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RESEARCH ARTICLE

Modifying the Release of Gentamicin from Microparticles Using a PLGA Blend

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ABSTRACT

Carrier systems for local gentamicin (GS) treatment based on collagen sponges and polymethylmethacrylate beads show pharmacokinetic disadvantages in their GS-release profiles. Therefore, poly(lactic-co-glycolic acid) (PLGA) microparticles were devised. None of the five poly(α -hydroxy acid)s tested resulted in the desired antibiotic release over approximately one week. However, preparing microparticles from a 50/50 blend of Resomer[®] RG 502H, an uncapped variety, and Resomer RG 503, an endcapped polymer, yielded the targeted liberation profile. The mechanism of GS release was investigated by analyzing water uptake and polymer molecular weight. Release of GS from RG 502H particles occurred instantaneously and coincided with substantial water penetration. Particles prepared from RG 503 started out at a higher molecular weight and since the endcapped polymer takes up less water, the decrease in molecular weight was delayed. The threshold of collapse was reached after two weeks, which coincided with water penetration and GS release. For the 50/50 RG 502H/RG 503 blend, this process was delayed for two to three days. Hydrolysis occurred at the same rate as for RG 502H due to the high water content as a consequence of the uncapped polymer fraction and renders GS release over one week with release limited to 30% in the first two days due to the endcapped polymer fraction of higher molecular weight. Thus, the mixture of endcapped and uncapped Resomer exhibits a new quality for adjusting drug release from poly(α -hydroxy acid)s.

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Key Words: *Gentamicin; Microparticles; Molecular weight; Poly(lactic-co-glycolic acid); Water content*

INTRODUCTION

Despite a reduction in the risk of contamination due to improved materials, implant, and clean room techniques as well as preoperative antibiotic prophylaxis, infections still remain a feared complication in orthopedic and trauma surgery (1). The Polymethylmethacrylate (PMMA) beads loaded with gentamicin (GS) have been used successfully in the local treatment of soft tissue and bone infections (2,3). However, this device has to be removed in a second procedure posing the risk of additional pain, anaesthetic complications, and incurring extra costs. In vivo, PMMA beads slowly liberate GS resulting in exudate levels of approximately 30 mg/L within the first day postoperatively and provide local concentrations of approximately 5 mg/L for several weeks (4). These levels are well above the concentrations necessary to inhibit sensitive and moderately sensitive etiologically important pathogens, whereas resistant microbes [minimal inhibitory concentration (MIC) > 4 mg/L] may not be affected (5). The efficiency in tissue approximately 1–2 mm away from the implant, where the concentration will be much lower, is still unclear (6). Following implantation of PMMA beads, recurrence of infection most likely occurs within the first two weeks after surgery (7). A commercial collagen sponge loaded with GS was specifically designed as an adjuvant therapy for the treatment of bone and soft tissue infections (8). The biodegradable collagen sponge carrier should also enhance the postoperative tissue regeneration and eliminate the need of surgical removal. In vitro release tests under sink-conditions revealed that GS liberation is delayed only for a few minutes by the collagen carrier system (9). The maximum concentration of GS at the implant site is several times higher with the collagen devices as compared to the concentrations achieved with PMMA beads (10,11). However, the antibiotic levels decrease much faster, and in addition, the local blood supply affects the pharmacokinetics after application of the GS loaded sponge (5,12). In secluded areas with reduced blood flow, such as in cavities or bone, release and clearance of GS occurs slower than at well-perfused sites, where higher values for c_{\max} at earlier t_{\max} but shorter half-life are found. For example, the wound fluid concentrations after surgical revision of groin wound vascular graft infection decreased below the MIC for most

bacteria within 2–3 days and the MIC for resistant microbes within less than 24 hr (5,11).

