Biodegradable Branched Polyesters Poly(vinyl sulfonate-covinyl alcohol)-graft Poly(D,L-lactic-coglycolic acid) as a Negatively Charged Polyelectrolyte Platform for Drug Delivery: Synthesis and Characterization

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ABSTRACT: Biodegradable, negatively charged, branched polyesters, namely, poly(vinyl sulfonate-covinyl alcohol)-graft-poly(D,L-lactic-coglycolic acid), abbreviated as P(VS-VA)-g-PLGA, were synthesized by ring-opening polymerization using sulfonate-modified poly(vinyl alcohol) backbones as a platform for cationic drug delivery systems. The structures of sulfonate-modified backbones and the graft polyesters were characterized by NMR, FT-IR, GPC-MALLS, DSC, and viscosity measurements. By controlling the degree of sulfonate substitution of the backbone and the feed ratio of the backbone to the monomer, graft polyesters with different degrees of sulfonate substitution and different branch lengths were obtained. The random copolymer structure of PLGA and the successful grafting of PLGA to the P(VS-VA) backbone were confirmed by COSY NMR experiments. In vitro degradation studies demonstrated that the increased sulfonate substitution leads to a faster degradation rate; half-lives as fast as 8 days were observed. Nanoparticles were prepared from these amphiphilic graft copolymers by a solvent displacement technique and were characterized by particle size, polydispersity, zeta potential, and SEM. These novel biodegradable polyesters are promising candidates as negatively charged polyelectrolyte platforms for cationic drug delivery systems.

Introduction

Novel carriers for gene and drug delivery remain a major topic for the successful transfer of new therapeutic principles into the clinic.[1–3] For drugs, bioactive growth factors, and gene and cell delivery systems, each requirement differs with regard to their application.[4–8] The aliphatic polyesters polylactide (PLA), polyglycolide (PGA), and their copolymers are of great interest for applications in biological and biomedical areas due to their desirable properties of biodegradability, biocompatibility, and permeability.[9–11] Moreover, as nanocarrier systems, the formulation of PLGA nanoparticles can be scaled to industrial manufacture.[12] However, the lack of hydrophilic and functional groups leads to microencapsulation and stability issues during storage or under in vitro and/or in vivo release conditions, such as undesired discontinuous or polyphasic release patterns, high initial burst release, low efficiency of drug encapsulation, or relatively weak interactions between drug and polymer.[13,14]

To solve these problems, many approaches have been investigated, among which two major modifications to the polymer properties have been proposed. First, hydrophilic segments were introduced, such as poly(ethylene glycol) (PEG),[15–17] dextran,[18–20] chitosan,[21] cellulose derivatives,[22,23] poly(*N*-vinyl-2-pyrrolidone) (PVP),[24] and poly(vinyl alcohol) (PVA).[25,26] Increased hydrophilicity of PLGA results in faster water uptake and swelling of the polymer matrix,[27,28] causing faster and more prolonged drug release during the initial pore diffusion phase.[29–32] Second, polyelectrolyte functional groups, such as amine and sulfonic acid groups, were introduced into the brushlike graft PLGA backbone. These modifications affect the colloidal stability of carrier systems by imparting positive or negative surface charges and increasing protein or drug loading of carriers by electrostatic interactions,[8,33–35] and these functional groups also accelerate the degradation rate by enhancing the hydrophilic character of the polyester.[19,36,37]

However, current limitations have included difficulties in controlling the amounts of the functional groups introduced to the amphiphilic polyester systems. The synthesis of well-defined aliphatic amphiphilic polyesters with precisely controlled functionalities has been pursued extensively. Compared with amphiphilic block copolymers, the amphiphilic graft copolymers have multiple grafting points, and their properties can be easily varied by simply adjusting the graft densities and the chain lengths of the branches.[38] Poly(vinyl alcohol) (PVA) has shown good protein compatibility, mucoadhesive properties, and temperature stability during bulk polymerization with lactide and glycolide. PVAs with molecular weights less than 15 000 g/mol can be considered as biocompatible since they will be eliminated from the body by renal excretion.[37,38] The hydrophilic and lipophilic properties of the matrix can be manipulated by adjusting the grafting degree of the PVA backbone with PLGA chains.[25,26] The introduction of charged functional groups, such as amine or sulfobutyl structures, into the PVA backbone was shown to lead to carrier systems possessing positive and negative surface charges, respecitively.[41,42] The ability to modify not only the backbone but also the length of the PLGA side chains results in an extremely adaptable polymer system. Previously, it was demonstrated that uncharged and charged PVA−PLGAs having side chain lengths higher than 10 have potential for the formation

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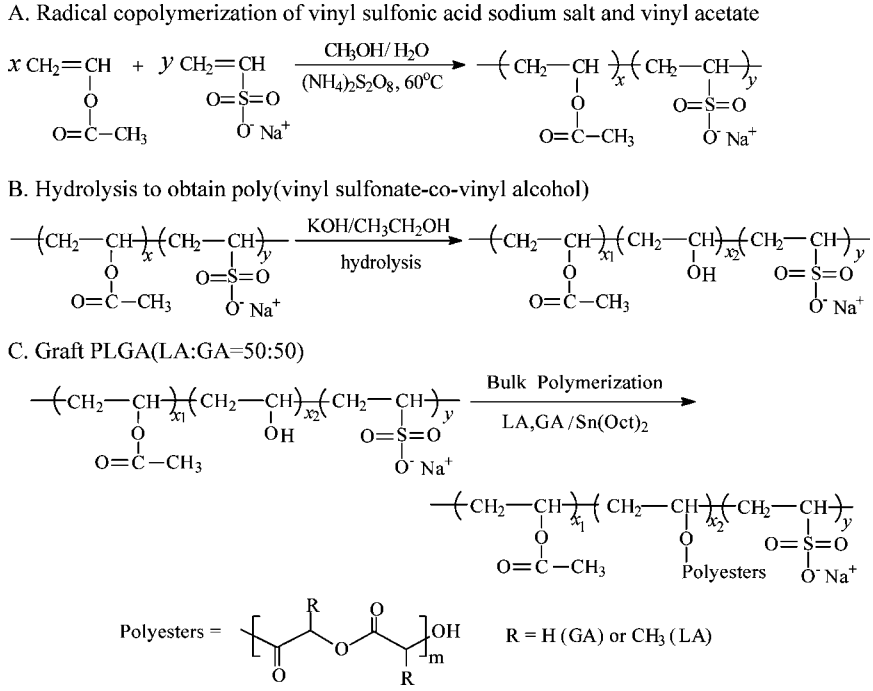
Scheme 1. Synthesis of Poly(vinyl sulfonate-covinyl alcohol)-g-PLGA



of microparticles and nanoparticles, whereas water-soluble polyesters with PLGA side chain lengths less than 3 are capable of forming defined nanocomplexes with oppositely charged proteins.[3,17,25,42] These polymers allow the preparation of nanoparticles and self-assembling colloidal systems with defined surface properties even without the use of surfactants.[39,40] However, the introduction of charged groups is limited by the activation efficiency of PVA, and hence the degree of substitution is difficult to control. The negatively charged poly(2-sulfobutyl vinyl alcohol)-g-PLGA system was especially affected by this particular limitation, because the degradation of these polyesters is still relatively slow and cannot be modified to achieve degradation times between 1 and 20 days due to the lack of sulfonic functional groups.

