Pure Appl. Chem., Vol. 84, No. 10, pp. 2121–2133, 2012.
http://dx.doi.org/10.1351/PAC-CON-11-11-04

Green polymer chemistry: Living oxidative polymerization of dithiols*

Emily Q. Rosenthal-Kim[1,‡] and Judit E. Puskas[2]

[1]Department of Polymer Science, University of Akron, Akron, OH 44325, USA; [2]Department of Chemical and Biomolecular Engineering, University of Akron, Akron, OH 44325, USA

Abstract: Reduction sensitivity and mild synthetic conditions make disulfide-bonded materials ideal for degradable biomaterial applications. Both the degradation and the synthetic advantages of disulfide-bonded biomaterials have been applied to drug delivery vesicles, protein conjugation, and hydrogel biomaterials, but the synthetic advantages are rarely seen in the creation of biopolymers. A greener and highly efficient oxidative system is presented for the polymerization dithiols to high-molecular-weight poly(disulfide) polymers. The application of this system to 2-[2-(2-sulfanylethoxy)ethoxy]ethanethiol (DODT) produced corresponding degradable poly(disulfide) polymers with molecular weights as high as M_n = 250 000 g/mol and with a polydispersity index (PDI) as low as 1.15.

Keywords: biodegradation; biomaterials; polymerization; redox reactivity; sulfur; sustainable chemistry.

INTRODUCTION

Records of the antiseptic and healing properties of sulfur date back thousands of years. With its long history in alchemy and medicine, it is fitting that sulfur-containing polymers were among the first plastic and rubber materials synthesized. Today, biomedical research connects the medicinal branch of sulfur chemistry to the polymer branch of sulfur chemistry. The unique properties of sulfur–sulfur bonds make them both biologically significant and synthetically useful.

Disulfide-bonded drug delivery systems have been employed in the biomedical field for 50 years owing to their selective sensitivity to reductive environments found within biological systems [1–3]. The synthetic benefits of disulfide bond formation were often overshadowed by the utility of their facile biodegradation. Increased demand for new biomaterials has brought renewed interest in polymeric materials not only containing disulfide bonds but also created by disulfide bonds. The need for greener synthetic routes places disulfide bond formation at the forefront of synthetic strategies as the application of green chemistry practices follow inherently with the mild reaction conditions available in disulfide bond synthesis.

THIOL-DISULFIDE REACTIONS

When casually speaking of thiol oxidation reactions, it is often omitted that thiol groups are not the active participants in disulfide formation. Instead, the accepted mechanism provides that sulfhydryl

Pure Appl. Chem.* **84, 2065–2133 (2012). A collection of invited papers based on presentations at the International Symposium on Ionic Polymerization (IP 2011), Akron, OH, USA, 10–15 July 2011.
‡Corresponding author

groups should be in their deprotonated state in order to react [4]. As a rule, thiols are more acidic than their alcohol analogues, allowing deprotonation to occur under milder conditions. For example, the pK_a of ethyl mercaptan is 10.5 [5], while the pK_a of ethyl alcohol is 16 [6]. Once deprotonated, the thiolate anion is open to a spectrum of processes that lead to disulfide formation [7]. At one end of the spectrum is a single electron transfer process to a reducible species [8]. The oxidation, which results in the formation of the thiyl radical, has been shown to be the rate-determining step [9]. Thiyl radicals then couple rapidly with each other to form the disulfide bond. At the other end of the spectrum, the nucleophilic thiolate anion may attack another disulfide bond resulting in the formation of a new disulfide and a new thiolate anion. The use of electron spin resonance (ESR) spin-trapping experiments have also suggested that the sulfur–hydrogen bond may be cleaved homolytically in the presence of certain enzymes, thereby forming the thiyl radical in one step [7]. Base-catalyzed thiol oxidation begins a self-promoting cycle that is pictured in Fig. 1.

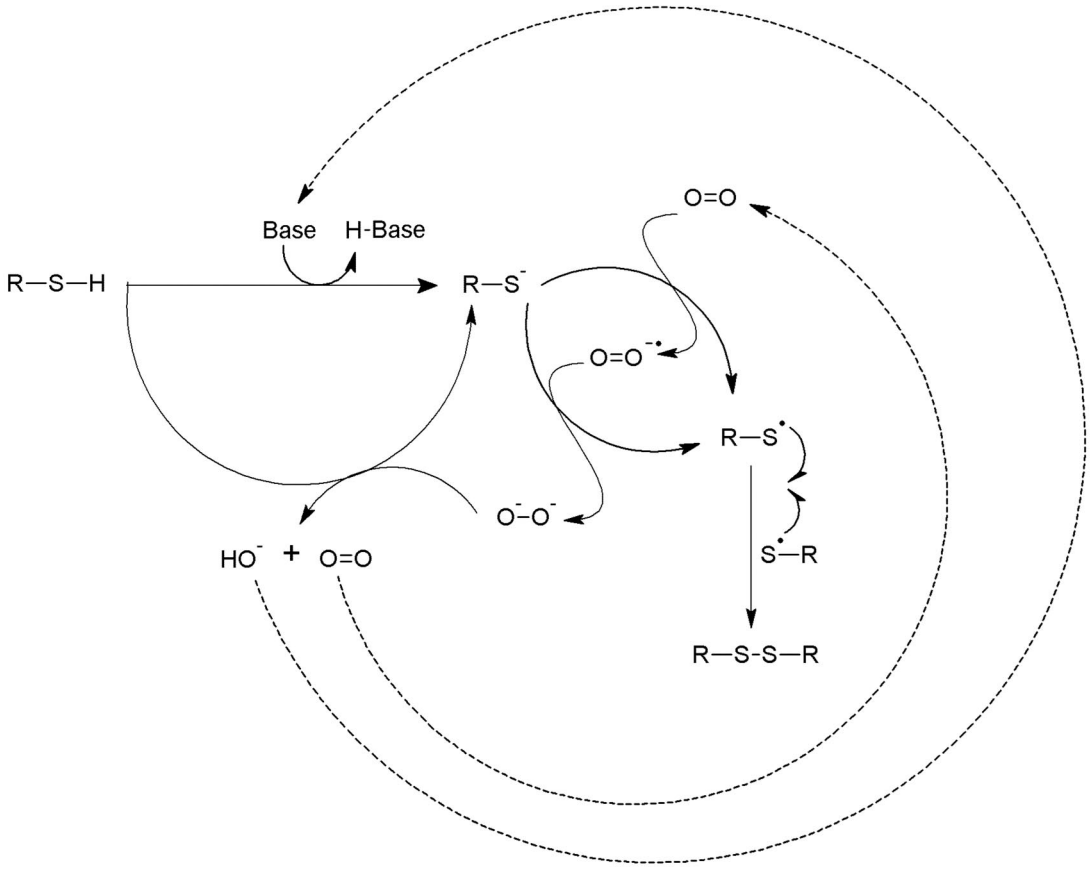

Fig. 1 Base-catalyzed thiol oxidation.

