Arborescent Polystyrene-*graft*-poly(2-vinylpyridine) Copolymers as Unimolecular Micelles. Synthesis from Acetylated Substrates

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ABSTRACT: Branched copolymers with a dendritic structure were synthesized by grafting poly(2-vinylpyridine) (P2VP) side chains onto linear and arborescent polystyrene substrates of generations G0−G2 randomly functionalized with acetyl groups. The P2VP side chains were generated in tetrahydrofuran using 1,1-diphenyl-3-methylpentyllithium as the initiator. The living polymer was then titrated with a solution of acetylated substrate to yield the copolymer. The grafting yield reached 92−96% in the 0−25 °C range with LiCl as a reactivity modifier for the P2VP anions. Arborescent copolymers with $M_w \approx 5000$ or 30 000 P2VP side chains were synthesized. The copolymers with short side chains contain 82−92 mol % P2VP units. For the long side chain systems, the P2VP content reaches over 97 mol %. These materials are characterized by a very compact structure, a narrow molecular weight distribution ($M_w/M_n = 1.06-1.08$), and a roughly geometric increase in branching functionality and molecular weight for successive generations. The copolymers derived from the linear polystyrene substrate aggregate when dissolved in aqueous HCl solutions. In contrast, copolymers based on G0−G2 substrates give unimolecular micellar solutions under the same conditions.

Introduction

Solubilization by amphiphilic block copolymers is based on their ability to form micelles via dynamic equilibrium with nonassociated species. The topology and colloidal stability of these micelles depends on factors such as solvent composition, temperature, concentration, and pH.[1] Amphiphilic dendrimers and dendritic−linear block copolymers can either aggregate into micelles in analogy to block copolymers or exist as unimolecular micelles, depending on their structure and composition.[2] The static, covalently bonded structure of a unimolecular micelle is advantageous when colloidal stability is required under a wider range of conditions than attainable with block copolymer micelles. Another interesting feature of unimolecular micelles is that they have no critical micelle concentration, unlike small molecule or block copolymer amphiphiles. One limitation of dendrimer micelles is that their size is typically less than ca. 10 nm. The synthesis of unimolecular micelles of controllable morphology with a larger size is of interest for some applications. For example, in the intravenous controlled delivery of drugs, a particle size in the 10−100 nm range can increase the residence time in the bloodstream.[3]

Arborescent polymers have a highly branched structure combining features of dendrimers and star polymers.[4] The hydrodynamic diameter of these molecules ranges from 10 to 200 nm, thus extending significantly the size attainable with dendrimer micelles. Amphiphilic copolymers containing poly(ethylene oxide)[5] or poly(2-vinylpyridine)[6] (P2VP) segments grafted onto a hydrophobic arborescent polystyrene core have been synthesized. Arborescent polymers are prepared from successive grafting reactions of well-defined polymeric building blocks onto substrates with randomly distributed coupling sites. The synthesis relies on the introduction of chloromethyl grafting sites along a linear polystyrene chain as a first step. Living polystyryl anions are then coupled with the linear substrate to generate a comb-branched (generation 0 or G0) polymer. Repetition of the chloromethylation and coupling reaction cycles leads to higher generation (G1, G2, etc.) arborescent polymers. This "graft-upon-graft" approach yields macromolecules with a high branching functionality and molecular weight in a few reaction steps, while maintaining a narrow molecular weight distribution (MWD). A serious limitation of previous arborescent copolymer syntheses is that the chloromethylation reactions rely on highly toxic chloromethyl methyl ether for the introduction of coupling sites on polystyrene substrates.

A new procedure was introduced recently for the synthesis of arborescent polystyrenes based on substrate functionalization with acetyl chloride, a more innocuous reagent than chloromethyl methyl ether.[7] We are now reporting the synthesis of amphiphilic copolymers incorporating P2VP segments of different molecular weights grafted onto acetylated linear, comb-branched (G0), G1, and G2 polystyrene substrates. In this study, the grafting reaction is optimized with respect to the reaction temperature and the presence of additives. The grafting yields obtained are higher for acetylated substrates than for chloromethylated substrates in most cases. Characterization results for the copolymers by size exclusion chromatography, static light scattering, and ^{1}H NMR spectroscopy are discussed. Dynamic light scattering measurements show that the P2VP amphiphilic copolymers form unimolecular micellar solutions in water upon protonation.

Experimental Section

Solvent and Reagent Purification. Acetyl chloride (Aldrich, 99+%) was distilled under nitrogen. *sec*-Butyllithium (Aldrich, 1.3 M in cyclohexane) and *tert*-butyllithium (Aldrich, 1.7 M in pentane) were used as received. The exact concentration of the *sec*-butyllithium solution was determined to be 1.37

