Biodegradable Brushlike Branched Polyesters Containing a Charge-Modified Poly(vinyl alcohol) Backbone as a Platform for Drug Delivery Systems: Synthesis and Characterization

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ABSTRACT: Novel biodegradable, positively charged, brushlike branched polyesters, namely, poly[vinyl-3-(dialkylamino)alkylcarbamate-*co*-vinyl acetate-*co*-vinyl alcohol]-graft-poly(D,L-lactide-*co*-glycolide), abbreviated as (P[$VACB_{0-70}$−VAc_{0-36}−VA_{15-195}−$VPLGA_{75-240}$]), were synthesized using water-soluble amine-modified poly(vinyl alcohol) backbones in a ring opening polymerization as a platform for parenteral delivery systems of hydrophilic macromolecular drug substances such as proteins and DNA. The structure of theses graft-polyesters was studied by NMR. Modifying side chain length and charge ratio, the solubility of the polymers could be designed to range between water-soluble and lipophilic behavior. The polymers' compact, branched architecture and their molecular mass were determined by GPC-MALLS. By differential scanning calorimetry (DSC) and wide-angle X-ray scattering (WAXS) the amorphous character of the polyesters was demonstrated. The amphiphilic nature of the polyesters allowed drug entrapment by electrostatic interactions as a function of amine modification. Amine-modified graft-polyesters were shown to possess unusually short degradation times and merit further investigation as a platform for biodegradable delivery systems.

Introduction

Branched polymers differ significantly in their physicochemical properties from their linear counterparts or polymer networks.[1] They are usually defined as possessing branch points in the polymer chain leading to more than one end group. Their architecture affects melt rheology, mechanical properties, crystallinity, and solution behavior.[1] Branched polyesters may also offer favorable properties for the design of drug delivery systems, such as enhanced biodegradability and controlled release of drug substances; for a recent review, see Dailey et al.[2]

Biodegradable branched polyesters have not been studied to a great extent. Branched poly(ethylene adipate) and poly(butylene succinate) showed increased biodegradation with increasing degree of branching.[3] Further, branched polyesters consisting of a non-polysaccharide backbone and poly(D,L-lactide-*co*-glycolide) (PLGA) side chains have recently been reported.[4−6] Previously, it was demonstrated that uncharged and negatively charged PVA-*g*-PLGA (PVA = poly(vinyl alcohol)) copolymers having chain lengths higher than 10 have potential for the formation of micro- and nanoparticles, whereas water-soluble polyesters with PLGA side chain length < 3 are capable of forming defined nanocomplexes with oppositely charged proteins.[6−9]

Drug delivery of hydrophilic macromolecular drug candidates such as peptides, proteins, RNA, and DNA is generally considered as the Achilles' heel for their therapeutic or clinical application.[10] These molecules are rapidly degraded by enzymes and cleared from the blood circulation requiring frequent injections or infusions.[11−13] Their size and enzymatic lability usually prevent uptake through epithelium of the gastrointestinal tract and thus necessitates parenteral administration.[14] To overcome these biopharmaceutical problems, carrier systems stabilizing hydrophilic macromolecular drug substances are subject of intensive research efforts.[9,15−23] In this context nanoscale carriers such as nanoparticles and nanocomplexes have found increasing attention since they allow intravenous application.[15,24] Controlled and sustained release can be accomplished using microspheres and implants from biodegradable polymers.[10,17,23,25] Co-polyesters of lactic and glycolic acid, PLGA, are suboptimal for protein and DNA delivery due to inactivation by the acidic microenvironment and uncontrolled burst release due to poor compatibility between lipophilic polymers and hydrophilic drug candidates.[14,26−29]

To address these problems associated with linear PLGA, we hypothesized that modification of the polyester structure would allow manipulation of polymer solubility and degradation behavior in a vast range. A brushlike branched structure was considered beneficial for accelerating degradation since fewer cleavage steps are necessary to generate water-soluble degradation products.[5,8] As hydrophilic and water-soluble backbone, we selected PVA with a molecular weight of 15 000 g/mol (M_w/M_n = 1.3), which is considered as biocompatible and can be eliminated from the body by renal excretion.[30−32]

Amino functions were covalently coupled to PVA in a polymer-analogous reaction using carbonyl diimidazole, CDI, introducing cationic charges under physiological conditions. These modifications should affect colloidal stability of carrier systems by imparting positive surface charges on one hand[33] as well as increasing protein or DNA loading of carriers by electrostatic interactions on the other hand.[34,35] Also acceleration of PLGA degradation by base catalysis was thought to allow the design parenteral delivery systems which are eliminated from the body within 1 week. Due to their lower cytotoxicity secondary and tertiary amino functions, the corresponding diamines were coupled to PVA via the hydrolytically stable

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Table 1. Characterization of Branched Polyesters: Molar Mass, T_g, Side Chain Length, and Degree of Amine Substitution

