# Synthesis of Poly(ethylene oxide sulfide) with Large Molecular Weight by $O_2$ Gas Oxidation

**Heebeom Koo, Geun-woo Jin, Yan Lee, Heejung Mo, Min Yi Cho, and Jong-Sang Park***

*School of Chemistry & Molecular Engineering, Seoul National University, Seoul 151-742, Korea*
**E-mail: pfjspark@plaza.snu.ac.kr*
*Received October 8, 2005*

**Key Words :** Poly(ethylene glycol), PEG, Poly(ethylene oxide sulfide), PEOS, Disulfide

Poly(ethylene glycol)(PEG) is widely used in many fields (drug delivery,[1] hydrogelation,[2] protein PEGylation,[3] etc.). However, its usage in a controlled release system was limited because its backbone is nondegradable. Many degradable PEG molecules were developed to overcome this weakness,[4] including our recent work concerning poly(ethylene oxide sulfide)(PEOS).[5] The PEOS polymer consists of many ethylene oxide monomers linked by the biodegradable disulfide bonds.[6] The disulfide bonds are degraded into thiols responding to the reduction potential in cytosol developed by the high concentration (5 mM) of a reduced form of gultathione. On the contrary, disulfides are very stable in the cell exterior, the oxidative environment. These characteristics have been used in the development of polymers which can be degraded after uptake into the cell.

However, the PEOS polymers with shorter ethylene oxides (PEOS-2 with monomers derived from triethylene oxides) have small molecular weights as shown in Table 1. The PEOS-2 polymers with small molecular weights are very sticky so that the fabrication of the polymer is uncomfortable. For example, when we made the nanoparticles with PEOS-2, they aggregated easily due to the sticky property of the polymer. This will be one obstacle for various applications in chemical or medical fields.

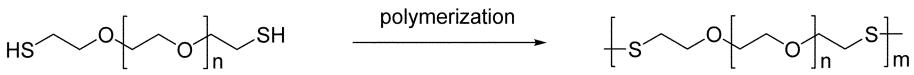
Thus, in this paper, we developed other polymerization methods to obtain a PEOS having larger molecular weight ($M_n$, $M_w$) than the DMSO/water polymerization in the previous paper.

DMSO oxidation is a typical disulfide bond formation method for the formation of the polymers.[7] In the previous paper,[5] we used 20% DMSO/water condition for the polymerization of the PEOS. In case of the PEOS having high oxygen number (PEOS-12, 36), both monomers and polymers are hydrophilic, so total polymerization proceeds in a homogeneous phase. But in case of the PEOS having low oxygen number (PEOS-2), the monomers are hydrophilic, whereas the polymers become hydrophobic after their hydrophilic thiol groups change into disulfide bonds by the oxidation. For this reason in the case of low oxygen number polymers, as the molecular weights of the polymers increase, they become insoluble in the DMSO/water condition. It was supposed that this can be one reason for the small molecular weights and large PD (polydispersity. $M_w / M_n$) values of PEOS-2.

For this reason, we tried to use DMSO/ethanol/chloroform (1 : 2 : 2) solvent in the substitute of 20% DMSO/water solvent in PEOS-2 polymerization. In the DMSO/ethanol/chloroform (1 : 2 : 2) solvent, both monomers and polymers of the PEOS are soluble, and polymerization can proceed in a homogeneous phase. PEOS-2 polymerized in this condition has very small PD value, but the molecular weights ($M_n$, $M_w$) are much smaller than that in 20% DMSO/water condition (Table 1).

Afterwards, we tried PCC (pyridinum chlorochromate) as the oxidant which is used in many organic oxidation reactions.[8] In this polymerization, both the monomers and PCC are dissolved in methylene chloride and stirred for 48 h at room temperature. PEOS-2 polymerized by PCC has small PD value, but small molecular weights ($M_n$, $M_w$) as in the case of using DMSO/ethanol/chloroform (1 : 2 : 2) solvent.

