

## Efficient, Greener Synthesis of Disulfide Polymers and Disulfide Crosslinked Networks

Emily O. Rosenthal, Judit E. Puskas.

The University of Akron.

**Statement of Purpose:** Disulfide bonds are reducible under specific biological conditions. The oxidizing extracellular environment favors disulfide bond maintenance,<sup>[1]</sup> while the reducing environment found within the cell cytosol cleaves disulfide bonds.<sup>[1,2]</sup> For this reason, disulfide bonds are often employed as conjugating or crosslinking agents in polymeric biomaterials, however they are rarely seen as repeating units within a polymer backbone.

Disulfide bonds can be formed by the oxidation of thiols. Common methods include exposure to oxygen,<sup>[7]</sup> hydrogen peroxide<sup>[8]</sup> or dimethyl sulfoxide.<sup>[11]</sup> Recently, Park and coworkers synthesized disulfide polymers with number average molecular weights of  $6.1 \times 10^4$  g/mol in 50 hours.<sup>[12]</sup> They used a similar method to create a disulfide crosslinked hydrogel using a trithiol crosslinker.<sup>[13]</sup> We report a synergistic combination of base-catalyzed air and hydrogen peroxide oxidation leading to an efficient, greener synthesis of disulfide polymers and disulfide crosslinked networks.<sup>[14]</sup>

**Methods:** In a typical polymerization reaction, 2-[2-(2-sulfanylethoxy)ethoxy] and triethylamine (1:1.25 equivalent ratio) are reacted for 10 minutes in an open reactor while bubbling in air. To the bulk mixture, 2.0 equivalents of hydrogen peroxide (3% aqueous solution by weight) are added in 10-15 aliquots of equal volume and then reacted for an additional 2 hours. The rate of hydrogen peroxide addition is paced in order maintain an average reaction temperature above 50°C but below 60°C. Typical conversions for the polymerization reactions are 90%.

Network formation was achieved by reacting the dithiol monomer and the trithiol, trimethylolpropane tris(mercaptopropionate), in a 100:1 ratio with 1.0eq. of triethylamine under bulk conditions for 10 minutes. Then 3mL of thiol/amine solution was added to 5.0mL ethyl acetate and 3.0mL THF. Hydrogen peroxide (24mL of 3% aq. by weight) is added in 1mL at a rate of 1mL every 5 seconds while swirling. Air was bubbled into the beaker for 5 minutes. After 72hours the disulfide crosslinked polymer disk was removed from the solution. The addition of solid sodium phosphate (0.6mmol) increased the conversion from 59% to 92%.

Degradation of the polymer dissolved in THF/H<sub>2</sub>O (5:2) was achieved using the dithiol-specific reducing agent dithiothreitol (35.75mM). Aliquots (10mL) were taken at timed intervals and analyzed by NMR.

Analysis by nuclear magnetic resonance (<sup>1</sup>H and <sup>13</sup>C) was performed using a Mercury 300MHz instrument. Molecular weights of soluble products were determined by size exclusion chromatography (SEC). The data from the SEC was processed using Astra Version 5.3.4.14. The dn/dc value for the polymer was determined based on 100% mass recovery of the sample.

**Results:** Polymers with number average molecular weights exceeding  $2.30 \times 10^5$  g/mol were synthesized by the new method and degraded using dithiothreitol.

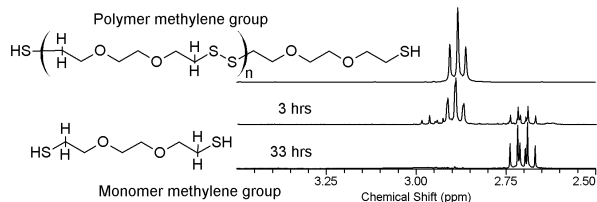


Figure 1. The reduction of the polymer by DTT is demonstrated by the NMR peaks of the sulfur-adjacent methylene group indicated on the drawn structures.

During a 72 hour solvent-swelling test, the network gained over 4.5 times its dry mass when exposed to THF, while it did not swell in hexanes or water. However, the gel exposed to water did become opaque, suggesting surface water adsorption and a surface-eroding degradation route.

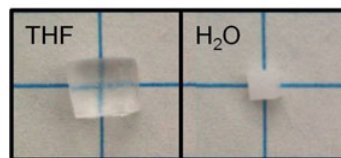


Figure 2. The crosslinked network is shown after 72 hours of exposure to THF and water.

**Conclusions:** The new method produces high molecular weight polymers that may be reduced using a dithiol-specific agent indicating both the presence of disulfide bonds and the potential for biodegradation. The method is fast and efficient, reaching number average molecular weights of over 230,000g/mol in less than 3 hours with conversions of 90%. It also offers a new route to crosslinked networks.

**Acknowledgements.** This material is based on work supported by the National Science Foundation under DMR-0509687.

### References:

- 1) Saito, G. *Adv Drug Delivery Rev.* 2003; 55:199–215.
- 2) Cerritelli, S. *Biomacromolecules.* 2007; 8:1966-1972.
- 7) Choi, W. J. *Polym. Sci., Part A: Polym Chem.* 1998; 36:79-84.
- 8) Whistler, R.L.; *J. Polym. Sci., Part A-1.* 1967;5:2111-2117.
- 11) Goethals, E.J. *Macromol Chem.Phys.* 1968; 119: 249-251.
- 12) Koo, H. *Bull Korean Chem. Soc.* 2000; 26:2069-2071
- 13) Koo, H. *Int. J. Pharm.* 2009; 274:58-65.
- 14) Puskas, J.; Rosenthal, E. *UA Patent Disclosure* 2010.