Appendix C

COPOLOLYMERS OF ACRYLAMIDES AND TRIPHENYLMETHANE LEUCO (MALACHITE GREEN) MONOMERS: THEIR SYNTHESIS AND PHOTORESPONSIVE BEHAVIOR

C.1 Introduction

Our studies of azobenzene-functionalized poly(ethylene glycol) (PEG) (Appendices A and B) indicated that a photoresponsive chromophore that undergoes a larger change in dipole moment after irradiation than azobenzene would be more useful for controlling polymer aggregation in solution (Appendix A) and wetting properties on surface-immobilized polymer adlayers (Appendix B). Malachite green dyes (also called triphenylmethane leuco dyes) change from a neutral, colorless form to a cationic, green form when irradiated with UV light (Figure 1). When the cationic form of the dye recombines with the anion, the dye discolors and returns to a neutral form.[1] Copolymers synthesized using the vinyl derivative of malachite green chromophores bearing two different anions are reported in the literature: triphenylmethane leucohydroxide vinyl monomer (VN-MG-OH) and triphenylmethane leucocyanide vinyl monomer (VN-MG-CN).

The literature on malachite green-functionalized polymers, however, is much less developed than that of azobenzene-functionalized polymers. Many of the published papers study networks formed when vinyl malachite green monomers are copolymerized with acrylamides and crosslinking agents.[2-5] Unfortunately, data from the synthesis of hydrogel networks is difficult to generalize to linear polymerizations. Information such as the relative reactivity of the two monomers is obscured. Only two papers focus on linear copolymers of acrylamides and malachite green vinyl monomers. One fails to examine fundamental properties such as the molecular weight of the polymers and the possibility that branching may occur during the polymerization.[6] The other does report the molecular weight of the two polymers produced.[7] The authors, however, did not examine both the suspiciously low molecular weights of the polymers and the very large dependence of yield on the mol% of the malachite green monomer in the feed; they did succeed in their goal of synthesizing a polymer to produce *in situ* photoactivated pH jumps.

The lack of well-characterized materials in the literature required initial studies of the feasibility of using these comonomers to produce copolymers of well-defined branching, length, and composition. Our intent was to synthesize vinyl monomers containing malachite green chromophores by previously published procedures and prepare linear copolymers of these monomers with acrylamides. Such polymers would enable studies of their photoresponsive aggregation using rheological and light scattering methods. Ultimately, it was hoped that these monomers could be used in a surface-polymerization technique to form photoresponsive brushes. Given our intent in creating brushes, our attention was directed to monomers suitable for growing chains from a surface, specifically acrylamide copolymers.

Low conversions were the focus of our synthetic efforts to guarantee that the resulting polymers contained low branching and as homogenous a distribution of comonomers as possible. Before attempting to synthesize the random copolymer of VN-MG-OH and N,N-dimethylacrylamide (DMAAm), PDMAAm homopolymer was synthesized to find the optimal conditions for achieving high molecular weight, linear polymers. A relatively low reaction temperature was used to maximize the molecular weight.[8] Low polymer conversions were used for two reasons, the first reason being that low conversions minimize polymer branching. The branching density, ρ, (number of branches per monomer molecule polymerized) can be related to the extent of reaction, *p*, (the fraction of monomers incorporated into chains) and the polymer transfer constant, C_p: [8]

$$\rho = -C_p\left[1 + \left(\frac{1}{p}\right)\ln(1-p)\right]$$

Using a value of 6.1×10^{-3} for this system (a value calculated for PDMAAm at 50º C) [9], assuming a molecular weight of 100K, a system polymerized to $p = 0.1$ will have one branched chain for every 303 chains, while a system polymerized to $p = 0.9$ will have one branched chain in every 10. Also, since these homopolymerizations are being used to determine optimal conditions for copolymerization, it is important to keep in mind that the composition of copolymers in a free radical polymerization changes with conversion: polymers formed early in the polymerization process contain more of the reactive monomer than polymers formed late in the polymerization process. Since the copolymerization of VN-MG-OH and DMAAm is not a documented copolymerization, the reactivity ratios are unknown. Low conversions, whatever the reactivity ratio, will guarantee a minimum of drift in composition between polymers created at the beginning and polymers created at the end of the reaction. Once the optimal conditions for producing high molecular weight PDMAAm were determined, copolymerizations with VN-MG-OH were attempted at the same conditions.