**Table 1**. The molecular weight and PD of PEOS-2 polymerized by various oxidation methods




**1** : n = 1
**2** : n = 11

**3** : n = 1, PEOS-2
**4** : n = 11, PEOS-12

| Polymerization method[a] | $M_n$ / $M_w$ | PD[b] |
|---|---|---|
| 20% DMSO/water solution | $7.3 \times 10^3$ / $2.5 \times 10^3$ | 3.4 |
| DMSO/ethanol/chloroform (1 : 2 : 2) | $2.1 \times 10^3$ / $2.7 \times 10^3$ | 1.2 |
| PCC in methylene chloride | $2.1 \times 10^3$ / $3.1 \times 10^3$ | 1.5 |
| $O_2$ gas in $NH_3$/MeOH | $6.1 \times 10^4$ / $1.4 \times 10^5$ | 2.3 |

[a]The detailed synthetic methods are in Experimental section. [b]PD value : $M_w/M_n$.

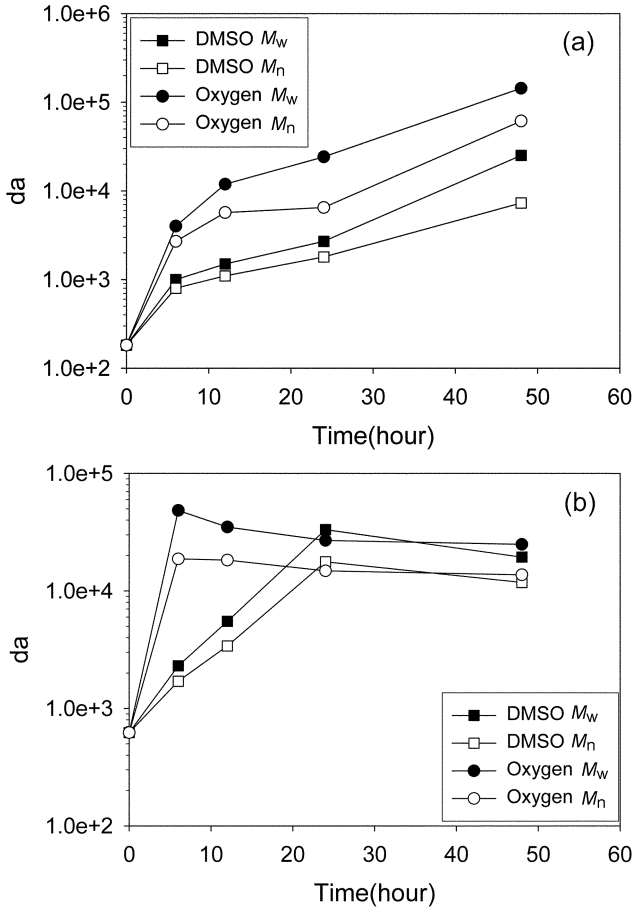



**Figure 1**. The time course of the molecular weight ($M_n$, $M_w$) of PEOS-2 (a) and PEOS-12 (b) polymerized by DMSO/water and $O_2$ gas oxidation.

We also tried the $H_2O_2$ oxidation method[9], but the molecular weight is similarly small (data not shown).

The next polymerization method for PEOS polymerization was $O_2$ gas oxidation.[10] The deacetylated monomer of the PEOS-2 was stirred in $NH_3$/MeOH solution under $O_2$ pressure. Through the total polymerization process, oxygen was supplied by an oxygen balloon connected to the stopper of the round bottom flask. As shown in Table 1, PEOS-2 polymerized by $O_2$ oxidation has 5-10 fold larger molecular weights ($M_n$, $M_w$) than that polymerized by the previous method. The comparison of the polymerization time course of PEOS-2 between DMSO and $O_2$ oxidation is shown in Figure 1a.

