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Biodegradable comb polyesters. Part II. Erosion and release properties of poly(vinyl alcohol)-*g*-poly(lactic-*co*-glycolic acid)

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Abstract

Poly(lactic acid) (PLA) and its random copolymers with glycolide (PLG) were modified by grafting onto hydrophilic macromolecular backbones, such as poly(vinyl alcohol) (PVA), to increase both hydrophilicity and to manipulate the polymer structure. The resulting branched PVA-*g*-PLG offers the possibility to manipulate physico-chemical properties, such as molecular weight and glass transition temperature. The degradation and erosion rates required for continuous release of hydrophilic macromolecules differs significantly from linear PLG. Microspheres were prepared to investigate the release of hydrophilic dextran as a function of polymer structure.

A reduction of the poly(lactic-*co*-glycolic acid) chain lengths in PVA-*g*-PLG caused a change in erosion profiles from bulk erosion to a surface front erosion mechanism, when the molecular weight of the PLG side chains was below 1000 g/mol, which is equal to water-solubility when cleaved from the backbone. Drug release rates from microspheres were significantly influenced by the polymer structure. A reduction of the PLG chain lengths led to increasing erosion controlled release rates, while an increase of the molecular weight of the core PVA resulted in a more diffusion controlled release mechanism. Release profiles could be adjusted over a broad range from ca. 14 days to 3 months. In combination with the possibility of avoiding accumulation of acidic breakdown products in the delivery device, PVA-*g*-PLG are of particular interest for parenteral delivery systems containing proteins, peptides or oligonucleotides.

Keywords: Poly(vinyl alcohol)-*g*-poly(lactic-*co*-glycolic acid); Biodegradable polyesters; Microspheres

1. Introduction

Aliphatic polyesters, such as poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) and their random copolymers poly(lactide-*co*-glycolide) (PLG) are widely used for parenteral drug delivery systems [1,2]. While drug delivery systems for parenteral administration of peptides have become commercially available, hydrophilic macromolecular drugs, such as proteins, still present a formidable challenge for continuous or infusion-like drug release both under in-vitro and in-vivo conditions. Possible interactions between proteins and hydrophobic matrix polymers can lead to deactivation and denaturation of these sensitive molecules. Therefore, the search for new biomaterials allowing protein, antisense, oligonucleotide or gene delivery remains an ambitious goal [3,4].

Successful delivery of these hydrophilic, macromolecular drugs from microspheres or implants strongly depends on the properties of the polymers used for encapsulation, affecting water uptake, thermo-mechanical properties, rates of biodegradation (cleavage of chemical bonds leading to a reduction of molecular weight) and erosion (mass loss) [5–8]. The erosion mechanism for linear PLA and PLG is controversially discussed in the literature [8–11]. Autocatalysis by acidic degradation products, which are retained in the polymer matrix, is thought to accelerate degradation of PLG chains inside the delivery device. Consequently, the protein in the polymeric matrix is exposed to a microenvironment of increasing acidity. In combination with elevated temperatures and hydrophobic surfaces, sensitive proteins are known to become inactivated [12].

In spite of considerable efforts, drug release rates often deviate from an ideal “infusion like” profile, generated by zero-order release kinetics. Polyphasic drug release profiles can be modified either by formulation approaches or by selection of more appropriate biodegradable polymers. While the release properties of biodegradable microspheres can be influenced to a limited extent by formulation ameters, polymer modifications provide a broader spectrum of control during the erosion phase.

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Two strategies have been proposed for modifications of polyesters with regard to parenteral protein drug delivery [5,13]: for one, increasing polyester hydrophilicity should result in faster water uptake and swelling of the polymer matrix, affecting protein release during pore-diffusion phase [14,15]. Or secondly, grafting short PLG chains onto hydrophilic backbone molecules should lead to graft polymers with accelerated erosion behavior, because the degradation products become soluble in water after few cleavage steps [16,17].

The aim of this study was to investigate the effect of grafting hydrophobic PLG onto poly(vinyl alcohol) (PVA) with respect to both physico-chemical properties of the branched PVA-*g*-PLG, as well as functional properties as biodegradable carriers, namely degradation and release properties [5,18–22].

2. Experimental

2.1. Polymer synthesis

The following designation will be used to specify different PVA-*g*-PLGs, *aa-bb-cc*, the first digits (a) designate the molecular weight of the PVA backbone in g/mol × 1000, followed by the degree of hydrolysis (b) and (c) the relative amount of backbone hydroxyl groups per carboxylic acid repeating unit in mol%. The synthesis and characterization of PVA-*g*-PLG was reported earlier [22]. Briefly, ring-opening polymerization of lactide and glycolide in the presence of the backbone PVA was carried out in melt, using stannous octoate as catalyst. All reactions were performed under anhydrous conditions under a dry nitrogen atmosphere. Complete monomer conversion was achieved after a polymerization time of 3 h at 130°C. Properties of the purified PVA-*g*-PLGs are summarized in Table 1.

For comparison, D,L-PLG (RG503) with a molar lactide/glycolide ratio of 1:1 and a weight average molecular weight (M_w) of 40 000 g/mol was obtained from Boehringer Ingelheim (Germany).

2.2. Analytical methods

Size exclusion chromatography (SEC): 0.5% (w/v) polymer solutions were injected into a thermostatted (35°C) Merck–Hitachi system (columns: Lichrogel PS mix and Lichrogel PS 40, 10 μm) with a differential refractometer (RI 71) as detector. Chromatograms were obtained with dichloromethane (DCM) in case of high molecular weight polymers, HMW-PVA-*g*-PLG, and acetone for lower molecular weight polymers, LMW-PVA-*g*-PLG, at a flow rate of 1 ml/min. Molecular weights were estimated using third-order universal calibration relative to poly(styrene) reference materials (Merck). For combined SEC and *static light scattering (SLS)* analysis a MiniDawn light scattering detector (Wyatt Technology Corporation) (100 μl K5 cell, laser wavelength 690 nm, laser power 30 mW, three angles of detection (45, 90 and 135°)) was added to the above system.

Differential scanning calorimetry (DSC): was conducted in nitrogen atmosphere using a DSC7 calorimeter (Perkin–Elmer) in sealed aluminum pans, relative to indium and gallium standards. Thermograms covered a range of −20 to 200°C with heating and cooling rates of 10°C/min. Glass transition temperatures (T_g) were determined from the second run.

Nuclear magnetic resonance spectroscopy (NMR): was performed at 35°C with 6% (w/v) polymer solutions in different deuterated solvents, like d-chloroform, d_6-DMSO and d_6-acetone. 400 MHz ^{1}H- and 100 MHz ^{13}C-NMR spectra were recorded with a Jeol GX400 Delta N FT spectrometer, 500 MHz ^{1}H- and 125 MHz ^{13}C-NMR spectra as well as 2D COSY (two dimensional *co*rrelation *s*pectroscop*y*) ^{1}H-, ^{1}H- and inverse gated decoupling ^{13}C-NMR experiments (to suppress nuclear Overhauser effect) with a Jeol LA500 Eclipse + Delta FT spectrometer.

Scanning electron microscopy (SEM): was performed on gold sputter-coated samples using a SEM 501S (Hitachi).