A synthetic advantage of using sacrificial disulfides to create new disulfides is that it eliminates the potential for over-oxidation. Stronger oxidizing agents may not stop at the disulfide, but instead continue to the sulfonic acids [9,10]. The progression from thiol to disulfide to sulfonic acid is demonstrated in Fig. 2. The intermediate is not stable and has only been isolated under specific experimental conditions [10]. Potential for over-oxidation has prevented hydrogen peroxide from common use in disulfide synthesis [9].

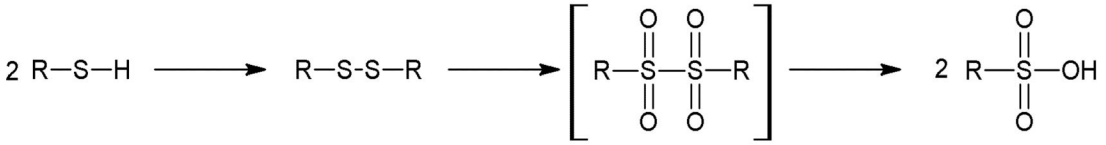

Fig. 2 Thiol oxidation past the disulfide bond.

Oxidoreductases comprise a class of enzymes responsible for most oxidation–reduction reactions. The class includes sulfhydryl oxidases and peroxidases, which both have the ability to oxidize thiols, as well as thioredoxin (TrxR) and glutathione reductase (GR), which are described later. In the 1946 publication, Randall demonstrated that the oxidation of thiols by hydrogen peroxide was catalyzed by horseradish peroxidase [11]. Horseradish peroxidase has since established itself as an efficient catalyst in organic chemistry [12]. Sulfhydryl oxidases are a subclass of enzymes that specifically oxidize thiols using a flavin cofactor. The flavin cofactor and two sulfur-containing residues work collaboratively to form a disulfide bridge from two thiol-containing residues in the protein substrate.

Flavins are often attached to enzymes as cofactors, however, they are also known to oxidize thiols to disulfides on their own [9,13]. Yano et al. have developed a highly oxidizing flavin derivative that shows particular effectiveness toward thiols (Fig. 3) and nitroalkanes [13]. The effect of pH on the reaction rate was studied, and the highest rate constant was seen at a pH of 9.5, which corresponded to the pK_a of the thiol (mercaptoethanol, pK_a 9.5) [14]. Oxidation back to the original flavin derivative requires the conversion of oxygen to hydrogen peroxide [14]. Holden and Main showed that riboflavin (vitamin B_2) showed a negligible catalytic effect on the oxidation of thiols under anaerobic conditions [15].

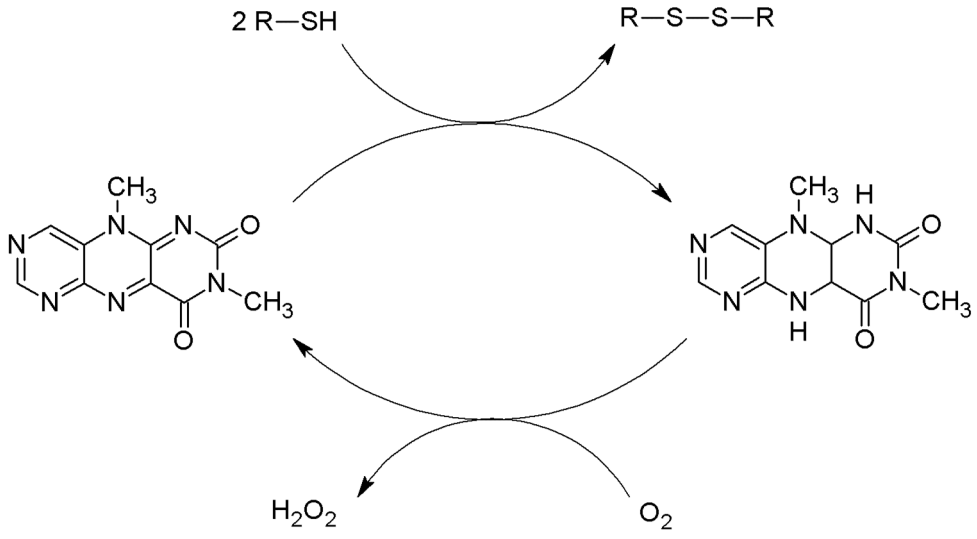

Fig. 3 Thiol oxidation by flavin compounds.

Metal ions in biological complexes have been shown to catalyze the oxidation of thiols by oxygen better than their nonbiologlical counterparts. The Fe(III) ion in heme, for example, increased the rate of oxidation by seven times over ferric sulfate [9]. Within a cell, the purpose of metal complexes, like heme and vitamin B_{12}, is to absorb and transfer oxygen, which makes them ideal catalysts for oxidative reactions.

The reverse reaction of thiol oxidation is disulfide reduction. One of the most widely used disulfide-reducing agents is dithiothreitol [16]. Upon oxidation, the dithiol compound forms an intramolecular disulfide bond that results in a stable six-membered ring (Fig. 4). Dithiothreitol has significant synthetic utility for maintaining thiol functionality by preventing oxidation [17–19]. Another commonly used reducing agent is tris(2-carboxyethyl) phosphine or TCEP [1,20,21]. Both reductants may be applied to selectively break protruding disulfide bridges in proteins without affecting internal disulfide bridges that may result in protein denaturation [20]. Approximations of in vivo degradation rates may use glutathione (GSH) solutions as the reducing agent [1].

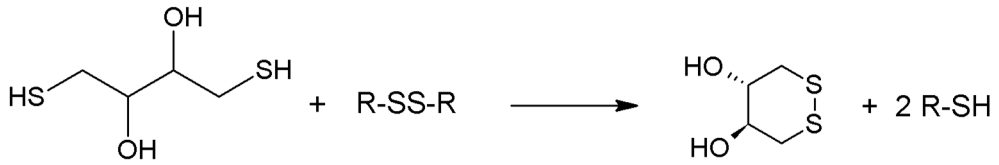

Fig. 4 Oxidation of dithiothreitol by a disulfide.

