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Highly Tailorable Materials based on 2-Vinyl-4,4-dimethyl Azlactone: (Co)Polymerization, Synthetic Manipulation and Characterization

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ABSTRACT: Through rigorous spectroscopic characterizations, including *in situ*, real-time monitoring, and size-exclusion chromatography (SEC) we describe the functionalization of polymers and copolymers based on vinyl dimethyl azlactone (VDMA), as well as modification of the VDMA monomer using efficient ring-opening strategies. Specifically, we demonstrate modification of VDMA-based materials by "pegylation", base-catalyzed ring-opening hydrolysis, and nucleophilic addition of short alkyl chains, fluorescent markers, and motifs used to specifically bind proteins. All of these functionalizations take advantage of the susceptibility of the pendant azlactone ring of VDMA to undergo nucleophilic attack. Polymers as well as copolymers incorporating vinyl pyrrolidone were synthesized by conventional free radical polymerization and thoroughly characterized by FTIR, ^{1}H NMR, ^{13}C NMR, SEC, thermogravimetric analysis and differential scanning calorimetry prior to modification. The variety of conjugations and ease of transformations enabled by use of the reactive yet hydrolytically stable VDMA-based materials inspires a broad range of applications for these soft materials.

Introduction

Conventional free radical polymerization is one of the most common and useful methods for making polymers. Industrially, radical polymerization is probably the most prominent polymerization technique employed because reactions can be conducted under relatively undemanding conditions, a large range of monomers can be used because the systems are tolerant to functional groups, copolymerization is reasonably facile, and set up is simple (e.g., bulk, solution, emulsion).[1]

There continues to be considerable research activity pertaining to stimuli-responsive and reactive polymers for myriad applications in biotechnology, including drug delivery vehicles,[2−4] gene therapy agents,[5] as well as biomaterial coatings.[6] Polymers based on 2-vinyl-4,4-dimethylazlactone (VDMA) offer compelling potential because VDMA can be polymerized under a variety of conditions,[7] including controlled polymerization techniques to generate well-defined materials and retain the reactive azlactone moiety,[8−10] and because VDMA undergoes several different reactions based on the nature of the modifying moiety.[7,10] For instance, the azlactone ring of VDMA can undergo nucleophilic addition when reacted with alcohols and primary (1°) amines.[7] In these cases, the vinyl group is retained, thereby generating acrylamide monomers, which have potential as stimuli-responsive materials. Michael addition reactions (e.g., enamines, imides, *N*-heterocycles, secondary amines, thiols, and carbon acids) at the C=C double bond with retention of the azlactone have been reported.[7] The products from these Michael addition reactions are modified azlactones that can be used to modify polymers containing alcohol or primary amine functionalities.

VDMA is prepared from *N*-acylamino acids; its synthesis was first reported by Iwakura et al. in 1967.[11] Heilmann and co-workers at 3M pioneered much of the work pertaining to VDMA-containing materials[7] and they continue to evaluate the properties of the reactive VDMA-containing polymers in a variety of applications.[12−16]

In this contribution, we describe our efforts to synthesize and characterize polymers and copolymers containing VDMA using conventional free-radical polymerization and our efforts to subsequently modify VDMA and VDMA-containing polymers. Specifically, via rigorous characterization we demonstrate successful pegylation of VDMA as well as the functionalization of pVDMA using dansylcadaverine, N_α,N_α-bis(carboxymethyl)-L-lysine hydrate, and via base-catalyzed hydrolysis. These studies demonstrate both the versatile nature of VDMA-based polymers and the ability to markedly change substrate properties upon functionalization.

Experimental Section

Materials. Benzene (ReagentPlus, thiophene free, ≥99%; Aldrich) was dried with calcium hydride, distilled under reduced pressure and stored over nitrogen. 1-Vinyl-2-pyrrolidone (VP; ≥99%, 0.01% sodium hydroxide as inhibitor; Aldrich) and styrene (ReagentPlus, ≥99%; Aldrich) were individually treated with calcium hydride overnight, distilled under high vacuum, and stored at reduced temperature over dry nitrogen. 2,2'-Azobis(2-methylpropionitrile) (AIBN; 98%, Aldrich) was recrystallized from anhydrous methanol at least three times, dried in vacuo, and stored under a blanket of dry nitrogen at 3 °C. 2-Vinyl-4,4-dimethyl azlactone, (VDMA; Isochem North America, LLC) was fractionally distilled under reduced pressure and the middle fraction (∼70%) was used. *n*-Hexylamine ($HxNH_2$, Fluka, ≥98.0%), N_α,N_α-bis(carboxymethyl)-L-lysine hyrdrate (LH; Aldrich, ≥ 97.0%), dansylcadaverine (DC, Aldrich, ≥ 99.0%), sodium hydride (NaH, 95%), and hexaethylene glycol monomethyl ether (TCI America) were used as received.

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Instrumentation. Solution ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopy was performed on a Varian Unity 500 wide bore multinuclear spectrometer. Samples were placed in 5 mm o. d. tubes with sample concentrations of 5% and 10% (w/v), respectively. Chloroform-*d* ($CDCl_3$), D_2O, and DMSO-d_6 were used as solvents using residual solvent peaks as internal standards.

Size exclusion chromatography (SEC) was performed using a Waters Alliance 2695 Separations Module equipped with a Polymer Laboratories PLgel 5 μm guard column (50 × 7.5 mm) three Polymer Laboratories PLgel 5 μm mixed-C columns (300 × 7.5 mm; linear range of molecular weight, 200−2 000 000 g mol^{-1}) in series, a Waters Model 2414 refractive index detector (λ = 880 nm), a Waters Model 2996 photodiode array detector, a Wyatt Technology miniDAWN multiangle light scattering (MALS) detector (50 mW GaAs laser, λ = 660 nm), and a Wyatt Technology ViscoStar viscometer with tetrahydrofuran (THF) as the mobile phase at a flow rate of 1.0 mL/min. Absolute molecular weights (viz. weight average molecular weight, M_w) were determined when dn/dc values were known. In some cases, dn/dc values were calculated using Astra V software (Wyatt Technology) assuming 100% mass recovery of the eluted polymer samples correlated to the sample concentration; the RI detector was used as the concentration detector. In cases where dn/dc values were deemed inaccurate (e.g., copolymers having nominally ≥50% VP content), molecular weights and molecular weight distributions were determined by conventional calibration against low polydispersity index (PDI) polystyrene standards (162 to 6.04 × 10^6 g mol^{-1}).

Aqueous size exclusion chromatography (AQSEC) was performed using an Agilent 1100 Series quaternary pump system and Agilent 1200 Series Micro Well Plate Autosampler equipped with Tosoh Biosciences, LLC columns, TSKgel Super AW guard (particle size, 7 μm) 4000 (particle size, 6 μm; pore size, 450 Å), 3000 (4 μm; 150 Å),and 2500 (4 μm; 25 Å) in series, an Agilent 1200 Series UV−vis photodiode array detector, a Wyatt Technology HELEOS multiangle light scattering (MALS) detector (50mW GaAs laser, λ = 660 nm) with quasielastic light scattering (QELS) option, and a Wyatt Technology Optilab rEX refractive index detector (λ = 690 nm) with an eluent consisting of 20% acetonitrile/80% 0.05 M Na_2SO_4(aq) (v/v) at a flow rate of 0.3 mL/min. Data were collected using Astra V software (Wyatt Technology).