To this end, a new method for the introduction of sulfonate groups to the PVA−PLGA system was developed. The polyelectrolyte backbones were obtained by the radical copolymerization of vinyl acetate and vinyl sulfonic acid sodium salt and the subsequent hydrolysis, as shown in Scheme 1. The resulting poly(vinyl sulfonate-covinyl alcohol) (P(VS-VA)) backbones were then grafted with PLGA by ring-opening melt polymerization using $SnOct_2$ as catalyst through the hydroxyl groups. On the basis of this strategy, we designed sulfonate modified amphiphilic polyesters with different sulfonic acid substitutions and different PLGA side chain lengths by controlling the feed ratios of radical copolymerization and the feed ratios of the modified backbone to the monomer, thus allowing the design of new carrier systems suitable for negatively charged polyelectrolyte platforms for drug delivery. To establish structure–function relationships, a panel of 12 different polymers was synthesized by systematically modifying sulfonic acid substitution and PLGA side chain length. These novel polyesters were also characterized in terms of their degradation behavior and the formation of nanoparticles.

Experimental Section

Materials. Vinyl acetate (VAc) (Fluka, Steinheim, Germany) was purified by stirring over CaH_2 and vacuum distillation before polymerization to remove inhibitors. Vinylsulfonic acid sodium salt solution (VSA) (Fluka, Steinheim, Germany) was used as an aqueous solution, 30% w/w. Ammonium peroxy-disulfate ($(NH_4)_2S_2O_8$, APS) (Fluka, Steinheim, Germany) was used as received. D,L-Lactide (S-grade) and glycolide (S- and A-grades) (Boehringer Ingelheim, Germany) were recrystallized twice from dry ethyl acetate (refluxed over calcium hydride) and dried for 48 h in vacuo directly before use. The melting points were 125–126 °C and 82–83 °C, respectively. Tin(II) 2-ethylhexanoate (Aldrich, $Sn(Oct)_2$) was used as received. All other chemicals and solvents were of analytical grade.

Synthesis of Poly(vinyl sulfonate-covinyl acetate). The copolymerization between vinyl acetate and vinylsulfonic acid sodium salt was carried out in an aqueous/methanol medium using APS (5% mol) as an initiator. The vinyl acetate was added as methanol solution (0.2 g/ml), and the vinylsulfonic acid was added as 30% aqueous solution. For example, to synthesize P(VS-VAc)-4, a 500 mL round-bottom flask with a gas inlet and a magnetic stirring bar was filled with 34.27 g of VS as 30% aqueous solution, 10 g of VA in 50 mL of methanol, and 2.21 g of APS. The reaction mixture was kept stirring at 60 °C under N_2 for 24 h. Then the crude copolymers were kept under a vacuum to distill off the unreacted vinylacetate (bp 72 °C), which would be detrimental to the subsequent hydrolysis reaction. The unreacted vinyl sulfonic acid sodium salt was eliminated by ultrafitration, which was performed four times on each sample (initial concentration: 10 g of polymer in 250 mL of water), by using an Amicon stirred ultrafiltration cell model 8010 (Amicon Corp., Beverly, Massachusetts, USA) equipped with a YM1 filter membrane (Amicon, cut off = 1000 g/mol). Finally, the crude copolymer solutions were concentrated by rotary evaporation and dried by lyophilization (Edward Freeze-Dryer Modulyo, standard conditions). The polymers were obtained as slightly yellowish hygroscopic powders. Yields: > 85%.

Hydrolysis to Obtain Sulfonate-Modified Poly(vinyl alcohols) (P(VS-VA)). The hydrolysis reactions were carried out under basic conditions, and the amount of potassium hydroxide (KOH) used was 0.5 mol ratio to VAc units in the feed. For example, to obtain P(VS-VA)-2, 10 g of P(VS-VAc) (VAc units: 0.093 mol) was dissolved in 100 mL of water with stirring in a 500 mL round-bottom flask, and 93 mL of KOH methanol solution (0.5 mol/L) was added dropwise, whereupon P(VS-VA) precipitated within 30 min. The mixture was kept refluxing overnight at 60 °C. The crude copolymers were then purified by precipitation into methanol and washed with methanol several times until alkali was

Table 1. Experimental Conditions and Resulting Properties of the P(VS-VA) Backbone

backbone	feed[a]	S^b (%)	S^c (%)	copolymer composition[d] (VS%)	M_n[e] (kg/mol)	M_w[e] (kg/mol)	M_w/M_n[e]	intrinsic viscosity[f] (dl/g)	T_d[g] (°C)
P(VS-VA)-2	25.7/20.0/1.67	9.9	10.4	29	1.341	2.717	2.026	0.162	500
P(VS-VA)-4	34.3/10.0/2.21	16.2	16.3	48	1.157	2.244	1.939	0.065	494
P(VS-VA)-6	77.1/10.0/3.31	17.9	20.1	70	1.102	2.013	1.827	0.053	456
P(VS-VA)-8	102.8/5.0/6.63	21.3	22.7	84	1.045	1.908	1.826	0.040	421

[a] Feed composition (grams): *m*(30% VSA aqueous solution)/*m*(VAc)/*m*(APS). [b] From sulfur elemental analysis. [c] Theoretical sulfur content calculated from the feed on the assumption that the degree of hydrolysis is 100%. [d] Copolymer vinyl sulfonate unit composition (%) calculated from ^{1}H NMR data. [e] M_n, M_w, and polydispersity measured by GPC-MALLS. [f] Determined with an Ubbelohde viscosimeter from aqueous 0.5 N $NaNO_3$ solutions at 25 °C with five different concentrations. [g] Thermal decomposition temperatures (T_d), determined by TGA.

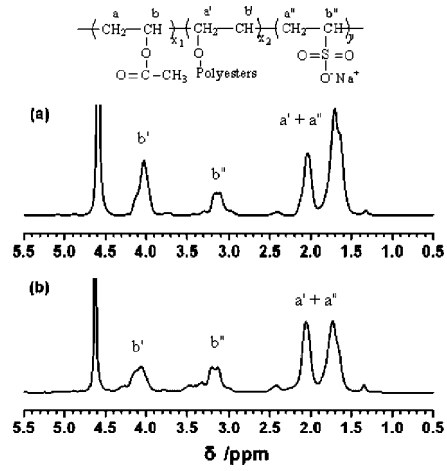


Figure 1. ^{1}H NMR spectra of the P(VS-VA) backbone in D_2O: (a) P(VS-VA)-2; (b) P(VS-VA)-4.