polyester	feeding[a]	M_n[b] (kg mol^{-1})	M_n[c] (kg mol^{-1})	M_w[c] (kg mol^{-1})	M_w/M_n[c]	T_g[d] (°C)	side chain[e,f]	amine[g]
P(12)-10	0.40/2.19/1.76/0.09	200	200	260	1.3	30.8	10.8	4.0
P(68)-10	0.75/2.09/1.69/0.16	140	280	800	2.8	11.5	7.4	22.7
M(7)-10	0.60/3.72/3.00/0.13	230	180	250	1.4	27.8	12.4	2.3
M(7)-20	0.30/3.72/3.00/0.08	380	230	300	1.3	31.1	20.9	2.3
M(13)-1	2.00/1.19/0.96/0.25	55	66	160	2.4	39.1	2.2	4.4
M(13)-2	1.30/1.55/1.25/0.16	67	69	140	2.0	29.5	3.0	4.4
M(13)-10	0.60/3.58/2.89/0.13	230	330	630	1.9	23.6	12.4	4.4
M(13)-20	0.30/3.58/2.89/0.07	390	340	590	1.7	25.7	21.6	4.4
M(32)-1	2.00/1.03/0.83/0.25	nd	63	87	1.4	nd	nd	10.8
M(32)-2	2.00/1.34/1.08/0.17	63	77	230	3.0	nd	(2.7)	10.8
M(32)-10	0.60/3.09/2.48/0.15	190	360	950	2.7	18.5	10.0	10.8
M(32)-20	0.30/3.09/2.49/0.09	330	770	1900	2.5	20.7	19.0	10.8
M(69)-10	0.60/2.15/1.73/0.16[h]	220	390	790	2.0	32.9	13.4	23.0

[a] Feed composition (mass/grams): *m*(modified PVA)/*m*(lactide)/*m*(glycolide)/*m*($SnOct_2$). [b] Estimation of M_n based on backbone substitution and side chain length from ^{1}H NMR data. [c] M_n, M_w, and polydispersity measured by GPC-MALLS. [d] Glass transition temperature T_g of the second run, method: heating and cooling −10 to +200 °C, 10°/min. [e] nd = not determined. [f] Average side chain length calculated from ^{1}H NMR data. [g] Degree of amine substitution of PVA backbone. [h] A 3 mL aliquot of dry NMP was added to the reaction tube, and the reaction temperature was reduced to 100 °C.

urethane bond.[36,37] As a consequence of partial protonation under physiological conditions, these amines will enhance the hydrophilic character of the polyester. Results on charge-modified PVA backbones were communicated earlier, such as structure and tacticity[38] as well as complex formation with DNA.[39] These amine-modified PVAs were found to be non-cytotoxic in biocompatibility tests using L929 at relevant concentrations for drug delivery.[39]

Finally, PLGA was grafted onto the amine-modified PVA backbone in a ring opening polymerization (ROMP) in bulk, thus introducing lipophilic side chains. On the basis of this strategy, we designed amphiphilic polymers with a broad range of solubility characteristics ranging from water soluble to insoluble thus allowing the design of new carrier systems suitable for macromolecular drug candidates. To establish structure−function relationships a panel of 52 different polymers was synthesized by modifying amine substitution and PLGA side chain length systematically, and their entrapment properties and degradation behavior were characterized.

Experimental Section

Materials. (Diethylamino)propylamine (DEAPA; purum, >98%), (dimethylamino)propylamine (DMAPA; purum, >98%), (diethylamino)ethylamine (DEAEA; purum, >98%), poly(vinyl alcohol) (MW, 15 000; degree of polymerization, 300 (P = 300); degree of hydrolysis, 86−89%), carbonyl diimidazole (purum, ∼97%), *N*-methylpyrrolidone (NMP; absolute), dimethylacetamide (DMAc; for HPLC, 99.8%), and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU; puriss., absolute, over molecular sieve) were purchased from Fluka GmbH (Germany) and used as received. D,L-Lactide (S-grade) and glycolide (S- and A-grades) (Boehringer Ingelheim, Germany) were recrystallized twice from ethyl acetate. Tin(II) 2-ethylhexanoate (Aldrich; $Sn(oct)_2$) and lithium bromide (extra pure; Merck) were used as received. Tetrahydrofuran (THF; BASF, Germany) was dried over sodium and distilled under nitrogen before use.

Synthesis of Amine Carbonylimidazoles. (*N*-(Dialkylamino)-alkyl)-1*H*-imidazole-1-carboxamide were synthesized as follows: Into a rigorously dried 100 mL round-bottomed flask equipped with gas inlet, a septum cap, and magnetic stirrer, 90 mL of dry THF was distilled under nitrogen gassing. Then 16.72 g of carbonyl diimidazole (CDI; 0.10 mol) were dissolved and the diamine (1:1 molar ratio) was injected slowly using a syringe keeping the reaction temperature < 55 °C.[40]

After stirring for 16 h at room temperature the resulting imidazole/amine−carbonylimidazole solution was isolated by evaporating THF using a rotavapor. The resulting oily, slight yellow mixture was used without further purification after the amount of amine−CI was quantitated by ^{1}H NMR spectroscopy. Yields: >90%

Synthesis of Amine-Modified Poly(vinyl alcohols). Poly(vinyl (dialkylamino)alkylcarbamate-*co*-vinyl acetate-*co*-vinyl alcohol). For example, to synthesize M(13), a 250 mL round-bottomed flask with gas inlet and magnetic stirring bar was rigorously dried, filled with 12.00 g of PVA (0.81 mmol) and 200 mL of anhydrous NMP and heated to 80 °C to dissolve PVA. After complete dissolution 2.39 g of *N*-(3-(dimethylamino)propyl)-1*H*-imidazole-1-carboxamide (12.2 mmol) was added and finally 0.17 g of DMPU (10 mol % of the amine-CI) were injected. The mixture was heated to 80 °C under stirring for 4.5 days (Table 1).

The resulting amine-modified poly(vinyl alcohol) was purified by ultrafiltration using an YM1 membrane (cutoff, 1000; Millipore). During filtration the solvent was substituted by demineralized water. After filtration of 2.5 L of water, the solvent volume in the cell was adjusted to ca. 50 mL. The solution was frozen at −20 °C and dried by lyophilization (Edward Freeze-Dryer Modulyo, standard conditions). The polymers were milled and stored until use at 40 °C in a vacuum to minimize water uptake during storage. The polymers are obtained as slightly yellowish hygroscopic powders. Yields: ∼74%.