2.3. In-vitro degradation of polymer films

Polymer films cast from 5% (w/v) DCM or acetone solutions on Teflon® plates were allowed to dry for 72 h. Residual solvents were then removed in vacuo at room temperature, at 30°C and finally 40°C for 2 days until constant weights were obtained. Films were cut into 50 × 10mm^2 slabs with a thickness of 50–100 μm. Samples of known weight (ca. 50 mg, $n = 2$) were immersed in 5 ml of isotonic phosphate buffered saline solution (PBS, pH 7.4, 0.15 M, periodically replaced during incubation experiments) in sealed glass test tubes and stirred in a rotating thermostat (Rotatherm, Liebisch) at 30 rpm and 37°C. At preset intervals samples were recovered and frozen at −20°C for 4 h, then freeze-dried in vacuum (0.4 mbar) for 48 h followed by secondary drying at room temperature in vacuum for one week. Molecular weights (GPC, GPC–SLS), mass loss (gravimetry), film morphology (SEM), thermal properties (DSC) and structure (NMR) were investigated. Water uptake was determined gravimetrically on film specimens after the water on the surface was removed by blotting. It was calculated by the following equation: Water uptake(%) = 100−(Mass(t)wet/Mass(t)dry × 100).

2.4. Microsphere preparation and marker release

Microspheres were prepared at 4°C using a modified W/O/W double-emulsion-technique [12]. Briefly, 250 μl of an aqueous FITC-dextran (40 000 g/mol, Sigma) solution (1% (w/v)) was emulsified in 1.5 ml of a DCM solution containing 500 mg of the different polymers using an Ultraturrax homogenizer Type18/10 (Janke & Kunkel, Staufen) at 20 000 rpm for 30 s. The resulting emulsion was injected with a syringe (18 G needle) in 200 ml of an aqueous solution (0.5% (w/w)) of PVA (Mowiol 18-88, Hoechst AG),

Table 1
Physicochemical properties of the PVA-*g*-PLG

Number	Polymer	M_w PVA (kg/mol)	PVA-OH:lactone (feed) (mol:mol)	LA:GA (NMR) (mol%)	M_w (SEC) (kg/mol)[a]	End:chain (NMR) (%)[b]	M_n (kg/mol) (feed)[c]	M_n (kg/mol) (NMR)[c]	M_n (kg/mol) (SLS)[d]	T_g (°C)	OH (mol%)[e]
1	HMW158801	15	2.6:100	50.8:49.2	155	1.60	1517	1254	1563	39.7	–
2	MMW158805	15	13.0:100	51.2:48.8	84.1	5.99	307.7	360.3	–	37	–
3	LMW158810	15	26.0:100	50.0:50.0	51.9	9.60	171.1	237.8	182.8	34	53
4	LMW158830	15	78.5:100	50.0:50.0	–	19.68	64.99	134.4	140.4	34.2	46
5	LMW068010	6	23.6:100	50.0:50.0	71.5	8.20	62.77	83.57	–	37	68
6	HMW207401	20	2.19:100	51.8:48.2	229	2.0	2011	1135	–	41.1	–
7	LMW207410	20	21.9:100	52.0:48.0	70.4	10.07	216.9	308.7	–	35	75
8	Linear PLG[f]	–	0:100	50.0:50.0	40.0	–	–	–	–	45	–

[a] DCM as eluent.
[b] PLG (end:chain) groups ratio, calculated from ^{1}H NMR.
[c] Calculated assuming complete conversion of PVA-OH.
[d] Values obtained by combined SEC–SLS analysis using acetone as eluent.
[e] Unesterified PVA-OH groups, calculated from inverse gated decoupling ^{13}C NMR.
[f] RG503, supplied by Boehringer Ingelheim, Germany.

emulsified with a high-speed homogenizer (Ultraturrax T25, Janke & Kunkel, Staufen) at 8000 rpm for 30 s and then stirred for 2.5 h at 200 rpm to allow solvent evaporation. Microspheres were collected by centrifugation (1.5 min, 1000 rpm, RC-5B from Sorvall–Du Pont Instruments, Bad Homburg) washed three times with 150 ml of distilled water, freeze-dried and stored at 4°C. For release studies a known amount of microspheres (ca. 40 mg, $n = 3$) was immersed in phosphate buffer saline solution (PBS, pH 7.4, 0.15 M) in sealed glass test tubes at 37°C. Loading and release were assayed by fluorescence spectroscopy using a LS 50 B luminescence spectrometer (Perkin–Elmer, Ueberlingen) at 493/515 nm.

Sizes and size distributions of the microspheres were analyzed by dispersing ca. 10 mg of the samples in an aqueous solution of Tween®20 (0.1% (w/v)). The measurements were carried out in triplicate by laser light scattering using a Malvern Mastersizer X (Malvern Instruments, UK).

3. Results and discussion

The branched structure of PVA-*g*-PLG can be adjusted by variation of feed composition and reaction conditions [22]. Using PVA as backbone, polymers with higher molecular weights are obtained (e.g. polymers 1 and 6, Table 1), than those accessible with linear PLG under similar reaction conditions [22]. Low content of PVA in the feed, corresponding to few polymerization propagation centers, yielded PVA-*g*-PLG with very high M_w (HMW-PVA-*g*-PLG). In this case up to ca. 300 PLG chains are connected to each PVA molecule on average, depending on the degree of PVA polymerization. The theoretical number average molecular weight (M_n), calculated from feed composition, e.g. 1 517 000 g/mol for polymer 1 (Table 1), assuming complete conversion of the PVA hydroxyl groups (PVA-OH), and the M_n determined from NMR analysis by comparison of the signal intensities of the PLG chain and end groups, 1 254 000 g/mol, were in reasonable agreement. These data suggest that steric hindrance inhibits complete conversion of all PVA-OH groups. Thus, a single polyester chain connected to a PVA-OH group consisted of ca. 64 repeating units, equivalent to a chain M_n of ca. 4000 g/mol. This value was found to be in good agreement with light scattering data.

The resulting molecular weights of the branched PLG were directly related to the amount of backbone incorporated, e.g. polymers 1–4 in Table 1. More PVA-OH groups present during the polymerization resulted on average in shorter PLG chains grafted onto PVA, designated as LMW-PVA-*g*-PLG. A hydrophilic PVA backbone onto which up to ca. 300 short PLG chains are grafted characterizes the structure of these polymers. For polymer 3 (Table 1) the number average molecular weight calculated from feed composition was 171 000 g/mol, whereas NMR analysis (237 000 g/mol) and combined SEC and SLS

analysis (182 000 ± 50 000 g/mol) yielded slightly higher values, indicating an incomplete conversion of PVA, possibly due to limited solubility in the melt of the monomers. An average PLG side chain consisted of ca. six dimers, equivalent to a chain $M_n < 1000$ g/mol. Moreover, increasing molecular weights of the PVA backbone caused a proportional increase in M_w of the resulting graft polymers due to an increase of the PLG chain number per molecule (polymers 5, 3 and 7).

SEC analysis calibrated with linear poly(styrene) standards allows a relative estimate for the M_w of the branched PVA-*g*-PLG. Their three-dimensional architecture yielded smaller hydrodynamic volumes in solution [22] and caused a significant underestimation of absolute molecular weights. Therefore, a combination of SEC and SLS as well as NMR spectroscopy was used for polymer characterization.

Fig. 1a gives a typical example of the polymer structure determined by ^{1}H NMR spectroscopy as a function of PLG chain lengths. The signals of the PLG chain groups resonate at $\delta = 4.85$ ppm (methylene groups of the glycolic acid residues), $\delta = 5.2$ ppm (lactyl C*H*) and $\delta = 1.55$ ppm (lactyl CH_3). In contrast to spectra of linear polyesters, new signals of the corresponding hydroxyl terminated end groups were found at $\delta = 4.25$ ppm (methylene end groups of the glycolic acid residues), $\delta = 4.35$ ppm (lactyl end C*H*) and $\delta = 1.45$ ppm (lactyl end CH_3). Their signal intensity was a function of PLG chain lengths. Shorter chains caused a corresponding increase in intensity. The assignment of the hydroxyl terminated lactyl units is in excellent agreement with literature data [23–25] and could be confirmed by the cross signals in 2D COSY ^{1}H–^{1}H spectrum (Fig. 1b). The lactic acid chain coupling (1.55 ppm/5.2 ppm) can be seen as clearly as the coupling of their corresponding end groups (1.45 ppm/4.35 ppm).