A Wyatt Technology Optilab rEX refractive index detector (λ = 658 nm) was also used in conjunction with a Harvard Apparatus PHD 2000 Infusion syringe pump to determine dn/dc values off-line. Sets of solutions having concentrations ranging from 0.2 to 2 wt % were prepared and allowed to equilibrate for several days prior to measurement. The dn/dc values were calculated using Astra V software.

Fourier-transform infrared (FTIR) spectra were collected using a Bruker Optics Vertex 70 spectrometer. Spectra were collected as the average of 16 scans with 4 cm^{-1} resolution and corrected for background. Polymer samples were analyzed as pressed KBr pellets (256 scans were used to acquire the KBr background) while liquid monomers (VP and VDMA) were analyzed as thin films between two NaCl plates (background of two salt plates; 256 scans). ATR−FTIR spectra were collected using a Harrick Scientific MVP Star accessory equipped with a diamond internal reflection element. All spectra were acquired in the double-sided, forward−backward mode and Mertz phase correction. Interferrograms were trunctated with the Blackman-Harris 3-term apodization function with a zero-filling factor of 2.

To monitor functionalization (i.e., disappearance) of azlactone groups in real-time, *in situ* ATR−FTIR spectra were collected using a Bruker Optics Matrix-MF spectrometer equipped with a silver halide fiber optic cable with diamond composite ATR reflection element and an internal mercury−cadmium-telluride (MCT) detector. A background spectrum of pure solvent was obtained from the average of 256 scans collected before functionalization. Reactions were monitored in real-time by collecting spectra (average of 16 scans) every minute over the spectral range of 4000−2250 and 1900−650 cm^{-1} with 4 cm^{-1} resolution. Spectra were acquired in the double-sided, forward−backward mode and Mertz phase correction. Interferrograms were trunctated with the Blackman-Harris 3-term apodization function with a zero-filling factor of 2. Reactions were monitored in real time using the CHROM option in OPUS 6.0 software.

A TA Instruments Q1000 differential scanning calorimeter (DSC) was used to evaluate thermal transitions of the (co) polymers. Samples were first equilibrated at −50.0 °C and then heated to 200.0 °C at a ramp rate of 10.0 °C/min. Samples were subsequently cooled to −50.0 °C at 10.0 °C/min. Finally, the samples were heated to 200.0 °C at C 10.0 °C/min. Glass transition temperatures are reported from the second heating as the midpoint of the heat flow derivative curve. The DSC was calibrated using indium standard (In; melting point, $T_{m,In}$ = 156.6 °C; provided by TA Instruments) according to the manufacturer's recommendation, which includes baseline and temperature calibrations. Additionally, standard thermal gravimetry experiments were performed on a TA Instruments Q5000IR TGA. Samples were heated from ambient temperature to 500.0 at 10.0 °C/min.

Synthetic Procedures

Conventional Free-Radical (Co)Polymerizations. In a typical reaction, AIBN (0.36 g, 2.2 mmol) was first added to a single-neck 100 mL Airfree round-bottom reaction flask equipped with a Teflon-coated magnetic stir bar and rubber septum. Dry benzene (42.0 mL) was then added and subsequently, the comonomers, VP (9.0 mL, 0.084 mol) and VDMA (9.0 mL, 0.067 mol), were transferred via syringe while a dry, inert nitrogen environment was maintained. Three consecutive freeze−pump−thaw cycles were used to remove dissolved oxygen. Next, the reaction vessel was placed in an oil bath thermostatted at 65 °C and allowed to react for 65 min and then the polymerization was quenched by immersing the flasks in liquid nitrogen. VDMA-containing copolymers were isolated by precipitation: copolymer solutions were reconstituted with THF (∼5−10%) and then slowly added dropwise (using a separatory funnel) to a 7-fold excess mixture of hexanes/THF (hexanes: THF = 3:1) chilled to −30 °C. These conditions were chosen to preserve the azlactone functionality, but still fractionally remove residual VP. The isolated (co)polymers were then dried under vacuum and analyzed. ^{1}H NMR spectroscopy was used to evaluate copolymer composition where mole fractions of VDMA and VP were determined from the integrated areas under the peaks centered at 1.37 and 3.23 ppm, respectively.[16] The peak centered at 1.37 ppm represents the six methyl protons associated with the VDMA ring [$C(CH_3)_2$], and the peak centered at 3.23 ppm represents the two methylene protons adjacent to the nitrogen of the pendant VP ring (N−CH_2). The results of the (co)polymerizations are summarized in Table 1.

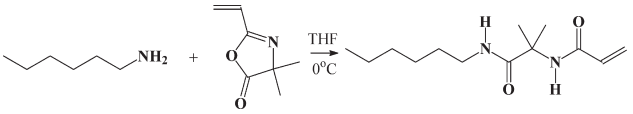
Monomer Modification Using Primary Amines as Nucleophiles. In a typical reaction, 40.0 mL THF was added to a 250 mL 3-neck round-bottom flask equipped with a Teflon-coated stir bar, rubber septa and fiber optic FTIR probe. After the contents were equilibrated to 0 °C using an ice bath, a pure solvent background was collected using the Bruker Optics Matrix-MF spectrometer. Next, 3.1642 g of undistilled VDMA (22.73 mmol; contains ∼500 ppm of 2,6-di-*tert*-butyl-4-methylphenol as an inhibitor) was added and the flask allowed to equilibrate to 0 °C. To establish a baseline, acquisition of FTIR spectra commenced upon addition of VDMA; reactions involving the azlactone ring can be monitored using the azlactone peak (C=O of azlactone ring at 1825 cm^{-1}). Subsequently,

Table 1. 1-Vinyl-2-pyrrolidone (VP)/2-Vinyl-4,4-dimethyl Azlactone (VDMA) (Conventional) Copolymerization Characteristics

	monomer feed (mol %)		copolymer composition (mol %)[a]						
entry	VDMA	VP	VDMA	VP	M_w[b] (kg/mol)	PDI[b]	M_w[c] (kg/mol)	PDI[c]	dn/dc[d]
A	44.2	55.8	60.3	39.7	232	2.45	305	1.95	0.067
B	20.9	79.1	30.7	69.3	184	2.25	534	1.93	0.047
C	8.1	91.9	15.1	84.9	226	2.03	4376	1.91	0.010
D	100	0	100	0	152	2.06	184	1.47	0.084
E	0	100	0	100	N/A	N/A	N/A	N/A	N/A

[a] Copolymer composition determined by ^{1}H NMR. [b] Weight average molecular weight, M_w, and polydispersity index, PDI, determined by conventional size exclusion chromatography calibration using polystyrene standards. [c] M_w and PDI determined by multiangle light scattering (MALS). [d] dn/dc determined by Astra V software assuming 100% mass recovery.

Scheme 1. Reaction Scheme of *n*-Hexylamine and 2-Vinyl-4,4-dimethyl Azlactone, VDMA.



1.2270 g of *n*-hexylamine (12.13 mmol) chilled to ~4 °C was slowly added over a period of 2 min. The reaction was allowed to reach completion, as evidenced by a constant intensity of the azlactone peak (1825 cm^{-1}). A second addition of 1.0630 g (10.50 mmol) of *n*-hexylamine, also chilled to ~4 °C, was slowly added until the reaction again reached completion. In general, reactions were monitored until the azlactone peak (1825 cm^{-1}; left integration limit, 1860 cm^{-1}; right integration limit, 1796 cm^{-1}) disappeared into the baseline indicating consumption of azlactone by *n*-hexylamine (or nucleophile).