Grafting of Poly(vinyl alcohol) with Lactide and Glycolide (Synthesis of Poly(vinyl 3-(dialkylamino)alkylcarbamate-*co*-vinyl acetate-*co*-vinyl alcohol)-graft-Poly(lactide-*co*-glycolide). In a glovebox under anhydrous conditions modified poly(vinyl alcohol) was very carefully mixed with D,L-lactide and glycolide (lactide/glycolide = 1:1) in the respective molar ratios (1:1, 1:2, 1:10, and 1:20). The molar ratio was calculated on the basis of the average number of free hydroxyl groups in the PVA backbone and lactic and glycolic acid (1, 2, 10, 20) in the feed. Then the catalyst tin(II) 2-ethylhexanoate (12% (w/w) for 1:1 and 1:2 or 22% (w/w) for 1:10 and 1:20) was added (Table 2) and thoroughly mixed. The mixture was transferred into a nitrogen-filled, rigorously dried round-bottomed flask with gas inlet. Bulk polymerization at 150 °C was carried out for 3 h in a preheated oil bath.[6]

Polyesters with short side chains were dissolved in methanol or water and then treated by ultrafiltration as described above (yields ∼ 59%). Polyesters with long side chains were dissolved in acetone or dimethyl sulfoxide (DMSO) and purified by precipitation into a mixture of 2-propanol/water (yields ∼ 80%). The isolated colorless to yellowish polymers were dried at 20 °C in vacuo.

Nomenclature. The polyesters consist of four different components. The source-based IUPAC nomenclature for these polymers suggests the following designation, e.g., poly(vinyl 3-(dialkylamino)alkylcarbamate-*co*-vinyl acetate-*co*-vinyl alcohol)-graft-poly(D,L-lactide-*co*-glycolide). As abbreviation A(x)−y is used. A is the abbreviation of the diamine component (P for DEA*P*A, M for D*M*APA, and E for DEA*E*A), x the total average number of amine functions on the PVA backbone, and y the polyesters side chain length (lactic and glycolic acid monomers) calculated from the feed.

Sample Characterization. ^{1}H and ^{13}C NMR spectroscopic data were collected using a JEOL Eclipse+ 500 and a Joel GX 400 D at frequencies of 500 and 400 MHz for ^{1}H NMR and 126 and 101

MHz for ^{13}C NMR, respectively, at 50 °C in DMSO-d_6 (eurisotop, <0.02% HDO + D_2O). A 40−50 mg sample was used for each measurement. ^1H NMR was performed with 64 scans. The ^{13}C NMR was performed with 4096 and 60 000 scans.

The amine substitution (AS) was evaluated by calculating the ratio between the integral of the CH_3-end group of the amine and the integral of the methylene protons of PVA on the basis of the degree of polymerization. $(100/(I_{PVA}/2))(I_{Amine}/3) = DS$; $DS \times 3 = AS$.

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. $[(I_{cent\_LA\_CH} + (I_{cent\_GA}/2)/(I_{end\_LA\_CH} + (I_{end\_GA}/2))] + 1 = SCL$ or $[((I_{cent\_LA\_CH_3}/3) + (I_{cent\_GA})/2)/((I_{end\_LA\_CH_3}/3) + (I_{end\_GA})/2)] + 1 = SCL$.

Differential scanning calorimetry (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 at a heating rate of 10 °C/min. Glass transition temperatures were calculated from the second run.

Gel permation chromatography 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, and an AS-2000A autosampler, a Merck T-6300 Column Thermostat, a Wyatt DAWN EOS MALLS detector (normalized with PSS (Polymer Standard Service, Mainz) PMMA 200k standard). The separation was performed using an Optilab DSP together with a PSS SDV linear M (8 × 300 mm, 5 μm) column connected to a precolumn of the same type (8 × 50 mm, 5 μm). The measurements were performed at 60 °C column temperature at a flow rate of 0.5 mL/min using dimethylacetamide (DMAc) and 2.5 g lithium bromide/L (LiBr) as eluent. The molecular weights of the samples were determined using the Wyatt software Astra V4.73. First d*n*/d*c* was determined over the whole signal area. Then the molecular mass was calculated using this d*n*/d*c* value (third-order fit).

GPC (Figure 6). For these investigations a combination of two columns, a Lichrogel PS 40 and a Lichrogel PSMix (each 8 × 300 mm, 10 μm, Merck Hibar PrePacked Column), and dichloromethane with an addition of 0.3% triethylamine as eluent was used. The other components of the GPC arrangement stayed the same; only the Optilab DSP was substituted against a differential refractometer Ri-71.

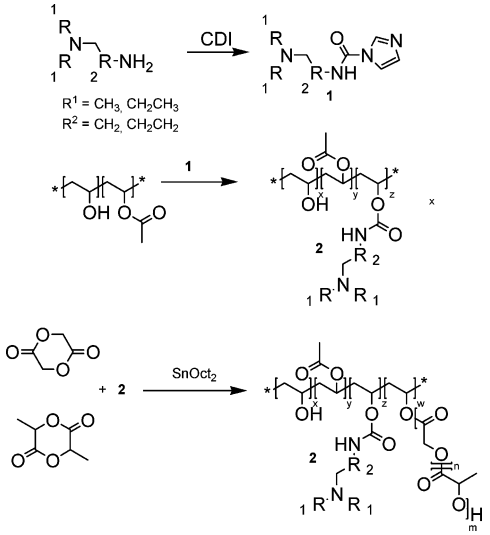
Wide-Angle-X-ray Diffraction. Wide-angle X-ray diffraction (WAXD) was performed to determine the crystallinity. Cu Kα radiation generated by a Siemens D5000 was used for WAXD of powdered polyester samples.

