Appendix A

AGGREGATION OF AZOBENZENE-FUNCTIONALIZED POLY(ETHYLENE GLYCOL) MICELLES

A.1 Introduction

Azobenzenes undergo a reversible, *trans* to *cis* isomerization when illuminated with UV light (Figure 1). The lowest energy conformation of the molecule is its *trans* form, but when exposed to UV light (generally near 340 nm, depending on the substitutions made on the aromatic rings) the molecule undergoes an isomerization to its *cis* form. The *cis* isomer reverts to the *trans* isomer either due to thermal energy or due to irradiation of light in the blue range (generally around 435 nm). The isomerization causes a change in dipole moment from less than 0.5 Debye in the *trans* form to 3.1 Debye in the *cis* form, a change roughly equivalent to switching between carbon tetrachloride and acetone[1].

The change in dipole moment between the *cis* and *trans* isomers of azobenzene has been exploited to grant water-soluble polymers photresponsive properties. Polymer solutions with reversible, photoresponsive changes in LCST[2, 3] and viscosity[4] have been produced when a monomer with an azobenzene side-chains is copolymerized with a water-soluble monomer. Photoresponsive changes in aggregation have been more difficult to achieve in aqueous solutions. Kopeček and coworkers synthesized random copolymers of an azobenzene-bearing monomer and a water soluble comonomer.[5] Polymers above a threshold mol% of azobenzene side-groups formed photoresponsive aggregates, the most dramatic change in aggregation number being a change from 311 before irradiation to 2 after irradiation. These photoresponsive aggregates were not, however, formed in pure water. They were formed by either dialysis of an ethanol solution of the polymer against water or by dripping a concentrated ethanol solution of the polymer into water. Only the structures formed by the dripping method showed photoresponsive deaggregation. Furthermore, the deaggregation observed was only weakly reversible. Zhao and coworkers also report finding light-responsive deaggregation of copolymers of a water soluble monomer and a second monomer with an azobenzene side chain.[6] Their aggregates were formed, however, by adding water to a dioxane solution of the polymer. A third paper with similar materials, Gan and coworkers, also found that UV irradiation did not change the aggregation of diblock copolymers where the hydrophobic block contained azobenzene pendent groups.[7]

Rather than use side-chain functionalized polymers, we investigated aggregation of PEG end-functionalized with azobenzene molecules. By varying the length of the PEG chain coupled with the azobenzene, we hoped to quantify the ratio of hydrophobic (azobenzene) to hydrophilic (PEG) material needed to induce photoresponsive changes in aggregation. Sadly—within the range of functionalized PEG molecules we could produce—aggregation occurred with both the *trans* and the *cis* forms of azobenzene. No photoresponsive changes in aggregation were observed.

A.2 Experimental

Azobenzeze-Functionalized PEG Synthesis. Azobenzene-functionalized PEG was prepared from the reaction of alcohol-functionalized azobenzenes with tosylated PEG. To synthesized tosylated PEG, 5g of PEG (either PEG diol or PEG mono-methyl ether) was dissolved in toluene and dried under vacuum. The polymer was dissolved in anhydrous dichloromethane. 9 g of *p*-toluenesulfonyl chloride and 5 ml of anhydrous triethylamine were added to the flask. These masses of reagent were used irregardless of the molecular weight of the PEG. The flask was topped with argon and stirred overnight at room temperature. The dichloromethane was evaporated in vacuum, and the products were redissolved in ethyl acetate. The solution was filtered to remove the insoluble triethylamonium chloride salt and the filtrate placed in the freezer overnight where the polymer precipitates from solution. The precipitate was filtered, and the process repeated two more times. After the final precipitation, the product was dried in vacuum to give a white, waxy product. NMR verified the substitution of the tosyl group on the polymer. The NMR indicated the presence of some untreated *p*-toluenesulfonyl chloride and some triethylamonium chloride, but a small amount of these impurities were tolerated and removed in the final purification.

Substitution of the azobenzene for the tosyl group was accomplished overnight in anhydrous acetonitrile at 35°C in the presence of cesium carbonate under argon. A ten-fold molar excess of Az-OH was used. While azobenzenes are thermally stable, they are cleaved by methyl-substituted aromatics (in this case, the tosyl group) at elevated temperatures.[8] For this reason, a higher temperature was avoided even if it would increase substitution. A gel formed as the reaction progresses, and this gel was broken by addition of methanol and vigorous stirring before the cesium carbonate was filtered. Once filtered, the methanol and acetonitrile were removed under vacuum, being careful never to heat the solution above 35°C. Precipitation in cold diethylether was used to separate away most of the unreacted azobenzenes, but precipitation alone could not yield a sample free of all small-molecule contamination. Therefore, after precipitation, the solids were redissolved in methanol, and the product separated using a 2.5 by 50 cm Econo-Column (BIO-RAD Life Sciences Research, Hercules, CA) filled with Sephadex LH-20 (Amersham Biosciences, Piscataway, NJ) in methanol. After the columns, the methanol was evaporated, and the polymer dried overnight under vacuum. The product was a deep yellow solid.