The most common sulfur-containing biomolecules either contain or are derivatives of the amino acid cysteine, which has a pendant thiol group. GSH, a cysteine-containing tripeptide, is the most abundant reducing agent found in biological systems. Part of its reducing strength stems from the stability of its disulfide conformation which is able to hydrogen bond in a β-sheet-like manner with the disulfide bridge above the plane of the molecule (Fig. 5) [22].

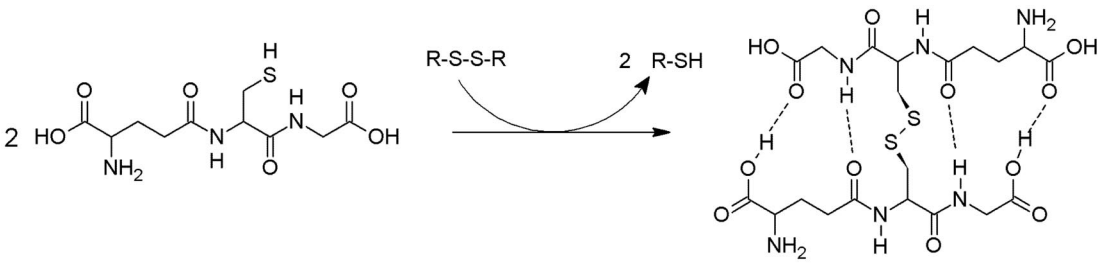

Fig. 5 GSH reduces disulfide bonds and forms GSSG with β-sheet structure.

GSH is also a cofactor in many redox enzyme systems. For example, the GSH enzyme system (Fig. 6) includes the enzymes GR and the selenoprotein glutathione peroxidase (GPx), as well as GSH, glutathione disulfide (GSSG), and hydrogen peroxide [23,24]. It is particularly important in the regulation of the concentration of thiols, disulfides, and hydrogen peroxide within cells [24]. Thioredoxin (Trx) enzymes, which are common to a wide variety of organisms, are another example of enzymes that catalyze the reduction of disulfide bridges. The Trx system consists of the enzyme Trx, and thioredoxin reductase (TrxR or TR). As the name suggests, the role of TR is to reduce the disulfide bond in Trx. As a result of its reduction, Trx itself becomes a strongly reducing enzyme that will transfer electrons to the disulfide bridge of another protein creating two new thiol groups [25]. Bindoli includes a third enzyme in the Trx system, peroxiredoxin, as it is one of many enzymes and proteins that may receive electrons from reduced Trx. It is specifically included for its importance in the regulation of cellular hydrogen peroxide concentrations [24].

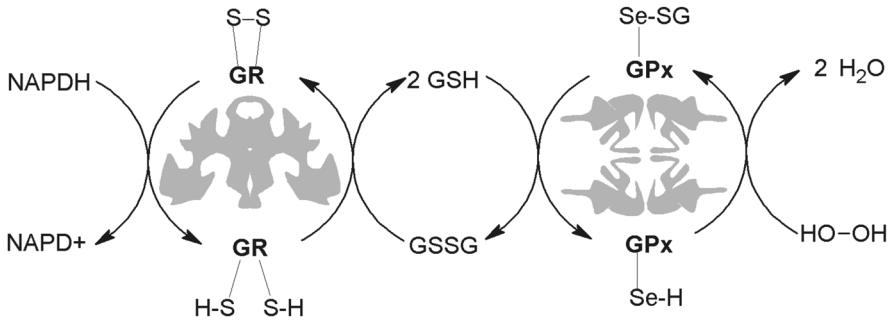

Fig. 6 Diagram of the GSH enzyme system [23,26,27].

BIOLOGICAL REDOX ENVIRONMENTS

The body regulates many different redox environments simultaneously, often separated by only a phospholipid bilayer. Regulating the pH level of the biological environment is one way to control its redox potential. Another crucial method of regulating the cellular oxidation potentials is through the concentration of thiols and disulfides [24]. The body uses a constant exchange between thiols and disulfides, called "shuffling", to signal and regulate changes within or to biological environments [28]. The disulfide bond represents a stable oxidation state for sulfur atoms, and for this reason, it is a crossroads for many biochemical pathways [29]. As seen in the enzyme systems, hydrogen peroxide is another critical molecule in the regulation of cellular redox potentials and is intimately connected to the reactions of sulfur-containing biomolecules.

In general, extracellular environments are more oxidizing than reducing and intracellular environments favor reduction over oxidation. Elevated concentrations of GSH and the disulfide reducing enzymes found within cells are largely responsible for the variation. Extracellular environments have concentrations of free thiol-containing molecules in the micromolar range, while they are found in the cytosol at millimolar concentrations [30]. Cytostolic GSH concentrations in human tissues are reported in the 1–10 mM range [31], and the GSH/GSSG ratio is in the range of 100:1 [30]. The intracellular environment also contains Trx in micromolar concentrations which further promote intracellular reduction of disulfides [32]. Plasma, as an example extracellular environment, contains GSH in concentrations of around 3.5 μM and free cysteine at about 12 μM [33]. Here the ratio of non-protein bound cysteine to cystine is about 1:8, showing that the dominant species is already in its oxidized form [33]. The difference in free thiol levels inside red blood cells is not typical of that found in other cell types. In red blood cells, GSH is found in levels of 6–8 μmol/g hemoglobin and free cysteine is found in levels of 39–57 nmol/g hemoglobin [34].

Distinct redox environments also exist within the cells. Lysosomes have a pH of about 5.5 and are particularly reducing [35]. Endosomes contain more free cysteine, and also have a depressed pH of about 5.8. The endoplasmic reticulum is responsible for protein synthesis and contains an oxidizing environment. Some extracellular environments favor reduction, such as that surrounding the lower respiratory tract which has GSH levels of over 300 μM [36]. While the difference in thiol concentration is the driving force between reduction-sensitive drug delivery, it is important to remember that reduction reactions still occur in the extracellular milieu. Of the non-protein bound glutathiones (GSH and GSSG) found in plasma, a 2:1 majority are found in their reduced state, and are therefore subject to oxidation in the presence of a disulfide species [33]. This mechanism for the slow degradation of disulfide materials in the extracellular environment could lead to potential applications in long-term biodegradable implants. The great range in the potential for redox-sensitive materials stems from the great variation in redox environments found within organisms. The thiol-disulfide reactions present an opportunity to take advantage of biomolecules and enzymes already designed for sulfur redox reactions.