CHN analysis was performed with a Hewlett-Packard autoanalyzer 185.

Dye Entrapment Assay. Ca. 30 mg of polyester accurately weighed was dissolved in 5 mL of acetone (M(13)-1 and M(13)-2 were solved in 5 mL of water). A 1 mL aliquot of the solution was transferred into an Eppendorf cup (1.5 mL) and centrifuged (14 000 U/min, 10 min, Eppendorf centrifuge 5415C). Then 0.5 mL of this solution was injected into 5 mL of 10.9 mg of Rose-Bengal dye (sodium salt, negatively charged) in water. After 10 min 0.5 g of sodium chloride was added. After 10 min during that, the solution was stirred or shaken from time to time. A 1 mL aliquot was transferred into an Eppendorf cup and centrifuged (14 000 U/min, 10 min, Eppendorf centrifuge 5415C). The absorption of the supernatant was measured using an UV/vis-spectrophotometer (wavelength, $\lambda = 550$ nm; Shimadzu UV-160; quartz cuvettes, 1 mL).

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 a temperature of 4 °C, the samples were recovered and disks 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.

Scheme 1. Schematic Presentation of the Synthesis of Branched Polyesters



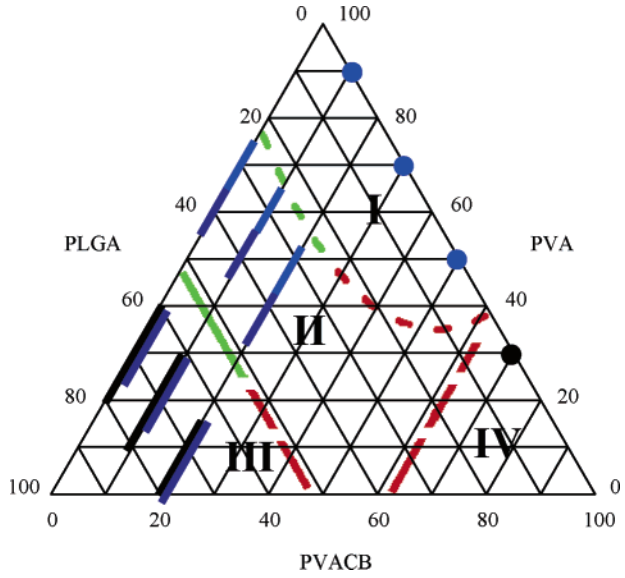
To determine the in-vitro degradation profiles, weighed film samples (ca. 30 mg, $n = 3$) were placed in 10 mL of phosphate buffered saline (PBS, pH 7.4, 0.15 M) and kept at 37 °C in an incubator. Glass vials were shaken once a day. The pH of the degradation buffer was periodically monitored and was found to be >6.8 after the study period of 21 days for all polymers tested. After 2, 7, 14, and 21 days, samples were recovered and blotted dry with Kimwipes and the wet weight was measured gravimetrically. Wet samples were then frozen at −80 °C, 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) × 100/(original mass)).

Results and Discussion

Using the three-step synthesis outlined in Scheme 1 branched polyesters containing a positively charged backbone were readily accessible in good yields. Two different types of polymers were generated. The hydrophilic branched polyesters with short PLGA side chains (molar ratio 1:1 and 1:2) designated as type I and more lipophilic polyesters with longer side chains (molar ratio 1:10 and 1:20; type II). In all cases yellow, amber, or dark red colored solidified melts were obtained. The reason for discoloration is under investigation. The discoloration intensifies with increasing amine substitution in the backbone and decreasing SCL. Purification of type I polymers was achieved by ultrafiltration, whereas type II compounds were subjected to precipitations. The results are summarized in Table 1. This table displays a number of polymers which are exemplary for the 52 synthesized polymers. Further information about all other polymers can be found in the Supporting Information.

Solubility. To investigate the solubility properties two different polymer types were synthesized. The hydrophilic branched polyesters with short PLGA side chains (molar ratio 1:1 and 1:2) designated as type I and more lipophilic polyesters with longer side chains (molar ratio 1:10 and 1:20; type II). As expected the solubility of both polymer types differed drastically. While type I polyesters were insoluble in organic solvents such as acetone and dichloromethane, they readily dissolved in water, methanol, or acetic acid. This means that branched polyester can be designed to possess solubility properties not found in linear PLGA, where water solubility occurs only for oligomers < 800.[41] Elongation of the polyester side chains lead to more lipophilic polymers soluble in organic solvents such as acetone

Scheme 2. Ternary Diagram Visualizing the Solubility of Branched Polymers and Their Composition: Area I, Completely Water Soluble and Hydrophilic; Areas II and IV, Amphiphilic; Area III, Lipophilic Water Insoluble



and DMSO. An increase in amine substitution caused the opposite effect, resulting in higher solubility in polar solvents.

In Scheme 2 the solubility behavior of branched polyesters was visualized as a ternary diagram. Four areas can be distinguished, separated by green and red lines. Green lines show observed solubility behavior, whereas red lines define extrapolations. Area I contains only highly hydrophilic polymers soluble in water and mixtures of water and alcohols, but not soluble in methanol. Nongrafted backbones, with and without amine substitution, fall into this category. Amine-modified PVA with a degree of substitutions up to 50−60% were readily soluble in water and alcohol/water mixtures.

Grafting with PLGA or amine substitution > 60% led to amphiphilic polymers, shown as areas II and IV. The polymers of both areas showed a higher solubility in acidic water caused by an increased protonation of the amine functions reacting as weak bases. With increasing PLGA graft ratio solubility in organic solvents such as methanol increased. Elongation of the polyester side chains yielded polymers which were completely insoluble in water (area III). The graft polyesters in this section were soluble in acetone. The polymer consists of three different main components: lipophilic PLGA side chains, the PVA backbone, and the diamine, both are hydrophilic. An elongation of the PLGA side chains increased the lipophilicity. This led to a solubility shift from hydrophilic, water-soluble polymers to lipophilic, water-insoluble ones.