Polymer Characterization. Nuclear magnetic resonance (NMR) proton spectra were collected on a 300 MHz Varian spectrometer equipped with an automated sampler using 32 scans and a 2 second delay time. Gel permeation chromatography (GPC) was performed using THF (flow rate = 0.9 μL min^{-1}), a Waters 410 differential refractometer, and two Polymer Laboratories 30 cm long PLgel 10 mL analytical columns connected in series (calibrated with polystyrene standards).

Irradiation and UV-Vis. Surfaces were illuminated with UV light using an Oriel Instruments (Stratford, CT) arc lamp equipped with a 500 W Hg-Xe lamp. An Oriel liquid filter was used to remove IR light; water was circulated through the liquid filter cooling jacket using an aquarium pump. To isolate UV light, a high pass filter (Schott WG 320) and a band pass filter (Hoya U 360) were used, both purchased from Edmunds (Barrington, NJ). These colored filters were submerged in water in a home made filter holder to prevent overheating. The sample was placed in a quartz cuvette for irradiation. A magnetic stir bar was placed in the cuvette, and the cuvette placed over a stir plate during irradiation.

Cis to *trans* isomerization of azobenzenes was followed using a UV-Vis spectrometer (Beckman, model DU640) to monitor absorption at 348 nm. This is the maximum absorption of the *trans* isomer, and the *cis* isomer has approximately zero absorption at this wavelength.[9]

MALS. The refractive index increment (dn/dc) was measured for the azobenzene-functionalized PEG samples since this parameter is needed for light scattering measurements. The index of refraction of samples ranging in concentration from 3.92×10^{-4} g/ml to 2.05×10^{-2} g/ml was measured on a refractometer (Bausch & Lomb). The value of dn/dc was calculated from the slope of the index of refraction versus concentration curve. For pure PEG, a value of 0.134 was used to calculate polymer molecular weight.[10] Tethering azobenzenes to PEG increases dn/dc, with its relative importance increasing as PEG length decreases (Table 1).

Static light scattering measurements were performed on a Wyatt Technology (Santa Barbara, CA) Dawn EOS light scattering instrument. A diode laser operating at 685nm (outside the absorbance range of azobenzenes) was used for the scattering experiments.

The MALS was operated in batch mode using scintillation vials. Calibration of the 90º detector was performed with a toluene sample passed through a 0.02 µm syringe filter. Other detectors were calibrated to the 90º detector using a polystyrene standard (M_w = 29,300) in toluene. All samples were filtered through a 0.2 µm syringe filter prior to measurement to remove dust.

To operate the instrument using scintillation vials, each measurement was repeated four times with the scintillation vial rotated approximately 40º after each measurement. At each position the data was collected for three minutes. Among the four measurements, the lowest was chosen for analysis since artifacts due to impurities on the vial or dust in the solution always act to increase scattering intensity. Scattering data was analyzed using Astra 4.90.08 (Wyatt Technoogies.) Briefly, the software averages data at each scattering angle (θ) during the three minutes collection time and calculates the excess Rayleigh ratio (R_θ). This data is then plotted using the Zimm method: K^*c/R_θ is poltted versus $\sin^2(\theta/2)$, where c is the concentration and K^* is the "optical constant." K^* is equal to $4\pi^2 n^2 (dn/dc)^2 \lambda^{-4} N_A^{-1}$, where n is the index of refraction of the solvent, λ is the wavelength of the scattered light, and N_A is Avogadro's number. The intercept gives the inverse of the molecular weight of the scatterer.

A.3 Results

Azobenzene-Functionalized PEG Synthesis. For all three azobenzene-functionalized PEG molecules, NMR showed no unreacted tosylates present and GPC showed a monomodal distribution (Figure2, a-c). The percent modification was calculated from NMR and is greater than 80% for all samples (Table 1).

Azobenzene Photoisomerization. The time for the azobenzene molecules to reach a stationary state increased as the concentration of the polymer increased (Figure 3.) To determine whether the azobenzenes would revert to their *trans* forms in the time frame of the light scattering experiments, the *cis* to *trans* relaxation of an azobenzene-functionalized PEG solution was also measured (Figure 4). Exposed to the ambient light of the laboratory, the half life for the *cis* to *trans* isomerization was 1.7 hours. When the sample was kept in the dark, however, the half life increased to 23 hours. It can therefore be assumed that minimal change in the ratio of the *trans* to *cis* states occurred during the time it takes to filter the irradiated sample into a scintillation vial and load it into the MALS instrument during which time it is exposed to ambient light (~5 minutes) and during the scattering experiment during which time it is in the dark (~12 minutes).