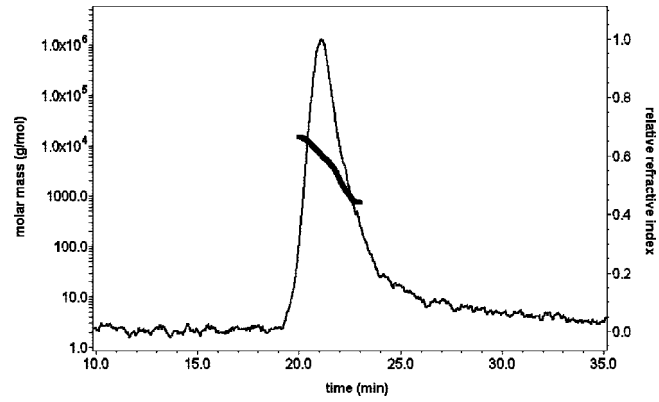


Figure 2. GPC−MALLS chromatogram of P(VS-VA)-2 measured by MALLS and a refractive index detector. The elution time correlates with the calculated molecular mass sequence.

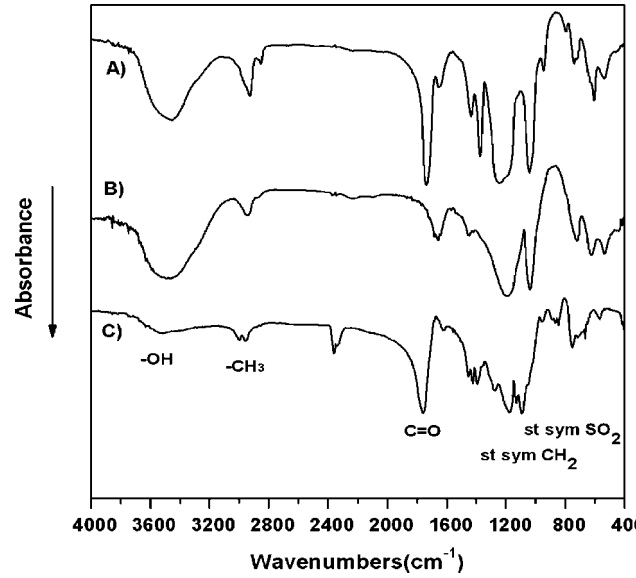


Figure 3. Representative FT-IR spectra: (a) before hydrolysis (P(VS-VAc)-8), (b) after hydrolysis (P(VS-VA)-8), and (c) after grafting (P(VS-VA)-g-PLGA(8–10)).

no longer detectable. Finally, the yellow powders were dried under a vacuum at 50 °C to constant weight. The polymers were obtained as slightly yellowish hygroscopic powders and kept in a desiccator over P_2O_5 under argon. Yields: >95%.

Grafting of Sulfonate-Modified Poly(vinyl alcohol) with Lactide and Glycolide. Under argon, the P(VS-VA) backbone was charged into a rigorously dried nitrogen 250 mL round-bottom flask, then the flask was degassed at 80 °C in a vacuum line for at least 2 h to eliminate traces of water in the sample. After cooling to room temperature, the required amount of freshly recrystallized D,L-lactide and glycolide (lactide/glycolide 1:1) and the $SnOct_2$ solution in toluene was added under nitrogen in the following weight ratios to the backbone: 5:1, 10:1, and 15:1. The amount of $SnOct_2$ added was 1/2000 mol ratio to the lactones. After thoroughly mixing, the flask was degassed at 50–55 °C in a vacuum line for at least 2 h to eliminate the toluene, purging three times with dry argon. Finally, the flask was immersed into a preheated oil bath at 150 °C, and the reaction was allowed to proceed for 12 h at 150 °C. The crude products were dissolved in acetone (50 mL) and precipitated in 500 mL of cold water two times. The obtained polyesters were collected by filtration, washed with water, and dried at 35 °C in vacuo over P_2O_5 for at least 48 h until constant weight was obtained. Yield ∼ 80%.

Nomenclature. The polyesters consist of four different backbone components and three different PLGA chain lengths. The following nomenclature is used to characterize the polymers: P(VS-VA)-g-PLGA(X-Y). The first number in parentheses (*X*) designates the feed ratio of VS in the copolymerization of vinyl sulfonic acid and vinyl acetate. The numbers 2, 4, 6, and 8 correspond to the molar feed ratios of 20%, 40%, 60%, and 80% vinyl sulfonate, respectively, thereby corresponding to the molar feed ratios of 80%, 60%, 40%, and 20% vinyl acetate, respectively. The second number in parentheses (*Y*) describes the weight ratio of grafted polyester chains to the backbone. The numbers 5, 10, and 15 represent the weight ratios of P(VS-VA):PLGA in the feed of 1:5, 1:10, and 1:15, respectively.

Polymer Characterization. Polymer structures were characterized with Fourier transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectroscopy. FT-IR spectral studies were carried out with a Nicolet 510P FT-IR spectrometer in the range between 4000 and 400 cm^{-1}, with a resolution of 2 cm^{-1}. All powder samples were compressed into KBr pellets for the FT-IR measurements.

NMR Experiments. P(VS-VA) backbones were dissolved in D_2O, and P(VS-VA)-g-PLGA polyesters were dissolved in $CDCl_3$. ^{1}H (400.13 MHz) and ^{13}C (100.21 MHz) spectra were recorded on a Bruker DRX-400 spectrometer. COSY experiments were performed on a Bruker DRX-500 spectrometer. Amounts of 40 to 50 mg of sample were used for each measurement. The HMBC experiment was optimized for C−H coupling of 8 Hz, without decoupling during acquisition, and HMBC data were acquired with 512 points in t_2. The number of increments for t_1 was 256. 64 scans and 4

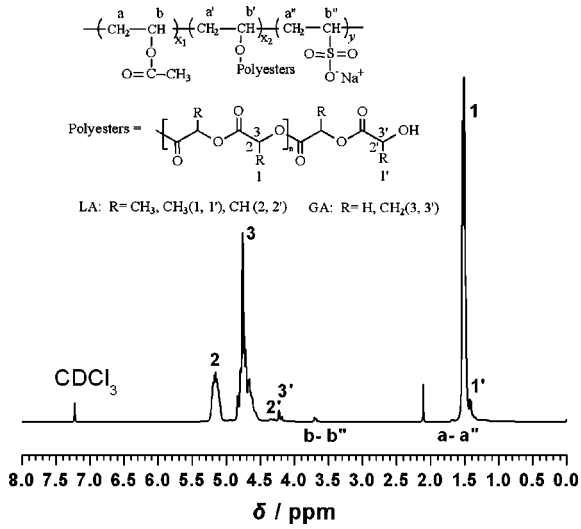


Figure 4. ^{1}H NMR spectra of P(VS-VA)-*g*-PLGA(8–10) in $CDCl_3$.