It should be noted that water-soluble branched polyesters yield nanocomplexes with oppositely charged drug substances by self-assembly as shown for insulin.[42] Also type II polymers offer promising features for generating nanospheres using the surface tension driven convection flow (Marangoni effect) to gently encapsulate for instance DNA.[43]

NMR Spectroscopy. The PLGA grafted polyesters were studied using NMR spectroscopy signals of the backbones, and the lactide/glycolide side chains could be identified in ^{1}H and ^{13}C NMR spectra and the grafting by PLGA could be demonstrated.[8,9] ^{13}C NMR spectroscopy and CHN analysis suggested that the amine-modified PVA backbone remained intact during the grafting procedure. The signal of the urethane carbonyl carbon at 155.4 ppm was detected in polyesters with a high degree of amine substitution. In comparison to the corresponding

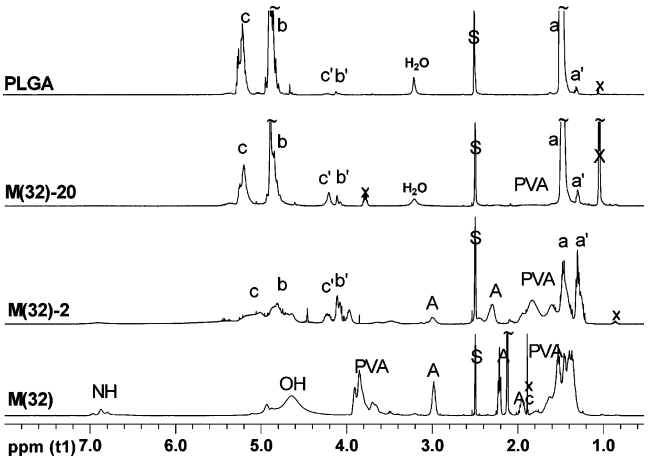


Figure 1. Differences in the ^{1}H NMR spectra of amine-modified PVA M(32), a PVA-*g*-PLGA M(32)-2 with short side chain length, a PVA-*g*-PLGA M(32)-20 with long side chain length, and a linear PLGA. Signals of PVA could be recognized in both polyester spectra, but its intensity decreases as expected from feed. Therefore, the signals of the polyester parts of the polymer show an opposite run.

PVA backbone the signals of protons bound to the amine group (NH) and especially the signal of the protons in direct vicinity to the amino function (A) shifted downfield compatible with an ionization of the amino group within the polyesters (compare spectra of M(32) and M(32)-2 in Figure 1). This ionization could be caused by free lactic and glycolic acid molecules during synthesis or purification. In Figure 1 M(32) a polymer of area 1 is compared to M(32)-2 and M(32)-20 polymers of areas II and III (Figure 1, Scheme 2). The fourth spectrum was obtained from a linear PLGA. The comparison shows that the signal intensities of PVA were decreasing with increasing graft densities of PLGA. In case of M(32)-20 the spectrum has more similarities to the spectrum of the linear PLGA than with the spectrum of M(32). Nevertheless this spectrum showed signals both of the backbone (PVA) and PLGA end groups (c′,b′,a′), which were of higher intensity than in the spectrum of linear PLGA indicating shorter PLGA chains for M(32)-20. Integration of the polyester signals (a (CH_3), b (CH_2), c (CH)) demonstrated a nearly 1:1 lactide/glycolide ratio in the side chain. Comparison with the end groups (a′, b′, c′) allowed calculation of mean side chain lengths of the polyesters (Table 1). These values correlated well with stoichiometric calculations and were more accurate at higher side chain lengths (SCL). The average of the SCL in 1:1 polymers was found to be ca. 2 and that of 1:2 ca. 3.

Only the polyester P(68)-10 showed a SLC close to 7, possibly caused by an inhibition effect on the ring opening polymerization or steric effects caused by the amine groups. In the case of competition between tertiary amino functions and lactide/glycolide during binding to the catalytic center of tin, the propylamine groups seemed to have a higher affinity.

In agreement with van der Velden et al. the signals of the PVA methine protons in direct vicinity to free hydroxyl groups resonated in the area between $\delta = 4.00$ and 3.33 ppm.[44] In this region no other signals of the polymer were detected, and therefore it could be used for reference calculations. Four different chain lengths were calculated from the feed (SLC 1, 2, 10, and 20). Due to these values and on the basis of the degree of polymerization of PVA(P=300), the following composition could be calculated on the basis of ^{1}H NMR spectroscopy: chain length 1, P[$VACB_{0-70}$−VAc_{0-36}−$VA_{165-195}$−$VPLGA_{75-105}$]; chain length 2, P[$VACB_{0-70}$−VAc_{0-36}−$VA_{120-150}$−$VPLGA_{105-135}$]; chain length 10, P[$VACB_{0-70}$−VAc_{0-36}−VA_{30-90}−$VPLGA_{180-225}$]; and chain length 20,