Light Scattering. Before UV irradiation the molecular weight of the Az-PEG$_{1100}$-Me aggregates was 17,800 g/mol (the average of measurements made at the three different concentrations); this gives an aggregation number of approximately 14. The molecular weight of the Az-PEG$_{2000}$-Me aggregates jumped to 136,000 g/mol, an aggregation number of 62. Finally, the molecular weight of the Az-PEG$_{5000}$-Me aggregates was close to that of the Az-PEG$_{2000}$-Me aggregates at 134,000 g/mol. The aggregation number of the Az-PEG$_{5000}$-Me micelles was approximately 26.

For most samples, decrease in aggregate molecular weight was observed with MALS after UV irradiation (Figure 5). Unfortunately, unmodified PEG showed the same decrease in molecular weight when examined with light scattering after UV irradiation (Figure 6). Also, MALS measurements of the azobenzene-functionalized PEG samples were repeated after relaxation of azobenzenes back to their *trans* isomers (confirmed with UV-Vis spectroscopy.) These measurements did not show a return back to the same molar mass as the unirradiated sample; thus, the change cannot be attributed to reversible photoisomerization of the azobenzene group.

A.4 Discussion and Conclusion

Intuitively, one would expect the aggregation number of azobenzene-functionalized PEG to decrease with increasing PEG length as long as the hydrophobe size remains constant. As the size of the PEG block grows, the entropic cost of stretching the polymer to confine it within an aggregate grows. Part of this entropic cost of stretching can be mitigated by allowing the polymer head-group to take up more space, thereby reducing the total number of polymers in the aggregate.

This trend—decreasing aggregation number with increased PEG molecular weight—is not, however, observed in the azobenzene-functionalized PEG system. The aggregation number increases as one moves from a surfactant with a 1100 g/mol PEG chain to a surfactant with a 2000 g/mol PEG chains. Only as one moves from a 2000 g/mol PEG chain to a 5000 g/mol PEG chain is the expected decrease in aggregation number observed. The reason for the anomalously low aggregation number measured from Az-PEG1100-Me is not clear. Aggregation has been observed in other PEG surfactant systems or mixed PEG and surfactant systems that does not follow the traditional micelle model. PEG interacts with the alkyl group of ionic surfactants such as SDS, for example, and these interactions appear hydrophobic in nature.[11] In PEG surfactant systems where the hydrophobe is a small fluoro-alkyl group (6 carbons), formation of dimers rather than micelles has been observed.[12] It is possible that such irregular self-assembly is occurring in the Az-PEG1100-Me system due to interactions between the PEG chains and the azobenzene molecules or due to azobenzene-azobenzene interactions that are not strictly hydrophobic in nature.

The lack of photoresponsive aggregation observed in the azobenze-functionalized system could perhaps be remedied by using a mixed solvent system such as the ethanol/water system used by Kopeček and coworkers[5] or the dioxane/water system used by Zhao and coworkers.[6] This approach was not pursued in our study, however, since our goal was to design useful material for aqueous systems in which the presence of organic solvents may be detrimental. Rather than looking to mix solvents, we decided to explore a second chromophore, malchite green, that changes from a neutral molecule to a charged molecule upon UV irradiation and would therefore be expected to have a larger change in solubility than the *trans* to *cis* isomerization of azobenzene.