Sadly, we learned that malachite green has disastrous consequences for free-radical polymerization. Since the predominant methods of forming copolymer brushes from surfaces are variants of radical polymerization, we therefore abandoned this avenue of research.

C.2 Experimental

Materials. All reagents were purchased from Sigma-Aldrich and used as received unless otherwise noted.

Malachite Green Vinyl Monomer Synthesis. Malachite green vinyl monomer (VN-MG-OH) was synthesized following a published procedure (Scheme1)[7] with the following changes: The magnesium turnings (1.01g) were placed in 5mL of anhydrous THF at the beginning of the synthesis. Heat, rather than a trace amount of iodine, was used to prompt formation of the Grignard reagent once the bromostyrene (6mL) was added. Anhydrous THF—rather than anhydrous benzene—was used as the solvent for Michler's ketone (5.1g). Four liquid/liquid extractions were performed after the reaction: two with 15 mL of 10% ammonium chloride and two with 20 mL of water. The organic phase was then purified on an alumina column with THF after the liquid/liquid extractions. Finally, the collected solution was concentrated by evaporation, and hexane was added until crystals began to form.

PDMAAm homopolymer polymerization. DMAAm was distilled over calcium hydride, and AIBN was recrystallized from methanol (2g AIBN, 40mL methanol). The desired amount of initiator and monomer was placed inside a Schlenk tube, and three freeze/thaw cycles were performed. Initially, polymerization was performed in THF as solvent. Then, the amount of THF was reduced, which ultimately lead to eliminating the THF altogether. The tube was then placed in an oil bath maintained at 45ºC for the desired reaction time. At the end of the reaction time, the contents of the Schlenk tube were transferred to a round bottom flask and hexane added to precipitate the polymer. The resulting polymer was precipitated twice in hexane and twice in diethyl ether. The precipitate was dried in vacuum overnight before analysis.

MG-VN-co-PDMAAm Synthesis. Copolymers were formed using the same procedure as with PDMAAm homopolymer. VN-MG-OH were soluble in DMAAm, so the monomer was used as the solvent. To test the reactivity of the chromophore independent of the vinyl group, malachite green (MG-OH) was also added to DMAAm using the same polymerization technique.

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. Multi angle light scattering (MALS) was performed on a Wyatt Technology (Santa Barbara, CA) Dawn EOS in micro-batch mode. Polymer solutions were mixed at a minimum of four different dilutions, a syringe pump was used to flow the solutions through the system, and a Zimm plot was constructed to find the molecular weight.

C.3 Results

Malachite Green Vinyl Monomer Synthesis. The ^{1}H NMR of the VN-MG-OH synthesized matched the peaks reported in the literature(Figure 2).[7] Typical yield: 4.8%.

PDMAAm homopolymer polymerization. Initially, PDMAAm was produced using the same conditions used for copolymerization of VN-MG-OH in the paper by Nakayama and Matsuda;[7] in their work the polymerization was performed at 60ºC for 6 hours. These conditions led to autoacceleration within 5 minutes. Lower reaction temperatures (45ºC) and shorter reaction times ($\leq$ 2 hours) were therefore examined (Table 1).

Six different reaction conditions were tested for the homopolymerization of PDMAAm during which autoacceleration did not occur (Table 1). In all runs—save the first—the conversion was maintained below 12% as desired. As expected, molecular weight of the polymer increases as the ratio of monomer concentration to initiator concentration is increased. Note that using an atypically low reaction temperature for AIBN and atypically high monomer concentration (bulk), high molecular weight polymer was synthesized: 2×10^6 g mol^{-1} (trial 6 in Table 1). Prospective studies of the effects of association on rheology would benefit from such high molar mass.

MG-VN-co-PDMAAm Synthesis. No copolymer was produced when VN-MG-OH and DMAAm were reacted under the same conditions that were found to be optimal for the homopolymerization of DMAAm (Table 2, trial 1). Only increasing the ratio of initiator to VN-MG-OH ten-fold and adding an extra hour of reaction time lead to the synthesis of a measurable amount of polymer (Table 2, trials 2 and 3). When reaction time was further increased (to 5 rather than 3 hours) and initiator doubled, the polymer produced was crosslinked (trial 4).

To test whether the inhibition observed when VN-MG-OH was present it the reaction tube was due to the monomer itself or due to an impurity present from the synthesis, it was attempted to homopolymerize DMAAm in the presence of malachite green (MG-OH) purchased from Sigma-Aldrich. THF was needed to solubilize the chromophore. PDMAAm could not be produced in the presence of MG-OH (trials 5 and 6).