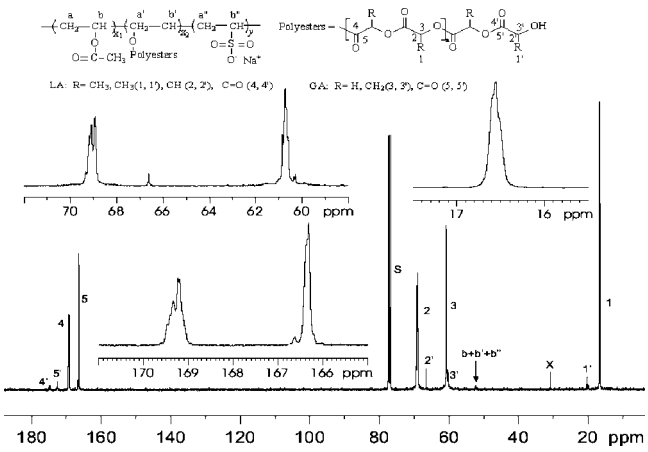


Figure 5. ^{13}C NMR spectrum of P(VS-VA)-g-PLGA(8–10). The main signals contain not just single peaks but partially resolved multiplets, which reflect the microstructures of the random copolymer PLGA. The assignment was done according to c-edited HSQC and HMBC spectra. X stands for impurity and S for solvent.

dummy scans were used for HMBC. A relaxation delay of 1 s was used for all 1D experiments and 2 s for all 2D experiments. The typical experiment time was about 12 h for HMBC. All the measurements were performed at room temperature.

The composition of the P(VS-VA) backbone was calculated by comparing integrals of characteristic peaks of the C**H**−OH group at 3.8–4.4 ppm and C**H**−SO_3Na group at 2.8–3.6 ppm in the ^{1}H NMR spectrum of the backbone.

The side chain length (SCL) was calculated using the integrals of the lactide and glycolide end groups (I_{end}) and their central groups (I_{cent}) and adding one for the end group. SCL = $[(I_{cent\_LA\_CH} + (I_{cent\_GA}/2))/(I_{end\_LA\_CH} + (I_{end\_GA}/2))] + 1$.

Differential Scanning Calorimetry (DSC). DSC was performed on a Perkin-Elmer DSC7 calibrated against Indium and Gallium. Using ca. 5 mg of polymer, the sample was scanned between −10 and 200 °C in a nitrogen atmosphere at a heating rate of 10 °C/min. Glass transition temperature values (T_g) were taken from the midpoints of the transition zones from the second run.

Thermogravimetric Analysis (TGA). TGA was carried out on a thermogravimetric analyzer TGA 7 with a thermal analysis controller TAC 7/DX from Perkin-Elmer using a sample weighing approximately 5 mg. Thermograms were recorded within the temperature range of 50–750 °C at a scanning rate of 20 °C/min. All experiments were performed under nitrogen in platinum crucibles.

Intrinsic Viscosities. They were determined using an Ubbelohde viscosimeter (Schott Geräte, Germany) as aqueous 0.5 N $NaNO_3$ solutions for the backbone or as acetone solutions for the grafted polyesters at 25 °C with at least four different concentrations.

Sulfur Analysis. Sulfur analysis of the P(VS-VA) backbone was performed by the Schoeniger method (barium perchlorate titration using thorin as the indicator).[43]

Gel Permation Chromatography (GPC). GPC in combination with a multiangle-laser light-scattering detector (GPC−MALLS) was employed for the determination of absolute molecular weights and weight distributions using a Duratec DDG-75 degasser, a Merck-Hitachi L-6000 pump, a Merck T-6300 Column Thermostat, and a Wyatt DAWN EOS multiangle-laser light-scattering detector (normalized with PSS (Polymer Standard Service, Mainz, Germany) PMMA 200k standard). For the measurement of backbones, the separation was performed using an Optilab DSP together with a PSS SDV linear M (8 × 300, 5 μ) column connected to a precolumn of the same type (8 × 50, 5 μ). Phosphate buffered saline (PBS, pH 7.4, 0.15 M) solution was used as a mobile phase with a flow rate of 1.0 mL/min. The measurements were carried out at 40 °C with an injection volume of 20 μL (3 mg/mL), and the sample solution was filtered through a 0.2 μm filter before the injection. The dn/dc = 0.063 mL/mg was determined over the whole signal area, which was measured from P(VS-VA)-2. The molecular weights of the samples were determined using the Wyatt software Astra V4.73. The MALLS (DAWN DSP, Wyatt Technology Co.) detector was operated at a laser wavelength of 690.0 nm. For the measurement of the polyesters, the separation was performed using an Optilab DSP together with a PSS GRAM linear M (8 × 300, 5 μ) column connected to a precolumn of the same type (8 × 50, 5 μ). Dimethylacetamide (DMAc) and 5 g/L of lithium bromide (LiBr) were used as the mobile phase with a flow rate of 0.5 mL/min at 60 °C. The dn/dc = 0.024 mL/mg was determined over the whole signal area, which was measured from P(VS-VA)-g-PLGA(6–10). The MALLS (DAWN DSP, Wyatt Technology Co.) detector was operated at a laser wavelength of 632.8 nm.

Degradation Studies of Polymer Film Samples. Polymer films were cast from a 5% (w/v) solution in dichloromethane using Teflon molds. After 72 h of drying at 4 °C, the samples were recovered, and discs with a diameter of 17 mm were punched from the polymer films in a semidry state using a cork bore. Residual solvents were then removed in vacuo at room temperature until constant weights were obtained.

To determine the in vitro degradation profiles, weighed film samples (ca. 30 mg, n = 3) were placed in 10 mL of phosphate buffered saline (PBS, pH 7.4, 0.15 M) and kept at 37 °C in an incubator. Glass vials were shaken once a day. The pH values of the PBS solutions were tested every two days by means of pH-stat. If the pH values deviated more than 0.2 from the original buffer pH, the PBS solutions were changed. After 2, 7, 14, 21, and 28 days, samples were recovered and blotted dry with Kimwipes, and the wet weight was measured gravimetrically. Wet samples were then frozen at −80 °C and freeze-dried in vacuo for ca. 72 h at room temperature until constant masses were obtained. Polymer mass loss was calculated from the following formula: mass loss (%) = 100% – (mass (dry)/original mass).