A.5 Tables and Figures

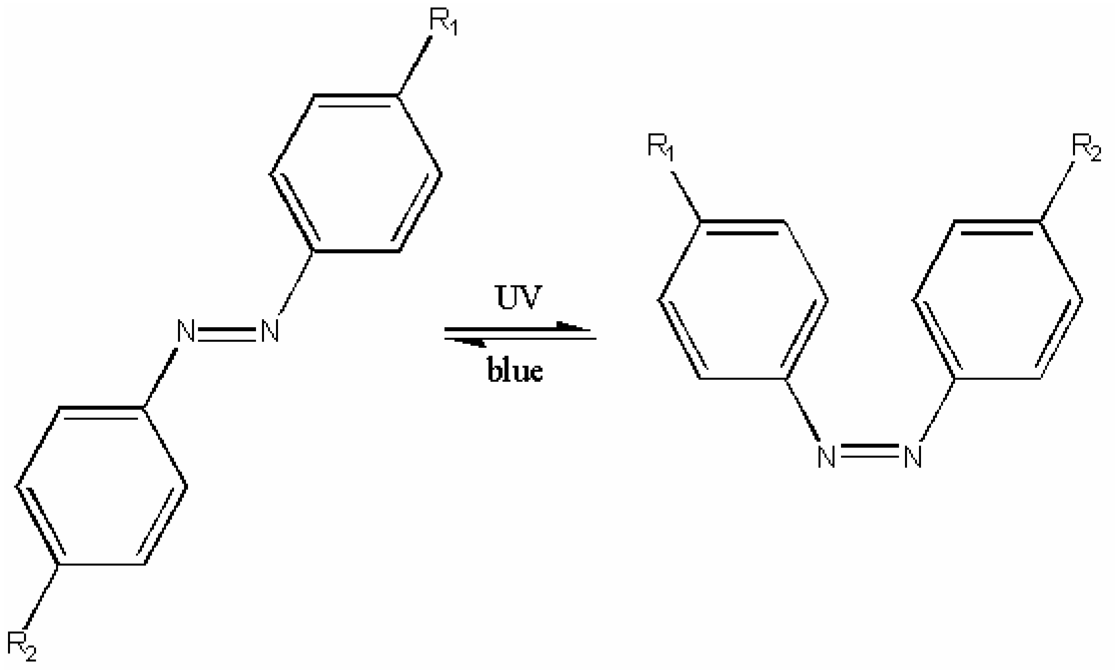

Figure A.1 Photoisomerization of azobenzene. *Trans* azobenzenes are planar, while the *cis* azobenzenes are crescent shaped. Concomitant with the change in shape is a change in length: the distance between 4 and 4’ carbons in the *cis* form of azobenzene is approximately .35 nm shorter than in the *trans* form. [1]

sample	percent substitution	dn/dc (ml/g)
Az-PEG1100-Me	93	0.162
Az-PEG2000-Me	95	0.135
Az-PEG5000-Me	87	0.130

Table A.1 Percent conversion of end-groups for azobenzene-functionalized PEG calculated from ^{1}H NMR and dn/dc values calculated from index of refraction measurements. To determine conversion, the integration of the final two hydrogens on the PEG chain (δ = 3.65 ppm) were compared with the integration of the methyl peak (δ = 3.4 ppm). The incremental index of refraction was measured at 627 nm.

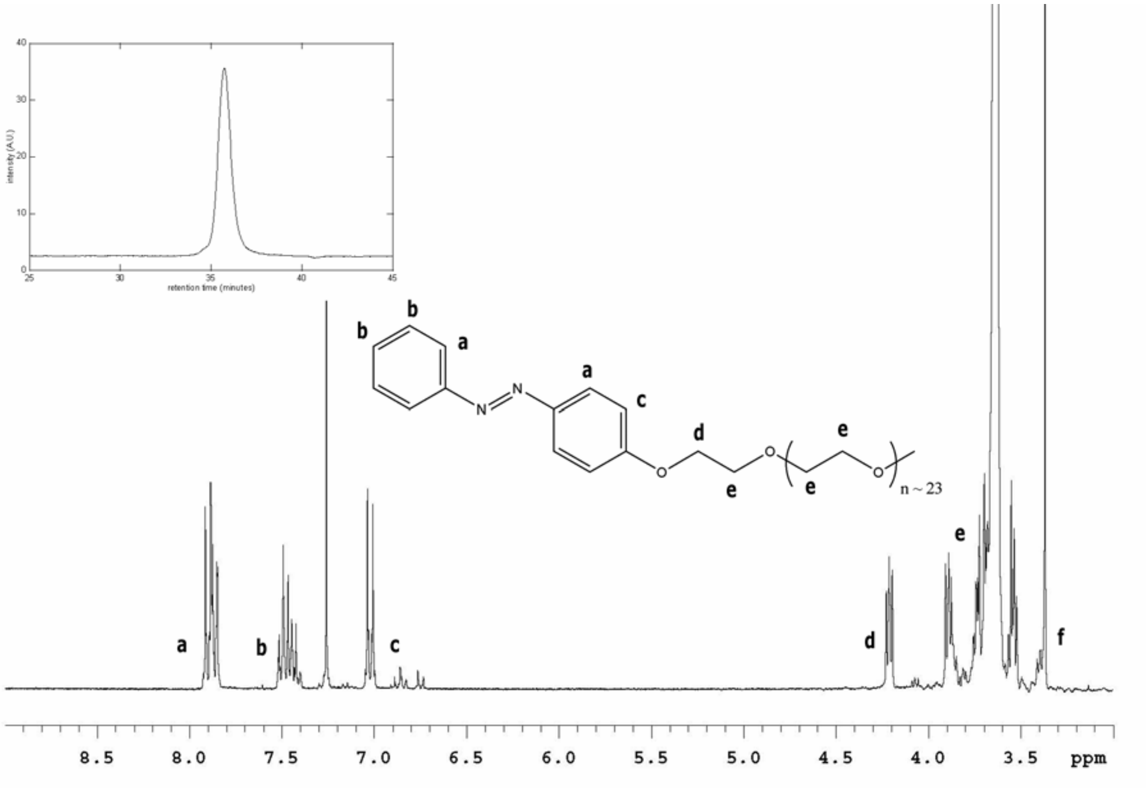

Figure A.2a NMR Spectrum and GPC trace of Az-PEG$_{1100}$-Me.