The release of GS could be further sustained when used as a salt with reduced solubility (13) or if the collagen sponge containing the antibiotic is additionally coated with resorbable poly(α -hydroxy acid) (14). Septacin[®] is an implant currently under development based on a polyanhydride matrix with GS release over several weeks (15). Implants based solely on poly(α -hydroxy acid)s have been developed, which deliver GS over several weeks (16–19). Furthermore, particles prepared by drying and subsequent grinding from a poly(lactic acid) (PLA) solution with GS suspended exhibited a two-phase release of a high-initial burst followed by liberation for more than two months (20,21). As an alternative, the addition of anionic polymers such as alginic acid or pectin was described, which could result in aminoglycoside antibiotic derivatives with low solubility (22).

According to Ipsen and Wachol-Drewek, a delivery system that provides higher local concentrations of GS for approximately 7–10 days would be desirable (23,24). Thus, the goal of this study was a biodegradable local delivery system with controlled release of GS for approximately one week. This was to be achieved by GS loaded PLA or poly(lactic-co-glycolic acid) (PLGA) microparticles locally delivering 200 mg GS per dose corresponding to the GS content of the commercial collagen sponge (Sulmycin[®] implant). The microparticles should be spread onto the open wound during surgery or injected into the infection site. As an alternative, the spheres could be further processed to be embedded into a porous carrier system such as a collagen sponge and the resulting composite may be implanted in the course of an operative procedure.

MATERIALS AND METHODS

Materials

Polymers used were poly(D,L-lactic acid) Resomer R 104 lot 250972 (R 104), Resomer R 202 lot 15027 (R 202), Resomer R 202 H lot 34038 (R 202H), poly(D,L-lactic-co-glycolic acid 50:50) Resomer RG 502H lot 270604 (RG 502 H), and Resomer RG 503 lots 34032, 211824, 211794, and 223808 (RG 503) (Table 1). All polymers were provided by Boehringer Ingelheim, Ingelheim, Germany,

Table 1

Molecular Weight Weight-Average (M_w), Molecular Weight Number-Average (M_n), Polydispersity Index (pi), and Glass Transition Temperature (T_g) of Polymers Used

Polymer	M_w [Da]	M_n [Da]	pi	T_g [°C]
Resomer [®] R 104	6,200	3,800	1.6	27
Resomer [®] R 202	16,200	7,200	2.3	44
Resomer [®] R 202 H ^a	12,900	7,200	1.8	47
Resomer [®] RG 502 H ^a	13,500	6,700	2.0	46
Resomer [®] RG 503	36,200	16,300	2.2	48

^aUncapped.

and stored desiccated and protected from light at 2–8°C. Gentamicin sulfate was obtained from Innocoll GmbH, Saal/Donau, Germany, and throughout this article the term GS describes the sulfate form.

Methods

Preparation of Microparticles

Microparticles were prepared via solvent evaporation technique. Sonication of a vortexed mixture (Vortex-Genie 2, Model G-560 E, Scientific Industries, Bohemia, NY) of a polymer solution in methylene chloride CH_2Cl_2 (Roth, Karlsruhe, Germany) and an aqueous GS preparation cooled on ice was performed using a Branson Sonifier[®] model B-15 equipped with a 3-mm diameter microtip (Heinemann, Schwäbisch Gmünd, Germany). A measured volume of 2 mL of 1% PVA (100.000 D; Fluka, Buchs, Switzerland) solution was added to the primary emulsion under vortexing, and after 20 sec, the mixture was added to 50 or 100 mL of a 0.3 or 1% PVA solution. The system was stirred at 500 rpm [magnetic stirrer Variomag Multipoint HP6 (H&P Labortechnik GmbH, München, Germany)] for solvent evaporation and hardening. The particles were allowed to settle, washed three times with 10 mL water for 10 min, and were subsequently lyophilized [placed on pre-cooled shelf of –45°C, frozen for 4 hr, dried for 10 hr at –45°C/0.12 mbar, heated to 20°C/0.12 mbar within 3 hr, and dried for 3 hr at 20°C/0.12 mbar using a Christ Model Delta Lyophilizer (Christ, Osterode, Germany)]. Microparticles were stored desiccated and protected from light at 20°C.