Since trial 2 alone gave a usable amount of polymer, this sample was further tested. Polymer from trial 2 was diluted with distilled water (polymer concentration = 1.36×10^{-4} g/mL). The solution immediately turned from colorless to green in the ambient light of the laboratory. The published extinction coefficient[1] was used to estimate the mol percent of MG-VN in the copolymer from the absorbance measured at 620nm; the chromophore accounted for 0.77mol% of the monomers in the copolymer. It was observed that the absorbance of the polymer solution did not decrease after three days in the dark. When dialyzed against 9.4 m*M* sodium hydroxide the polymer turned colorless in less than 15 min. Since the pH of distilled water is ~5 (the measured pH of this first polymer solution), a second solution was made in pH 7 buffer. This solution showed the same behavior: the polymer turned green spontaneously under ambient conditions and did not return to its colorless form even after two years in a dark drawer wrapped in aluminum foil.

C.4 Discussion and Conclusions

Despite the ease with which DMAAm is homopolymerized, it proved difficult to form a copolymer with VN-MG-OH. The inhibition of PDMAAm polymerization by VN-MG-OH and the crosslinking observed during longer reaction times indicates that VN-MG-OH is a chain transfer agent. To rule out the possibility that an impurity introduced during synthesis was responsible for the problems during polymerization, MG-OH (without the vinyl group) was purchased from Sigma-Aldrich. Since it was shown that

PDMAAm would not polymerize in the presence of MG, it was concluded that MG is an excellent chain transfer agent, and the copolymerization attempted was unachievable. Since alkyl-substituted amines are good transfer agents,[8] it is likely the methyl-substituted amines on MG-OH make it an excellent transfer agent.

Although it is never mentioned in the literature that malachite green is a transfer agent, this realization explains many of the observations made in previous investigations of malachite green containing copolymers. The predominance of work done on acrylamide networks containing malachite green monomers over reports of linear copolymers is probably due to the fact that the crosslinking caused by the malachite green monomer simply adds to the crosslinking caused by the nominal crosslinking agent. Of the two papers that focus on linear copolymers, only the work of Nakayama and Matsuda provides sufficient information to asses the potential role of the chromophore as a transfer agent. Two striking, unexplained observations are consistent with the role of malachite green. First, the large concentration of initiator used in their synthesis would lead to homopolymerization of DMAAm under the same conditions. The autoacceleration of DMAAm polymerization results from the rapid increase in viscosity due to formation of very long chains; in Nakayama and Matsuda, by contrast, the chain lengths remain low, consistent with the presence of a chain transfer agent. Second, the large dependence of polymer molecular weight on mol% of VN-MG-OH in the feed can be explained by an increase in the amount of chromophore increasing chain termination by transfer reactions. The ability of malachite green to function as a chain transfer agent precludes synthesis of the large, linear copolymers desired for our studies.

In addition to these synthetic difficulties, the small amounts of copolymer produced also illuminated further limitations of this chromophore. While the copolymer did change from its colorless, neutral form to a colored, charged form in water, it would not change back in the dark. This was true in both unbuffered solution at pH 5 and buffered solution at pH 7. Only in NaOH solution where a large excess of hydroxide ions were present was the reverse reaction possible. Irie and Kunwatchakun also report that hydrogels formed with VN-MG-OH only showed reversible switching below a threshold pH,[5] but in their studies this threshold was approximately pH 6. Even at pH 6.6 a three-fold reversible increase in swelling was observed after UV irradiation. The reason for the discrepancy between Irie's ability and our ability to observe reversible switching near neutral pH is unknown. Perhaps the local environment within an acrylamide network subtly changes the switching characteristics. Perhaps their pH meter was broken. Since the reversible switching we desired was only possible at extreme pH, malachite green was not a feasible chromophore for our studies, irregardless of the synthetic difficulties.

An alternate approach was possible: use of the cyanide rather than the alcohol form of the molecule. Irie and Kunwatchakun showed that hydrogels containing the cyanide form of malachite green could undergo reversible switching at pH values as low as pH 4. Also, these VN-MG-CN gels also showed greater swelling ratios than their VN-MG-OH counterparts, increasing in mass 17 times with UV irradiation at pH 6.5. Furthermore, polymers produced with the alcohol form of malachite green could be changed into the cyanide form using dialysis against potassium cyanide.[5] It was decided, however, that a material that responds to light by releasing highly toxic anions should not be synthesized.