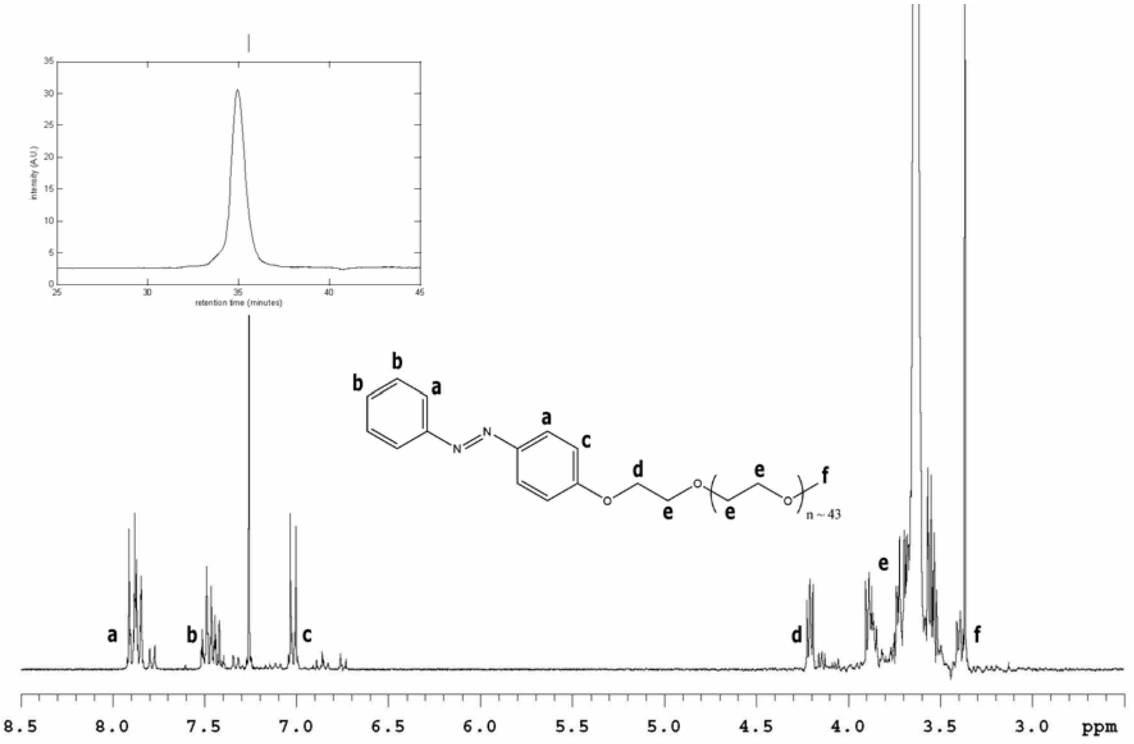

Figure A.2b NMR spectrum and GPC trace of Az-PEG$_{2000}$-Me.