Drug Incorporation

Microparticles of 50–100 mg were dissolved in 10 mL CH_2Cl_2 . The GS was extracted five times with

10 mL water each. The extracts were collected and the volume adjusted to 100 mL. The GS was quantified via absorbance after derivatization with *o*-phthaldialdehyde (Fluka) and thioglycolic acid (Fluka, Buchs, Switzerland) (25). Samples of volume 500 μ L were mixed with 300 μ L isopropanol and 200 μ L labeling reagent (50 mg *o*-phthaldialdehyde, 50 μ L thioglycolic acid, 1.25 mL methanol, and 11.2 mL 0.4 M borate buffer pH 9.5). After incubation for 45 min at room temperature, samples were analyzed at 332 nm.

Release Studies

Microparticles of 100 mg were incubated in 5 mL phosphate buffered saline (PBS) containing 0.2% sodium azide (Merck, Darmstadt, Germany) at 37°C in a shaking bath at 50 strokes per min. Tests were performed with three replicates. Different PBS pH 7.4 formulations described in the literature and pharmacopoeias were tested (PBS 1: 5.2 g K_2HPO_4 /1.415 g KH_2PO_4 per liter; PBS 2: 3.4 g NaH_2PO_4 /16.0 g Na_2HPO_4 per liter; PBS 3: 8.0 g NaCl/0.2 g KCl/1.15 g Na_2HPO_4 /0.1 g KH_2PO_4 per liter; PBS 4: 0.94 g Na_2HPO_4 /0.19 g KH_2PO_4 /8.0 g NaCl per liter; PBS 5: 6.8 g KH_2PO_4 /1.57 g NaOH per liter). The PBS 1 was used if not stated otherwise. At designated time points, 3 mL of the release medium was exchanged and the GS concentration was determined as described above.

Particle Size Analysis

For particle size analysis, microparticles were redispersed in 20 mL water containing 0.2 g Tween 80 and within 5 min at 22°C, the mean volume diameter was measured by laser diffractometry using a Coulter LS 230 (Coulter Electronics, Krefeld, Germany) and analyzed based on the Fraunhofer model.

Viscometry

The viscosity of polymer solutions was determined with capillary viscosimeters type 51630 I, II, and III (Schott, Hofheim, Germany).

Water Uptake Studies

Microparticles of 100 mg were incubated in PBS at 37°C and at designated time points, particles were removed and vacuum dried for 24 hr. Drug-free microparticles were used, in order to avoid secondary effects due to incorporated drug. The water content (%) was calculated as $100(m_0 - m_1)/m_1$, with m_0 the mass after removal of excess water on a filter paper and m_1 the mass after additional drying.

Molecular Weight Analysis

Polymer molecular weight was determined using a Phenogel linear mixed 50×7.80 mm guard column and a Phenogel $5 \mu\text{m}$ mixed MXL 300×7.80 mm column (Phenomenex, Hösbach, Germany) on a Perkin Elmer system (Perkin Elmer, Überlingen, Germany) consisting of a series 200 pump and an autosampler ISS-200 equipped with an RI detector ERC 7515A (ERC, Alteglofsheim, Germany). For analysis of M_w , M_n , and PI , TURBOCHROM-software 3.11 and a SEC software from Perkin Elmer was utilized, calibrated with polystyrene standards (Polymer Standard Service, Mainz, Germany) in the range 484–125,000 Da. Microparticles of 50 mg were dissolved in 5 mL tetrahydrofuran (THF), $100 \mu\text{L}$ injected, and eluted with THF at 1 mL/min.

Residual Solvent Analysis

Microparticles of 50–100 mg were dissolved in 2 mL DMSO (Fluka) and analyzed with a GC 8500 (5 min at 40°C and heated to 250°C at $25^\circ\text{C}/\text{min}$, p_{He} : 70 kPa) (Perkin Elmer) equipped with a headspace sampler (120°C sample temperature, 15-min cycle, 5-min wait, 5-min pressure adjustment, 0.1-min injection time, 0.2-min wait, p_{He} : 80 kPa), and an OPTIMA-1 column of $25 \text{ m} \times 0.32 \text{ mm} \times 0.25 \mu\text{m}$ (Macherey & Nagel, Düren, Germany) and a flame ionization detector (FID) (PerkinElmer, Shelton, CA). Calibration was performed with CH_2Cl_2 in DMSO.