DISULFIDE BONDS IN BIOMATERIALS

In addition to reducing naturally occurring disulfides, cysteine, GSH, and enzymes also reduce disulfide bonds from foreign sources. The biomedical field began taking advantage of this for drug delivery in the 1970s with the development of immunotoxins that are bifunctional molecules containing a targeting antibody attached to a drug or toxin [3,37]. Sterically hindered disulfide bonds were developed to deter premature disulfide reduction and proposed the opportunity to tune the release rate of the drugs [3,37]. The susceptibility of disulfide bonds into reducing conditions was later applied to prodrugs that became active upon reaching the reductive environment surrounding some tumors [38]. This ability to control the release rate of drugs into the body was soon eclipsed by the quick release of drugs into the cell upon endocytosis.

Nanoparticles and microparticles in the form of micelles, liposomes, and polyplexes have all been synthesized to exploit the reduction sensitivity of disulfide bonds for delivery [1,39,40]. Using disulfide bonds to link poly(ethylene oxide) (PEG) chains to hydrophobic polymers, ionic polymers or proteins have been of particular interest because of the unique cloaking ability of the hydrophilic polymer. PEG-surrounded particles may move within an organism without eliciting a negative immune response because the hydration sphere renders them undetectable by macrophages [41]. The PEG shell allows the drug, protein, or nucleotide to reach its target, however, removal of the protective layer is necessary to release the cargo. In some examples, disulfide bonds play an additional role by adding stability to the particle structure [19,42]. Self-assembled vesicles are sensitive to salt concentrations and shear and may rupture prematurely, but post-self-assembly disulfide cross-linking of the core or inner corona deters early rupture and subsequent cargo loss without interfering with the vesicle configuration [19]. Protein conjugation to PEG, or PEGylation, via disulfide bonds is a natural fit because of the existence of exposed thiol or disulfide bonds on protein surfaces providing a location for direct attachment. Disulfide conjugation may be achieved under mild conditions without causing protein denaturation [20]. Protein PEGylation demonstrates the dual usefulness of disulfide bonds; they may be biologically reduced and synthesized under biologically tolerated conditions.

Cell encapsulation into hydrogel materials is another process that demonstrates the unique synthetic potential of disulfide bonds. Hyalauronan [43,44], gelatin [45], chitosan [46], and PEG hydrogels [47] with disulfide cross-links have all been used to trap and/or grow cells. Hisano and co-workers trapped islets in a poly(acrylamide) hydrogel by forming disulfide bonds in a bioinspired process [48]. The thiol-functionalized acrylamide polymer was oxidized around dispersed cells using oxygen and 3,3'-dithiodipropionic acid. Disulfide bond formation upon exposure to oxygen did not occur quickly enough to maintain viable cells, so oxidizing disulfide molecules were added to couple the pendant thiol groups. Cystamine, another disulfide compound, caused more rapid cross-linking, but it was showed to be highly cytotoxic even in trace amounts [48]. From a synthetic viewpoint, the reactivity of aminothiols, aromatic thiols, and their disulfide counterparts is appealing, however, with the increased reactivity comes increased cytotoxicity [49,50]. Hemolysis from the production of "active oxygen" during thiol-disulfide shuffling reactions [49,50] and the click reaction of thiols with the double bonds in unsaturated fatty acids or vitamins [51] are all concerns associated with reactive thiol species. Although thiol and disulfide compounds are found universally in nature, not all are tolerated by living organisms and careful selection of compounds is needed to mitigate potential toxicity of vestige synthetic residues.

The advantage of using biocompatible reaction conditions available in disulfide bond synthesis has been widely demonstrated in the production of drug delivery vesicles and hydrogel biomaterials. However, the advantage of mild reaction conditions is rarely applied beyond the coupling and cross-linking existing polymers. The synthesis of polymers with disulfide bonds in the backbone offers both reduction sensitivity and the possibility of biologically tolerated reagents.

POLY(DISULFIDE) POLYMER SYNTHESIS

The first polysufide polymers were first introduced in the patents of J. C. Patrick and N. M. Mnookin in the late 1920s [52–54] resistant properties of the polymers. In Patrick and Mnookin's method of polysulfide synthesis, α,ω-dihalogenated linear organic compounds are reacted with polysulfide salts in an aqueous medium (Fig. 7) [52–54]. Disulfide and polysulfide bonds of the sulfide salt are created in situ prior to the reaction with the dihalide [52]. The rank of the sulfur bonds may be controlled to varying specificity, but exclusive formation of disulfide bonds desired for biomaterial applications is not expected [55,56]. The synthetic methods developed at Thiokol Corporation remain industrial standards for polysulfide polymer preparation.

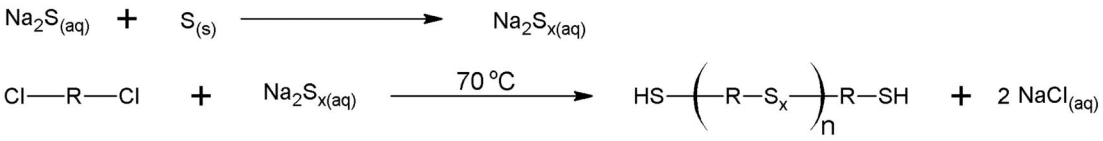

Fig. 7 Thiokol method of polysulfide polymer synthesis.

The oxidation of thiols to disulfides by the oxygen is a well-known reaction [9], and it has been applied to the synthesis of poly(disulfide) polymers with varying degrees of success since the 1940s [57]. Oxidative polymerization of dithiols requires the addition of a catalyst to achieve high molecular weights within a reasonable time, particularly when an air atmosphere is used rather than a pure oxygen atmosphere. In early work by Marvel, the creation of poly(disulfide) polymers was an annoying side reaction in his synthesis of polythioethers, but in the late 1950s, he and Olson published academic research on the purposeful synthesis of poly(disulfide) polymers [58]. Taking a different approach from the scientists at Thiokol, they selected α,ω-alkanedithiols as the starting material. The dithiols were suspended in an aqueous solution of lauric acid and potassium hydroxide. Compressed air was bubbled through the emulsion for one to four days. Several other oxidizing agents (bromine, nitric acid, and ferric chloride) were investigated, but they found that air oxidation produced the best results [58]. They also discovered the utility of selenious acid as a catalyst for the reaction [58]. Lauric acid and potassium hydroxide were added to create surfactant molecules in a one-pot process, however, hydroxide ions may also promote the formation of disulfide groups and a slight stoichiometric imbalance could shift the equilibrium toward the desired disulfide product. Later that decade, Bonsignore, Marvel, and Banerjee studied the effect of temperature on the oxidative polymerization of dithiol-functionalized siloxane monomers and concluded that high molecular weights are promoted by elevated temperatures not exceeding 80 °C, as above this temperature some dithiol monomers underwent cross-linking reactions to form gel [59]. Dimethylsulfoxide (DMSO), a common thiol oxidizer, was used by Goethals and Sillis in the presence of heat to create linear poly(disulfide) polymers with molecular weights of up to M_n = 10 500 g/mol [60].

