New Biodegradable Polymers Based on Previously Unknown Functional Groups for Drug and Gene Delivery


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ABSTRACT

We recently developed the synthesis of the first polysulfenamides, polydiaminosulfides, and polydisulfidediamines ever reported in the literature. These polymers are based on functional groups previously unknown in macromolecular chemistry. The sulfenamide (RSNR₂), diaminosulfide (R₂NSNR₂), and disulfidediamine (R₂NSSNR₂) functional groups are all stable in organic solvents, but they degrade by hydrolysis in water. The rates of hydrolysis under acidic conditions of these functional groups is orders of magnitude faster than esters which is the most commonly used functional group for polymers in medicine. These polymers were investigated for their ability to act as carriers for drugs in new drug delivery systems. No evidence of toxicity was observed in in vitro experiments with HEK-293 cells or in vivo studies in mice.

Keywords: sulfenamide, diaminosulfide, disulfidediamine, polymer, nanoparticle

1 INTRODUCTION

A common method to deliver drugs or nucleic acids to desired cells is to encapsulate them in a polymeric nanoparticle or within a nanomicelle whose walls are typically composed of a polymer. Many of the polymers used in this field are based on ester, anhydride, amide, or other bonds that degrade in vivo to harmless side products. The motivation to use a biodegradable polymer is strong because if a polymer does not degrade it may accumulate in an organ such as the heart, lung, or kidneys and cause a toxic response. We recently developed three new polymers based on functional groups that were previously unexplored in macromolecular science but that readily degrade in the presence of water (Figure 1). The motivation for this work is that it is often observed that the integration of new functional groups into polymer science opens up new opportunities to take advantage of their reactivities and properties. The polymers that we developed are all stable in organic solvents but degrade to release different products in water. In this article and the presentation we will describe the synthesis of these polymers, their fabrication into particles, and their key properties as they relate to drug delivery.

2 POLYSULFENAMIDES

The sulfenamide functional group (R₂NSR) was initially studied to explore the synthesis of polymers based on sulfur and nitrogen. These polymers were synthesized by the reaction of a secondary diamine and an activated dithiol as shown in Figure 2. The activated dithiol was synthesized by the room temperature reaction of a dithiol with N-chlorosuccinimide. The resulting product was stable and purified by recrystallization or by column chromatography on silica gel.

Figure 1. Examples of the three polymers that we recently synthesized are shown.

Figure 2. A dithiol was activated by reaction with N-chlorosuccinimide and then polymerized with a secondary diamine.

The resulting polysulfenamides possessed molecular weights up to 6,300 g mol⁻¹ and were solids. The
polysulfenamide shown in Figure 2 was used in initial work to fabricate particles for drug delivery applications. These particles were fabricated according to an oil-in-water-in-oil double emulsion and possessed smooth surfaces (Figure 3). These particles were also readily loaded with drugs or with dyes. In Figure 4 a confocal microscope image of a microparticle loaded with FITC-dextran is shown. This confocal microscopy image of a cross-section of the particle demonstrates that the dye was evenly distributed throughout the particle.

Figure 3. Example of microparticles fabricated from polysulfenamides. The scale bar in both images is 5 µm in length.

Figure 4. A cross-section of a microparticle of a polysulfenamide loaded with FITC-dextran to appear green was obtained using confocal microscopy.

The particles were studied for any toxicity and for their ability to transfect cells. Microparticles loaded with FITC-dextran to appear green were exposed to human embryonic kidney (HEK-293) cells. These particles were readily taken into the cells as shown in Figure 5 and other optical images. The toxicity of these particles was investigated using in vitro studies with HEK-293 cells and in vivo experiments in mice. The particles were added to HEK-293 cells at concentrations of 1-1000 µg per mL of growth medium. After 4 h, the cell viability was probed using a MTS assay. Notably, no toxicity was observed even at the highest concentration.

These particles were also injected into mice to investigate any in vivo toxicity. The mice were injected by an intraperitoneal injection and the levels of two liver enzymes – alanine aminotransferase (ALT) and aspartate aminotransferase (AST) – were investigated at days 0, 2, 9, 16, and 23 (Figure 6). The levels of these two liver enzymes can be used to detect liver inflammation, necrosis, and cellular degeneration; they are approved FDA assays for the determination of preclinical toxicity. Importantly, the levels of these enzymes did not increase compared to control experiments in mice that were injected with PBS buffer or microparticles of poly(lactic-co-glycolic acid) which is known to be a “safe” polymer.