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M by the procedure of Lipton et al.[8] 1,1-Diphenylethylene (DPE, Aldrich, 97%) was purified by adding enough *n*-butyllithium solution (Aldrich, 2.5 M in hexanes) to obtain the deep red 1,1-diphenylhexyllithium coloration and reduced pressure distillation. Lithium chloride (99.99+%, Aldrich) was first oven-dried at 110 °C and further dried by azeotropic distillation with dry tetrahydrofuran. Nitrobenzene (Aldrich, 99%) was distilled under reduced pressure. Tetrahydrofuran (THF; Caledon, reagent grade) was distilled from sodium benzophenone ketyl under nitrogen. *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (TMEDA; Aldrich, 99%) and 2-vinylpyridine (2VP; Aldrich, 97%) were first purified by stirring with CaH_2 and distillation under reduced pressure. 2-Vinylpyridine was further purified on a vacuum line prior to polymerization using three freezing−evacuation−thawing cycles in the presence of CaH_2, followed by slow distillation to an ampule. TMEDA was further purified by the same technique after titration with *tert*-butyllithium to a persistent yellow end point. Reagent ampules, used in the polymerization and grafting procedures, were prepared with the help of high-vacuum techniques and filled with nitrogen. The ampules were equipped with poly(tetrafluoroethylene) stopcocks and ground glass joints and mounted directly on the glass polymerization reactor.[5]

Preparation of Acetylated Polystyrene Substrates. The synthesis of the linear, comb-branched (G0), G1, and G2 partially acetylated polystyrene substrates used in the current investigation was reported previously.[7] The substrates had $M_w \approx 5000$ polystyrene core and side chains.

Synthesis of Polystyrene-*graft*-poly(2-vinylpyridine) Copolymers. The synthesis of a comb-branched copolymer (PS−P2VP5) is described as an example. Ampules containing 2-vinylpyridine (15.0 g, 0.143 mol in 25 mL of THF), DPE (0.65 g, 3.6 mmol in 20 mL of THF), acetylated linear polystyrene (1.65 g, 3.6 mequiv of acetyl functionalities), and LiCl (0.58 g, 15 mmol in 50 mL of THF), a THF line from the drying still, and a septum inlet were mounted on the reactor. After evacuation, flaming, and filling of the reactor with nitrogen, purified THF (200 mL) and the contents of the LiCl and DPE ampules were added. After cooling to −78 °C, *sec*-butyllithium was added dropwise to scavenge impurities, followed by 2.2 mL (3.0 mmol, for a calculated side chain $M_w \approx 5000$) to generate the bright red initiator 1,1-diphenyl-3-methylpentyllithium, in situ. After 5 min the 2-vinylpyridine solution was added, resulting in an immediate color change from bright red to dark red. After 30 min, a sample of side chains was removed with a syringe and terminated with degassed methanol. The remaining polymer solution was warmed to 0 °C and titrated with the acetylated polystyrene solution over ca. 30 min until a residual pale red coloration was observed. The reactor was warmed to room temperature, leading to further fading of the color. Residual anions were terminated with degassed water. The grafting yield estimated by GPC analysis (vide infra) was 95%. Nongrafted P2VP side chains were removed from the crude product by precipitation fractionation using THF/methanol (4/1 v/v) as solvent and *n*-hexane as nonsolvent. The purified copolymer, recovered by precipitation in *n*-hexane and dried under vacuum, had an absolute $M_w = 7.2 \times 10^4$ and a P2VP content of 92 mol %.

Polymer Characterization. A size exclusion chromatography (SEC) system calibrated with linear polystyrene standards was used for routine characterization of the polystyrene substrates, the P2VP side chains, the raw grafting products, and the fractionated graft copolymers. The instrument, operated at room temperature, consisted of a Waters 510 HPLC pump, a 500 mm × 10 mm Jordi DVB Mixed-Bed linear column (molecular weight range 10^2-10^7), and a Waters 410 differential refractometer detector. THF at a flow rate of 1 mL/min served as eluent for the analysis of polystyrene samples. TMEDA (5% v/v) was added to the mobile phase for the analysis of P2VP side chains and the graft copolymers to minimize adsorption of P2VP on the column.[9]

The absolute weight-average molecular weight (M_w) of the polystyrene substrates, the P2VP side chains, and most arborescent copolymers was determined by SEC-MALLS (multiangle laser light scattering) analysis using a Wyatt Dawn DSP-F instrument operating at 632.8 nm. The SEC system consisted of a Waters 590 programmable HPLC pump coupled with Waters Ultrastyragel columns (10^4, 10^5, and 10^6 Å pore sizes) operated at 25 °C. THF at a flow rate of 1 mL/min served as the mobile phase. Polymer concentration measurements in the eluent were accomplished with a Waters 2410 DRI detector. Copolymer samples based on the generation G2 polystyrene substrate could not be analyzed by SEC-MALLS because the samples were not eluted from the SEC column. Their absolute M_w was determined using static light scattering measurements on a Brookhaven BI-200 SM light scattering goniometer equipped with a BI-2030AT 201-channel correlator and a Lexel 2 W argon ion laser operating at 514.5 nm.[5] The M_w was determined by Zimm extrapolation to zero angle and concentration for a series of measurements at 25 °C for 6−8 THF solutions at angles ranging from 30° to 150°. The refractive index increment (dn/dc) of the copolymers was determined at 25 °C using a Brice-Phoenix differential refractometer equipped with 510 and 632 nm interference filters. Dynamic light scattering measurements were also carried out on the Brookhaven BI-200 SM instrument at a temperature of 25 °C and a scattering angle of 90°. The copolymer samples were dissolved either directly in 0.1 N HCl or first in THF before dilution with 10 volumes 0.1 N HCl. The final concentration of the samples was 0.02−0.2% w/v, depending on the generation number. The correlator was operated in the exponential sampling mode, the last four data acquisition channels being used for baseline measurements. The translational diffusion coefficients used in the hydrodynamic diameter calculations were determined from first-order as well as second-order analysis of the normalized electric field correlation function $|g^1(\tau)|$.