In the 1960s, Whistler and Hoffman investigated the polymerization of thiosugars, whose biomedical applications are still of interest today [61]. Using the work of Marvel and Olson as a starting point, they polymerized dithiol-functionalized sugars using three different oxidative reagents: oxygen, ammonium persulfate, and hydrogen peroxide (Fig. 8) [62]. The oxygen oxidation was performed in both aqueous emulsion and pyridine solvent systems using selenium dioxide as the catalyst. Hydrogen peroxide and ammonium persulfate oxidations were only performed in pyridine. Using oxygen, they were able to obtain oligomers with an average of 23 repeating saccharide units [62].

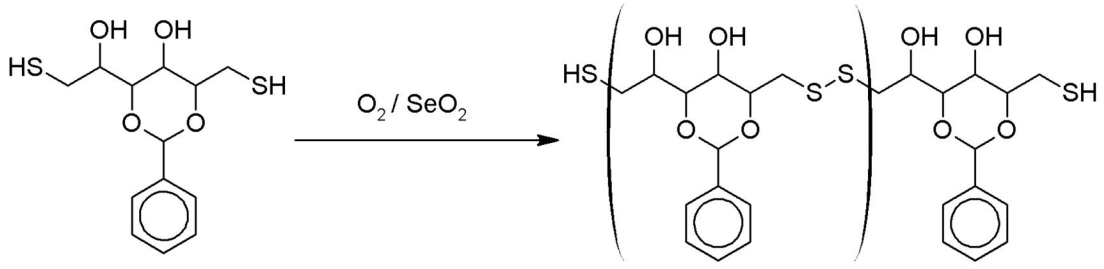

Fig. 8 Oxidative polymerization of glucitol derivative [62].

Choi et al. [63] polymerized large, bisphenol dithiol monomers with oxygen in the presence of triethylamine (Fig. 9). In this one-pot process, the monomers were synthesized in tetrahydrofuran before triethylamine (2.5 equiv) was added to the reaction vessel to catalyze the oxidation step. The reaction was then stirred under an oxygen atmosphere for 100 h at ambient temperature. Kinetic studies of one of their monomers showed that 80 % conversion was reached after 10 h. The polymerizations without triethylamine resulted in M_n = 3700 g/mol [63], which corresponds to about 5 repeating units. The same monomer polymerized with the addition of triethylamine yielded a polymer with M_n = 20 200 g/mol. A copper catalyst was applied by Meng et al. to the oxygen oxidation of a wide variety of dithiol monomers including aliphatic, aromatic, and arylene dithiols and their copolymers [64–66].

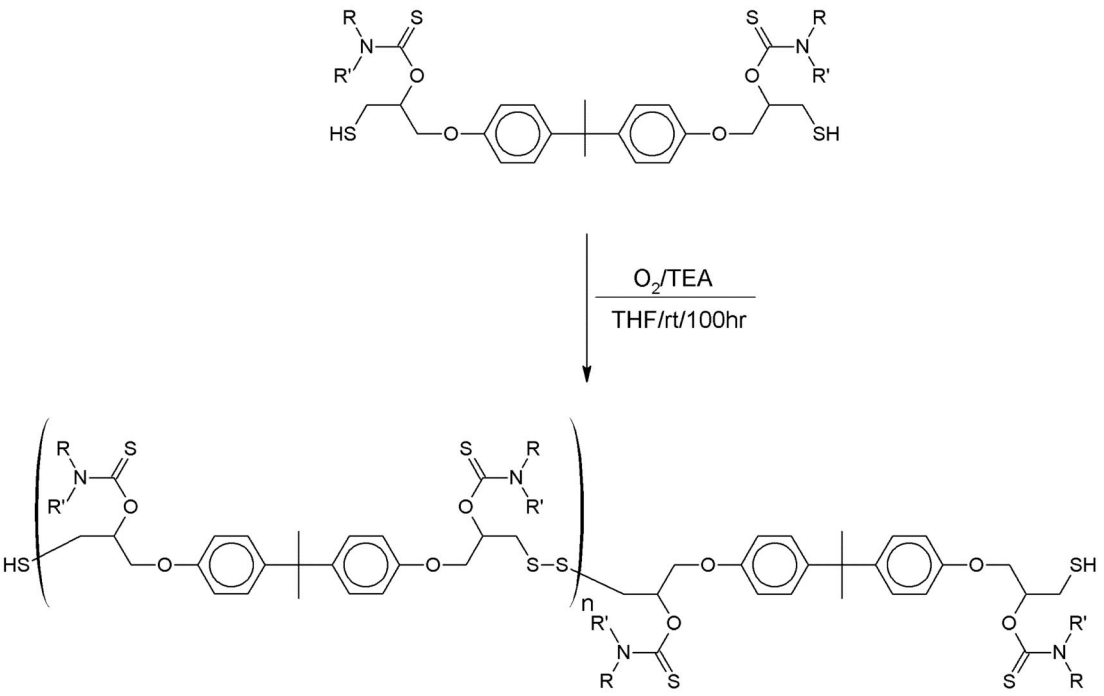

Fig. 9 Oxidative polymerization of thiol-functionalized bisphenol derivatives.

In 2005, Park et al. used a pure oxygen atmosphere to polymerize α,ω-thioacetate functionalized PEG oligomers with 2–36 ethylene glycol units to linear polymers specifically for their research into biomaterials [67,68]. The monomers and oligomers were dissolved in an ammonia/methanol solvent

mixture and exposed to pure oxygen gas atmosphere while stirring. Using this method, they were able to reach molecular weights of M_n = 61 000 g/mol (based on PEG standards) in a 50-h period [67]. Adding the same monomer to a DMSO water solution produced polymers with M_n = 7000 g/mol [67]. While the linear polymers produced were sticky and unprocessable, they later showed that disulfide-bonded hydrogels could be created in vivo by mixing their α,ω-thioacetate-functionalized PEG (M_n = 600 g/mol) with a trifunctional thioacetate cross-linker [69]. Initial cross-linking in 20 % DMSO produced an injectable fluid that finished cross-linking upon exposure to 40–50 °C heat. A subcutaneous injection of the hydrogel precursor was cross-linked to a solid gel in vivo by the application of a heating pad at 43 °C for 10–20 min [69].