"PEGylation" of VDMA: Monomer Modification Using Hexaethylene Glycol Monomethyl Ether. *Synthesis of 2-(2-(2-(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethyl 2-(Acrylamido)-2-methylpropanate*. In a typical reaction, 20.0 mL of dry THF was added to a 100 mL 3-neck round-bottom flask equipped with a Teflon-coated stir bar, rubber septa and fiber optic FTIR probe. The contents were equilibrated to 0 °C using an ice bath and a pure solvent background was collected using the Bruker Optics Matrix-MF spectrometer. Next, 1.9258 g of hexaethylene glycol monomethyl ether (6.4982×10^{-3} mol) was added to the flask, and acquisition of FTIR spectra began. Spectra were collected every 30 s. Subsequently, 0.2008 g (8.367×10^{-3} mol) NaH were added to generate the sodium alkoxide of hexaethylene glycol monomethyl ether in situ while monitoring the FTIR spectrum for 30 min. At this point, 1.0725 g (7.7075×10^{-3} mol) of undistilled VDMA (contains ~500 ppm of 2,6-di-*tert*-butyl-4-methylphenol as an inhibitor) were added to the flask and real-time monitoring of the reaction continued for 75 min. In this case, the C=C (1600 cm^{-1}; left integration limit, 1623 cm^{-1}; right integration limit, 1578 cm^{-1}) and azlactone (1825 cm^{-1}; left integration limit, 1860 cm^{-1}; right integration limit, 1796 cm^{-1}) peaks were evaluated in real time using both peak areas and peak heights to demonstrate preservation of C=C and consumption of the azlactone, respectively.

Polymer Modification Using Primary Amines as Nucleophiles. In general, 1.0693 g of pVDMA (M_n = 125.2 kg/ mol, PDI = 1.47) was dissolved in 3.6628 g of THF. Two successive additions of *n*-hexylamine were made over several minutes and the reaction was monitored in real-time and *in situ* using remote probe ATR−FTIR spectroscopy. In all cases, dry THF was used as the background (256 scans). First, 131.4 mg *n*-hexylamine (1.09 equivalents) were added and the reaction was allowed to proceed until no change in the FTIR spectrum was observed. Next, a second injection of 159.4 mg n-hexylamine (1.33 equivalents; total, 2.42 equivalents) was made and the reaction was again allowed to proceed until no change in the FTIR spectrum could be observed. At this point, the reaction was deemed complete. Polymers were precipitated into hexanes (5x excess), filtered, and dried under vacuum prior to analysis. The isolated polymer (*n*-hexylamine-modified pVDMA) had M_n = 60.4 kg/ mol and PDI = 2.53 with respect to polystyrene standards. Functionalizations of pVDMA-containing polymers with dansylcadaverine and N_α,N_α-bis(carboxymethyl)-L-lysine hyrdrate followed similar protocols, with deviations noted where results are discussed.

Base Catalyzed Hydrolysis of pVDMA. Hydrolysis of pVDMA was performed using a 1.0 N aqueous solution of sodium hydroxide (NaOH(aq)) while monitoring the reaction using real-time, *in situ* remote probe ATR−FTIR spectroscopy. In all cases, dry THF was used as the background scan. In a typical reaction, 196.0 mg pVDMA (M_n = 125.2 kg/ mol, PDI = 1.47) were dissolved in 3.7130 g THF. Next, 0.7 mL NaOH(aq) (0.5 equivalents) were injected and the reaction proceeded for 12.5 min. Subsequently, 0.7 mL NaOH(aq) (0.5 equivalents; total, 1.0 equivalents) were added and the reaction was monitored for an additional 10 min.

Results and Discussion

Conventional (Co)Polymerization. A set of VDMA-containing (co)polymers were made via conventional free radical polymerization of VP and VDMA. The data obtained for a series of copolymers synthesized having various comonomer feed ratios as well as for homopolymers of VDMA and VP are summarized in Table 1.

As shown in Table 1, for all cases the amount of VDMA incorporated into the copolymers exceeds the feed, suggesting that VDMA is more reactive than VP; however the copolymers were analyzed after multiple precipitations that remove unreacted monomers. As a result, monomer conversion and final polymer yields were not assessed. Additionally, monomer reactivity ratios were not determined for these reactions because conversion was not limited.

Molecular weight characteristics were evaluated using both conventional calibration against polystyrene standards as well as by SEC−MALS. For copolymers that contain higher amounts of VP, the molecular weights determined via SEC−MALS are higher than those determined by conventional calibration. For example, the agreement between the molecular weights obtained by conventional calibration and SEC−MALS are quite good for entry A of Table 1; however, because VP and THF are nearly isorefractive, there is considerable disagreement in the M_w values obtained by these two methods as VP content increases because the calculated dn/dc values determined by Astra are in error. The dn/dc determined off-line for pVDMA (Entry D) is 0.0835 mL/g,

which is in complete agreement with the dn/dc determined by 100% mass recovery.

Thermal Analysis. Differential scanning calorimetry (DSC) measurements were made to determine glass transition temperature (T_g) of the VP−VDMA copolymers and homopolymers. A T_g = 179.1 °C was measured for PVP and a T_g = 106.4 °C was measured for pVDMA. The experimentally measured T_g values and predictions from the Fox equation for p(VP-*co*-VDMA) (entries A−E, Table 1) plotted as a function of copolymer composition (determined by ^{1}H NMR spectroscopy) is provided in the Supporting Information (Figure S1). The experimentally measured copolymer T_g s are nearly equivalent to the predicted values.

Results obtained from thermogravimetric analysis (TGA) qualitatively reflect copolymer composition; however, TGA of pVDMA indicates multiple transitions, possibly due to reaction of azlactone group, and these are also observed in measurements on the copolymers. TGA results indicate a propensity for the polymers and copolymers to absorb up to 7% of their weight in moisture. The onset of degradation for PVP occurs at approximately 400 °C while the onset of degradation for all copolymers and pVDMA occurs at approximately 240 °C. The onset of a second decomposition is observed at ∼340 °C in all copolymers and pVDMA, after which rapid decomposition occurs.

Monomer Modification. *Modification of VDMA Using n-Hexylamine*. In the first set of experiments, VDMA was reacted with *n*-hexylamine (*n*-HxNH$_2$) in dilute solution and the reactions were monitored via mid-IR remote probe ATR−FTIR spectroscopy (as described in the Experimental Section). Scheme 1 illustrates the reaction of VDMA with *n*-hexylamine, which yields N-(2-(hexylcarbamoyl) propan-2-yl) acrylamide (HCPAm).

The partial ATR−FTIR spectra shown in Figure 1 illustrate the diminution of the azlactone peak (1825 cm^{-1}) during the course of the reaction. Additionally, the peak height profiles for the azlactone (black, 1825 cm^{-1}) and the amide II (red, 1537 cm^{-1}) peaks are isolated and shown in the inset figure to show the two-step addition of *n*-HxNH$_2$ (indicated by the arrows) and reaction. As expected, the azlactone peak disappears as the amide appears simultaneously. Retention of C=C is confirmed by the presence of the peak at 1600 cm^{-1} and via ^{1}H NMR spectroscopy.