C.5 Figures, Schemes and Tables

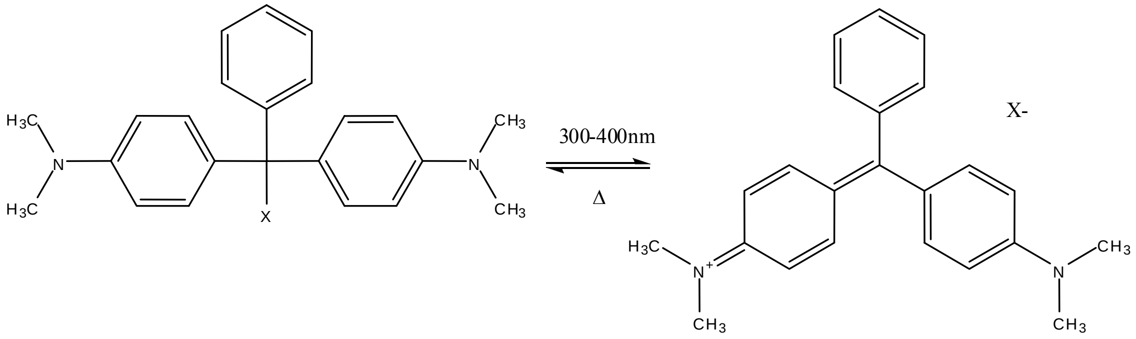
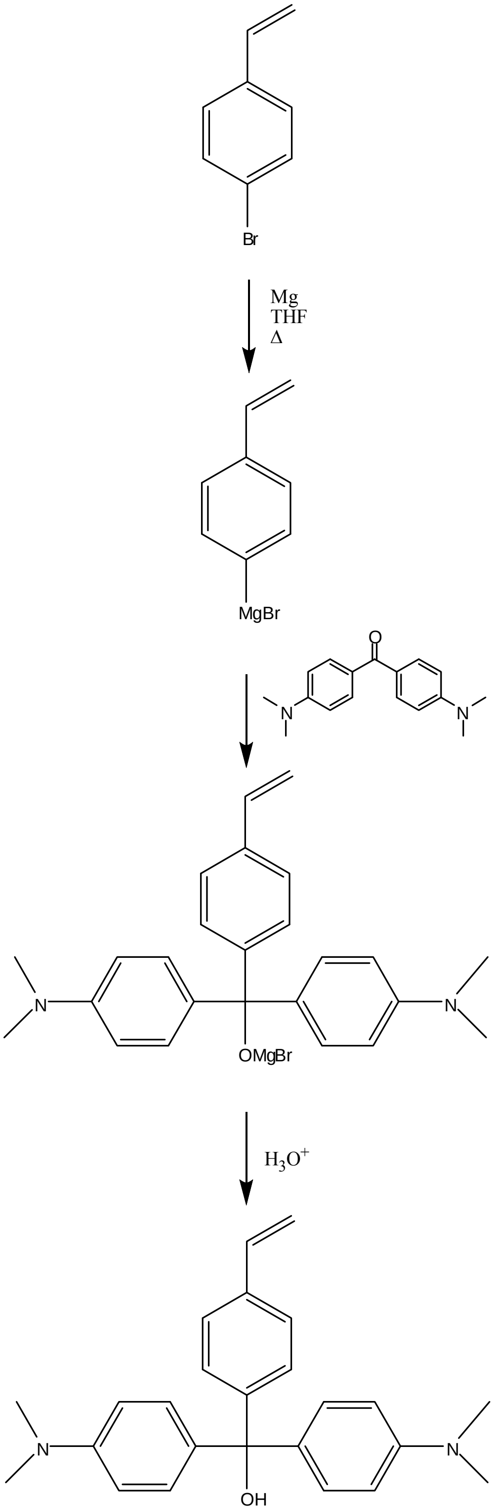


Figure C.1 Photoresponsive behavior of triphenylmethane leuco dyes (malachite green dyes) where X = OH^- or CH^-. The cationic form of the dye is deeply colored and results from UV irradiation of the neutral form. The reverse reaction is thermally activated.



Scheme C.1 Synthesis of VN-MG-OH.