Telechelic oligomers with dithiol functionality have also been oxidized to form high-molecular-weight polymers with multiple disulfide bonds in the backbone. Using reversible addition-fragmentation chain transfer polymerization (RAFT), Whittaker et al. and You et al. synthesized polystyrene with thiol functional groups at either end [70,71]. Whittaker et al. oxidized their oligomers (M_n = 3700 g/mol) using Fe(III)Cl_3 in DMF to reach M_n = 14 500 g/mol in 72 h at 60 °C [70]. Cyclization of these polymer was achieved under dilute conditions. **[ER-217]** You et al. used iodide in THF to oxidize their dithiol oligomer precursors (3500 g/mol) to polymers with M_n = 35 000 g/mol in 36 h at room temperature [71]. You et al. applied the same technique to the synthesis poly(disulfide) polymers from dithiol oligomers from acrylic acid and dithiol 2-(dimethylamino)ethyl methacrylate [71]. Tsarevsky and Matyjaszewski employed atom transfer radical polymerization (ATRP) to synthesize α,ω-bromine-functionalized polystyrene. The terminal bromine atoms were then replaced by thiol groups using thiodimethylformamide [72]. The dithiol-functionalized polystyrene (M_n = 16 000 g/mol) were oxidized using Fe(III)Cl_3 in DMF at 60 °C for 22 h to produce polymers reaching M_n = 39 500 g/mol [72].

LIVING OXIDATIVE POLYMERIZATION VIA CYCLIC INTERMEDIATES

During an effort to explore the synthetic advantages of thiol oxidation, we discovered a new living polymerization involving cyclic intermediates [73,74]. This new living polymerization is based on a synergistic oxidizing system for the polymerization of dithiol monomers. A combination of air and dilute hydrogen peroxide (3 % by weight) efficiently polymerizes dithiol monomers in the presence of triethylamine, a recyclable catalyst, at ambient temperatures [73]. This new system represents the first time that all three components have been combined for the polymerization of dithiols. The system was applied to the polymerization of 2-[2-(2-sulfanylethoxy)ethoxy]ethanethiol (DODT) (Fig. 10) [74].

The structure of the polymer product was verified by ^{1}H NMR, ^{13}C NMR, Fourier transform infrared (FTIR), and matrix-assisted laser desorption/ionization with time-of-flight (MALDI-TOF) mass spectroscopy [68,69]. All methods supported the formation of polymer with repeating units connected exclusively through disulfide bonds. The degradation of the polymer back to the original monomer by dithiothreitol further verified that the repeat units were connected by only disulfide bonds. The dn/dc value of the polymer was determined experimentally in order to determine the absolute molecular weight by size exclusion chromatography (SEC) [74].

Using the new polymerization method, we were able to reach M_n of 250 000 g/mol in 2 h with a molecular weight distribution (MWD) under 1.5. This narrow MWD is typical of all polymers synthesized by this method and starting at room temperature. We found that performing the reaction in an ice bath decreased the MWD to 1.15 while still reaching a M_n of 113 000 g/mol in 25 min. At this temperature, about half of the polymer product was oligomeric. The refractive index trace from SEC analysis shows the high-molecular-weight peak with narrow distribution separated from a series of overlapping oligomeric peaks [74]. MALDI-TOF showed that the low-molecular-weight fraction of the polymers contained only cyclic oligomers (2–14-mers) with no linear species in that range.

The self-promoting nature of base-catalyzed thiol oxidation allows for multiple pathways to the reactive thiolate anion (see Fig. 2). Additionally, the process regenerates oxygen, thereby assuring that the oxidant is always in supply to create the thiyl radical. The narrow MWD achieved in this method

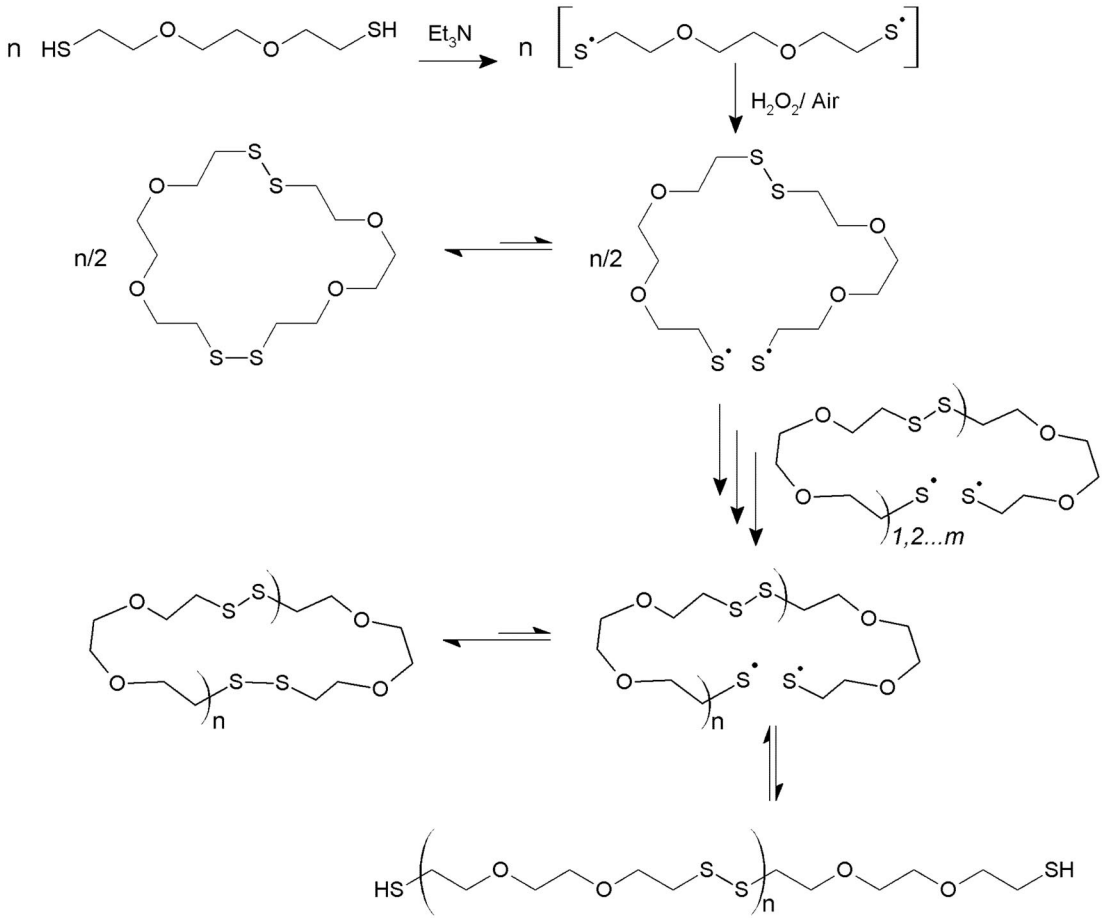

Fig. 10 Living dithiol polymerization via cyclic intermediates.

points to radical recombination as the dominant step in the polymerization. The product precipitates from the aqueous reaction medium as soon as it begins to form, forcing all reactive sulfur species into near bulk concentrations. We believe that near bulk conditions are responsible for the high molecular weights reached with this new oxidizing system.