Figure 5. Confocal laser microscopic image of HEK-293 cells after 24 h incubation at 37 ºC. These images show several different characteristics of the cells. The actin is stained red, the nucleus is stained blue, and the polysulfenamide particles were loaded with green dye. In the lower right is a merged image to demonstrate that the particles are readily taken into these cells. Scale bar, 20 µm.

Figure 6. The levels of ALT and AST enzymes in mice at 0, 2, 9, 16, and 23 days after injection. There are five data points for each day. These data points correspond to injection of mice with PBS buffer, 10 mg of poly(lactic-co-glycolic acid) microparticles, 5 mg of polysulfenamide microparticles, 10 mg of polysulfenamide microparticles, and 15 mg of polysulfenamide microparticles.

The rates and products of degradation of sulfenamides were investigated. The degradation of a small, water-soluble molecule containing a sulfenamide bond was investigated under neutral conditions, in the presence of 8 molar equivalents of acetic acid, or in the presence of 9 molar equivalents of sodium hydroxide (Figure 7). The degradation of the sulfenamide group was slowest under basic conditions and it was fastest under acidic conditions. The rate of hydrolysis under acidic conditions was at least...
two orders of magnitude faster than the hydrolysis of ester bonds under identical conditions. This result is important because most polymers used in medicine are based on ester bonds, and the fast hydrolysis of these bonds under acidic conditions is desirable for many applications.

![Graph showing degradation over time](image)

**Figure 7.** The degradation of a molecule containing a sulfenamide bond was studied in water under acidic, neutral, and basic conditions. This molecule degraded very rapidly (orders of magnitude faster than an ester bond) under acidic conditions.

### 3 POLYDIAMINOSULFIDES AND POLYDISULFIDEDIAMINES

We also developed the synthesis of the first polydiaminosulfides and polydisulfidediamines as new targets for delivery vehicles for drugs. These functional groups were not used in the synthesis of polymers prior to our work, so their synthesis was first investigated.

The synthesis of polydiaminosulfides began with the synthesis of a new sulfur reagent as shown in Figure 8a. In this synthesis, S₂Cl₂ was reacted with dimethylamine to yield a stable product ((CH₃)₂NSSN(CH₃)₂) in high yield that was purified by distillation. This product was activated by reaction with SO₂Cl₂ followed by addition of dimethylamine to yield ((CH₃)₂NSN(CH₃)₂). Importantly, both steps of this synthesis could be scaled up to yield >50 g of product within several days without the need for costly and time consuming column chromatography.

The polymerization of secondary diamines with ((CH₃)₂NSSN(CH₃)₂) were completed at 60-85 °C for 24-72 h. The polymerizations went to high conversions (>98%) and yielded polymers with molecular weights up to 12,400 g mol⁻¹. These polymers were stable in organic solvents, but they degraded rapidly under acidic conditions.

![Diagram of polymerization](image)

**Figure 8.** a) The synthesis of a monomer with one sulfur atom. b) The polymerization of the product of a) and a secondary diamine yielded a polydiaminosulfide.

Microparticles were fabricated from these polymers and studied for their ability to be taken into cells and for any toxicity. These nanoparticles were readily internalized into HEK-293 cells (Figure 9), and they displayed no *in vitro* toxicity even at loadings of 1 mg of particles per mL of medium.

![Microparticles](image)

**Figure 9.** Microparticles of polydiaminosulfides were loaded with FITC-dextran to appear green and exposed to HEK-293 cells. The nuclei were stained blue and the cytoplasm/cell membrane was stained red. This image demonstrates that the microparticles were internalized into the cells.

The synthesis of polydisulfidediamines required the synthesis of a monomer that would transfer two S atoms. The synthesis of this monomer was straightforward and completed as shown in Figure 10a. The monomer was synthesized in >50 g quantities, and it was purified by recrystallization. This monomer was stable and possessed no odor.

The polymerization reactions to yield polydisulfidediamines were completed at room temperature for 24 h. Secondary diamines were reacted with the monomer synthesized as shown in Figure 10a to yield polydisulfidediamines with molecular weights up to 11,400 g mol⁻¹.
Figure 10. a) The synthesis of a new monomer was completed in high yields and purified by recrystallization. b) The polymerizations to yield polydisulfidediamines proceeded at room temperature.

4 CONCLUSIONS

We developed the synthesis of three new polymers based on bonds between sulfur and nitrogen. Although these three new polymers possess similar looking functional groups, the differences between these groups lead to differences in reactivities, stabilities, and products of hydrolysis with water. Each of these three polymers has potential to be used in drug delivery and other applications as shown in the figures above and in other work.

REFERENCES