3.1. Degradation and erosion properties of PVA-g-PLG

Factors contributing to degradation properties of PLG and PLA by random hydrolytic ester bond cleavage are molecular weight, copolymer composition and crystallinity [20]. Branched PVA-*g*-PLG offer additional possibilities to manipulate the hydrophilicity and hence the water penetration by incorporation of the hydrophilic backbone PVA. Using PVA backbones with different degrees of polymerization (5, 3 and 7) leads to PVA-*g*-PLG with a different number of PLG chains grafted per backbone molecule.

Secondly, by variation of the feed composition, polymers with relatively short PLG chains attached to the hydrophilic PVA backbone can be synthesized. These short PLG chains should be more rapidly cleaved and transported from degrading device due to their water-solubility. Therefore, the molecular weight of the PVA-*g*-PLG was varied with respect to the PLG chain lengths (e.g. polymers 1–4, Table 1).

As described above, the physico-chemical properties of HMW-PVA-*g*-PLG are dominated by the long PLG chains and should be comparable to linear PLG of high molecular weight. Consequently, erosion and degradation profiles of HMW-PVA-*g*-PLGs were comparable to those, typically observed for bulk erosion of linear PLG as demonstrated in Fig. 2a. The mass loss of HMW-PVA-*g*-PLG was characterized by an initial lag phase without detectable erosion, followed by a sigmoid decay of the polymeric matrix. It seems that the very high weight average molecular weight (M_w) of the HMW-PVA-*g*-PLG does not affect the rates of hydrolytic bond cleavage in a significant manner; graft polymer and PLG show similar degradation rates in spite of large differences in M_w. The duration of the lag phase, however, was found to be influenced both by the molecular weight of the graft polymer and the molecular weight of the PVA backbone. Shorter lag times seen with HMG-PVA-*g*-PLG suggest that PVA increases polyester hydrophilicity. The PLG, not containing a PVA backbone, yielded a half-time of erosion ($t_{1/2}$) of ca. 46 days, whereas branched PVA-*g*-PLG with a 15 000 g/mol PVA backbone reached a $t_{1/2}$ of 39 days and with a 20 000 g/mol backbone a $t_{1/2}$ of 33 days was obtained. This change in polymer hydrophilicity affected the initial water penetration rate as depicted in Fig. 2c. Branched HMW-PVA-*g*-PLG shows reduced lag time in mass loss, because the rapid water penetration leads to an accelerated rate of ester bond hydrolysis. In case of linear PLG, high M_w materials cause prolonged lag phases without detectable changes in mass, delaying drug release [26].

Monomodal SEC traces of the HMW-PVA-*g*-PLG became multimodal during in-vitro degradation experiments (Fig. 2b). Since PVA is hydrolytically stable, chain cleavage only occurs in the branches, yielding in a mixture of more than one degrading species. Comb and linear breakdown products, as well as unchanged comb polyesters during the first days of incubation, rendered a quantitative interpretation of these mixtures very difficult. The faster hydration of the hydrophilic backbone implies an initially preferred cleavage at the PLG-backbone bonds. But the long PLG chains may also undergo random and end chain scission of the ester bonds before they are cleaved from the backbone. The expected mixture of at least two different species was observed.

Compared to the HMW-PVA-*g*-PLG the observed degradation and erosion pattern of polyesters with shorter PLG chains (LMW-PVA-*g*-PLG) changed significantly as shown in Fig. 3a. No initial lag phase of mass loss was observed and the polymer chain cleavage occurred almost at constant rate. As described above, an average PLG side chain consisted of ca. six dimers, equivalent to a chain M_n below 1000 g/mol. Linear degradation products will become immediately water-soluble [27]. Consequently, no entrapment of breakdown products inside the polymeric matrix was expected. The amphiphilic character of the LMW-PVA-*g*-PLG and especially their degradation products resulted in a reduced solubility in DCM. Therefore, the eluent for SEC–SLS analysis was changed to acetone.

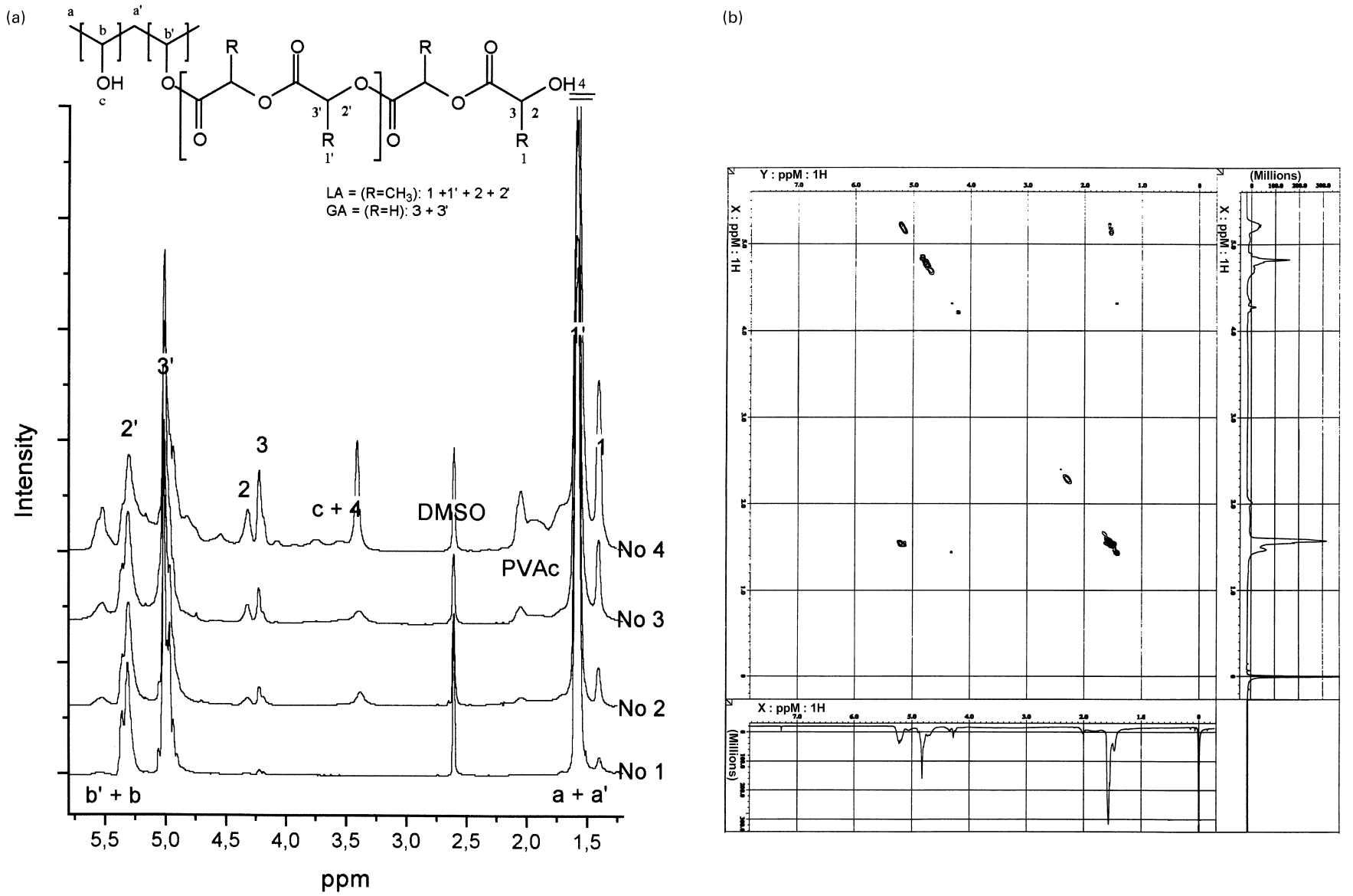

Fig. 1. (a) ^{1}H NMR spectra of PVA-*g*-PLGs with different PLG chain lengths (polymers 1–4, Table 1). (b) 2D COSY ^{1}H–^{1}H NMR spectrum of LMW-PVA-*g*-PLG (polymer 3, Table 1).