The conditions used in the polymerization are environmentally benign, as triethylamine can be easily trapped and recycled and the side products are only oxygen and water. Polymerization reaches conversions of over 90 % in 2 h leaving little residual monomer, which could be added to subsequent polymerization feed stocks. The catalytic effect of triethylamine in cooperation with air and hydrogen peroxide serves as a benchmark for even milder catalytic reagents under investigation. Proton NMR and MALDI-TOF show no trace of triethylamine in the polymer after purification, but we acknowledge that its substitution for a more biocompatible reagent would be preferred. We also continue to use this simple system to elucidate the mechanism of this highly efficient polymerization.

CONCLUSIONS

The utility of disulfide bonds as reduction-sensitive coupling agents and crosslinkers has been fully developed by the biomedical field. The mild reaction conditions available to disulfide bond synthesis have been applied to the creation of some types of biomaterials, but only a few times to the formation

of poly(disulfide) biopolymers. Our new living polymerization produced high-molecular-weight poly(DODT), which was rubbery and reduction-sensitive. The mild reaction conditions of the polymerization were intended to achieve maximum efficiency with minimal solvents and energy consumption, and could potentially be used for a wide variety of dithiol monomers. The high molecular weights reached with this new system stand as a target for the many oxidizing biochemicals that are potential reagents in the synthesis of poly(disulfide) polymers.

ACKNOWLEDGMENTS

This material is based upon work supported by the National Science Foundation under Grant No. DMR-0804878. We also wish to thank the Goodyear Corporation for the donation of the 300 MHz NMR instrument used in this work and the Ohio Board of Regents and the National Science Foundation for funds used to purchase the 500 MHz NMR instrument used in this work.

REFERENCES

1. F. Meng, W. Hennick, Z. Zhong. *Biomaterials* **30**, 2180 (2009).
2. G. Saito, J. A. Swanson, K.-D. Lee. *Adv. Drug Delivery Rev.* **55**, 199 (2003).
3. P. E. Thorpe, P. M. Wallace, P. P. Knowles, M. G. Relf, A. N. Brown, G. J. Watson, R. E. Knyba, E. J. Wawrzynczak, D. C. Blakey. *Cancer Res*. **47**, 5924 (1987).
4. C. Jacob, I. Knight, P. G. Winyard. *J. Biol. Chem.* **75**, 1385 (2006).
5. J. P. Danehy, C. J. Noel. *J. Am. Chem. Soc.* **82**, 2511 (1960).
6. P. Ballinger, F. A. Long. *J. Am. Chem. Soc.* **82**, 795 (1960).
7. L. S. Harman, C. Mottley, R. Mason. *J. Biol. Chem*. **259**, 5606 (1984).
8. A. Holmgren. In *Methods in Enzymology*, **107**, K. M. Finn Wold (Ed.), pp. 295–350, Academic Press, New York (1984).
9. S. Oae. *Organic Sulfur Chemsitry: Structure and Mechanism*, CRC Press, Ann Arbor, MI (1991).
10. R. L. Augustine (Ed.). *Oxidation: Techniques and Applications in Organic Synthesis*, **1**, pp. 247–248, Marcel Dekker, New York (1969).
11. L. O. Randall. *J. Biol. Chem.* **164**, 521 (1946).
12. K. Faber. *Biotransformations in Organic Chemistry*, 5th ed., pp. 226, 378–381 Springer-Verlag, New York (2004).
13. Y. Yano, M. Oshima, S. Suto, M. Nakazato. *Chem. Commun.* **5**, 1031 (1984).
14. Y. Yano, M. Oshima, I. Yatsu, S. Sutoh. *J. Chem. Soc., Perkins Trans. 2* **6**, 753 (1985).
15. J. W. Holden, L. Main. *Aust. J. Chem*. **30**, 1387 (1977).
16. G. M. Whitesides, J. E. Lilburn, R. P. Szajewski. *J. Org. Chem.* **42**, 332 (1977).
17. X.-L. Wang, R. Jensen, Z.-R. Lu. *J. Controlled Release* **120**, 250 (2007).
18. Y.-M. Chen, B.-H. Huang, J.-J. Lin. *Polymer* **46**, 4619 (2005).
19. S. Matsumoto, R. J. Christie, N. Nishiyama, K. Miyata, A. Ishii, M. Oba, H. Koyama, Y. Yamasaki, K. Kataoka. *Biomacromolecules* **10**, 119 (2009).
20. S. Brocchini, A. Godwin, S. Balan, J. Choi, M. Zloh, S. Shaunak. *Adv. Drug Delivery Rev.* **60**, 3 (2008).
21. W. Shen, R. Lammertink, J. K. Sakata, J. A. Kornfield, D. A. Tirell. *Biomaterials* **30**, 2180 (2009).
22. R. P. Lyon, W. M. Atkin. *J. Am. Chem. Soc.* **123**, 4408 (2001).
23. G. E. Arteel, H. Sies. *Environ. Toxicol. Pharmacol.* **10**, 153 (2001).
24. A. Bindoli, M. P. Rigobello, G. Scutari, C. Cabbiani, A. Casini, L. Messori. *Coord. Chem. Rev.* **253** 1692 (2009).
25. A. Holmgren, M. Bjornstedt. In *Methods in Enzymology*, **252**, L. Packer (Ed.), pp. 199–208, Academic Press, New York (1995).
26. S. N. Savvides, P. A. Karplus. *J. Biol. Chem.* **271**, 8101 (1996).