The composition of the copolymers was determined from ^1H NMR spectra obtained in $CDCl_3$ on a Bruker AM-250 spectrometer.

Results and Discussion

Structurally well-defined linear P2VP homopolymers[10] and block copolymers with polystyrene,[9] poly(*tert*-butyl acrylate),[11] poly(*tert*-butyl methacrylate),[12] and poly(dimethylsiloxane)[13] blocks have been synthesized using living anionic polymerization techniques. Moreover, the preparation of star-branched poly(2-vinylpyridine) homopolymers[14] and comb-branched polystyrene-*graft*-poly(2-vinylpyridine) copolymers[15,16] has been reported. The range of structures synthesized in terms of branch number and branch size is, however, limited for most of these materials.

Arborescent polymers can be prepared with a low polydispersity and a wide range of branch sizes and branching functionalities from well-defined polymeric building blocks. By extending the method recently developed for arborescent polystyrenes,[7] P2VP anions can be grafted onto linear, G0, G1, and G2 acetylated polystyrene substrates. Scheme 1 describes the coupling reaction of P2VP anions with an acetylated linear polystyrene substrate as an example. The synthesis comprised three steps: the grafting substrate was acetylated to introduce coupling sites, 2-vinylpyridine was polymerized anionically to generate poly(2-vinylpyridinyl)lithium, and the living polymer was titrated with the acetylated polystyrene substrate to yield the graft copolymer. Each reaction step is discussed in more detail below.

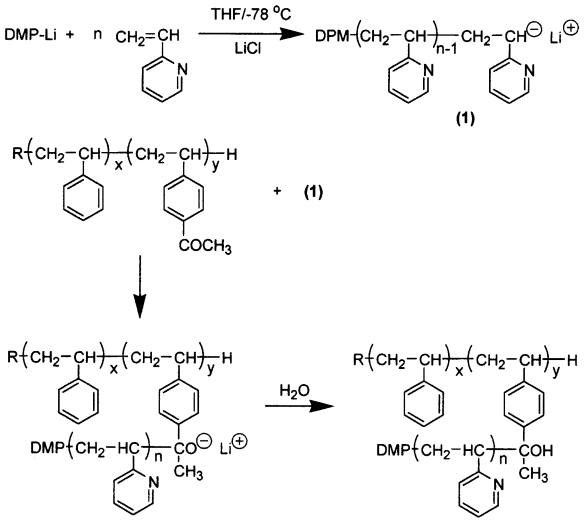
Polystyrene Substrates. The characteristics of the linear and branched polystyrene substrates used in the preparation of the graft copolymers are summarized in Table 1. The substrates, prepared by cycles of acetylation, grafting, and fractionation, are based on $M_w \approx 5000$ polystyrene core and side chains. The branching functionality of the polymers, defined as the number of

Table 1. Characteristics of Polystyrene Substrates[7]

	branches		substrates				
polymer	M_w [a]	M_w/M_n [a]	M_w [a]	M_w/M_n [a]	f_w [b]	CH_3CO-/mol % [c]	grafting sites
PS (linear)			5.1×10^3	1.07		25	12
G0PS	4400	1.09	5.3×10^4	1.08	11	22	100
G1PS	4500	1.07	4.3×10^5	1.08	84	27	980
G2PS	5000	1.08	3.9×10^6	1.09	690	20	6600

[a] Absolute values determined by SEC-MALLS measurements before acetylation. [b] Number of branches added in the last grafting reaction. [c] Acetylation level determined by ^{1}H NMR spectroscopy.

Scheme 1. Preparation of Polystyrene-*graft*-poly(2-vinylpyridine) Copolymer



chains added in the last grafting reaction, is calculated from the equation

$$f_w = \frac{M_w(G) - M_w(G-1)}{M_w^{br}} \qquad (1)$$

where $M_w(G)$, $M_w(G-1)$, and M_w^{br} are the absolute weight-average molecular weight of graft polymers of generation G, of the preceding generation, and of the side chains, respectively. The acetylation level of the substrates was maintained in the range 20−30 mol % to provide 10−15 coupling sites per side chain on average. The number of potential grafting sites introduced on the substrates, reported in Table 1, is calculated from the total molecular weight and the acetylation level.

2-Vinylpyridine Polymerization and Optimization of Grafting Conditions. The 2-vinylpyridine polymerization was carried out in THF at −78 °C using 1,1-diphenyl-3-methylpentyllithum, prepared in situ from *sec*-butyllithium and DPE, as the initiator. An additive (LiCl or TMEDA) was used in most cases to modify the reactivity of the macroanions in the subsequent grafting reaction. The characterization data for side chains removed before the grafting reaction (Tables 2 and 3) demonstrate that good control was maintained over the molecular weight and MWD under the conditions used.