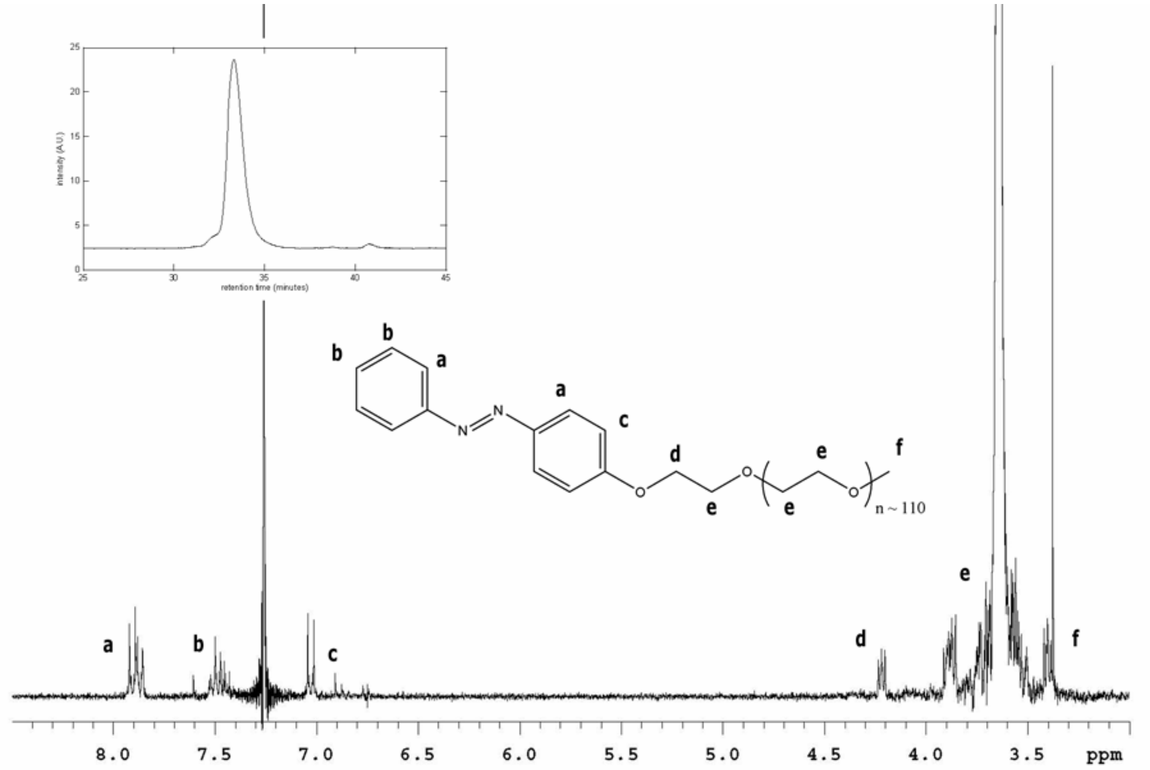

Figure A.2c NMR spectrum and GPC trace of Az-PEG$_{5000}$-Me.

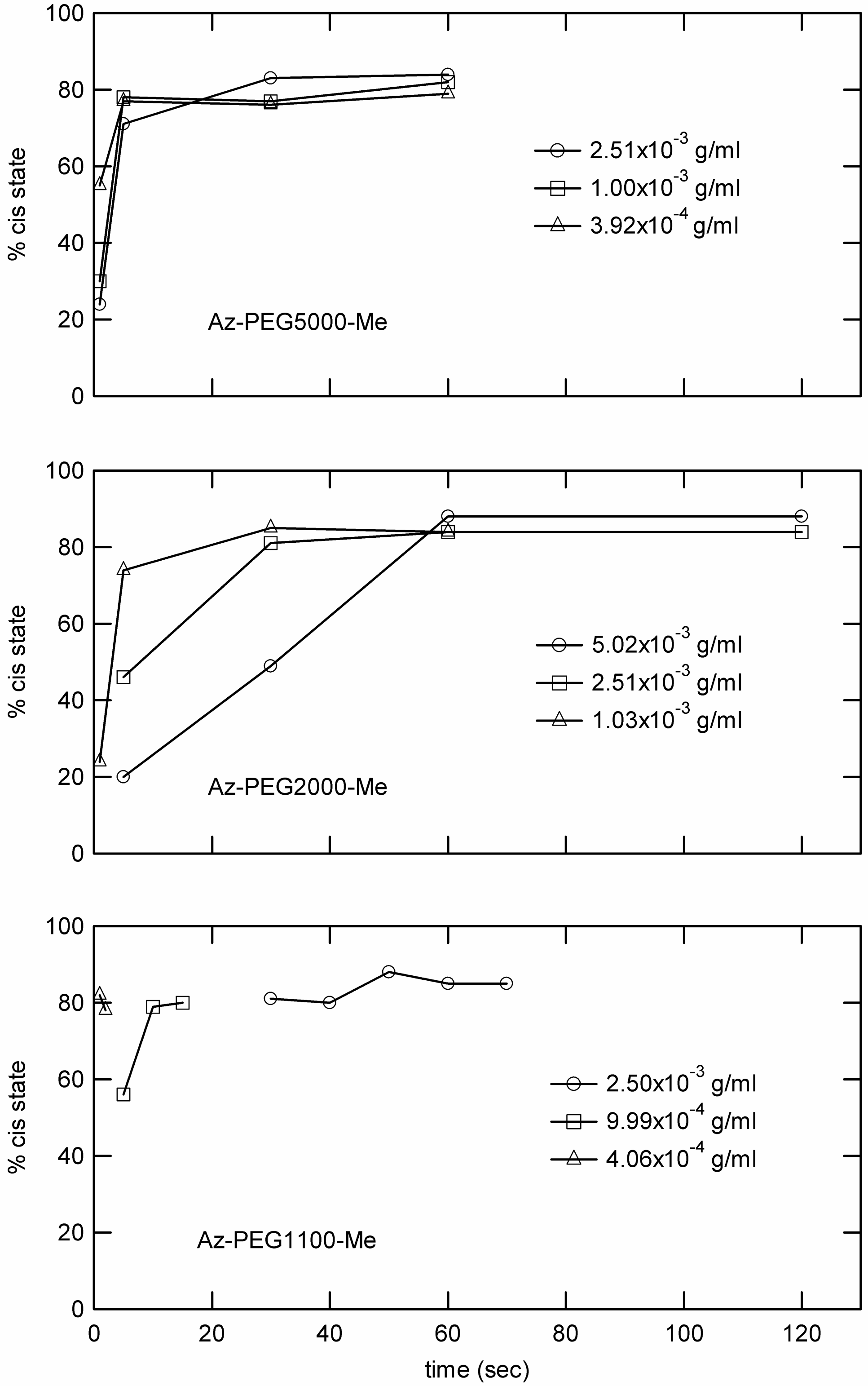

Figure A.3 Time to reach photostationary state for the azobenzene-functionalized PEG samples used for MALS study. The percentage of the *cis* form present at the photostationary state is calculated assuming the absorption of the *cis* azobenzene is zero.