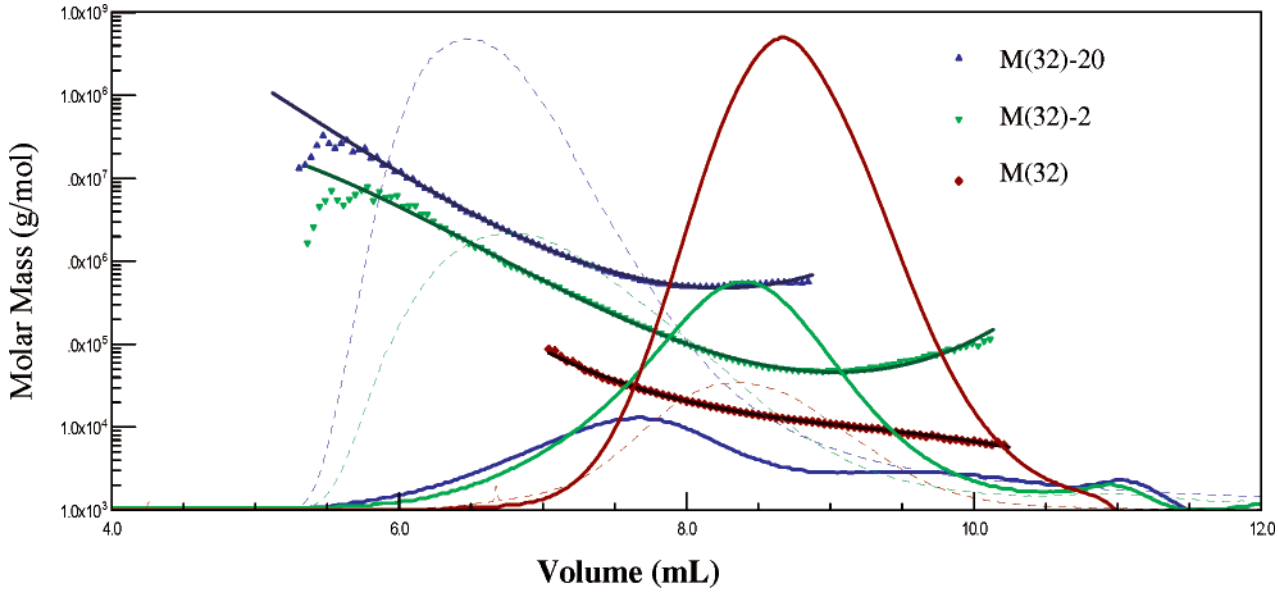


Figure 2. GPC elugrams of M(32), M(32)-2, and M(32)-20 measured by a MALLS and a refractive index detector. M(32) is the polymer with the lowest molecular mass, and M(32)-20 has the highest. The elution time correlates with the calculated molecular mass sequence.

P[$VACB_{0-70}$−VAc_{0-36}−VA_{15-105}−$VPLGA_{150-240}$]. In polymers of area 2, 25−35% for 1:1 and 35−45% for 1:2 of the monomer units in the PVA backbone carry a PLGA side chain. This means that a substantial number of hydroxyl groups in the backbone remained unmodified, i.e., 40−65% (type I). Presently we cannot fully explain this observation. It indicates that possibly due to sterical reasons no transesterification with the backbone hydroxyl groups appear within the investigated time frame. In polyesters of area III (type II) 50−80% of the backbone monomer units were carrying a polyester side chain.

Multiangle Laser Light Scattering. Using these measured chain length and the known structural composition of the backbone, a number average of the molar mass was calculated. Results of GPC-MALLS showed a reasonable agreement with these calculations. Conventional GPC cannot be used to estimate MW due to a lack of appropriate standards, necessary for this comparative method. GPC-MALLS were selected since it allowed measurement of absolute MW.

Development of a suitable GPC method for these polymers offered significant challenges.[45,46] The refractive index increment d*n*/d*c* 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, such as the polymers discussed here, require salt addition to the eluent to minimize unspecific interactions.[47] This will ensure that the polymer is in thermodynamic equilibrium with the surrounding solution. Last, a growing tendency to form aggregates with increasing amine substitution was observed. This could be a result of increasing amphiphilicity and therefore 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.

Against this background we developed a GPC method using dimethylacetamide + 2.5 g/L LiBr as eluent, dissolving all types of graft-polyesters and their respective backbones. In Figure 2 three GPC elugrams of a backbone, a polyester with short side and one with long side chains were shown. In every case nearly the same polymer amount was solved in 1 mL of solvent, but due to the decrease of d*n*/d*c* with increasing polyester side chain length a reduction of the RI signal was observed, whereas the light-scattering signal (LS) increased proportionally. Especially,

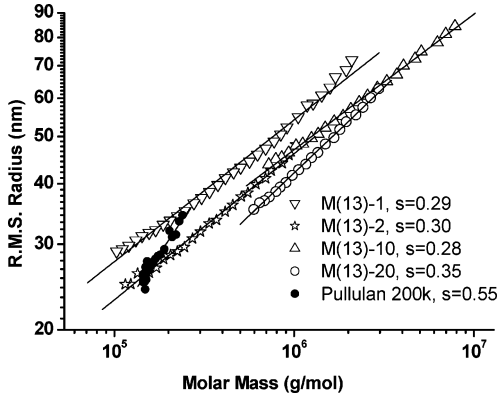


Figure 3. Double logarithmic plot of rms radius of gyration against molar mass. The small slopes express high branching: M(13)-1, s = 0.29; M(13)-2, s = 0.30; M(13)-10, s = 0.28; M(13)-20, s = 0.35. Random coil: pullulan, s = 0.55.

the volume−molar mass curve of the polyester showed some high molecular weight fractions causing asymmetric LS curves. Apart from this, polyesters showed monomodal distributions and the retention times of the polymers measured by the RI detector followed the expected sequence: Polymers with long SLC eluted first, then polymers with shorter SLC, and last the backbones. Further, the volume−molecular mass curve could be fitted well by a third-order function. In most cases the polydispersity was smaller than that mentioned in the literature for linear PLGAs initiated with tin(II) octoate ($M_w/M_n \approx 2.3-2.4$).[48]

Apart from molecular weight characterization, GPC-MALLS could further be used to obtain information about the molecular architecture of the polymers in solution. By plotting the rms (root-mean-square) radii of gyration as a function of molar mass in a double logarithmic way (Figure 3), the gross molecular conformation can be investigated according to

$$\log r_i = k + a \log M_i \qquad (1)$$

where r_i is a radius of gyration, k the interception on the y axis, a the slope, and M_i a molar mass.[46,49] The slope a contains information concerning the molecular conformation of the polyester.