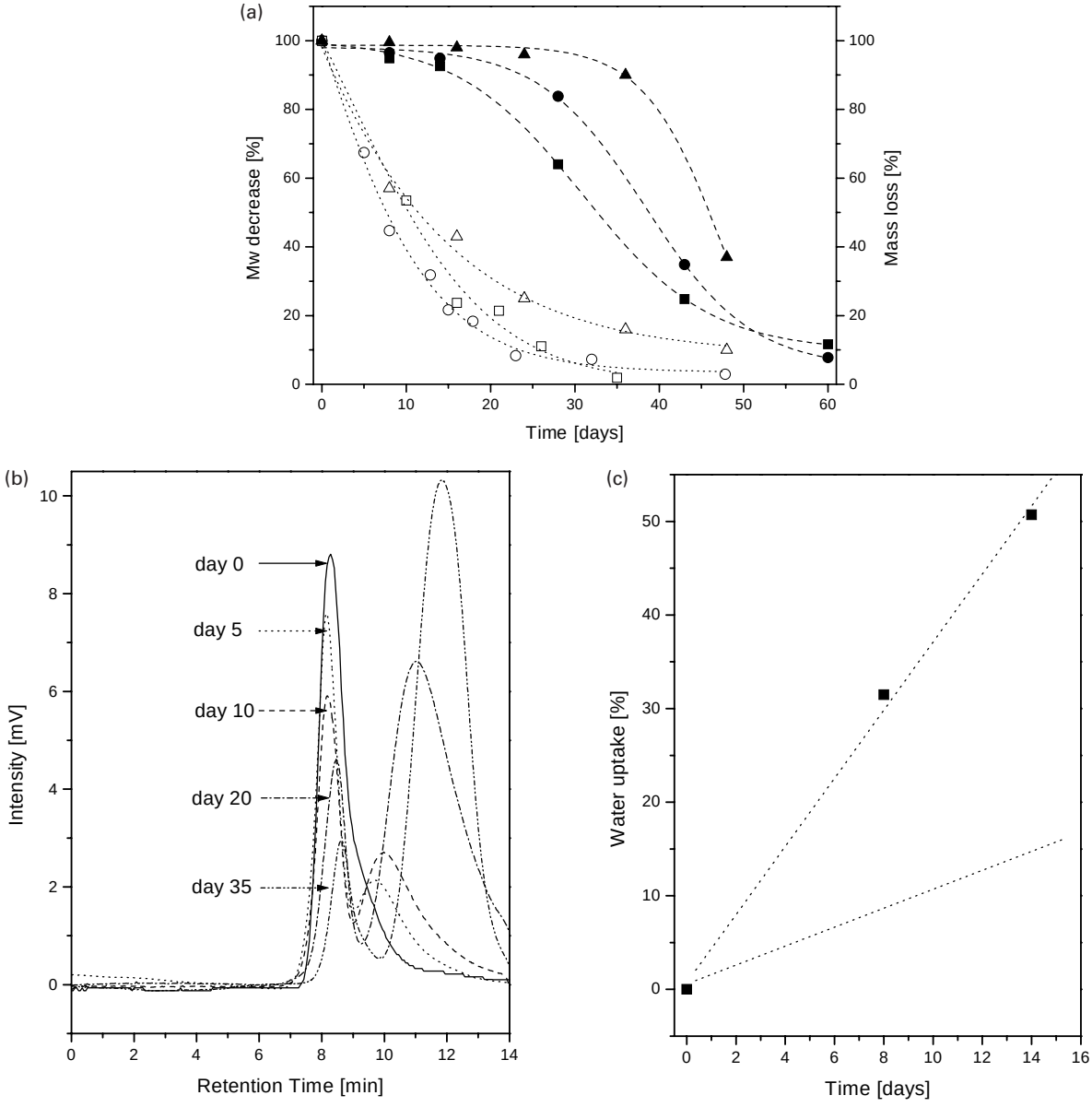

Fig. 2. (a) Mass loss (erosion, full symbols) and loss of molecular weight (degradation, open symbols) of HMW-PVA-*g*-PLGs compared to a linear PLG, polymer 1 (Table 1) (circle), polymer 6 (Table 1) (square), polymer 8 (Table 1) (up triangle). (b) SEC traces of HMW-PVA-*g*-PLG (polymer 1, Table 1) over time. (c) Water uptake of HMW-PVA-*g*-PLGs, polymer 1 (Table 1) (circle), polymer 6 (Table 1) (square).

Monomodal SEC traces of LMW-PVA-*g*-PLG remained monomodal over time, suggesting a more homogenous mechanism of degradation, as shown in Fig. 3b. No entrapment of oligomers and cleavage products occurred, since they would have been dissolved and detected in acetone. Short PLG chains and high amounts of the hydrophilic backbone PVA increased matrix hydrophilicity resulting in rapid water uptake of the polymer films (Fig. 3c), which can be explained by the amount of unmodified PVA hydroxyl groups as determined by inverse gated decoupling ^{13}C-NMR (Table 1). On average every second PVA-OH group was esterified. Compared to HMW-PVA-*g*-PLG the rates of water uptake were found to be two to eight times higher.

3.2. Hypothetical degradation mechanism

Fig. 4a outlines the different possibilities of chain scission during degradation of the graft PLG. A random and end scission of ester bonds leads to short linear hydroxyl terminated degradation fragments. As a consequence an increase of the total number of end groups, graft plus linear polymers, occurs. In contrast, a non-random scission in the vicinity of the connecting points of the side chains to the backbone results in a constant number of PLG end groups with time. ^{1}H-NMR analysis was used to monitor the structural changes of the bulk degradation products by comparison of the signal intensities of the PLG chain and end groups. HMW-PVA-*g*-PLGs showed a distinct lag time