Nanoparticle Preparation and Characterization. Nanoparticles were prepared by a solvent displacement technique, described in detail elsewhere.[44] Briefly, 10.0 mg of polymer was dissolved in 1 mL of acetone at 25 °C. The resulting solution was subsequently injected into a magnetically stirred (500 rpm) aqueous phase of 5 mL of filtrated and double distilled water (pH 7.0, conductance 0.055 μS/cm, 25 °C) using a special apparatus. The apparatus consists of an electronically adjustable single-suction pump which was used to inject the organic solution into the aqueous phase through an injection needle (Sterican 0.55 × 25 mm) at a constant flow rate (10.0 mL/min). The pump rate was regulated and constantly monitored by electric power control. After injection of the organic phase, the resulting colloidal suspension was stirred for 8 h under reduced pressure to remove the organic solvents. Particles were characterized and used directly after preparation.

Particle Size and Zeta Potential. The average particle size and zeta potential of the NPs were measured using a Zetasizer Nano

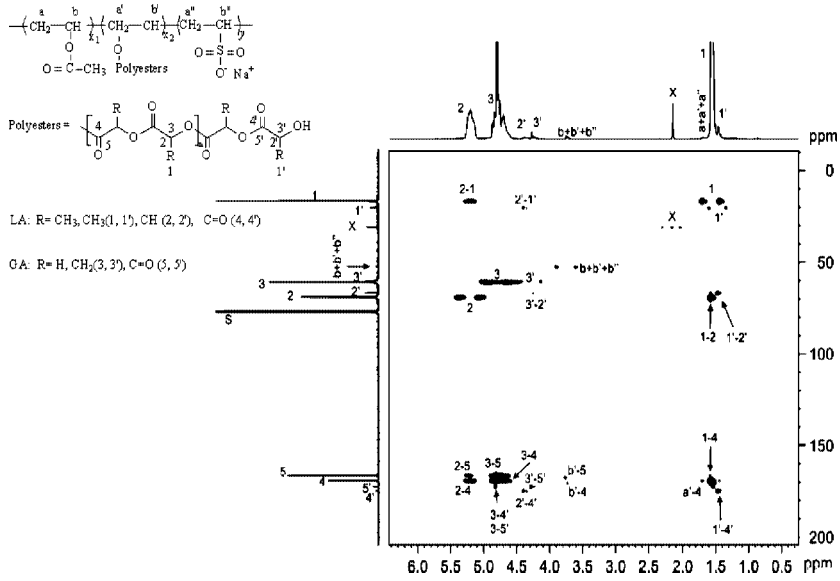


Figure 6. $^{1}H-^{13}C$ HMBC spectrum of P(VS-VA)-g-PLGA(8–10). The single cross peaks show multiple-bond H,C connections. These peaks are labeled in gray. Pairs of signals are for the one-bond C−H connections (assignment labels in black). X stands for impurity and S for solvent.

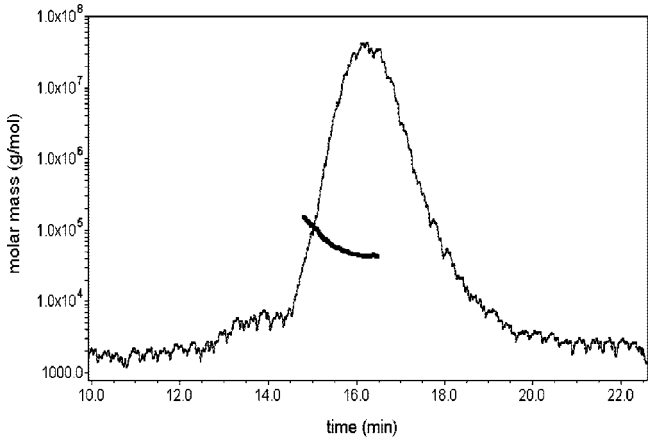


Figure 7. GPC−MALLS chromatogram of P(VS-VA)-g-PLGA(2–15) measured by MALLS and a refractive index detector. The elution time correlates with the calculated molecular mass sequence.

ZS/ZEN3600 (Malvern Instruments, Malvern, UK). Particle size and polydispersity were determined using noninvasive back scatter (NIBS) technology, which allows sample measurement in the range of 0.6 nm−6 μm. Freshly prepared particle suspension (800 μL) was placed in a green disposable zeta cell (folded capillary cell DTS 1060) without dilution. The measurement was carried out using a 4 mW He−Ne laser (633 nm) as light source at a fixed angle of 173°. The following parameters were used for experiments: medium refractive index 1.330, medium viscosity 0.88 mPa·s, a dielectric constant of 78.54, temperature 25 °C. Each size measurement was performed with at least 10 runs. All measurements were carried out in triplicate directly after NP preparation, and the results were expressed as the Z-average mean size ± SD.

After the size measurement, the zeta potential was measured with the M3-PALS Technique, a combination of laser Doppler velocimetry and phase analysis light scattering (PALS). A Smoluchowsky constant F (K_a) of 1.5 was used to calculate zeta potential values from the electrophoretic mobility. Each zeta potential measurement was performed automatically at 25 °C. All measurements were carried out in triplicate directly after NP preparation, and the results were expressed as mean size ± SD.

Scanning Electron Microscopy (SEM). Prior to SEM observation, nanoparticle suspensions were diluted 1/5 with ultrapure water, and a drop of diluted suspension was then directly deposited on a freshly polished aluminum sample holder. Samples were dried in a vacuum and subsequently sputter-coated with a carbon layer at 4–6 A for 30 s then with a gold layer at 2 A for 30 s at 5×10^{-5} Pa (Edwards Auto 306 Vacuum Coater, Edwards, Germany). Subsequently, the morphology of nanoparticles was observed at 3 kV using a scanning electron microscope (SEM, S-4200, Hitachi, Japan).

Results and Discussion

Synthesis of the P(VS-VA) Backbone. Sulfonate-modified P(VS-VA) backbones with different degrees of sulfonate substitution were readily accessible in good yields by radical copolymerization between vinyl actate and vinylsulfonic acid sodium salt and subsequent hydrolysis.

As biodegradable drug delivery carrier systems, the backbone of the polyester should be biocompatible and eliminated from the body by renal excretion as reported for PVAs with a molecular weight smaller than 15 000 Da.[39,40] To control the molecular weight of P(VS-VA) backbones, the amount of the APS was chosen as 5 mol % to the VSA and VAc monomers.

It was reported that the amount of KOH can be used to control the degree of hydrolysis.[44] The addition of a small amount of KOH, 0.004–0.2 mol ratio to VAc units, was enough for hydrolysis of nonmodified PVA. Taking into account the effect of sulfonic acid groups in the hydrolysis reaction, the amount of KOH used for this system was chosen as 0.5 mol ratio to VAc units in the feed to achieve a high degree of hydrolysis in this system.