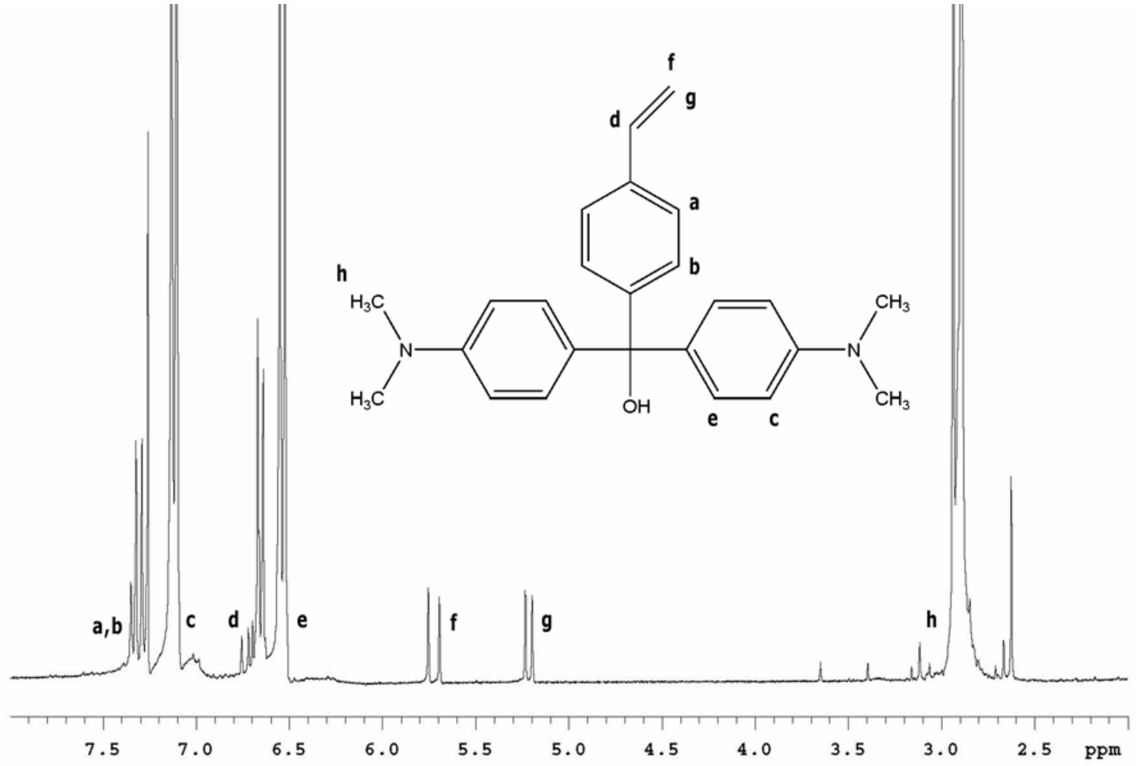


Figure C.2 NMR of VN-MG-OH.

trial	AIBN	DMAAm	THF	rxn. time	conversion	MW ($\times 10^6$)
	[g]	[mL]	[mL]	[hours]	[%]	[g/mol]
1	0.02	5.8	12	1	27.3	0.18
2	0.01	5.8	12	1	10.1	0.19
3	0.005	5.8	12	2	10.3	0.19
4	0.0025	6	6	2	11.7	0.3
5	0.001	9	6	2	5.2	0.44
6	0.001	15	0	1.33	7.14	2

Table C.1 Reaction conditions for the homopolymerization of PDMAAm. All reactions run at 45° C after three freeze/thaw cycles. Molecular weight was measured using MALS.

trial	chromophore used	chromophore (g)	AIBN (g)	DMAAm (mL)	THF (mL)	rxn. Time (hours)	yield (mg)
1	MG-VN-OH	0.5477	0.001	15	0	2	0
2	MG-VN-OH	0.5508	0.0115	15	0	3	58.5
3	MG-VN-OH	0.0481	0.001	1.5	0	3	11.5
4	MG-VN-OH	0.0502	0.002	1.5	0	5	177.6*
5	MG	0.22	0.0025	6	6	2	0
6	MG	0.2203	0.01	6	6	2	0

Table C.2 Reaction conditions for copolymerization of DMAAm and MG-VN. Homopolymerization of DMAAm in the presence of commercial malachite green was also attempted. All reaction performed at 45º C. *Sample was crosslinked.

C.6 References

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