For the grafting reaction to proceed in high yield, the macroanions must remain active ("living") during the time required to complete the reaction, and coupling must be favored over side reactions that cause premature termination of the chain ends. In the coupling reaction of acetylated polystyrene with polystyryllithium, proton abstraction from the acetyl functionality was identified as a major side reaction leading to the deactivation of ca. 40% of the living chains.[7] Avoidance of these side reactions requires the macroanions to be more stable, but still sufficiently nucleophilic for the coupling reaction. The stability and reactivity of macroanions is controllable to some extent by variation of the reaction temperature for the grafting reaction and/or the use of additives. These parameters were systematically investigated to maximize the grafting yield. For each test reaction, short ($M_w \approx 5000$) P2VP side chains were grafted onto a partially acetylated $M_w = 5100$ linear polystyrene substrate with an acetylation level of 25 mol %. Table 2 summarizes the results obtained for the test reactions under different conditions.

Table 2. Effect of Reaction Temperature and Additives on Grafting Yield[a]

temp/ °C	additive [b]	P2VP side chains M_w [c]	P2VP side chains M_w/M_n [c]	grafting yield (%) [d]	graft copolymer M_w [c]	graft copolymer M_w/M_n [c]
−78	none	5700	1.10	78	35 000	1.11
−30	none	5500	1.08	74	33 400	1.09
0	none	5300	1.07	73	40 600	1.10
25	none	5200	1.09	70	38 600	1.09
−78	LiCl	5600	1.08	90	37 300	1.08
−30	LiCl	5000	1.08	93	38 000	1.09
0	LiCl	5500	1.07	96	45 000	1.08
25	LiCl	6200	1.08	92	43 300	1.08
−30	TMEDA	5700	1.09	46	23 300	1.12

[a] All reactions in THF with linear polystyrene substrate (M_w = 5100, M_w/M_n = 1.07, acetylation level = 25 mol %). [b] 5 equiv additive (LiCl or TMEDA) used relative to the number of chain ends in the reaction. [c] Apparent values determined by SEC analysis based on a linear polystyrene standards calibration curve. [d] Fraction of side chains generated that are attached to the substrate.

The influence of reaction temperature on the *grafting yield*, defined as the fraction of side chains generated that are attached to the substrate, was examined at −78, −30, 0, and 25 °C, after polymerization of 2-vinylpyridine at −78 °C in THF either with or without a coordinating additive (LiCl or TMEDA). In the absence of additive, the grafting yield increases slightly from 70 to 78% as the reaction temperature is lowered from 25 to −78 °C. The increased grafting yield at lower temperatures likely reflects the reduced susceptibility of the acetyl protons toward abstraction by the macroanions. In the reaction between P2VP anions and chloromethyl functionalities, a slower coupling rate is observed at lower temperature.[6] In contrast, the grafting reaction of P2VP anions with acetyl functionalities proceeds rapidly at all temperatures investigated, as indicated by the immediate fading of the coloration as the substrate solution is added to the reactor.

Additives can either increase or decrease the stability and reactivity of P2VP anions. The effects of LiCl or TMEDA as additives for the grafting reaction were investigated for comparison with reactions without additives.

Table 3. Characteristics of Arborescent 2-Vinylpyridine Copolymers of Successive Generations[a]

sample	P2VP side chains		grafting yield (%)[c]	graft polymer					mol % P2VP[f]	coupling efficiency (%)[g]
	M_w[b]	M_w/M_n[b]		d*n*/d*c* (mL/g)	M_w[b]	M_w/M_n[b]	M_w[d]	f_w[e]		
PS−P2VP5	5600	1.07	95	0.167	7.2×10^4	1.06	3.9×10^4	12	92	100
G0PS−P2VP5	5400	1.06	89	0.155	5.3×10^5	1.08	1.3×10^5	88	88	88
G1PS−P2VP5	5900	1.08	86	0.149	4.3×10^6	1.07	4.1×10^5	650	87	66
G2PS−P2VP5	5300	1.07	77	0.166[h]	2.4×10^7			3790	82	57
PS−P2VP30	34900	1.06	86	0.168	3.9×10^5	1.06	1.5×10^5	11	>97	92
G0PS−P2VP30	35200	1.07	78	0.158	2.5×10^6	1.07	4.7×10^5	70	97	70
G1PS−P2VP30	35700	1.07	34	0.148	1.3×10^7	1.08	6.8×10^5	350	96	36
G2PS−P2VP30	32500	1.06	26	0.140[h]	6.1×10^7			1760	91	27

[a] All reactions in THF at 0 °C with 5 equiv of LiCl added. [b] Absolute values determined by SEC-MALLS or laser light scattering measurements. [c] Fraction of side chains generated attached to the substrate. [d] Apparent values determined by SEC analysis based on a linear polystyrene standards calibration curve; G2PS−P2VP5 and G2PS−P2VP30 not eluted from the column in SEC analysis. [e] Number of branches added in the last grafting reaction. [f] P2VP content determined by ^{1}H NMR spectroscopy. [g] Fraction of available coupling sites on the substrate consumed. [h] d*n*/d*c* at 510 nm; all other values determined at 632 nm.