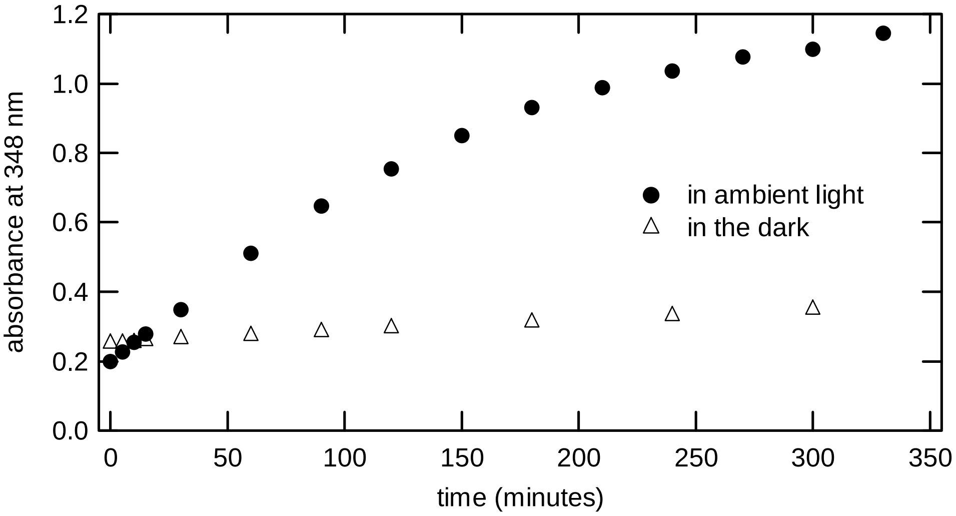

Figure A.4 Cis to trans relaxation of azobenzene-functionalized PEG. A solution of Az-PEG1100-Me (1.27×10-4 g/ml) was illuminated for 1 min before the relaxation was measured by monitoring the absorbance at 348 nm. The filled circles show relaxation when the sample is exposed to ambient light. This allows for both thermal relaxation and relaxation by absorbance of blue light to occur. The open triangles show the much slower relaxation of a sample in the dark in which thermal relaxation alone occurs.

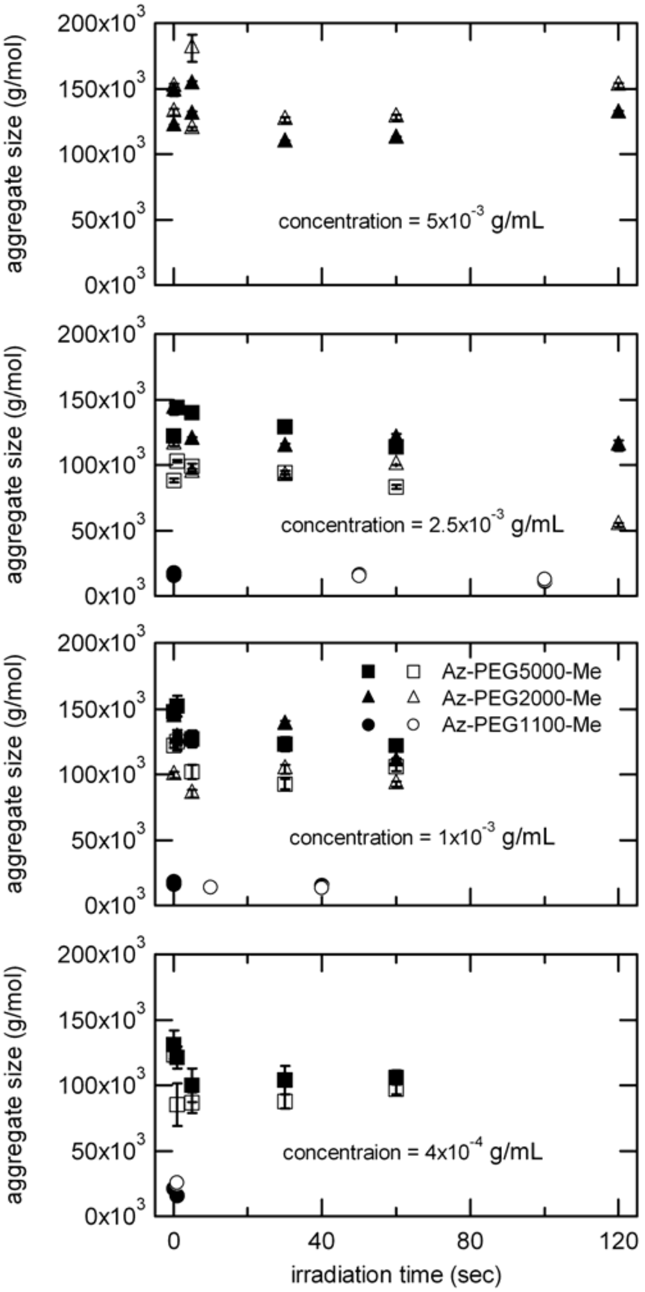

Figure A.5 Molecular weight of azobenzene-functionalized PEG aggregates measured by MALS. Filled symbols are measurements taken immediately after UV irradiation; open symbols are measurements takes after relaxation of azobenzenes back to trans form.