In the case of the PEOS-2, the difference in the molecular weights is considered to originate from two reasons. One is that $O_2$ gas is a stronger oxidant than DMSO in this condition. The other reason is the difference in the contact probability with the oxidant. During the polymerization in DMSO/water, as the polymer grows, it becomes insoluble in DMSO/water solvent. Afterwards, the reaction proceeds in the two-phase condition and the polymer would have lowered the contact probability with the oxidant, DMSO, and finally the rate of oxidation reaction would become slow. Thus, the PEOS-2 polymerized in this condition has small molecular weights.

On the other hand, oxidant, $O_2$ was supplied into the atmosphere of the reaction bottle containing the monomers. After the polymer grows and floats on the surface of the solvent, the contact probability with the oxidant would become higher. Afterwards, oxidation reaction could happen frequently, and the final polymer has much larger molecular weights.

Additional experiments were performed to support this assumption. Because both monomers and polymers of PEOS-2 can be solubilized in chloroform, the $O_2$ oxidation reaction in chloroform proceeds in a homogeneous phase. $M_w$ of the final PEOS-2 polymer in this condition is only a tenth of that in the $NH_3$/MeOH condition (data not shown). The results support our assumption that the contact probability is an important factor in determining the molecular weights.

The polymerization of hydrophilic PEOS-12 shows a different polymerization profile (Figure 1b). Both monomers and polymers of PEOS-12 are soluble in DMSO/water and $NH_3$/MeOH solution. Therefore, after 6 hour polymerization, the molecular weight of PEOS-12 of the $O_2$ oxidation is larger than that of DMSO oxidation only for the reason that oxygen is a stronger oxidant than DMSO.

After 6 hours, the $M_w$ of PEOS-12 decreased to about 30,000 in the $O_2$ oxidation. And $M_w$ in the DMSO/water oxidation decreased similarly to 30,000 after 48 hours. This result is probably explained by the sulfide exchange reaction and cyclization. Because PEOS-12 has a lower density in the thiol group than PEOS-2, the PEOS-12 would become short of thiols and so be oxidized faster. Thereafter the disulfide exchange reactions between the existing disulfide in the polymer would be dominant and the molecular weight could decrease. This result is similar to the scrambling reaction in general condensing polymerization.[11] Also, some cyclization of the polymers can be one factor in the molecular weight decrease. Because the polymerization reaction is slower in the DMSO oxidation, the start of the decrease in the

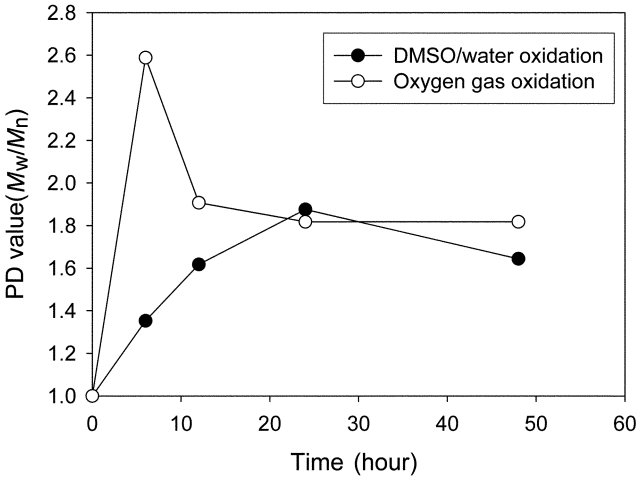



**Figure 2**. The time course of the PD (polydispersity) of PEOS-12 polymerized by DMSO/water and $O_2$ gas oxidation.

molecular weight is also slower. The change of the PD value of PEOS-12 is shown in Figure 2.

In conclusion, we developed a polymerization method to increase the molecular weight of PEOS-2 using oxygen. However the polymerization method does not work well in the polymerization of the hydrophilic PEOS-12. The high molecular weight PEOS-2 can be used in many research or medical fields as the form of the components of nanoparticles, copolymers, hydrogels and so on for controlled drug delivery.[12]

## Experimental Section

**The synthesis of PEOS-2 monomer.** Triethylene glycol-di-*p*-tosylate (3.668 g, 8.000 mmol) was dissolved in 30 mL N,N-dimethylformamide (DMF). Potassium thioacetate (2.284 g, 20.00 mmol) was added and stirred at room temperature for 7 hr. The DMF solution was concentrated to about 10 mL by evaporation and poured into 100 mL of water. The mixture was extracted three times by chloroform and the organic layer was collected, dried with $MgSO_4$ and evaporated. Thioacethyl triethylene glycol derivative (1.330 g, 5.000 mmol) was dissolved in 20 mL 2 M $NH_3$/MeOH and stirred overnight at room temperature. The solvent was removed by evaporation to give the PEOS-2 monomer.