After monitoring the reaction of VDMA with *n*-HxNH2 in THF, the product, HCPAm, was concentrated under reduced pressure and analyzed via ^{1}H and ^{13}C NMR spectroscopy. The structure of HCPAm is confirmed by the NMR spectra (see Supporting Information: Figure S2a (^{1}H NMR) and Figure S2b (^{13}C NMR)). The ratios of the integrated areas in the ^{1}H NMR spectrum are consistent with the proposed structure. On the basis of the gravimetric yield (100%) and conversion (100%) as well as data obtained from FTIR and NMR spectroscopies, the reaction appears quantitative. (^{1}H NMR (500 MHz, $CDCl_3$, δ): 6.64, 6.56 (s, 2H, NH), 6.28, 6.23 (d, 1H; CH), 6.15−6.06 (s, 1H; CH), 5.62−5.60 (q, 1H, CH) 3.25−3.20 (m, 2H, CH_2), 1.59 (s, 6H, CH_3), 1.52−1.45 (m, 4H, CH_2), 1.32−1.24 (m, 4H, CH_2), 0.85 (t, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 174.6 (C=O), 165.5 (C=C−***C***=O), 131.5 (***C***H=CH_2), 126.8 (***C***H_2=CH), 57.6 ((CH_3)***C***(NH)(C=O)), 40.1 (CH_2NH), 31.6 (CH_2), 29.5 (CH_2), 26.6 (CH_2), 25.3 ((CH_3)$_2$), 22.7 (CH_2), 14.1 (CH_3).)

HCPAm was subsequently polymerized in the absence and in the presence of VDMA (f_{VDMA} = 0.5) using conventional free-radical polymerization techniques under analogous polymerization conditions described for the polymerization of VDMA-containing (co)polymers. FTIR, NMR, and SEC analyses show clear evidence that HCPAm (co)polymerizes with VDMA (data not shown). This approach opens the possibility of making VDMA-based polymers functionalized with different substituents..

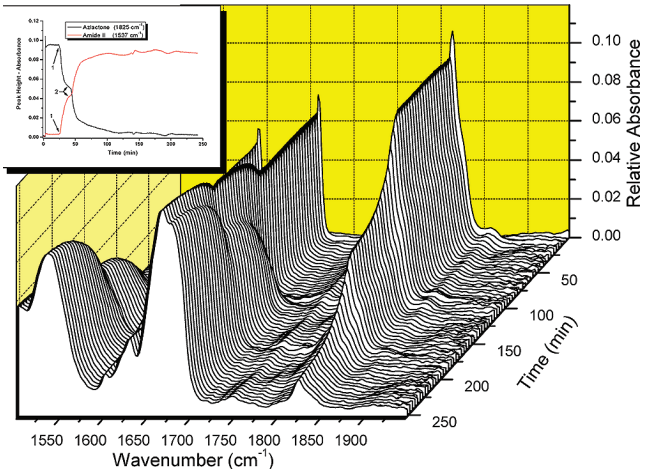


Figure 1. Partial ATR−FTIR spectra for the reaction of 2-vinyl-4,4-dimethyl azlactone (VDMA) with *n*-hexylamine (*n*-HxNH$_2$) in tetrahydrofuran (THF) at 0 °C. Reaction conditions: [VDMA]$_0$ = 0.528 M before addition of *n*-HxNH$_2$; [*n*-HxNH$_2$] = 0.491 M; THF volume, 40.0 mL.

PEGylation of VDMA: Monomer Modification Using Hexaethylene Glycol Monomethyl Ether. Modifying VDMA in order to generate "PEGylated" VDMA was achieved using NaH to form the sodium alkoxide of hexaethylene glycol monomethyl ether in situ; however, trialkylphosphines and cyclic amidines like 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) have demonstrated catalytic utility to generate acrylamide monomers.[7] Nucleophilic attack of the PEG−alkoxide on the azlactone ring yields an acrylamide−ester monomer that can be used in further (co)polymerization strategies. The product was simply filtered and concentrated under reduced pressure. The results of the NMR spectroscopy are included in the Supporting Information: Figure S3 shows both the ^{1}H (Figure S3a) and ^{13}C (Figure S3b) NMR spectra of the product, 2-(2-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethyl 2-(acrylamido)-2-methylpropanate. Again it is observed that the ratios of integrated areas of the ^{1}H NMR spectrum are consistent with the proposed structure. Furthermore, it can be seen that the reaction was highly efficient insofar that the ^{13}C NMR spectrum is very clean (i.e., no appearance of byproducts). On the basis of the gravimetric yield (100%) and conversion (100%) as well as data obtained from FTIR and NMR spectroscopies, the reaction appears quantitative. (^{1}H NMR (500 MHz, $CDCl_3$, δ): 6.28, 6.24 (s, 1H, CH), 6.16−6.08 (m, 1H; CH), 5.65−5.59 (s, 1H, CH), 4.30 (t, 2H, CH_2O(C=O)), 3.77−3.58 (m, 4H, CH_2CH_2), 3.54 (t, 2H, C***H***$_2$OCH_3), 3.37 (s, 3H, CH_3), 1.59 (s, 6H, (CH_3)$_2$)); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 174.8 ((C=O)O), 164.9 ((C=O)NH), 131.2 (***C***H=CH_2), 126.7 (***C***H_2=CH), 72.1 (***C***H_2OCH_3), 70.8 (CH_2), 69.2 (***C***H_2CH_2O(C=O)), 64.7 (CH_2O(C=O)), 59.2 (CH_3), 56.7 ((CH_3)***C***(NH)(C=O)), 24.9 ((CH_3)$_2$).)

Polymer Modification. *Polymer Modification Using Primary Amines as Nucleophiles*. A series of primary amines are used to modify the pendant azlactone rings of VDMA-containing polymers. We have chosen three vastly different amino-derivatives to incorporate functionality that inspire a broad range of applications for these materials.

n-Hexylamine Modification. When *n*-hexylamine was used as a nucleophile for modification of pVDMA, pHCPAm was

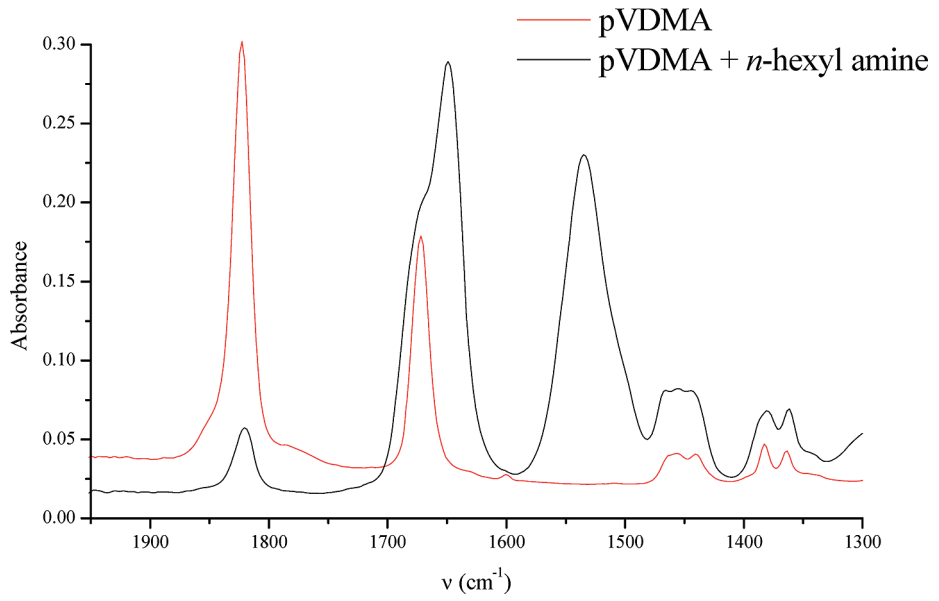


Figure 2. FTIR spectra of (a) poly(vinyl dimethyl azlactone) (pVDMA) in tetrahydrofuran (THF) (red) and (b) the reaction product of pVDMA with *n*-hexylamine in THF (black).