The addition of a common ion salt to an anionic polymerization reaction shifts the ion pair vs free ion equilibrium in favor of ion pairs.[17] These ion pairs are characterized by a lower nucleophilic character and thus a lower reactivity and higher stability. The addition of LiCl to the anionic polymerization of acrylates and methacrylates in polar solvents has been shown to increase the initiation efficiency and decrease the breadth of the molecular weight distribution.[18] The addition of LiCl to the polymerization of 2-vinylpyridine also does not have a detrimental effect on the living character of the anions and facilitates the preparation of block copolymers incorporating poly(*tert*-butyl acrylate) end blocks.[11] Experimentally, the grafting yield increases substantially with a 5-fold excess of lithium ions at all reaction temperatures investigated to reach a maximum of 96% at 0 °C. The increased grafting yield in the presence of excess LiCl presumably reflects the fact that the stabilization of P2VP macroanions reduces the rate of proton abstraction more than the rate of the coupling reaction.

To confirm the hypothesis that the increased grafting yield with LiCl results from the decreased concentration of free ions in the reaction, the dissociation equilibrium was also perturbed with TMEDA to increase the fraction of free anions. TMEDA can act as a ligand for lithium ions.[19] Consequently, the extent of ionization (and the nucleophilic character) of the P2VP macroanions should be enhanced in the presence of that additive. It was previously reported that the addition of TMEDA prior to grafting P2VP macroanions onto chloromethylated polystyrene increases the grafting yield, presumably due to increased nucleophilic character of the macroanions.[6] The situation is very different in the reaction with acetylated substrates since the addition of TMEDA decreases the grafting yield from 74% (Table 2, entry 2) to 46% (Table 2, entry 9) at −30 °C.

On the basis of the results obtained, grafting of LiCl-modified P2VP anions onto the acetylated polystyrene substrates in THF at 0 °C was selected as a standard procedure for the preparation of arborescent polystyrene-*graft*-poly(2-vinylpyridine) copolymers.

Arborescent Polystyrene-*graft*-poly(2-vinylpyridine) Copolymers. Using the conditions optimized for the preparation of comb-branched 2-vinylpyridine copolymers, two series of arborescent copolymers with either short ($M_w \approx 5000$, P2VP5) or long ($M_w \approx 30\,000$, P2VP30) P2VP side chains were prepared from acetylated polystyrene substrates of different generations. Characterization data for these copolymers are provided in Table 3. In keeping with previous nomenclature,[20] graft copolymer sample identification specifies their composition and structure. For example, G1PS−P2VP5 refers to a graft copolymer with $M_w \approx 5000$ P2VP side chains grafted onto a G1 (twice-grafted) arborescent polystyrene substrate.

When P2VP side chains are grafted onto randomly acetylated polystyrene substrates of different generations, the grafting yield (the fraction of the P2VP side chains generated that are attached to the substrate) ranges from 26 to 95% (Table 3). For both the P2VP5 and P2VP30 series, as the generation (or branching functionality) of the substrate increases, the grafting yield decreases. An important factor contributing to the deactivation of living P2VP anions under these conditions may be their reaction with residual protic impurities introduced with the substrate polymer solution, since it is more difficult to purify the higher generation substrates after acetylation. To reach the end point (complete discoloration of the living anions) in the grafting procedure, an excess of substrate (beyond the expected 1:1 acetyl to P2VP anion stoichiometry) must be used for the preparation of higher generation copolymers. This method, while ensuring full consumption of the anions (and maximum grafting yield), results in the introduction of a larger amount of protic impurities in the reaction. The grafting yield also depends on the length of the P2VP grafts: The yield is always lower for the P2VP30 than the P2VP5 side chains. This effect is most noticeable for the G2 substrates, with a decrease from 77% for G2PS−P2VP5 to 26% for G2PS−P2VP30. The concentration of living ends decreases when the molecular weight of the side chains increases, making the reaction more susceptible to deactivation by impurities. Similar variations were also observed with chloromethyl coupling sites in the preparation of arborescent polystyrenes[21] as well as polyisoprene[20] and P2VP[6] copolymers. Another factor that may have contributed to the deactivation of a fraction of the living chains is proton abstraction from the tertiary alcohol functionalities introduced in previous grafting reactions. The acetylated G0 substrate, for example, contains 11 *tert*-OH functionalities and 100 coupling sites (Table 1). This termination reaction could therefore account for the deactivation of up to 10% of the living anions. The situation is similar for upper generation (G1 and G2) substrates. While this factor may explain the loss of a portion of the living anions, it does not suffice by itself to explain the relatively low grafting yields observed for higher generations.

The *coupling efficiency*, defined as the fraction of coupling sites consumed in the reaction, follows similar

trends to the grafting yield, as shown in Table 3. Since the acetyl groups are presumably randomly distributed throughout the polystyrene substrate, all sites should have the same intrinsic reactivity. However, when the substrate is highly branched, the structure becomes more congested. As the grafting reaction proceeds, steric congestion leads to differential accessibility for the remaining acetyl sites, and the coupling efficiency decreases. The influence of reaction time on coupling efficiency was investigated in the synthesis of arborescent P2VP copolymers by grafting onto chloromethylated substrates.[6] For the reaction of chloromethylated G1PS with a 25% excess of living P2VP chains, increasing the reaction time from 1 to 6 h led to no significant improvement in coupling efficiency. The result was explained by increased steric congestion of the substrate upon grafting making a fraction of the coupling sites effectively inaccessible. The morphology of arborescent polystyrenes was probed using fluorescence quenching techniques, which demonstrated that the inner portion of the molecule is less accessible to quencher molecules than chains in the outer layer.[22] The fraction of less accessible material increased for higher generation polymers. Small-angle neutron scattering measurements on arborescent polystyrenes[23] also confirmed the high chain density in the core portion of the molecules relative to the outer layer. This differential accessibility effect explains the decrease in coupling efficiency observed for higher generation acetylated polystyrene substrates as well as for higher molecular weight (P2VP30) side chains.