27. E. S. Pilka, C. Johannson, K. Guo, O. Gileadi, A. Rojkowa, F. von Delft, A. C. W. Pike, K. L. Kavanagh, M. Sundstrom, W. H. Lee, S. Muller, B. D. Marsden, C. Bountra, U. Oppermann. *GPX3: Human glutathione peroxidase 3*, PDB Code: 2R37 (2007).
28. B. A. Shirley (Ed.). *Protein Stability and Folding: Theory and Practice*, Humana Press, New York (1995).
29. B. S. Mamathambika, J. C. Bardwell. *Annu. Rev. Cell Dev. Biol*. **24**, 211 (2008).
30. C. V. Smith, D. P. Jones, T. M. Guenther, L. H. Lash, B. H. Lauterburg. *Toxicol. Appl. Pharmacol.* **140**, 1 (1996).
31. A. Braunová, M. Pechar, R. Laga, K. Ulbrich. *Macromol. Chem. Phys.* **208**, 2642 (2007).
32. F. Q. Schafer, G. R. Buettner. *Free Radical Biol. Med.* **30**, 1191 (2001).
33. C. Carru, L. Deiana, S. Sotgia, G. M. Pes, A. Zinellu. *Electrophoresis* **25**, 882 (2004).
34. A. Zinellu, S. Sotgia, M. F. Usai, R. Chessa, L. Deiana, C. Carru. *Electrophoresis* **26**, 1963 (2005).
35. Y. Moriyama, M. Maeda, M. Futai. *FEBS Lett.* **302**, 18 (1992).
36. A. M. Cantin, S. L. North, R. C. Hubbard, R. G. Crystal. *J. Appl. Physiol.* **63**, 152 (1987).
37. D. Willner, P. A. Trail, S. J. Hofstead, H. D. King, S. J. Lasch, G. R. Braslawsky, R. S. Greenfield, T. Kaneko, R. A. Firestone. *Bioconjugate Chem.* **4**, 521 (1993).
38. P. D. Senter, W. E. Pearce, R. S. Greenfield. *J. Org. Chem*. **55**, 2975 (1990).
39. A. N. Koo, H. J. Lee, S. E. Kim, J. H. Chang, C. Park, C. Kim, J. H. Park, S. C. Lee. *Chem. Commun*. **48**, 6570 (2008).
40. S. Cerritelli, D. Velluto, J. A. Hubbell. *Biomacromolecules* **8**, 1966 (2007).
41. T. M. Allen. *Adv. Drug Delivery Rev.* **13**, 285 (1994).
42. S. Bauhuber, C. Hozsa, M. Breunig, A. Gopferich. *Adv. Mater*. **21**, 3286 (2009).
43. Y. Ji, K. Ghosh, X. Z. Shu, B. Li, J. C. Sokolov. G. D. Prestwich, A. F. Richard, M. H. Rafailovich. *Biomaterials* **27**, 3782 (2006).
44. X. Z. Shu, Y. Liu, M. C. Roberts, G. D. Prestwich. *Biomaterials* **3**, 1304 (2002).
45. X. Z. Shu, Y. Liu, F. Palumbo, G. D. Prestwich. *Biomaterials* **24**, 3825 (2003).
46. Z. M. Wu, X. G. Zhang, C. Zheng, C. X. Li, S. M. Zhang, R. N. Dong, D. M. Yu. *Eur. J. Pharm. Sci.* **37** 198 (2009).
47. J. Zhang, A. Skardal, G. D. Prestwich. *Biomaterials* **29**, 4521 (2008).
48. N. Hisano, M. Noriyuki, H. Iwata, Y. Ikada. *Biomed. Mater. Res*. **40**, 115 (1998).
49. R. Munday. In *Methods in Enzymology*, **251**, L. Packer (Ed.), pp. 117–120, Academic Press, New York (1995).
50. R. Munday. *Free Radical Biol. Med*. **7**, 659 (1989).
51. E. Cadenas. In *Methods in Enzymology*, **251**, L. Packer (Ed.), pp. 106–116, Academic Press, New York (1995).
52. J. C. Patrick. U.S. Patent 1890 191, Issued 6 Dec 1932.
53. J. C. Patrick. U.S. Patent 2553 206, Issued 15 May 1951.
54. J. C. Patrick, N. M. Mnookin, J. C. Patrick. U.S. Patent 1996 486, Issued 2 April 1935.
55. E. M. Fettes, J. S. Jorczak. *Ind. Eng. Chem. Res.* **42**, 2217 (1950).
56. K. Kishore, K. Ganesh. *Adv. Polym. Sci.* **121**, 83 (1995).
57. J. C. Patrick. U.S. Patent 2363 614, Issued 28 Nov 1944.
58. C. S. Marvel, L. E. Olson. *J. Am. Chem. Soc.* **79**, 3089 (1957).
59. P. V. Bonsignore, C. S. Marvel, S. Banerjee. *J. Org. Chem*. **25**, 237 (1960).
60. E. J. Goethals, C. Sillis. *Macromol. Chem. Phys*. **119**, 249 (1968).
61. Z. J. Witczak, J. M. Culhane. *Appl. Microbiol. Biotechnol*. **69**, 237 (2005).
62. R. L. Whistler, D. J. Hoffman. *J. Polym. Sci., Part A: Polym. Chem*. **5**, 2111 (1967).
63. W. Choi, F. Sanda, N. Kihara, T. Endo. *J. Polym. Sci., Part A: Polym. Chem*. **36**, 79 (1998).
64. Y. Z. Meng, A. R. Hllil, A. S. Hay. *Polym. Adv. Technol*. **15**, 564 (2004).
65. Y. Z. Meng, A. S. Hay. *J. Appl. Polym. Sci.* **74**, 3069 (1999).

66. Y. Z. Meng, S. C. Tjong, A. S. Hay. *Polymer* **42**, 5215 (2001).
67. H. Koo, G. Jin, Y. Lee, H. Mo, M. Cho, J.-S. Park. *Bull. Korean Chem. Soc.* **26**, 2069 (2005).
68. Y. Lee, H. Koo, G. Jin, H. Mo, M. Cho, J.-Y. Park, J. Choi, J.-S. Park. *Biomacromolecules* **6**, 24 (2005).
69. H. Koo, G. Jin, H. Kang, Y. Lee, H. Y. Nam, H.-S. Jang, J.-S. Park. *Int. J. Pharm*. **374**, 58 (2009).
70. M. Whittaker, Y.-K. Goh, H. Gemici, T. Legge, S. Perrier, M. Montiero. *Macromolecules* **39**, 9028 (2006).
71. Y. Z. You, D. S. Manickam, Q.-H. Zhou, D. Oupicky. *Biomacromolecules* **8**, 2038 (2007).
72. N. V. Tsarevsky, K. Matyjaszewski. *Macromolecules* **35**, 9009 (2002).
73. J. E. Puskas, E. Q. Rosenthal. U.S. Patent Application 20110218298, Filed 4 March 2011.
74. E. Q. Rosenthal, J. E. Puskas, C. Wesdemiotis. *Biomacromolecules* **13**, 154 (2011).