generated. This modification generates a more hydrophobic system in comparison to pVDMA. Additionally, this functionalization provides a means to compare the efficiency of functionalization with respect to the polymerization of HCPAm (described above). Real-time *in situ* ATR−FTIR spectroscopy was used to track the conversion of azlactone groups. Figure 2 shows the spectrum of pVDMA in THF prior to the addition of *n*-hexylamine as well as the spectrum of the final product after addition and reaction of a 2.4-fold excess of *n*-hexylamine. Comparison of the spectra indicates that the reaction does not proceed to completion as a small peak indicative of the azlactone remains at 1825 cm^{-1}. This suggests that while the azlactone is a highly reactive moiety, changes in polymer structure or solubility upon modification may alter the accessibility of the azlactone groups. Analysis via SEC indicates that the *n*-hexylamine-modified polymer has M_n = 60.4 kg/mol and PDI = 2.53 with respect to polystyrene standards. Furthermore, analysis of the FTIR spectra shown in Figure 2 suggests that ~84% of the azlactone groups were derivatized by *n*-hexylamine (1825 cm^{-1} peak areas and heights were calculated using left integration limit, 1860 cm^{-1} and right integration limit, 1796 cm^{-1}) and is confirmed by ^{1}H NMR spectroscopy..

Dansylcadaverine (DC) Modification. DC is often used as a fluorescent probe and marker in cell membrane studies.[17] While our focus does not extend to cell uptake studies, we select DC as a model biomolecule because of its utility in biology and because it has a single primary amine and is fluorescent. Previously we used DC in a drop-on-demand (DOD) inkjet process to attach DC onto a surface-tethered VDMA-containing copolymer scaffold,[16] demonstrating the utility of the azlactone moiety and the ease of functionalization. The trace shown in Figure 3 from real-time FTIR monitoring shows the diminution of the azlactone group (1825 cm^{-1}) upon functionalization of p(VP-*co*-VDMA) (entry A, Table 1) with DC in methylene chloride. In this experiment, a portion of DC was added to the copolymer solution, allowed to react and subsequently reach equilibrium, as inferred from the steady signal. A second portion was added and the reaction was again monitored to the equilibration point. The results demonstrate that functionalization occurs very rapidly. After the reaction of DC with p(VP-*co*-VDMA) (entry A, Table 1), FTIR analysis of the

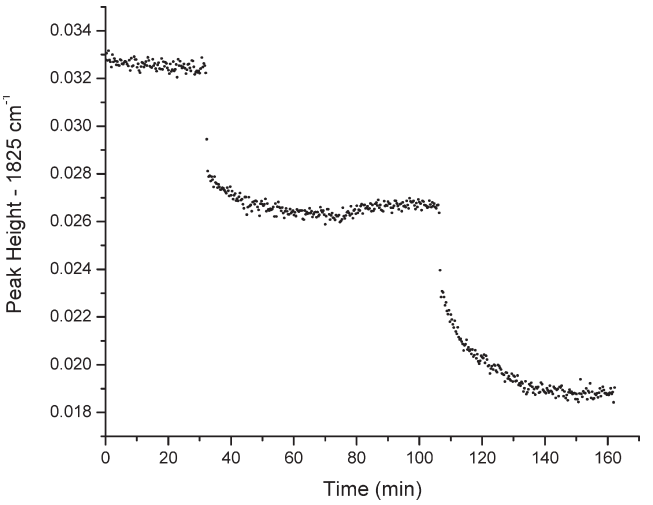


Figure 3. Reduction of the 1825 cm^{-1} peak height with time for poly (vinylpyrrolidone-*co*-vinyl dimethyl azlactone) p(VP-co-VDMA) (entry A, Table 1) reacted with dansylcadaverine (DC). Reaction conditions: 197.5 mg of p(VP-co-VDMA) dissolved in 5.571 g of methylenechloride ($MeCl_2$). DC dissolved in $MeCl_2$ at 5.0 wt %; 0.5 mL of DC solution added at times t = 28 min and t = 107 min.

product indicates that roughly 44% of the azlactone groups were derivatized. Further analysis via ^{1}H NMR spectroscopy (data not shown) indicated that 41% of the azlactone groups were derivatized (discussed below), which is in good agreement with FTIR analysis. Additionally, SEC−MALS analysis of the product indicates M_w = 474 kg/mol and PDI = 1.63, where dn/dc = 0.073 mL/g assuming 100% mass recovery.

Additionally, analysis of the recovered, functionalized polymer via SEC with UV detection provides substantial evidence of DC incorporation. Figure 4 shows that upon modification of p(VP-*co*-VDMA) with DC, there is relatively no change in elution time between the two copolymers with respect to the RI signal (Figure 4a); however, there is a dramatic difference between the UV chromatograms of the parent p(VP-*co*-VDMA) (Figure 4b) and the dansylated analog (Figure 4c). The incorporation (reaction) of DC into the copolymer is evident by the appearance of distinct peaks

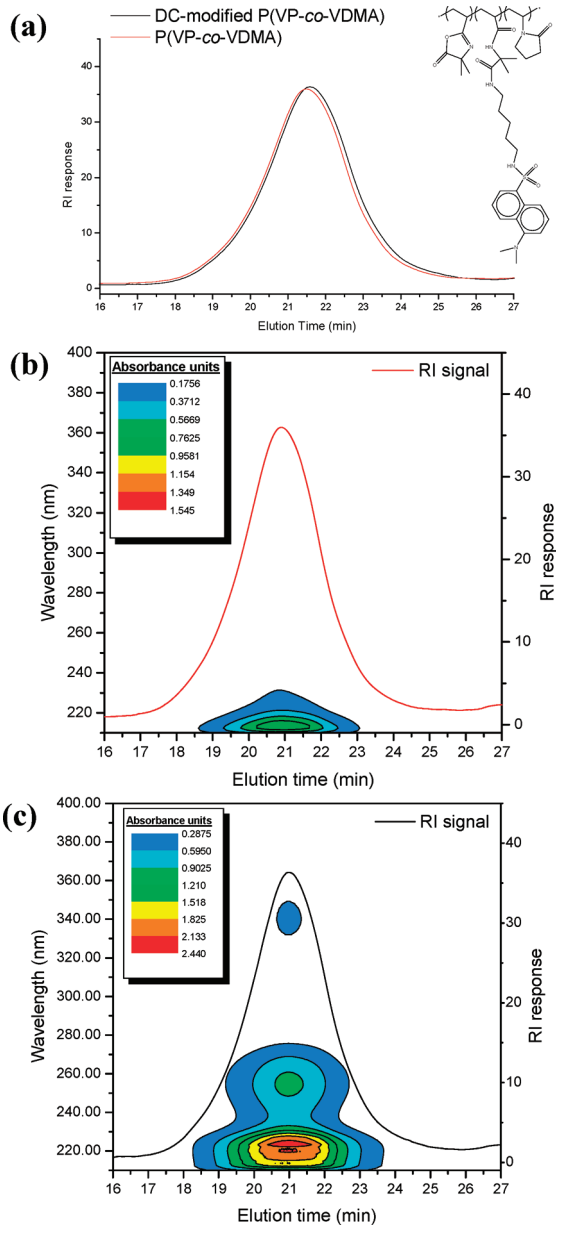


Figure 4. SEC analysis of dansylcadaverine (DC)-modified poly(vinylpyrrolidone-*co*-vinyl dimethyl azlactone) (p(VP-co-VDMA)): (a) comparison of RI signal for p(VP-*co*-VDMA) and DC-modified p(VP-*co*-VDMA) with proposed structure for DC-modified p(VP-*co*-VDMA); (b) UV and RI signals for p(VP-*co*-VDMA); (c) UV and RI signals for DC-modified p(VP-*co*-VDMA).

at 253.5 and 337.8 nm in the UV chromatogram (which is superimposed over the elutogram obtained by the RI detector). These peaks, which are clearly visible in Figure 4c, are attributed to the dansyl group, but absent in the UV chromatogram of the parent copolymer (Figure 4b).