The obtained P(VS-VA) backbones were characterized by NMR, FT-IR, GPC-MALLS, elemental analysis, and intrinsic viscosities. The reaction conditions and the properties of the obtained P(VS-VA) backbones are shown in Table 1. In this table, the sulfur element analysis results are in reasonable agreement with the theoretical sulfur content calculated from the feeds on the assumption that the degree of hydrolysis is 100%, which indicates that the composition of the P(VS-VA) backbone can be controlled by the feed ratio of radical polymerization.

The structure and the compositions of the backbone were studied by NMR. Figure 1 shows the ^{1}H NMR spectra of two representative P(VS-VA) backbones in D_2O; according to the literature,[45,46] protons and the corresponding signals are assigned in the spectra.

It is worth noting that no signals of the PVA backbone connected with acetyoxyl groups were visible after hydrolysis

Table 2. Physicochemical Properties of the P(VS-VA)-g-PLGA Systems

polymers[a]	feed[b]	PLGA units per chain [c]	M_n[d] (kg/mol)	M_w[d] (kg/mol)	M_w/M_n[d]	intrinsic viscosity[e] (dl/g)	T_g[f] (°C)
P(VS-VA)-g-PLGA(2–5)	1.00/2.77/2.23/0.0039	10.4	4.597	7.424	1.61	0.152	25.9
P(VS-VA)-g-PLGA(2–10)	1.00/5.54/4.46/0.0078	17.4	8.891	14.59	1.64	0.188	35.1
P(VS-VA)-g-PLGA(2–15)	1.00/8.31/6.69/0.0117	32.3	20.37	33.00	1.62	0.242	37.3
P(VS-VA)-g-PLGA(4–5)	1.00/2.77/2.23/0.0039	9.9	8.377	13.80	1.65	0.127	25.5
P(VS-VA)-g-PLGA(4–10)	1.00/5.54/4.46/0.0078	21.1	15.09	32.22	1.68	0.154	35.0
P(VS-VA)-g-PLGA(4–15)	1.00/8.31/6.69/0.0117	40.4	27.87	46.54	1.67	0.239	35.5
P(VS-VA)-g-PLGA(6–5)	1.00/2.77/2.23/0.0039	14.1	6.566	11.75	1.79	0.109	24.9
P(VS-VA)-g-PLGA(6–10)	1.00/5.54/4.46/0.0078	30.1	14.38	23.43	1.64	0.175	32.7
P(VS-VA)-g-PLGA(6–15)	1.00/8.31/6.69/0.0117	51.2	30.27	50.55	1.67	0.225	38.2
P(VS-VA)-g-PLGA(8–5)	1.00/2.77/2.23/0.0039	21.1	—	—	—	—	—
P(VS-VA)-g-PLGA(8–10)	1.00/5.54/4.46/0.0078	41.5	9.240	14.71	1.59	0.120	34.4
P(VS-VA)-g-PLGA(8–15)	1.00/8.31/6.69/0.0117	54.4	20.77	35.31	1.70	0.192	38.3

[a] The following nomenclature is used to characterize the polymers: P(VS-VA)-g-PLGA(*X*-*Y*). The first number in parentheses (*X*) designates the ratio of VSA in the copolymerization of vinyl sulfonic acid and vinyl acetate. The second number in parentheses (*Y*) describes the weight ratio of grafted polyester chains to backbone, i.e., the weight ratio of P(VS-VA):PLGA in feed = 1:5, 1:10, or 1:15). [b] Feed composition (grams): *m*(P(VS-VA))/*m*(lactide)/*m*(glycolide)/*m*($SnOct_2$). [c] Average side chain length calculated from ^1H NMR data. [d] M_n, M_w, and polydispersity measured by GPC-MALLS. [e] Determined with an Ubbelohde viscosimeter from acetone solutions at 25 °C with at least four different concentrations. [f] Glass transition temperature T_g calculated from the second run involving heating and cooling from −10 to +80 °C at 10°/min.

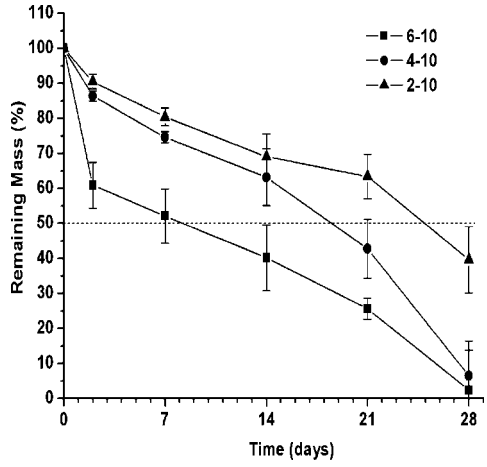


Figure 8. In vitro degradation profile of P(VS-VA)-g-PLGA. Mass loss of P(VS-VA)-g-PLGA(2–10), P(VS-VA)-g-PLGA(4–10), and P(VS-VA)-g-PLGA(6–10) incubated in PBS (pH 7.4, 37 °C). Increasing sulfonate substitution led to decreasing degradation times.

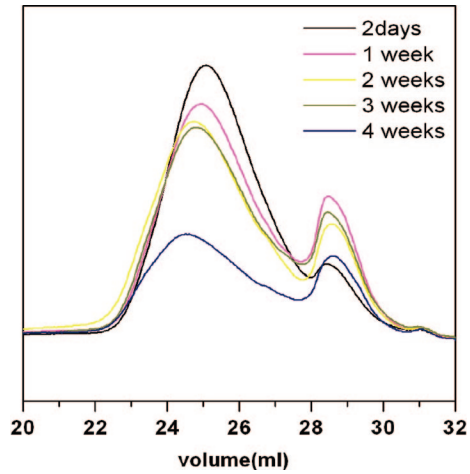


Figure 9. GPC profiles of P(VS-VA)-g-PLGA(2–10) during the following degradation study time points: 2 days, 1 week, 2 weeks, 3 weeks, and 4 weeks.

(the esterified $-\mathbf{CH}-OCOCH_3$ in the range of 4.9 ppm), indicating that the degree of hydrolysis was nearly 100%. For the methylene ($-\mathbf{CH_2}-$) protons, it was not possible to integrate the signals due to overlapping. Hence, it was not possible to determine the copolymer composition through the integrals of

Table 3. Particle Size, Distribution, and ζ-Potential of Nanoparticles

polymer type	size (nm)	polydispersity Index	ζ-potential (mV)
P(VS-VA)-g-PLGA (2–10)	120 ± 1	0.072 ± 0.003	−19.2 ± 0.2
P(VS-VA)-g-PLGA (4–10)	139 ± 0	0.087 ± 0.013	−25.9 ± 0.8
P(VS-VA)-g-PLGA (6–5)	151 ± 2	0.091 ± 0.017	−25.6 ± 0.4
P(VS-VA)-g-PLGA (6–10)	138 ± 1	0.085 ± 0.015	−30.6 ± 0.5
P(VS-VA)-g-PLGA (6–15)	134 ± 0	0.080 ± 0.016	−27.7 ± 0.5

the methylene protons. For methane ($-C\mathbf{H}-$) protons, the signal of the VSA group was sufficiently separated from the VA group, thus making it possible to calculate the copolymer composition of VS by comparing the integrals of characteristic peaks of the $C\mathbf{H}-OH$ and the $C\mathbf{H}-OCOCH_3$ groups near δ = 3.8–4.4 ppm and the $C\mathbf{H}-SO_3Na$ groups at δ = 2.8–3.6 ppm. The results are shown in Table 1, which shows that the actual compositions of the copolymers are in good agreement with the feed ratios of VSA and VAc. The VS compositions are a little higher than the feed ratios in each case, which is in agreement with the results from the sulfur elemental analysis.