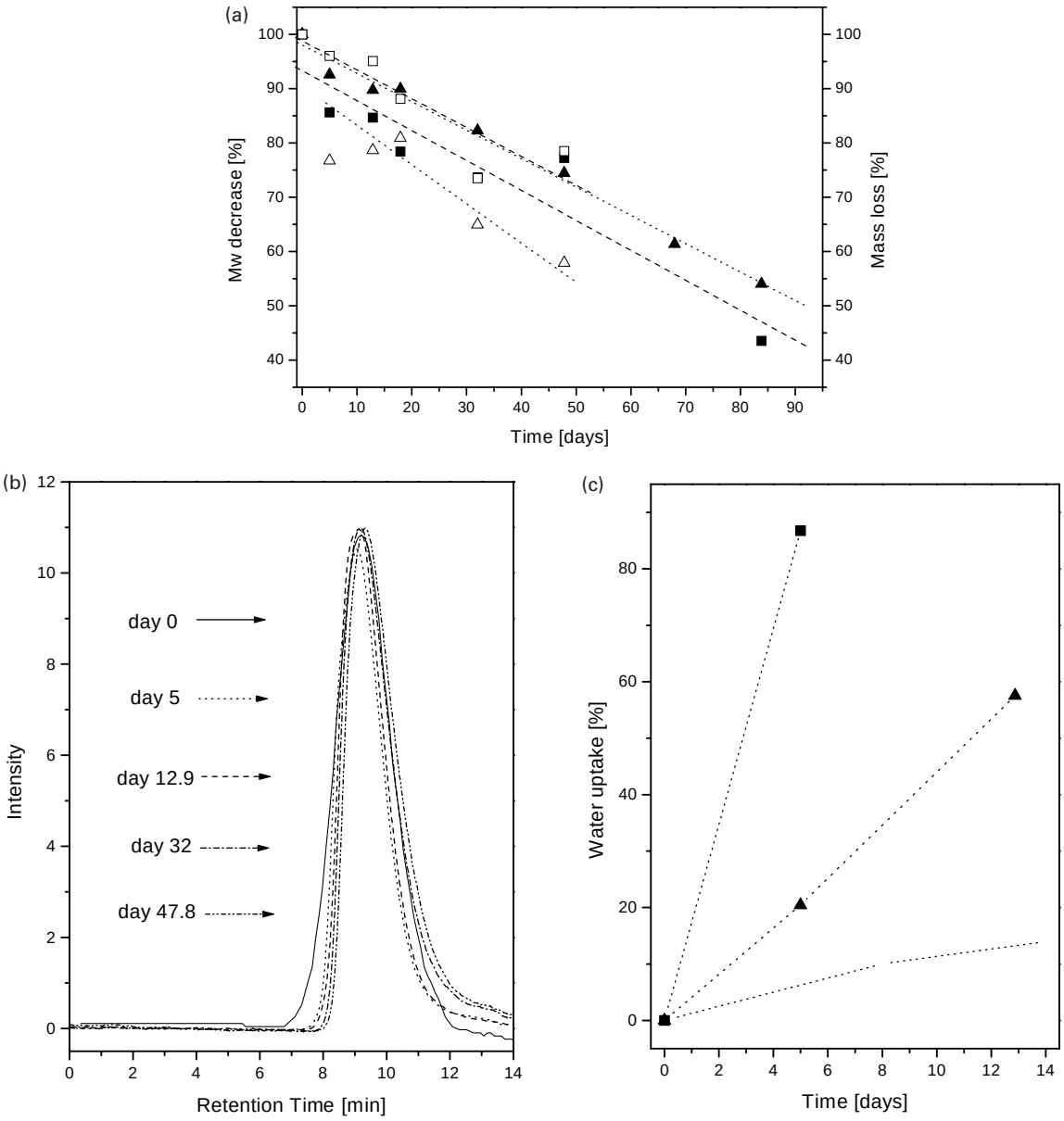

Fig. 3. (a) Mass loss (erosion, full symbols) and loss of molecular weight (degradation, open symbols) of LMW-PVA-*g*-PLGs, polymer 3 (Table 1) (square), polymer 4 (Table 1) (up triangle). (b) SEC traces of LMW-PVA-*g*-PLG (polymer 3, Table 1) over time. (c) Water uptake of LMW-PVA-*g*-PLGs compared to HMW-PVA-*g*-PLG, polymer 4 (Table 1) (square), polymer 3 (Table 1) (up triangle), polymer 1 (Table 1) (circle).

without visible changes, followed by an increase of the PLG (end:chain) groups ratio at later degradation stages (Fig. 4b). The absence of low molecular weight degradation products during the first weeks of polymer erosion, in combination with an exponential M_w decrease, seems to indicate a cleavage of long PLG chains directly at the PVA backbone. This degradation behavior of HMW-PVA-*g*-PLG deviates from the random chain scission, known for linear PLG. When samples had lost about 70% of their initial M_w, the increase of the ratio indicates that random chain scission begins to dominate the degradation pattern. By contrast, the PLG (end:chain) group ratio of LMW-PVA-*g*-PLG remained constant for more than 40 days under in-vitro conditions. Consequently, the short PLG chains are cleaved directly at the backbone and removed by dissolution in the buffer. This degradation mechanism differs from linear PLG, avoiding accumulation of acidic breakdown products in the device, which could be an advantage for drug delivery systems containing sensitive proteins.

3.3. Thermal properties and sample morphology

Thermo-chemical properties are an important factor for the degradation of polymers. In general, high glass transition temperatures (T_g) and/or high degrees of crystallinity reduce degradation rates of PLG. Branched PVA-*g*-PLG