Additional analysis by NMR spectroscopy further elucidates the structure of these modified polymeric materials and confirms the incorporation of DC. We have previously described the ^{1}H NMR characterization of p(VP-*co*-VDMA) in an earlier report.[16] The ^{1}H NMR spectrum of the DC-modified copolymer (proposed structure illustrated in Figure 4a) shows a distinct peak at 2.85 ppm attributed to N(C$\boldsymbol{H}_3$)$_2$, as well as broad peaks at 7.15 ppm, 7.49 ppm, 8.16 ppm, 8.32 ppm, and 8.49 ppm attributed to the naphthalene protons, and a peak at 9.06 ppm attributed to the amide (CONH) protons of the product. In order to determine the

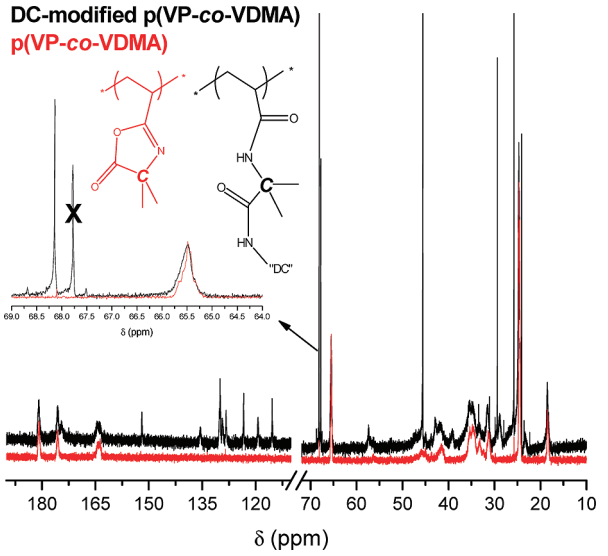


Figure 5. Comparison of ^{13}C NMR spectra for poly((vinylpyrrolidone-*co*-vinyl dimethyl azlactone) (p(VP-*co*-VDMA)) (red; entry A, Table 1) and dansylcadaverine (DC)-modified p(VP-*co*-VDMA) (black). The peak marked with an X in the inset is attributed to residual THF.

amount of azlactone groups functionalized with DC in the reaction, the peak at 2.85 ppm, which represents the six methyl protons of the N(C$\boldsymbol{H}_3$)$_2$ group, and the peak at 1.37 ppm, which represents the six methyl protons associated with the residual (i.e., unmodified) VDMA ring [C(CH$_3$)$_2$] and the six methyl protons associated with the ring-opened C(CH$_3$)$_2$ group (12 protons total), were analyzed. The ratio of the integrated areas of the peaks (N(C$\boldsymbol{H}_3$)$_2$: C(C$\boldsymbol{H}_3$)$_2$) indicated that 41% of the azlactone groups reacted with DC. Similarly a comparison of the ^{13}C NMR spectra illustrates the incorporation of DC as shown in Figure 5. The red spectrum represents unmodified p(VP-*co*-VDMA) (entry A, Table 1 and SEC results shown in Figure 4b). Upon modification with DC (black spectrum), new peaks appear between 114 − 153 ppm due to the carbons of the naphthyl ring. Additionally, DC modification alters the chemical shift of the N$\boldsymbol{C}$(CH$_3$)$_2$C=O carbon (position-4 of the heterocycle): unmodified p(VP-*co*-VDMA) has only one peak at 65.5 ppm, which is attributed to the N$\boldsymbol{C}$(CH$_3$)$_2$C=O carbon of the azlactone; however, after reaction with DC, the resulting copolymer still shows a peak at 65.5 ppm, but a second peak appears at 68.2 ppm attributed to the NH$\boldsymbol{C}$(CH$_3$)$_2$C=O carbon. See proposed structures in inset of Figure 5..

N$_\alpha$,N$_\alpha$-*Bis(carboxymethyl)-*L*-lysine Hydrate Modification.* The incorporation of N_α,N_α-bis(carboxymethyl)-L-lysine hydrate (LH) into polymers is an attractive method to facilitate the development of affinity membranes for the high-capacity purification of his-tagged proteins.[18] For example, Jain et al. successfully incorporated LH onto poly (hydroxyl ethyl methacrylate) (pHEMA) brushes made using surface initiated atom transfer radical polymerization (ATRP) through a multistep process. In their work, pHEMA brushes were first modified with *N*-hydroxy succinimide (NHS) ester approach to amidation chemistry, a nonsite specific biomolecule immobilization technique, and then these NHS ester modified brushes were exposed to LH. Despite its wide use, NHS ester modification has several limitations including hydrolytic instability at neutral conditions and the propensity to undergo unwanted side-reactions such as formation of hydrolytically unstable ring-opened conjugates and glutaride-bound conjugates.[19,20] To this end,

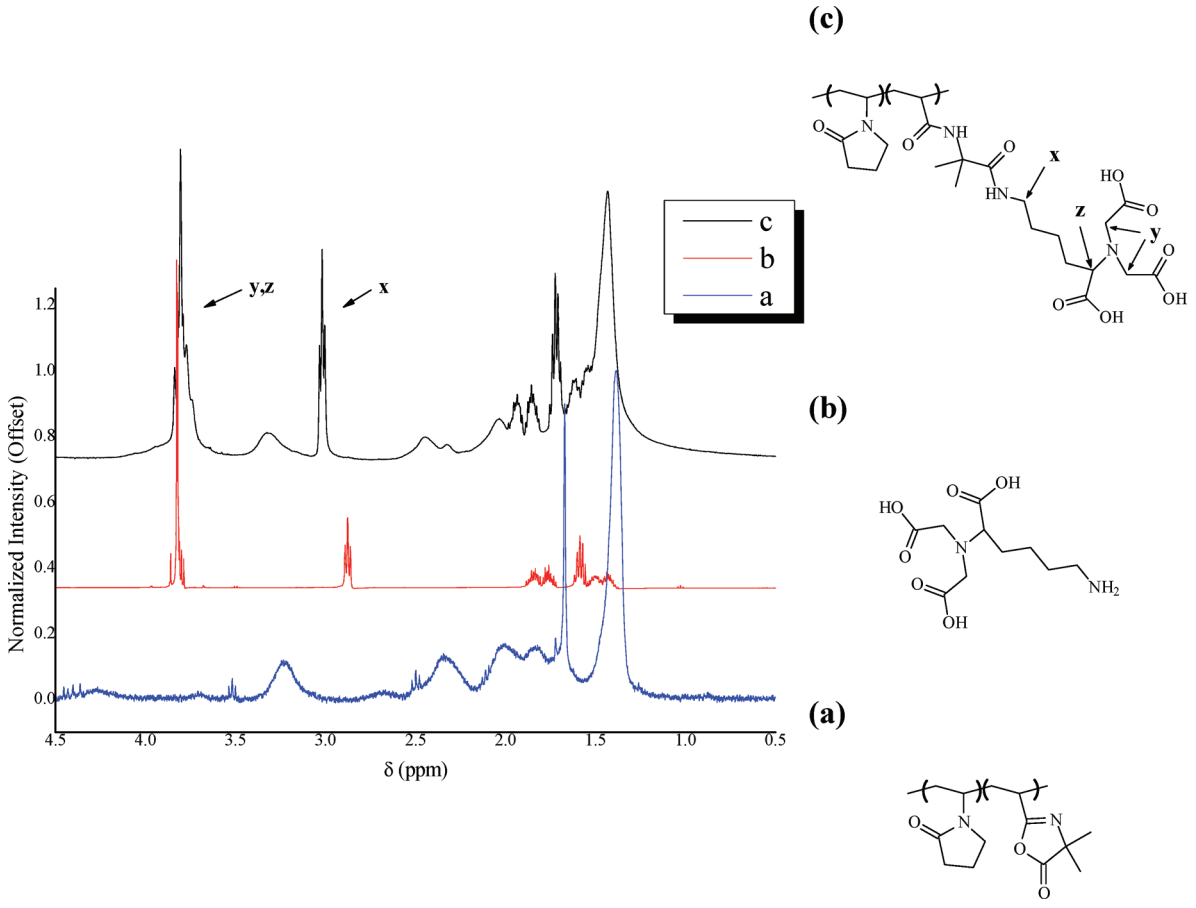
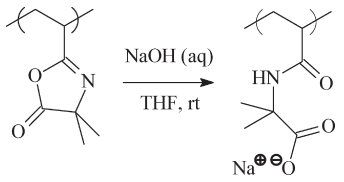