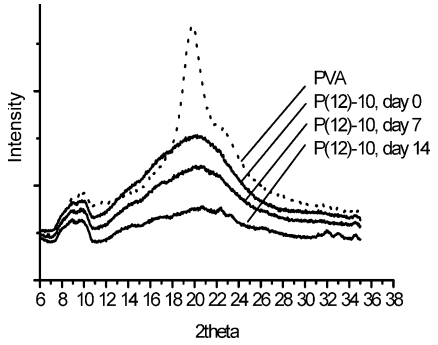
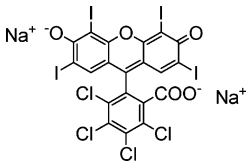


Figure 4. Wide-angle X-ray diffraction (WAXD) of the P(12)-10 after 0, 7, and 14 days of degradation showing only the amorphous halo. There is no crystallinity even after degradation.

Scheme 3. Rose-Bengal Sodium Salt



Most real random coils show slopes in the range of 0.55−0.60,[46,50] whereas branched polyesters yielded smaller slopes, suggestive of their more compact structure.[46] In Figure 3 a linear, unbranched pullulan was compared to M(13)-*y* polyesters. Pullulan showed a typical slope value of random coil polymers (0.55). In contrast the slopes of the polyesters were much smaller (0.28−0.35), demonstrating their highly branched, compact character.[46,49]

Differential Scanning Calorimetry. Apart from the molecular structure of branched polyesters in solution, their microstructure and thermal behavior was thought to be important for their performance as a drug delivery platform. DSC of the polyesters showed only one glass transition. In the first run relaxation processes superimposed with the glass transition (data not shown). The glass transition temperatures (T_g) of branched polyesters were found to be close to body temperature. Moreover, as known from literature,[51] it can be assumed that water uptake will rapidly reduce T_g below 37 °C and will ensure viscoelastic properties of the carrier system after its application. This has consequences both for influx of water into devices and a subsequent release of drug substances from the carrier. Also degradation of branched polyesters is affected by the rate of water influx. No phase separation was noted in the second DSC run (only one T_g). In contrast to PLLA/PVA blends, full miscibility of backbone and PLGA side chains was observed.[52]

Crystallinity. Neither DSC nor WAXD (wide-angle X-ray diffraction) show crystalline polymer segments. In WAXD only a halo indicative for amorphous materials could be observed (Figure 4). Further no crystallization appears during a degradation test on polymer films after 7 and 14 days of incubation. This means that despite the high plasticizing effect of water and the resulting chain flexibility, no organization of the side chains during degradation appears and could affect the degradation rate.

Drug Entrapment Assay. Rose-Bengal, RB, dye (Scheme 3) was used as a model drug to investigate the influence of amine substitution onto the complexation and encapsulation of hydrophilic macromolecular drugs such as peptides, proteins, and DNA.[53] The drug candidates are entrapped into carrier systems relying both on electrostatic and hydrophobic interactions.[42,43] In this screening assay electrostatic interactions appeared between the positively charged polymer and the negatively charged RB. Using branched polyesters having a (dimethylamino)propylamine substituted backbone and comparable side chain lengths, a clear decrease in the absorption of RB in the supernatant could be observed (Figure 5a). This means that with increasing degree of amine substitution the concentration of free Rose-Bengal dye in the supernatant decreased, suggesting that charges on the backbone were accessible for interactions and the degree of amine substitution is an important factor for the encapsulation or complexation efficiency of appropriate drug candidates in drug delivery systems. As expected an increase of the amine substitution led to enlarged loading capacity of the polymers.

Further, the side chain length of the polyester had also an influence on the encapsulation or complexation efficiency (Figure 5b). With increasing SLC the complexation of RB was reduced. In type II polyesters the steric hindrance and the shielding of positively charged amino groups were expected to be much higher than in type I polyesters. By manipulating polymer composition the loading efficiency of drug delivery systems can be optimized. By Oster et al. the encapsulation abilities of the polyesters have been demonstrated by investigating the interactions between P(68)-10 and DNA. An extraordinary transfection of L929 mouse fibroblasts could be examined.[43] Therefore, the abilities of the synthesized polyesters for drug delivery could be demonstrated.

Degradation Behavior. For drug delivery systems containing peptides, proteins, and DNA the parenteral route of administration is of primary importance, since hydrophilic macromolecular drug molecules are not efficiently transported across epithelial barriers. Therefore, these drug delivery systems should be both