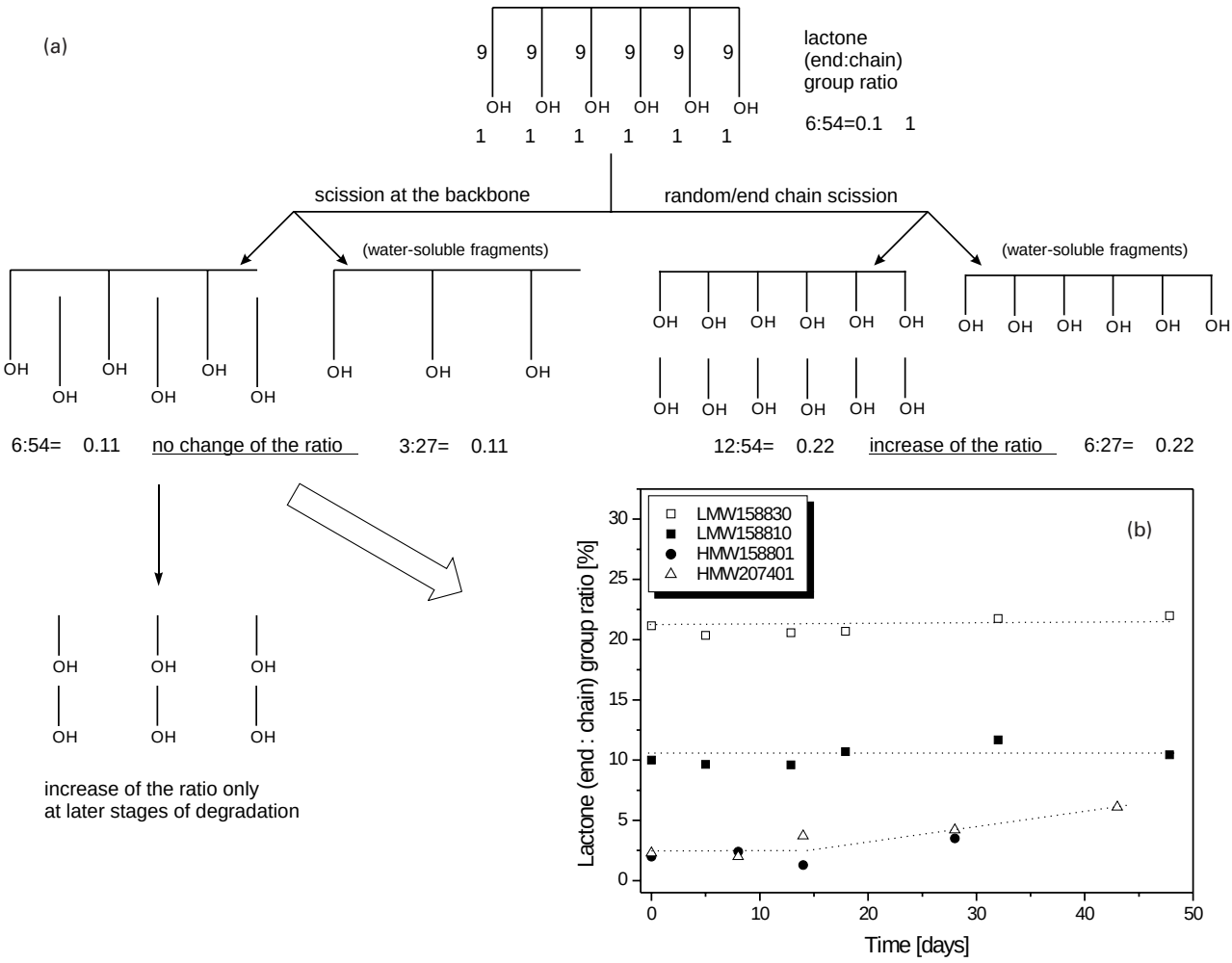

Fig. 4. (a) Possible chain scission mechanisms during degradation of PVA-*g*-PLGs. (b) Changes of the polymer structure with time as investigated by ^{1}H-NMR analysis indicating a non-random chain scission mechanism. HMW-PVA-*g*-PLGs—polymer 1 (Table 1) (full circle) and polymer 6 (Table 1) (open up triangle) vs. LMW-PVA-*g*-PLGs—polymer 3 (Table 1) (full square) and polymer 4 (Table 1) (open square).

and PVA-*g*-PLA have lower T_g as well as a reduced degree of crystallinity compared to linear polyesters [22]. A linear relation of PLG chain lengths and T_g was found, shorter chains led to decreasing values, which is in accordance with the free volume theory. With increasing thermal energy the chain ends will be able to rotate more readily than the rest of the chain. Consequently, the glass transition temperature is lowered. Using differential scanning calorimetry (DSC), the T_g of the degrading polymers in dry and hydrated state were investigated. The structure of the graft polyesters affected T_g of the dry samples during degradation and led to different decay rates of T_g for the HMW- and LMW-PVA-*g*-PLG. Fig. 5 outlines a fast and nearly constant T_g decrease of HMW-PVA-*g*-PLG films over the time, which is in accordance with the fast decay of M_w of this type of polymer and the increase of the number of more flexible end groups. In contrast, the T_g of the LMW-PVA-*g*-PLGs remained nearly unchanged over time. An explanation could be that in case of LMW-PVA-*g*-PLGs water-soluble breakdown products are immediately dissolved in the buffer and cannot cause an internal plasticizer effect. We observed only one T_g in all samples both in dry and hydrated state, suggesting that in PVA-*g*-PLG no phase separation occurred.

In the hydrated state T_g below 37°C were reached very rapidly. This means that under physiological conditions drug release and device degradation will occur in the rubbery state. While HMW-PVA-*g*-PLG (polymer 1, Table 1) exhibited a T_g of ca. 30°C after one day of incubation, only 15°C were determined for LMW-PVA-*g*-PLG (polymer 3, Table 1). In case of polymer 4 (Table 1) T_g was too low to be determined even after 30 min of incubation.

3.4. Morphology of PVA-g-PLG films

Visual inspection of PVA-*g*-PLG films incubated under in vitro conditions revealed changes of the morphology from a clear glassy to a white rubbery state as a function of polymer composition. While in the case of HMW-PVA-*g*-PLG samples this change was observed in the course of

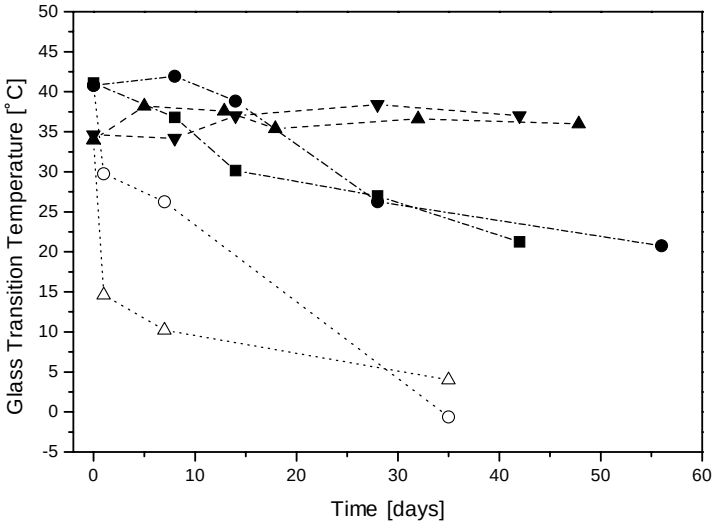

Fig. 5. Glass transition temperatures (T_gs) of the degraded films of the graft PLGs with incubation time; dry samples = full symbols, wet samples = open symbols. HMW-PVA-*g*-PLGs—polymer 6 (Table 1) (square) and polymer 1 (Table 1) (circle) vs. LMW-PVA-*g*-PLGs: polymer 3 (Table 1) (up triangle) and polymer 4 (Table 1) (down triangle).

several days, it occurred in a few hours with LMW-PVA-*g*-PLG. Morphological changes of the incubated films after freeze-drying were investigated by scanning electron microscopy (SEM). Fig. 6 shows a time-series of SEM photographs for HMW-PVA-*g*-PLG (polymer 1). No measurable changes in film thickness were found until the matrix was completely degraded. In the initially phase of degradation (Fig. 6a) pore development of different sizes