Figure 6. ^{1}H NMR analysis of (a) poly((vinylpyrrolidone-*co*-vinyl dimethyl azlactone) (p(VP-co-VDMA)) (entry A, Table 1; solvent $CDCl_3$/DMSO-d_6), (b) N_α,N_α-bis(carboxymethyl)-L-lysine hydrate (solvent D_2O/DMSO-d_6), and (c) p(VP-co-VDMA) modified with N_α,N_α-bis(carboxymethyl)-L-lysine hydrate (solvent D_2O/DMSO-d_6).

Scheme 2. Reaction Scheme for the Base-Catalyzed Hydrolysis of Poly (vinyldimethyl azlactone) (pVDMA).



we have used the azlactone moiety to conjugate LH onto VDMA-containing copolymers in one step.

While LH is soluble in aqueous media, it is insoluble in most organic media; however, we have found that it is sparingly soluble in DMF as well as in a THF:water mixture (1:1 by mass). On the other hand, we have found that pVDMA is quite hydrolytically stable in solvent water. As a result of these conflicting solubilities, modification using LH has been exclusively performed on VP−VDMA copolymers rather than pVDMA homopolymers, because the comonomer VP enhances solubility in aqueous solution. In this experiment, p(VP-*co*-VDMA) modification was achieved by two successive additions of 1.0 and 0.9 mL of a buffered LH solution (PBS buffer, 9.54 mg/mL; $[LH]_{stock\ solution}$ = 0.575 mol LH/l buffer, pH = 7.0, 266.7 mg p(VP-*co*-VDMA) (entry A, Table 1) dissolved in 3.949 g of a 1:1 THF/H_2O mixture). While the fluid gelled after both LH injections, gentle heating restored the contents to a liquid state. The entire reaction proceeded for 86 min after which the LH-derivatized VP−VDMA copolymer was dialyzed against deionized water to remove salts and residual

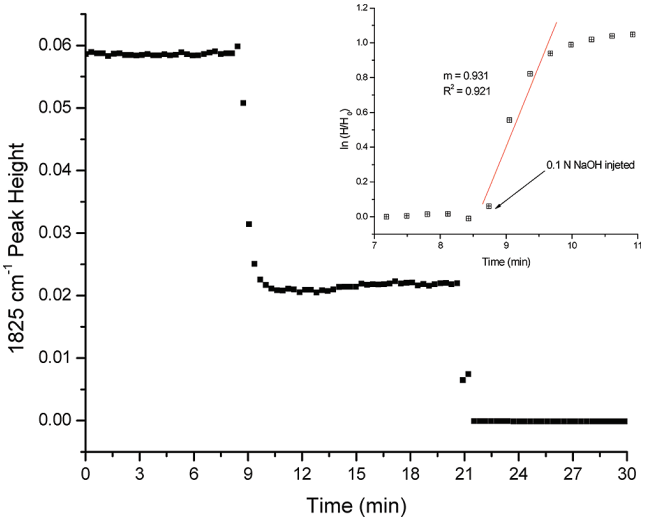


Figure 7. Two-step base-catalyzed hydrolysis of poly(vinyl dimethyl azlactone) (pVDMA): reduction of the 1825 cm^{-1} peak height with time for pVDMA (entry D, Table 1) reacted with 0.1 N NaOH(aq). The inset shows the first-order kinetic plot of base-catalyzed hydrolysis of pVDMA upon the first injection of NaOH(aq).

LH. Figure 6 shows the ^{1}H NMR spectrum of p(VP-*co*-VDMA) after modification with LH. The incorporation of the LH into the copolymer is apparent because of the peaks centered around 3.80 ppm, which are due to the CH_2 and CH protons (labeled *y* and *z*) directly adjacent to the tertiary N of the LH. In addition, a peak centered at 3.08 ppm, assigned to the CH_2 protons (labeled *x*) of the LH moiety adjacent to the

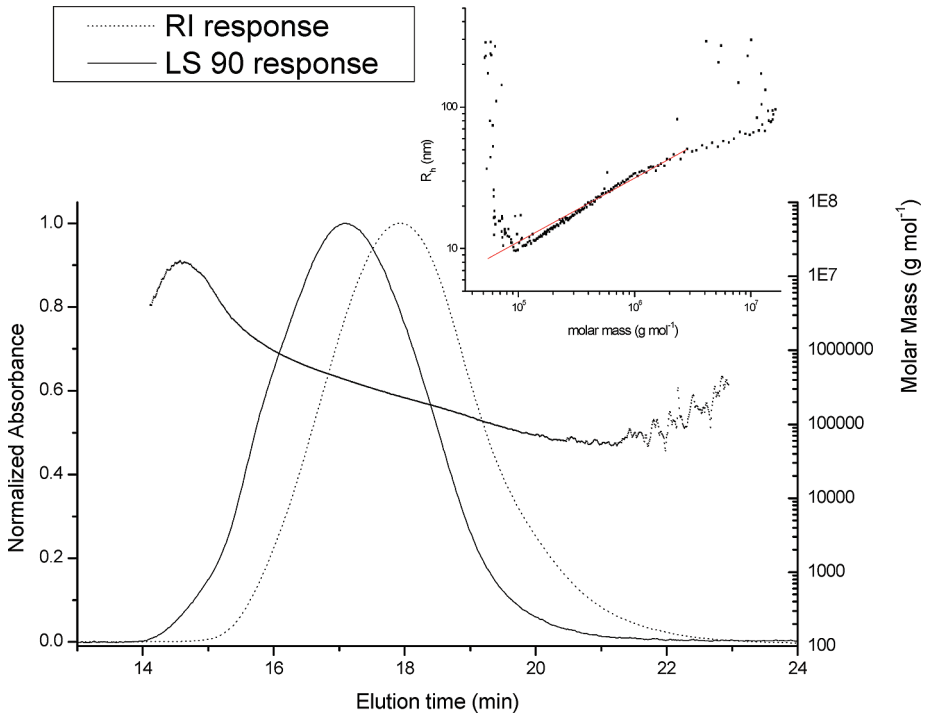


Figure 8. AQSEC analysis of hydrolyzed poly(vinyl dimethyl azlactone) (h-pVDMA) consisting of the chromatograms for the 90° light scattering signal (solid line) and the RI signal (dashed line) with molar mass data overlaid. The inset shows how the hydrodynamic radius varies with molar mass; a slope of 0.45 indicates that hydrolyzed h-pVDMA is a random coil.

