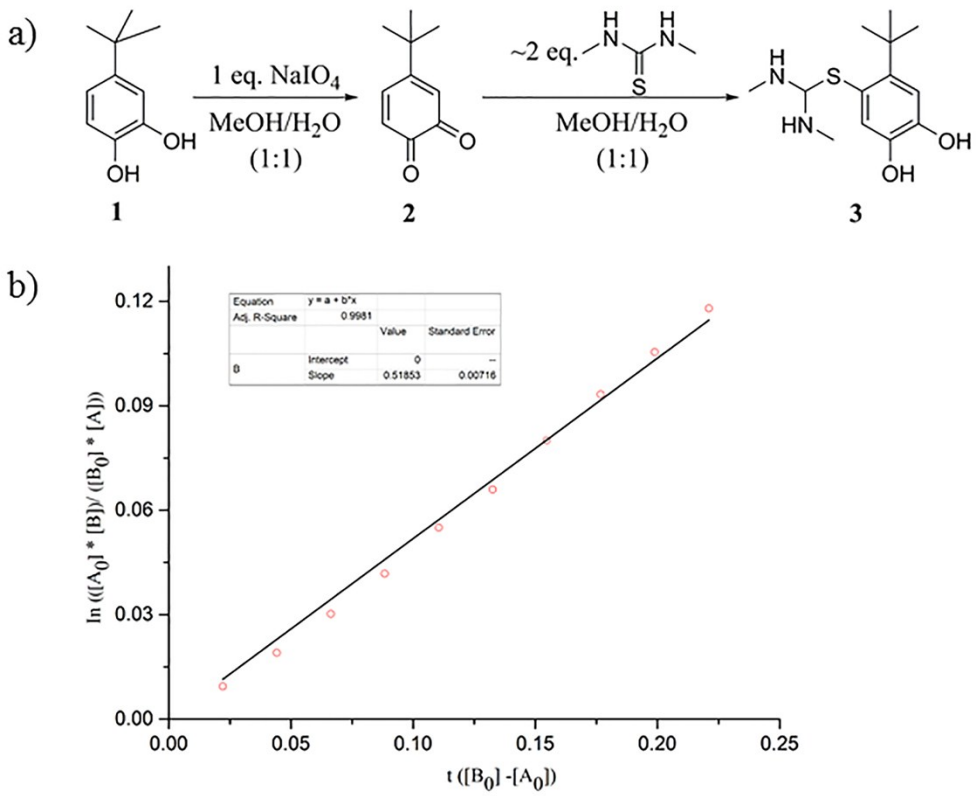

Figure S8. a) Michael addition reaction of 4-tertbutyl-1,2-quinone (**2**) with N,N'-dimethylthiourea. b) Determination of rate constant of NCSN-quinone reaction in MeOH/H_2O (1:1) at room temperature using decay in absorbance of **2** at 395 nm.

Unlike DOPA form quinone which is easy to self-polymerize, because of the significant steric effect of tert-butyl, the structure of 4-tert-butyl-1,2-quinone (**2**) is more stable. So the 4-tert-butylcatechol (**1**) was chosen as a simple model substrate to verify whether the NCSN group would react with quinone through Michael addition reaction, and to determine the reaction kinetics of this reaction. UV/Vis measurements were performed on a Cary 5000 UV-vis spectrophotometer (Agilent) using a 1-cm-path length quartz cuvette.

Firstly, at room temperature, 2 mL 500 mM of **1** in methanol/water (1:1) was added to the quartz cuvette, and then mixed with 0.88 mL 1.14 mM of sodium periodate ($NaIO_4$) (1 eq.). The value of UV absorbance at 395 nm (quinone peak) increased immediately, indicating **2** was formed through the oxidation reaction of 4-tert-butylcatechol. After 30 min, **1** was assumed to be totally oxidized to **2**, because the UV absorbance at 395 nm seemed constant. Next, 20 mL 107.6 mM of N,N'-dimethylthiourea solution (2.15 eq.) was added to the quartz cuvette, and then the decay in absorbance at 395 nm was

measured every 60 seconds.

Since only **2** absorbs at 395 nm, the time-dependent concentration of **2** was then calculated using the following equation:

$$Abs = \varepsilon_A * [A]$$

with Abs as the total absorbance at 395 nm, ε_A as the extinction coefficients of **2**, which can be calculated from the initial Abs and concentrations of **2**, and [A] as the time-dependent concentrations of **2** (mol/L).

From the conversion plots thus obtained, we calculated the second order rate constants of NCSN-quinone reaction according to this equation:

$$kt([B]_0 - [A]_0) = \ln[([A]_0 * [B])/([B]_0 * [A])]$$

with k as the 2nd order rate constant ($M^{-1}s^{-1}$), t as the reaction time (s), $[A]_0$ as the initial concentration of **2** (mol/L), $[B]_0$ as the initial concentration of N,N'-dimethylthiourea (mol/L).

In summary, we found that the NCSN group can react with quinone through Michael addition reaction, and the reaction rate constant was calculated to be about 0.5185 M^{-1} s^{-1}, roughly the same order of magnitude as traditional 'click' reactions such as strain-promoted cycloaddition of azides and cyclooctynes (typical values 0.01-1 M^{-1} s^{-1}). We believe that such fast Michael addition reaction between NCSN group and quinone would prevent the self-polymerization of quinones.

References

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