**The synthesis of PEOS-12 monomer.** Poly(ethylene glycol) ($M_n$ = 600) (6.000 g, 10.00 mmol) was dissolved in 20 mL dichloromethane. 4-(Dimethylamino)-pyridine (DMAP) (0.611 g, 5.00 mmol), *p*-toluene sulfonyl chloride (4.194 g, 22.00 mmol), and triethylamine (1.6 mL) were added and stirred at 0 °C for 3 hrs. The solvent was removed by evaporation and dissolved in 100 mL chloroform. The solution was washed three times with water and twice with 0.1 N HCl. The organic layer was dried with $MgSO_4$ and evaporated. Tosylated poly(ethylene glycol) (7.193 g, 8.000 mmol) were dissolved in 30 mL N,N-dimethylformamide (DMF). Potassium thioacetate (2.284 g, 20.00 mmol) was added and stirred at room temperature for 7 hrs. The DMF solution was concentrated to about 10 mL by evaporation and poured into 100 mL of water. The mixture was extracted three times with chloroform and the organic layer was collected, dried with $MgSO_4$ and evaporated. The product, thioacetyl poly(ethylene glycol) derivative (3.532 g, 5.000 mmol) was dissolved in 20 mL 2 M $NH_3$/MeOH and stirred overnight at room temperature. The solvent was removed by evaporation to give the PEOS-12 monomer.

**PEOS-2 and PEOS-12 polymerization with DMSO, PCC or $H_2O_2$.** For the first polymerization experiment, 2.00 mmol of the PEOS-2 monomer or PEOS-12 monomer was dissolved in 10 mL of 20% DMSO aqueous solution. For the DMSO oxidation in organic solvent, the monomer was dissolved in a mixture of DMSO (2 mL), ethanol (4 mL), and chloroform (4 mL). For the PCC oxidation, the monomer was dissolved in 20 mL of methylene chloride with pyridinium chlorochromate (PCC) (0.431 g, 2.000 mmol). For the $H_2O_2$ oxidation, the monomer was dissolved in 10 mL of $H_2O_2$. The molecular weight was measured after 48-hour polymerization at room temperature.

**PEOS-2 and PEOS-12 polymerization with $O_2$ gas.** 2.00 mmol of the PEOS-2 monomer or PEOS-12 monomer was dissolved in 10 mL of 2 M $NH_3$/MeOH or 10 mL of chloroform. Then the mixture was stirred under oxygen atmosphere for 48 hr at room temperature. After 6, 12, 24 and 48 hrs, the polymer solution was evaporated, washed with water and dried for the characterization.

**Size characterization.** $M_n$ and $M_w$ of the polymers were determined by Size Exclusion Chromatography [SEC] (Waters 600, column T23431A 02, T23431A 12 7.8 × 300 mm Column WAT044240 Waters) using a chloroform eluent and polyethylene glycol (PEG) standards. 2 mL of the sample solution (0.20 M) in reaction was collected and extracted three times with 5 mL of chloroform. The organic layer was concentrated to 2.00 mL and 20.0 $\mu$L of the solution was injected into SEC.

**Acknowledgements.** This work was supported by the SRC-Molecular Therapy Research Center at Sungkyunkwan University (R11-2000-080-10003-0), the Korea Health 21 R&D Project of The Ministry of Health & Welfare (0405-BO02-0205-0001), and the Gene Therapy Project of The Ministry of Science and Technology (M1053403004-05N3403-00410).

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