amide formed upon conjugation, is present. Furthermore, there is a slight upfield shift in the peaks labeled y and z, from 3.96 ppm (Figure 6b) to 3.80 ppm (Figure 6c) upon incorporation into the copolymer. Likewise, a downfield shift for the CH_2 protons (labeled *x*) from 3.00 (Figure 6b) to 3.02 ppm (Figure 6c) was observed after conjugation. Molecular weight information is currently unavailable due to solubility issues of the LH-modified copolymer..

Hydrolysis of pVDMA. As noted previously, pVDMA has proven to be hydrolytically stable in water at neutral pH conditions. For example, to assess the stability 185 mg of pVDMA (entry D, Table 1) powder was combined with 13 mL of deionized. After aging for 1 year, pVDMA remained insoluble in deionized water, with minimal hydrolysis (detailed below). Furthermore, the recovered polymer remained soluble in many organic solvents (e.g., THF, DMF, $CHCl_3$, $MeCl_2$, toluene, benzene) suggesting, at least qualitatively, that to a large degree bulk pVDMA resists hydrolysis, which is a considerable advantage compared to NHS-functionalized polymers. While it is possible that some of the azlactone groups underwent hydrolysis, the hydrophobic nature of the polymer seems to prohibit dissolution of the polymer into water, inhibiting the hydrolysis of the azlactone moiety. A minor amount of polymer (35 mg, 19% by gravimetry) was recovered from the aqueous phase first by decanting the solution, rinsing the organo-soluble portion with ~5 mL of DI water and combining the rinsings with the aqueous phase, and subsequently freeze-drying. The recovered material was analyzed by ^{1}H and ^{13}C NMR and ATR−FTIR spectroscopies, and these characterizations showed complete hydrolysis of azlactone groups. In contrast, when an inorganic base such as sodium hydroxide (NaOH) is used, hydrolysis proceeds rapidly and quantitatively. Scheme 2 illustrates the proposed reaction product for the hydrolysis of pVDMA.

Figure 7 shows the base-catalyzed hydrolysis of pVDMA in real-time using remote-probe ATR−FTIR spectroscopy by monitoring the peak height of the azlactone group at 1825 cm^{-1}. As indicated in the scheme and because of the insolubility of pVDMA in water, base-catalyzed hydrolysis was carried out in THF solutions. After a consistent baseline was established (t < 8 min), 0.7 mL of 0.1 N NaOH(aq) was injected to the polymer solution (196 mg of pVDMA in 3713 mg of THF). The rapid reaction is evidenced by the sudden drop in azlactone peak height (and peak area, not shown), after which a constant signal indicating equilibrium is attained. Upon the second injection of 0.7 mL of 0.1 N NaOH(aq), the signal indicative of the carbonyl of the azlactone ring quickly disappears into the baseline, indicating complete hydrolysis by ring-opening of the azlactone. The inset in Figure 7 shows a first-order kinetic plot for the first addition of NaOH(aq) used to catalyze the hydrolysis of pVDMA. An apparent rate constant, k_{app} = 0.931 min^{-1} was determined for the initial hydrolysis from a linear fit of the data between 8.7 and 9.7 min (error = 0.193%, standard deviation = 0.135).

Hydrolyzed pVDMA is insoluble in most organic solvents (i.e., THF, DMF, $CDCl_3$, etc.), which also is a good qualitative indication of the conversion to the ring opened form. As a result, ^{1}H and ^{13}C NMR spectra were recorded using D_2O:DMSO-d_6 (1:1) as a cosolvent system; DMSO provided an internal standard (DMSO peak at 39.39 ppm in D_2O[21]) in ^{13}C NMR measurements. While the ^{1}H NMR spectrum is useful in the analysis of hydrolyzed pVDMA, the ^{13}C spectrum provides more definitive information. A comparison of the ^{13}C NMR spectra of pVDMA and hydrolyzed pVDMA is provided in the Supporting Information (Figure S4) and the respective structures as well as the calculated (ChemDraw Ultra 8.0) and observed ^{13}C chemical shifts are listed in Table S1. (pVDMA: ^{13}C NMR (125 MHz, DMSO-d_6, δ): 181.4 (C=O), 163.8 (O−C=N), 65.5 ($\boldsymbol{C}(CH_3)_2$), 33.9, 35.4 (CH), 24.6 ($(CH_3)_2$), 20.9, 22.1 (CH_2); hydrolyzed pVDMA: ^{13}C NMR (125 MHz, D_2O: DMSO-d_6, δ): 183.1 ((C=O)OH), 177.0 ((C=O)NH), 59.5, 63.0 ($\boldsymbol{C}(CH_3)_2$), 42.0−45.1 (CH), 26.4 ($(CH_3)_2$), 36.9 (CH_2).)

In addition, aqueous size exclusion chromatography (AQSEC) was used to analyze the hydrolyzed pVDMA. Figure 8 shows the RI and 90° light scattering chromatograms overlaid with a plot of molar mass with elution time. A dn/dc = 0.1182 mL/g was determined (off-line) for hydrolyzed pVDMA. The separation of the AQSEC chromatograms as well as the sloping plot of molar mass in Figure 8 suggests that the hydrolyzed pVDMA has a broad molecular weight distribution. From the analysis, M_w = 315 kg/mol and PDI = 1.76. In addition the plot of hydrodynamic radius versus molar mass (inset of Figure 8) has a slope of 0.45, suggesting that hydrolyzed pVDMA is a random coil in the solvent system used.

Conclusions

Conventional free radical polymerization techniques have been employed to tailor copolymer composition of VDMA/VP copolymers. For these macromolecules, glass transition temperatures directly follow copolymer composition, as determined by DSC and ^{1}H NMR spectroscopy. Through a variety of functionalizations and subsequent characterization methods, we demonstrate the versatility of the azlactone moiety insofar that the monomer can be modified prior to polymerization as well as postpolymerization. Specifically, we have shown the ease of preparation of (co) polymers containing VDMA as well as modification of polymers and monomer through conjugation of a variety of nucleophiles (e.g., *n*-hexylamine, hexaethylene glycol monomethyl ether, dansylcadaverine, N_α,N_α-bis(carboxymethyl)-L-lysine hydrate, and water) thereby generating materials with predetermined and controlled functionality. Efforts to exploit this feature of these polymers are currently under investigation in our laboratories. In total, this work suggests considerable possibilities for creation of novel polymers for bioinspired applications.

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Supporting Information Available: Figures showing plots of glass transition temperatures as a function for copolymer composition (DSC analysis results) and NMR spectra of modified VDMA monomers, and hydrolyzed pVDMA and a table of observed and calculated ^{13}C NMR chemical shifts for pVDMA and hydrolyzed pVDMA also showing their structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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