According to the literature,[44–47] the reactivity of the VAc unit is very high; the chain transfer reaction can easily occur during the polymerization of VAc, which would result in branched structures that would be cleaved after hydrolysis. The reason for the relatively low composition of VAc units compared to the feed ratio is likely due to some degree of chain transfer

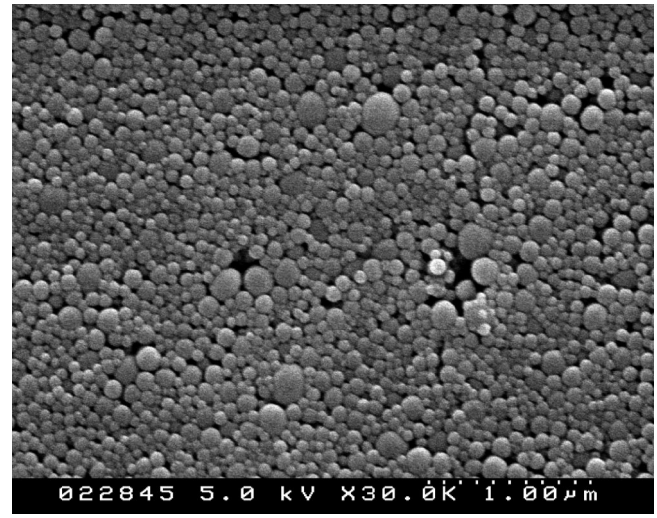


Figure 10. SEM image of P(VS-VA)-g-PLGA nanoparticles.

reaction of VAc units during the copolymerization, and the branched structure of small amounts of VAc units is cleaved after hydrolysis.

To accurately determine the molecular weights and distributions of the backbones, we combined GPC with MALLS. As shown in Figure 2, the GPC−MALLS chromatogram of P(VS-VA) backbones shows unimodal molecular weight distributions. The corresponding molecular weight data are shown in Table 1, which indicates that the water-soluble backbones can be easily eliminated from the body by renal excretion. Due to the increased solubility of the backbones with increasing VS composition, the intrinsic viscosity of the backbones decreased.

To graft PLGA to the backbone, the temperature stability of the backbone is important, since low temperature stability can lead to the destruction of the backbone during the polymer synthesis. TGA was used to determine the temperature stability of the backbones, and good temperature stability was demonstrated as the temperatures for the onset of mass loss are higher than 400 °C, as shown in Table 1.

Synthesis and Characterization of P(VS-VA)-g-PLGA. To establish structure–function relationships, a panel of 12 different polyesters was synthesized by using four different sulfonic substituted backbones and three different PLGA monomer feed ratios (1:5, 1:10, and 1:15). All graft polyesters were synthesized under rigorously anhydrous conditions. P(VS-VA) was carefully dried to avoid initiation by water, which could lead to a mixture of linear and graft products. The successful grafting of PLGA sequences onto the P(VS-VA) backbone was verified by FT-IR and NMR.

Figure 3 shows FT-IR spectra for the three synthesis steps: P(VS-VAc), P(VS-VA), and the grafted P(VS-VA)-g-PLGA, respectively. From the spectra, the broad peaks at wavenumbers ranging between 3000 and 3700 cm^{-1} represent the hydroxyl groups. After hydrolysis, the intensity of the hydroxyl groups became stronger, and then when it was grafted with PLGA, the intensity of this signal became comparatively weaker. The FT-IR peaks at wavenumbers around 1200 cm^{-1} and 1040 cm^{-1} emerged in all three spectra, which represent the CH_2 (st sym) and S═O (st sym) bonds in the backbone, respectively.[43] In addition, the intensity of the carbonyl peak at about 1710 cm^{-1} became weaker after hydrolysis according to the loss of the acetate units and became stronger again following grafting with PLGA. A very good correlation was observed between the relative signal intensities and the copolymer compositions. All these results suggest that the three reaction steps were carried out successfully.

To determine the microstructure of the sulfonate substituted P(VS-VA)-g-PLGA polyesters, HMBC (heteronuclear multiple-bond correlation) spectroscopy was used for complete assignment of the ^1H and ^{13}C spectra, as shown in Figures 4 to 6. Protons and the corresponding signals are assigned in the spectra.

In the ^1H NMR spectra (Figure 4), the signals for PLGA are $\delta = 1.45$ ppm (CH_3), $\delta = 4.76$–4.81 ppm (CH_2), and $\delta = 5.16$ ppm (CH). Several new signals appear in the spectra: $\delta = 1.65$ ppm (P(VS-VA) backbone −C**H**$_2$−) and $\delta = 3.34$ ppm (P(VS-VA) backbone −C**H**−), which can be confirmed by the observation of long-range ^1H, ^{13}C correlation peaks in the HMBC spectrum. The assignment of the hydroxyl terminated PLGA unit signals is at 4.4 ppm (lactide: terminal −C**H**(CH_3)OH), 4.2 ppm (glycolide: terminal –C**H**$_2$OH), and 1.28 ppm (lactide: terminal −CH(C**H**$_3$)OH). These assignments are in excellent agreement with literature data.[3,25,35] It is worth noting that signals of the methine protons of carboxylated lactyl end units (4.9 −5.0 ppm) and free lactic acid (4.0 ppm) cannot be detected in the spectra, indicating that under these reaction conditions no or less than 5% homopolymerization occurred.[25]

In the ^{13}C NMR spectrum (Figure 5), the signals of the terminal PLGA unit are at 20.38 ppm (**C**H_3), 60.34 ppm (**C**H_2), 66.74 ppm (**C**H), and 175.05 ppm (**C**O), respectively. The signals for the lactide chains appear at 169.6 ppm (**C**O), 69.04 ppm (**C**H), 60.84 ppm (**C**H_2), and 16.72 (**C**H_3).[25] The signal at 55 ppm comes from the –**C**HSO_3Na group of the backbone, which is in excellent agreement with literature data.[44] The main signals contain not just single peaks but partially resolved multiplets, which reflect the microstructures of the random copolymer PLGA. These assignments were confirmed by HMBC spectra.