There are other indications that the grafting reaction becomes limited by steric crowding in the reactions of G1 and G2 substrates. The amount of acetylated polymer required in the titration of the living polymers with the linear and G0 substrates is approximately as expected from the stoichiometry of the reaction. For substrates G1 and G2, however, a 50−100% excess of substrate is required to deactivate all of the living ends. This, again, points to surface overcrowding effects hindering the diffusion of the living chain ends to the acetyl sites. Surface overcrowding effects were also reported as a limiting factor in the preparation of dendrimers.[24]

Sample Characterization. Routine SEC analysis was used to characterize the polystyrene substrates, the P2VP side chains, and the graft products. Typical SEC characterization results obtained are shown Figure 1 for the synthesis of sample G0PS−P2VP5. Two peaks are present in the SEC trace for the raw grafting product (curve c). The leftmost (high molecular weight) peak corresponds to the graft copolymer. The rightmost peak has the same elution volume as the side-chain sample removed from the reactor prior to the reaction (curve b), corresponding to deactivated (nongrafted) side chains. A comparison of curves a and c indicates that no excess substrate is present in the raw product. This is a consequence of the colorimetric titration procedure used, allowing precise control over the stoichiometry of the reaction. Curve d demonstrates complete removal of the linear contaminant by fractionation. The SEC traces obtained for all graft polymers eluted from the column were symmetrical, in analogy to curve d of Figure 1.

The grafting yield (fraction of living chains grafted on the substrate) is conveniently determined from the SEC trace obtained using a differential refractometer

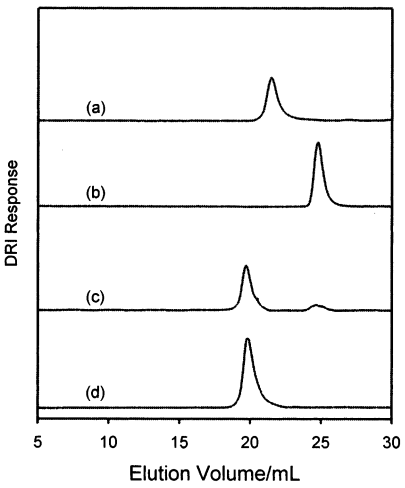


Figure 1. Preparation of sample G0PS−P2VP5: SEC traces for (a) G0PS substrate, (b) P2VP5 side chains, (c) crude product from the grafting reaction, and (d) fractionated copolymer.

(DRI) detector for the raw reaction product. Polystyrene and poly(2-vinylpyridine) have very similar refractive index increments in THF ($dn/dc \approx 0.19-0.20$ and 0.18−0.19, respectively)[25] and cause a similar response in the DRI detector. Under these conditions, the grafting yield is calculated as the ratio of the peak area for the graft polymer to the total peak area for the graft polymer and the linear P2VP contaminant. For example, the peak areas determined (in arbitrary units) for sample G0PS−P2VP5 (Figure 1c) were 79 770 and 9859 for the graft polymer and the nongrafted side chains, respectively, corresponding to a grafting yield of 79770/(79770 + 9859) = 89%. For graft copolymer samples that were retained on the SEC column (G2PS−P2VP5 and G2PS−P2VP30), the grafting yield was determined by a variation of the same method. In that case the graft polymer component is not detected, but a peak is observed for the nongrafted (linear) material. The response (peak area A_1) is first determined for a linear P2VP sample injected at a known concentration. The response A_2 is then measured for the nongrafted component when the raw product is injected at the same concentration. The grafting yield is then calculated as $1 - A_2/A_1$.

Comparison of the absolute molecular weights obtained from SEC-MALLS or static light scattering (Table 3, column 6) with the apparent values (column 8) obtained from the DRI detector indicates that the apparent molecular weights are strongly underestimated, especially for higher generations. This is a consequence of the highly compact structure of arborescent copolymers in solution relative to linear polymers. The MWD remains narrow ($M_w/M_n = 1.06-1.08$) for all generations characterized. Weight-average molecular weights ranging from $M_w = 7.2 \times 10^4-2.4 \times 10^7$ and $3.9 \times 10^5-6.1 \times 10^7$ for the P2VP5 and P2VP30 copolymer series, respectively, were obtained from SEC-MALLS or static light scattering measurements. The corresponding branching functionalities (f_w, calculated using eq 1) range from 12 to 3790 within the P2VP5 series and from 11 to 1760 within the P2VP30 series. While the analysis of copolymers by light scattering is subject to errors due to dn/dc fluctuations throughout the different sample fractions,[26] these errors are minimized when the refractive index increments for both components in the copolymer are close, such as in the present case. On that basis, the absolute M_w values