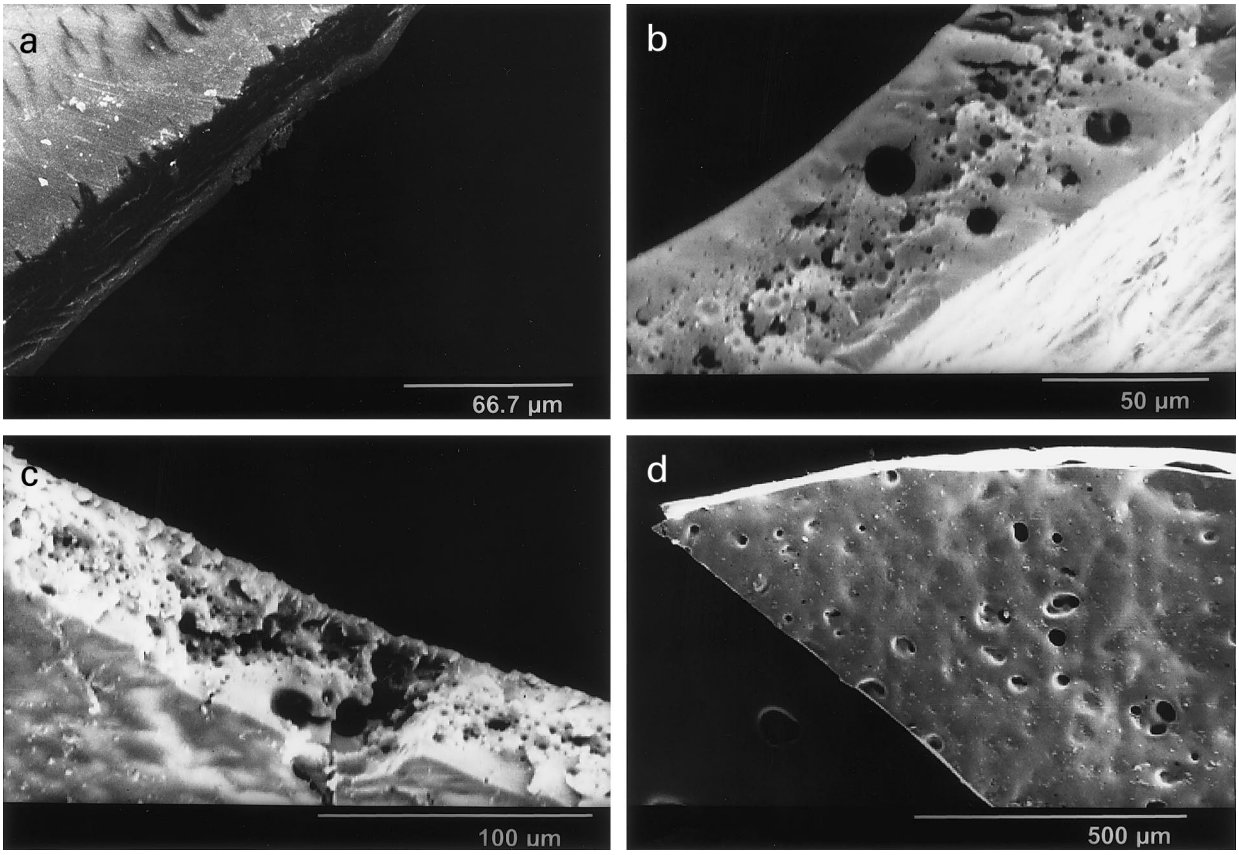

Fig. 6. Change of film morphology of HMW-PVA-*g*-PLG (polymer 1, Table 1) with time as investigated by SEM: (a) day 0; (b) day 5; (c) day 12.8; and (d) day 17.9.

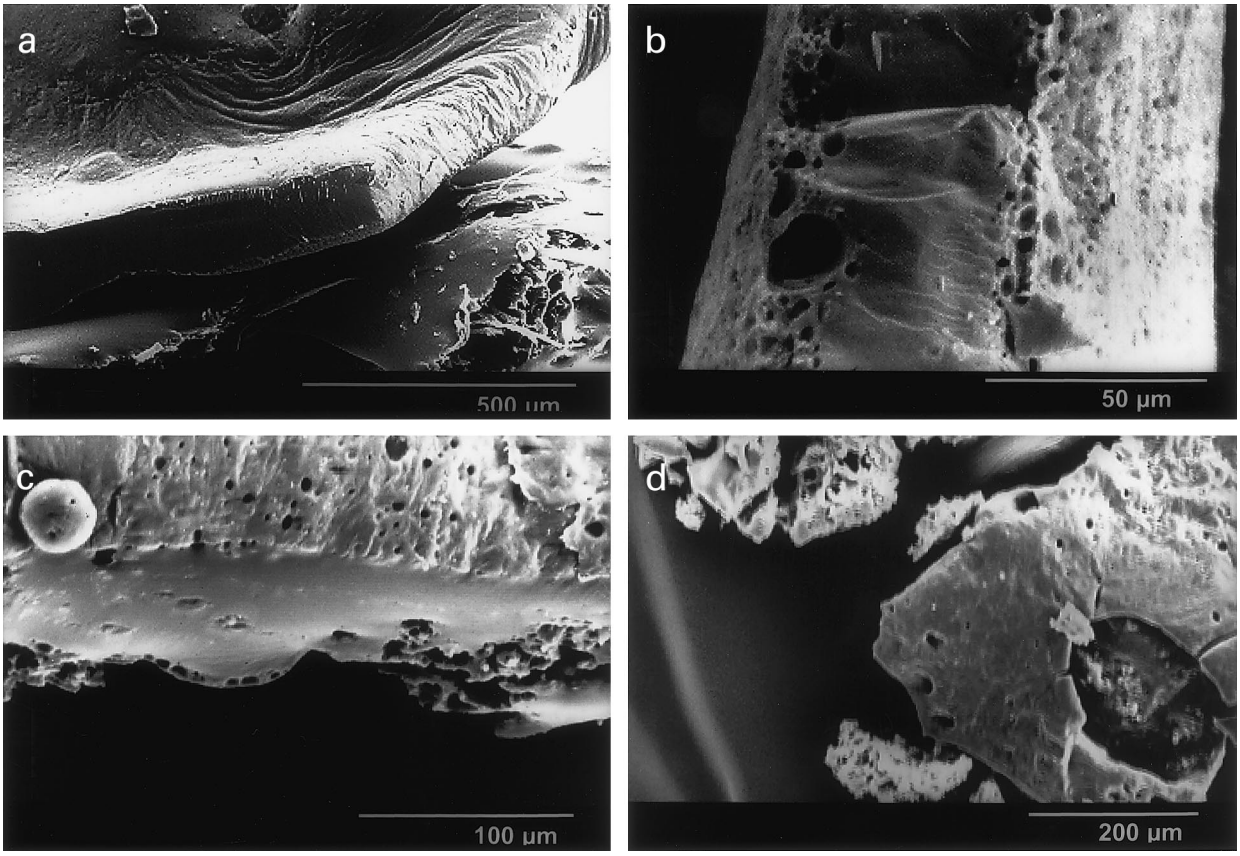

Fig. 7. Change of film morphology of LMW-PVA-*g*-PLG (polymer 5, Table 1) with time as investigated by SEM: (a) day 0; (b) day 5; (c) day 12; and (d) day 24.

and shapes was observed. The density of pores seemed to increase both with time and distance from the film surface (Fig. 6b after 5 days and Fig. 6c after 12.8 d), while the surface structure remained smooth. After ca. 18 days the surface layer was deteriorated and became rough and porous, as shown in Fig. 6d. This degradation pattern is compatible with a bulk degradation mechanism for the HMW-PVA-*g*-PLG, similar to PLG. The morphological changes in degrading film samples are in reasonable agreement with the observed erosion rates, for instance after ca. 2.5 weeks mass loss was detected (Fig. 2a).

By contrast, pore formation of LMW-PVA-*g*-PLG samples started from the matrix surface, moving into the center of the film, as outlined in Fig. 7. The initially smooth film surface (Fig. 7a) was found to be porous after 5 days (Fig. 7b). After 12 days (Fig. 7c) no major changes in the inside of the matrix occurred. Moreover, an irregularly shaped layer of eroded material around a core of intact polymer after more than 3 weeks of incubation (Fig. 7d) was observed. These data are compatible with a more surface front-like erosion mechanism, proposed for poly(anhydrides) [28,29].

3.5. Microsphere drug release

Graft PVA-*g*-PLGs with different chain lengths and different PVA M_w were used to prepare biodegradable microspheres containing fluorescently labeled dextran (FITC-Dex) as marker substance, to investigate the influence of polymer properties on drug release behavior. A modified W/O/W double emulsion method was employed to prepare microspheres at 4°C [12]. The microencapsulation with a theoretical drug loading of 1% (w/w) did not pose any technical problems. The encapsulation efficiency was in the range of 70–90% and yields were >60%. The average size was found to be ca. 10 µm for all polymers. FITC-dextran (M_w 40 000 g/mol), a fluorescent labeled hydrophilic polyglycan, is frequently used as a model compound for proteins. The typical biphasic release from linear PLG microspheres was found, as outlined in Fig. 8a. After an initial burst, the in-vitro release rate decreases to low values and recommences when the erosion of PLG becomes noticeable, leading to the characteristic sigmoid release profile. The in-vitro profiles of FITC-dextran from the PVA-*g*-PLGs were improved significantly (Fig. 8a). After small initial bursts, almost continuous release rates could be obtained. The same structure–property relationships found for the degradation and erosion behavior were also dominating the release of FITC-dextran from biodegradable PVA-*g*-PLG microspheres. At constant M_w of the PVA backbone, shorter PLG chain lengths resulted in a substantial increase of the release rates of the marker.