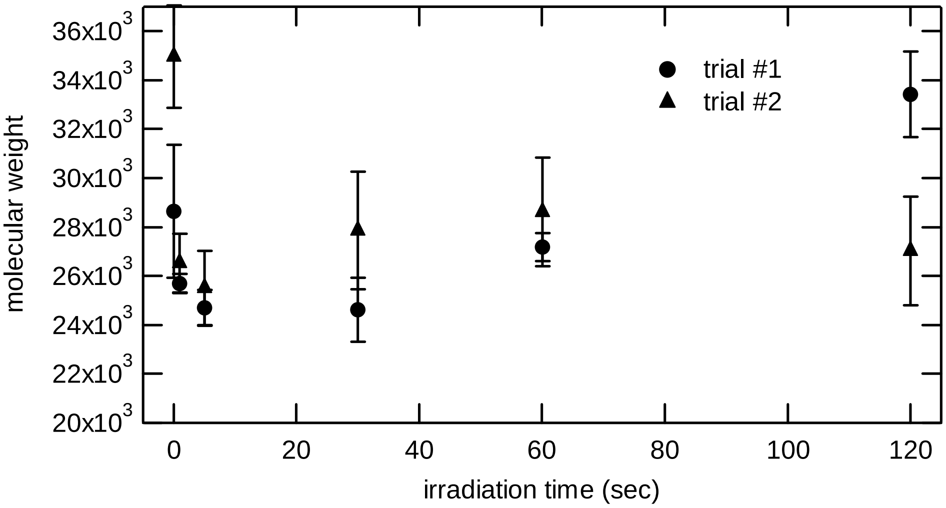

Figure A.6 Change of molecular weight of 35K PEG measured by MALS after UV irradiation.

A.6 References

1. Irie, M., Photoresponsive Polymers. *Advances in Polymer Science* **1990,** 94, 27-67.

2. Irie, M.; Kungwatchakun, D., Stimuli-Responsive Polymers - Photostimulated Reversible Phase-Separation of Aqueous-Solutions of Poly(N-Isopropylacrylamide) with Pendant Azobenzene Groups. *Proceedings of the Japan Academy Series B-Physical and Biological Sciences* **1992,** 68, (8), 127-132.

3. Kroger, R.; Menzel, H.; Hallensleben, M. L., Light Controlled Solubility Change of Polymers - Copolymers of N,N-Dimethylacrylamide and 4-Phenylazophenyl Acrylate. *Macromolecular Chemistry and Physics* **1994,** 195, (7), 2291-2298.

4. Pouliquen, G.; Tribet, C., Light-triggered association of bovine serum albumin and azobenzene-modified poly(acrylic acid) in dilute and semidilute solutions. *Macromolecules* **2006,** 39, (1), 373-383.

5. Konak, C.; Kopeckova, P.; Kopecek, J., Photoregulated Association of N-(2-Hydroxypropyl)Methacrylamide Copolymers with Azobenzene-Containing Side-Chains. *Macromolecules* **1992,** 25, (20), 5451-5456.

6. Wang, G.; Tong, X.; Zhao, Y., Preparation of azobenzene-containing amphiphilic diblock copolymers for light-responsive micellar aggregates. *Macromolecules* **2004,** 37, (24), 8911-8917.

7. Ravi, P.; Sin, S. L.; Gan, L. H.; Gan, Y. Y.; Tam, K. C.; Xia, X. L.; Hu, X., New water soluble azobenzene-containing diblock copolymers: synthesis and aggregation behavior. *Polymer* **2005,** 46, (1), 137-146.

8. Overberger, C. G.; Anselme, J.-P.; Lombardino, J. G., *Organic Compounds with Nitrogen-Nitrogen Double Bonds*. The Ronald Press Company: New York, 1966.

9. Kang, H. C.; Lee, B. M.; Yoon, J.; Yoon, M., Synthesis and surface-active properties of new photosensitive surfactants containing the azobenzene group. *Journal of Colloid and Interface Science* **2000,** 231, (2), 255-264.

10. Brandrop, J.; Immergut, E. H.; Grulke, E. A., *Polymer Handbook*. 4 ed.; John Wiley & Sons, Inc.: New York, 1999.

11. Nikas, Y. J.; Blankschtein, D., Complexation of Nonionic Polymers and Surfactants in Dilute Aqueous-Solutions. *Langmuir* **1994,** 10, (10), 3512-3528.

12. Zhang, H. S.; Pan, J.; Hogen-Esch, T. E., Synthesis and characterization of one-ended perfluorocarbon-functionalized derivatives of poly(ethylene glycol)s. *Macromolecules* **1998,** 31, (9), 2815-2821.