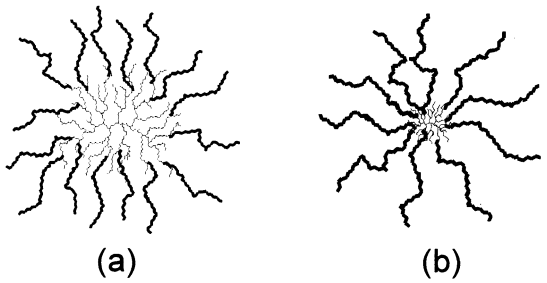


Figure 2. Comparison of structures obtained when a G1 polystyrene substrate is grafted with (a) short and (b) long P2VP side chains.

reported in Table 3 (column 6) are deemed to be reasonable estimates of the true values.

The composition of the copolymers, after removal of residual nongrafted P2VP side chains, was determined by ^{1}H NMR spectroscopy. The results obtained, summarized in Table 3, indicate that the P2VP content of the copolymers varies from 82 to 92 mol % and 91 to 97+ mol % for the P2VP5 and P2VP30 series, respectively. This shows that graft copolymers with a significant polystyrene component can be prepared if short (or fewer) P2VP segments are grafted. The polystyrene component becomes negligible when longer P2VP segments are used.

An alternate method to estimate the molecular weight of the copolymers is by combining the absolute M_w of the substrate (Table 1, column 4) with the copolymer composition determined by NMR analysis (Table 3, column 10). Considering the significant errors involved in NMR analysis for copolymers with low polystyrene contents (>90 mol % P2VP contents), these calculations have only been attempted for samples with a polystyrene content of at least 10 mol %. The molecular weight values thus obtained for samples G0PS−P2VP5, G1PS−P2VP5, and G2PS−P2VP5 are 4.4×10^5, 3.3×10^6, and 2.4×10^7, respectively, and compare favorably with the absolute M_w values reported in Table 3 (column 6).

Solution Properties. It is possible to control the composition of the copolymers by varying parameters such as the length and number of the P2VP segments and the size (generation) of the polystyrene substrate used. Variation in the structure of the copolymers provides a facile way to control their physical properties. Copolymers with short P2VP segments are equivalent to branched block copolymers with radial symmetry and should be suitable as unimolecular micelles (Figure 2a). When long P2VP segments are grafted onto the polystyrene substrates, the dimensions of the substrate are small relative to the outer branches, yielding a structure approximating a star-branched homopolymer (Figure 2b). The P2VP segments of the copolymers are easily protonated by acids, making the molecules freely soluble in aqueous solutions.

Solubilization of the molecules was demonstrated using dynamic light scattering measurements. Two different dissolution protocols were applied for comparison: The solid copolymers were either directly dissolved in 0.1 N aqueous HCl or first dissolved in THF before dilution with 10 volumes of 0.1 N aqueous HCl. The hydrodynamic diameters derived from first- and second-order analyses of the correlation function $|g^1(\tau)|$ are compared in Table 4. The uncertainties on the measurements are reported either as the standard deviation for a series of 10 values or ±1 nm, whichever is greater.

Table 4. Hydrodynamic Diameter of the P2VP Copolymers in 0.1 N HCl

	HCl dissolution[a]		THF/HCl dissolution[b]	
sample	d_{h1} (nm)[c]	d_{h2} (nm)[d]	d_{h1} (nm)[c]	d_{h2} (nm)[d]
PS−P2VP5	93 ± 50	42 ± 20	26 ± 13	15 ± 3
G0PS−P2VP5	30 ± 1	27 ± 1	29 ± 1	28 ± 1
G1PS−P2VP5	46 ± 1	43 ± 1	52 ± 1	51 ± 1
G2PS−P2VP5	119 ± 1	112 ± 1	102 ± 1	99 ± 1
PS−P2VP30	64 ± 5	40 ± 4	60 ± 2	43 ± 2
G0PS−P2VP30	85 ± 1	80 ± 1	77 ± 1	75 ± 1
G1PS−P2VP30	129 ± 1	119 ± 1	114 ± 1	111 ± 1
G2PS−P2VP30	160 ± 1	155 ± 1	136 ± 1	134 ± 1

[a] Direct dissolution in aqueous HCl. [b] Dissolution in THF followed by dilution with aqueous HCl. [c] From first-order analysis of the correlation function. [d] From second-order analysis of the correlation function.

For a monodisperse size distribution the results of first- and second-order analyses are expected to be identical, since the correlation function can be represented by a single-exponential decay.[27] The discrepancy between both results should increase as the size distribution broadens. For higher generation copolymers, the good agreement observed between the first- and second-order analyses suggests that the macromolecules exist as isolated (monomolecular) species in solution. This is the case even for solid samples directly dissolved in aqueous 0.1 N HCl. In contrast, the polymers derived from linear polystyrene substrates (PS−P2VP5 and PS−P2VP30) remain aggregated in solution irrespective of the dissolution protocol used. Initial dissolution in THF apparently decreases the average size of the species in solution, however.