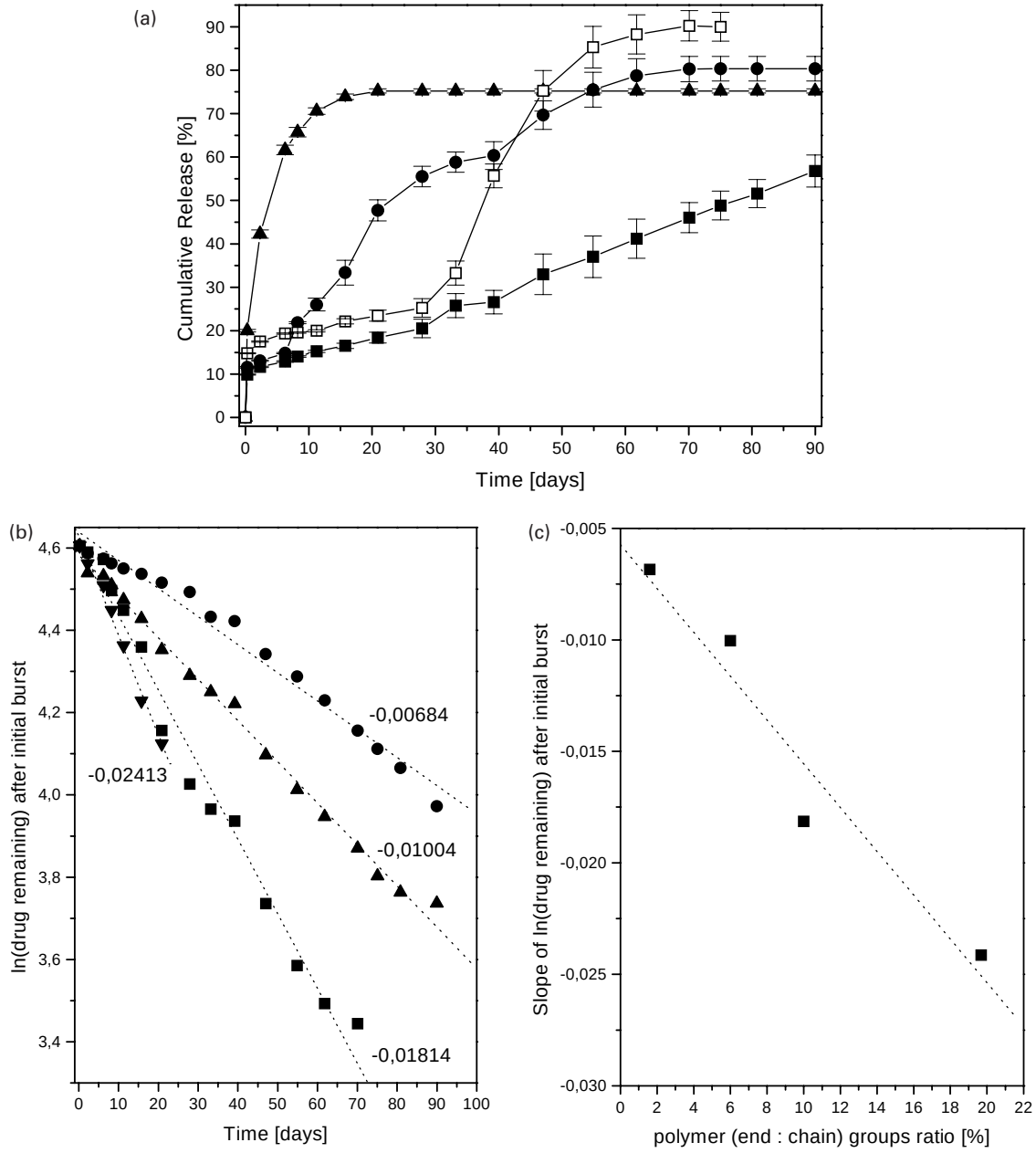

Fig. 8. (a) Cumulative release of FITC-dextran from microspheres prepared from linear PLG (polymer 8, Table 1) (open square), HMW-PVA-*g*-PLG (polymer 1, Table 1) (full square), LMW-PVA-*g*-PLG with 15 kg/mol backbone (polymer 3, Table 1) (full circle), LMW-PVA-*g*-PLG with 20 kg/mol backbone (polymer 6, Table 1) (full up triangle). (b) FITC-dextran release rates from microspheres as a function of PLG chain lengths, polymer 1 (Table 1) (circle), polymer 2 (Table 1) (up triangle), polymer 3 (Table 1) (square), polymer 4 (Table 1) (down triangle); and (c) slopes of the microsphere release rates (b) as a function of PLG chain lengths.

Whereas, a change of the ratio of hydrophobic PLG to hydrophilic PVA backbone at comparable PLG side-chain lengths resulted in a change from erosion controlled release to a pore diffusion type release with increasing M_w of the PVA backbone. In case of a 15 000 g/mol PVA backbone the maximum rate was observed after ca. 2 weeks, the polymer with a 20 000 g/mol PVA had already released nearly 80% of FITC-dextran at this time. These profiles can be explained by different hydrophilicity of the PVA-*g*-PLG. As investigated by inverse gated decoupling ^{13}C NMR ca. 50% of the hydroxyl groups of the 15 000 g/mol backbone were esterified, while in case of the 20 000 g/mol PVA, nearly 75% free OH groups were found, allowing fast hydration and pore formation. A nearly linear relation of dextran release and polymer hydrophilicity was found, as outlined in Fig. 8c, where the slope of the logarithmic rates of the drug remaining in the microspheres over time after the initial bursts (Fig. 8b) were plotted against the polymer composition as determined by ^{1}H-NMR. Shorter PLG chains led to increasing release rates of FITC-dextran. The

release was therefore directly related to the PLG chain lengths and polymer hydrophilicity, respectively. Obviously the drug release rates from microspheres differed to some extent from the film erosion rates. The surface front erosion mechanism generates a layer of partially eroded polymer around a core of intact polymer, influencing the diffusion of molecules. Moreover, this type of erosion mechanism is size dependent [28,29], which may lead to differences in film and microsphere erosion.

The structure modifications of the novel polyester PVA-*g*-PLG should allow the design of biodegradable parenteral drug delivery systems with release periods covering the range from ca. 30 to 90 days. The potential to switch the degradation mechanism from bulk to surface erosion is a feature of particular interest especially for the release of hydrophilic macromolecular drugs. Therefore, branched PVA-*g*-PLG could be of useful for parenteral delivery systems of proteins.

4. Conclusions

Manipulation of both the three-dimensional structure and the hydrophilicity of polyesters by grafting PLG chains onto PVA as backbone resulted in biomaterials with attractive properties, especially for the controlled delivery of hydrophilic proteins and peptides. It was possible to change the degradation and erosion profiles by parameters, such as PLG chain lengths and composition as well as PVA molecular weight in a systematic manner. The degradation mechanism can be switched from bulk to a surface front erosion behavior.

The transition from bulk to surface erosion seems to be mainly influenced by the PLG chain lengths. Grafting water-insoluble side chains onto PVA resulted in high molecular weight polymers exhibiting bulk erosion, while polymers bearing water-soluble PLG chains seem to erode by a different mechanism. The faster rates, accompanied by high water contents and porous structures allowed the design of drug delivery systems with almost zero-order kinetics covering the range from several weeks to several months. PVA-*g*-PLG are therefore potentially useful for parenteral drug delivery systems.

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