Using $^1H-^{13}C$ HMBC, the covalent bond between the P(VS-VA) backbone and PLGA was confirmed by the observed cross peaks between the carbonyl ^{13}C of the C**H**−SO_3Na group and the methine of the P(VS-VA) backbone, as shown in Figure 6. The single cross peaks show multiple-bond H,C connections, also indicating the random copolymer structure of the PLGA.

SEC Analysis. Conventional GPC analysis is not the method of choice to determine molecular weights since it always underestimates the M_w of the amphiphilic graft polyesters due to their smaller hydrodynamic volume in solution compared with the linear poly(styrene) reference material. Therefore, GPC-MALLS was selected to measure the absolute M_w.

The amphiphilic and negatively charged nature of the polyesters offered significant challenges to develop a suitable GPC method for these polymers.[48,49] The refractive index increment dn/dc of the polymers was measured to be <0.100, and hence small errors in sample preparation and/or measurements had a large influence on the results. Moreover, polyelectrolytes require salt addition to the eluent to minimize unspecific interactions.[37] A growing tendency to form aggregates with increasing amphiphilicity was observed. This could be a result of increasing amphiphilicity leading to an increasing ability of the polymers to form micellar structures. These aggregates led to high scattering signals in areas of small polymer concentration and finally inaccurate molecular weights. This led us to develop a GPC method using dimethylacetamide and 5 g/L of LiBr as eluent.

As shown in Figure 7, the GPC traces of the graft polyesters were monomodal, suggesting that no mixing of the graft and linear polyesters was formed. Compared to the molecular weight of the backbone, it is illuminated that the graft polymerization is successful and the –OH groups in the P(VS-VA) backbone are effective propagation centers.

The characteristics of the series of P(VS-VA)-g-PLGA polymers with different PLGA chain lengths are presented in Table 2. From the data listed in this table, it can be seen that with increasing VS content in the backbone the PLGA side length increased even at the same backbone/monomer feed ratios. This is due to the higher content of VS in the backbone and thus the lower content of –OH groups in the backbone, since the –OH groups in the P(VS-VA) backbones acted as co-initiators in the $SnOct_2$ catalyst system, as previously reported in the literature.[25,50]

Thermal Properties. DSC was used to determine the thermal properties of these polyesters. All DSC traces showed only one T_g; therefore, both components are totally miscible and do not lead to phase separation. The decrease in T_g with increasing backbone/PLGA ratio and increasing sulfonic composition can be explained by increased chain mobility.

Degradation Behavior. Drug release kinetics is affected by degradation rates; hence, determining the in vitro degradation profiles of novel polymers is of high importance. The introduction of sulfonic substitution in the backbone should lead to rapid swelling and an accelerated formation of water-soluble degradation products, thereby resulting in a faster degradation rate.

To test this hypothesis, we investigated the degradation of polymer films under in vitro conditions in phosphate-buffered saline as a function of time. The mass loss of the different polymers, also designated as polymer erosion, was studied in three samples differing in their degree of sulfonate substitution. For this study, branched P(VS-VA)-g-PLGA(2–10), P(VS-VA)-g-PLGA(4–10), and P(VS-VA)-g-PLGA(6–10) were selected. The results, shown in Figure 8, demonstrate that the polymer with the highest degree of sulfonate substitution, P(VS-VA)-g-PLGA(6–10), showed the highest erosion with a degradation half-life around 8 days. P(VS-VA)-g-PLGA(4–10) had a half-life of ca. 18 days, while P(VS-VA)-g-PLGA(2–10) had a half-life of ca. 25 days. In view of the high molecular weights of the branched polymers (Table 2), these can be considered as relatively short degradation times compared to linear PLGA.[51,52]

During degradation, oligomers are formed as shown in the GPC data (Figure 9). The main peak disappeared, and the concentration of small oligomeric compounds increased during these 4 weeks. Factors contributing to the degradation properties of these amphiphilic polyesters are random hydrolytic ester bond cleavage of PLGA side chains and diffusion or matrix swelling. The hydrophilic/hydrophobic balance affected the degradation behavior. Upon increasing the hydrophilicity of the polymeric matrix, the diffusion or matrix swelling rate increased, which led to accelerated degradation times. Higher degradation rates are advantageous for drug delivery applications, since the acidic microenvironment which can be harmful to proteins and which is known to be present in slow degrading PLGA systems will most likely be avoided.[51,52] The degradation mechanism is currently under investigation and will be reported elsewhere.

Preliminary Study of Nanoparticle Preparation. As potential candidates for negatively charged polyelectrolyte platforms for drug delivery, the properties of nanoparticles prepared with P(VS-VA)-g-PLGA were studied. Based on the amphiphilicity of the graft polyesters, these nanoparticles were prepared by the solvent displacement technique.[42] The properties of these nanoparticles were evaluated in terms of size, distribution, zeta-potential, and SEM images. As shown in Table 3, stable nanoparticle suspensions with narrow size distributions and high reproducibility were obtained. Increased sulfonate substitution degree of P(VS-VA)-g-PLGA caused a linear decrease in the zeta-potential. The particle size decreased with increasing PLGA grafting ratio. The size data shown in Table 3 are in good agreement with the SEM image in Figure 10, which also demonstrates that the morphology of the particles is regularly spherical.

Based on these preliminary studies, this new class of negatively charged polymers can be used as negatively charged nanocarriers as a polyelectrolyte platform for drug delivery. Related drug loading and release behavior studies are currently under investigation and will be reported elsewhere.

Conclusions

New stable poly(VS-VA) backbones with different sulfonate substitution were synthesized by radical copolymerization and subsequent hydrolysis. The copolymer compositions were calculated from the results of ^{1}H NMR and sulfur elemental analysis, which can be controlled by the feed ratios.

A series of biodegradable amphiphilic graft polyesters were successfully synthesized by grafting PLGA sequences onto sulfonate modified P(VS-VA) backbones through bulk melt ring opening polymerization using $SnOct_2$ as catalyst. Characterizations confirm the brushlike structure of the graft polymers. In vitro degradation of the sulfonate modified amphiphilic polyesters shows accelerated degradation with increasing sulfonate substitution. By controlling the feed ratio of the backbone to the monomers, graft copolymers with different branch lengths, degradation rates, and amphiphilicities can be obtained. Blank nanoparticles were successfully prepared by the solvent displacement technique, and SEM images demonstrated that these nanoparticles were regularly spherical in shape. The sulfonate-modified charge ratios of the backbones and the side chain lengths of PLGA can be modified to adjust the solubility and the amphiphilic nature of the polymers.

The amphiphilic and negatively charged nature of these polyesters is expected to allow drug entrapment by electrostatic interactions as a function of sulfonate modification. Further investigations on drug release and degradation mechanisms are currently underway.

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