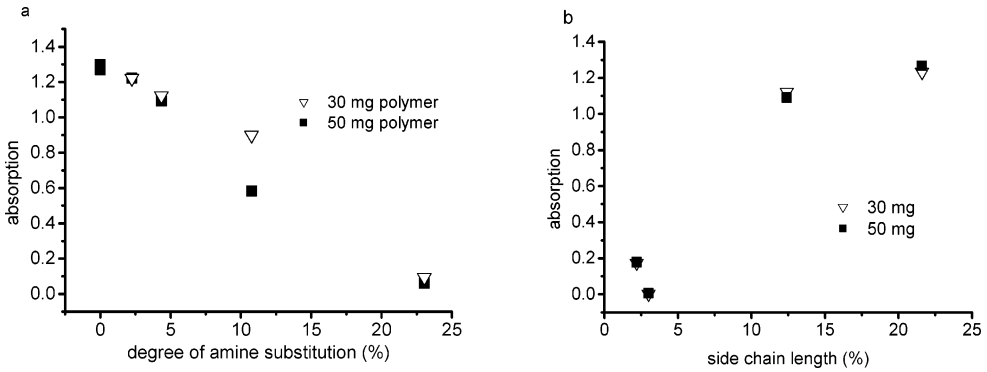


Figure 5. Drug entrapment assay: (a) Interaction of negatively charged Rose-Bengal dye (RB) and positively charged polyesters (M(*x*)-10) with similar side chain length and different degrees of amine substitution. Increasing amine substitution led to increasing RB−polymer interaction and therefore to a decrease in dye concentration in the supernatant. (b) Interaction of Rose-Bengal dye and polyesters (M(13)-*y*) having different side chain lengths and the same degrees of amine substitution. Elongation of the polyester side chains led to a shielding of the amine function and therefore to a decrease in dye complexation and finally a higher dye concentration in the supernatant.

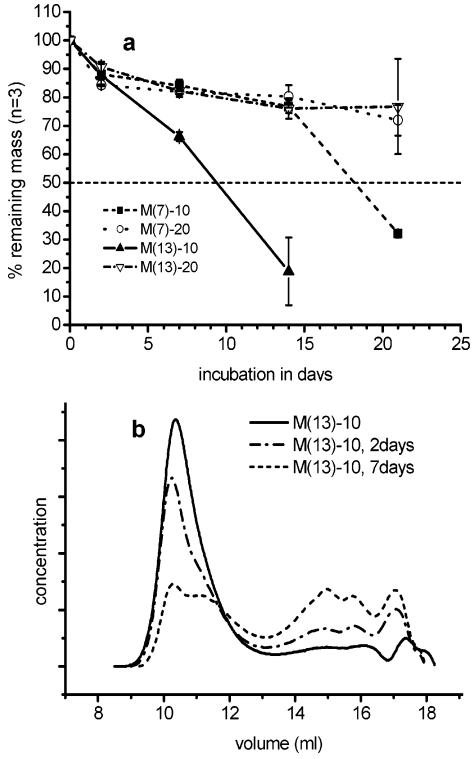


Figure 6. (a) In vitro degradation profile of branched polyesters. Mass loss of M(7)-10/20 and M(13)-10/20 as a function of incubation in PBS (pH 7.4; 37 °C). Increasing amine substitution led to decreasing degradation times. (b) GPC profile of M(13)-10 during degradation: days 0, 7, and 14. The main peak disappeared, and the concentration of small oligomeric compounds increased within these 2 weeks.

biocompatible and biodegradable to allow repeated administration to patients.[54] PLGA is known as a biocompatible material, but the residence time for the fastest degrading PLGA copolymers under in vivo conditions is around 5−6 weeks. Degradation occurs heterogeneously from "inside-out" by hydrolytic cleavage of the ester bonds leading to an acidic microenvironment.[55,56] This acidic microenvironment is detrimental for peptides, proteins, and DNA.[14] Thus more rapidly degrading biomaterials are of general interest.

Type II branched polyesters could present a delivery platform with which faster degrading polymer can be designed. 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 different polymers, also designated as polymer erosion, was studied in four samples differing in molecular weight and degree of amine substitution. For this study branched polyesters M(7)-10, M(7)-20, M(13)-10, and M(13)-20 were selected. The results, shown in Figure 6, demonstrate that the polymer with shorter SLC and higher degree of amine substitution, namely, M(13)-10, showed the highest erosion rate with a degradation half-life of 10 days. M(7)-10 had a half-life of ca. 20 days, while longer SLC reduced the degradation times significantly. In view of the high molecular weights of the branched polymers (Table 1) this can be considered as a relatively short degradation time as compared to linear PLGA.[41,57] During degradation oligomers are formed as shown in the GPC traces (Figure 6b) suggestive of bulk hydrolysis preceding mass loss possibly catalyzed by the amino groups. The degradation mechanism is currently under investigation and will be reported elsewhere.

Conclusion

The successful synthesis of amine-modified poly(vinyl alcohol)-graft-poly(lactide-*co*-glycolide)s was demonstrated by NMR and GPC-MALLS. Depending on the ratio of the three components (PVA, amine, lactide/glycolide), the solubility of the resulting graft-polyester could be modified within a large degree ranging from hydrophobic to water-soluble materials. Structure−property relationships were noted for polyesters with short (area II/type I) and long (area III/type II) SCLs. Elongation of the side chains caused an increase in hydrophobicity and therefore a shift in solubility to less polar solvents. Using GPC-MALLS the molecular weights and the three-dimensional architecture was investigated. Polyesters with long side chains (area III/type II) are characterized by molecular weights higher > 100 000 g/mol. A high degree of branching was demonstrated by multiangle laser light scattering of the polyesters. Small slopes in the molar mass−rms radius graph were found to be suggestive of a compact, highly branched structure.

The ability of the charge containing branched polyesters to interact with oppositely charged drug substances was studied using RB. It could be shown that Rose-Bengal loading was mainly affected by the degree of amine substitution and its accessibility.

Despite their high molar masses compared to linear PLGA, these branched polyesters showed relatively short degradation times. The amino function seemed to be an important factor for a fast degradation. Elongation of the side chains had the opposite effect.

The potential of amine-modified graft-polyesters as a platform for biodegradable delivery systems can be seen in the possibility to design branched polyesters for specific therapeutic applications requiring rapidly degrading devices. Further investigations regarding drug release and degradation mechanism are currently under way.

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Supporting Information Available: Table containing further characterization data for information about all other polymers in this work (pdf). This material is available free of charge via the Internet at http://pubs.acs.org.

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