Analysis of the correlation function using the CONTIN algorithm is often used to generate size distributions. The reliability of the CONTIN technique has been criticized,[28] particularly with respect to its sensitivity to baseline noise in the data. Despite its limitations, CONTIN analysis can still provide data useful to compare semiquantitatively the aggregation behavior of low- and high-generation arborescent copolymers. The size distributions in Figure 3 show the influence of the dissolution method (direct vs THF dissolution) and generation number (PS−P2VP5 vs G1PS−P2VP5) on the association of the copolymers in 0.1 N HCl. It is clear that while most of the low-generation copolymer molecules remain nonaggregated in solution (intensity maximum at $d = 12$ nm), some of the molecules also associate into a larger size population with a diameter of ca. 250−300 nm (Figure 3a). When the copolymer is dissolved in THF prior to protonation, the relative amount of aggregates obtained apparently decreases (Figure 3b). The origin of the discrepancy observed between first- and second-order correlation function analysis results can therefore be attributed unambiguously to aggregation of the molecules. It is also interesting to note that sample G1PS−P2VP5, with a higher branching functionality and lower interpenetrability than sample PS−P2VP5,[29] yields unimolecular micelle solutions without aggregates irrespective of the preparation method used (Figure 3c,d). Similar results were obtained for all copolymers based on G0−G2 substrates.

The core−shell morphology of the copolymers obtained by grafting short hydrophilic P2VP chains on the hydrophobic polystyrene core is interesting in terms of solubilization properties. The micellar properties of these materials, including their solubilizing ability for hydrophobic compounds, are currently under investigation and will be reported subsequently.

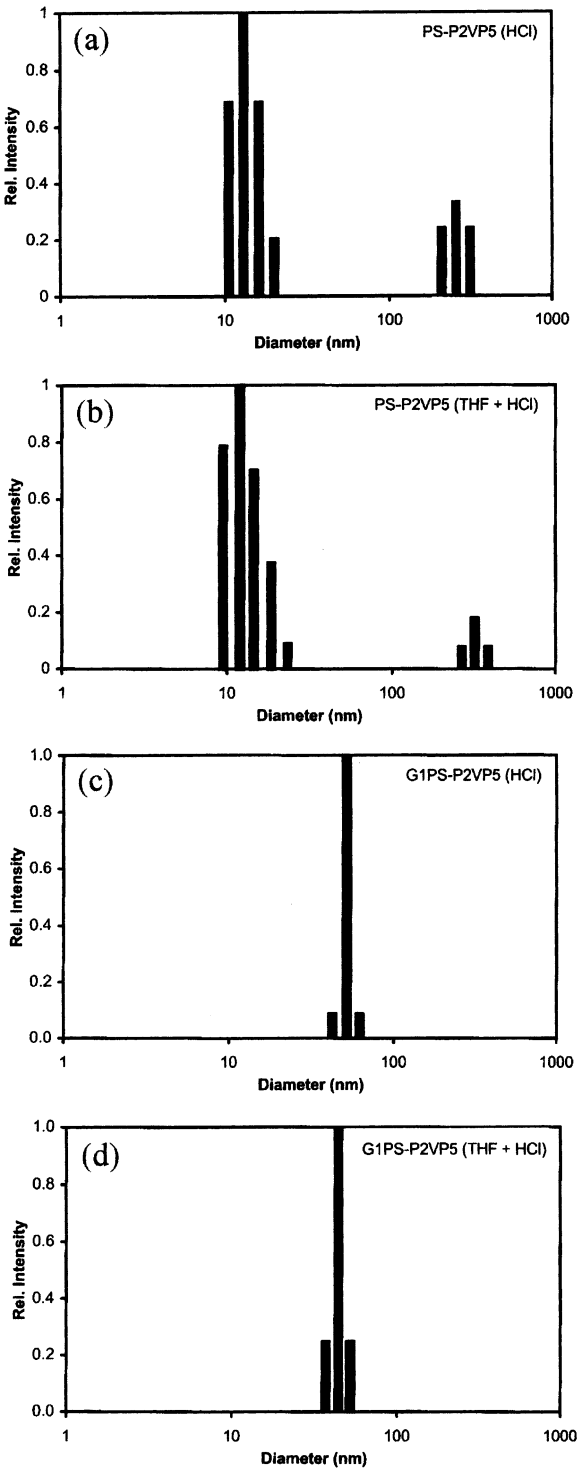


Figure 3. Hydrodynamic diameter distributions obtained by CONTIN analysis of the correlation functions.

Conclusions

Dendritic amphiphilic macromolecules, the arborescent polystyrene-*graft*-poly(2-vinylpyridine) copolymers, can be successfully synthesized by coupling living P2VP side chains with acetyl functionalities randomly distributed on polystyrene substrates. The grafting yield for the reaction between P2VP macroanions and acetyl sites improves substantially in the presence of LiCl. A narrow MWD is maintained for the graft copolymers over successive generations. Arborescent copolymers of different P2VP contents can be prepared by grafting P2VP side chains of different molecular weights onto acetylated polystyrene substrates of different generations (or branching functionalities). The polymers are freely soluble in diluted aqueous HCl solutions. Copolymers based on G0−G2 arborescent polystyrene substrates form unimolecular micellar solutions and should have interesting solubilization properties.

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