RESULTS AND DISCUSSION

Gentamicin Loading

At first, an appropriate processing technology for preparation of PLA and PLGA microparticles had to be evaluated. An important criterion, besides the release profile was an adequate incorporation rate, since the high dose of 200 mg hydrophilic GS should be delivered corresponding to the commercial 10×10 cm collagen sponge. An elevated GS loading of the microparticles helps to lower the concentration of acidic degradation products from poly(α -hydroxy acid)s, which may induce irritative effects (26), facilitates embedding into a porous carrier, and reduces costs. On the basis of the W/O/W-double emulsion technique, variations of different manufacturing parameters were tested in order to optimize the process (Table 2), and the resulting microparticles were evaluated by macroscopic appearance and GS incorporation. The RG 503 was used as a model polymer for this process development. The results of these studies indicated that:

- With increasing volume of the inner water phase, the loading efficiency decreased, as drug diffusion into the outer water phase during the second emulsification process is accelerated (27) and the microparticles showed higher porosity.
- Higher amounts of GS dissolved in the inner water phase caused the absolute drug loading to increase, whereas the incorporation efficiency slightly decreased as frequently described in the literature (27–29).

Table 2

Process Parameters Tested for Preparation of Poly(Lactic Acid) and Poly(Lactic-co-glycolic acid) Microparticles Loaded with Gentamicin via Double-Emulsion Technique (W_1 : Inner Water Phase, O : CH_2Cl_2 Phase, W_2 : Outer Water Phase)

Process Parameters	Variations
W_1 volume (μL)	200 ^a , 400, 600, 800
GM quantity in W_1 (absolute target load of microparticles) (mg)	30 (9%), 60 (17%), 90 (23%), 120 (29%) ^a
Use of gentamicin	Gentamicin base dissolved in W_1 Gentamicin sulfate dissolved in W_1 ^a Gentamicin sulfate suspended in O
Stirring time (min)	10, 30, 60 ^a , 120
Amount of polymer (mg)	300 ^a , 500
O volume (mL)	0.33, 0.66 ^a , 1 ^a , 5
W_2 volume (mL)	50, 100 ^a
PVA concentration in W_2 (%)	0.3 ^a , 1
Sonication time (sec)	60, 90 ^a , 120

^a Selected.

- Incorporation of GS-free base in solution was less, compared to the sulfate salt dissolved in the inner water phase and may be related to higher solubility of the free base in CH₂Cl₂ (38 mg/mL) as compared to GS sulfate (18 mg/mL) (30). Suspending GS sulfate in the organic polymer solution resulted in particles of irregular shape.
- Stirring time of 10 or 30 min was inadequate for hardening of the particles and additionally led to a high initial burst in GS release tests.
- The CH₂Cl₂ volume was limited at the lower end as the high viscosity of the polymer solution hindered homogeneous emulsification in the outer water phase. High amounts of organic solvent resulted in reduced incorporation rates and in inadequate microparticle formation.
- At 1% PVA in the outer water phase, residual fibers of the stabilizer after washing led to the aggregation of the final microparticles. Hence 0.3% was selected.
- Variation of the outer water phase volume, the polymer amount (based on a constant polymer:GS ratio), and the sonication time were without major effects with respect to GS incorporation and particle appearance.

Thus, the studies demonstrated that the volume of the inner water phase and the organic phase were the critical factors within the microparticle manufacturing process. The conditions optimized for RG 503 were used to

evaluate the different PLA and PLGA polymers for preparation of microparticles loaded with GS.

Gentamicin Loaded Microparticles from Different Poly(Lactic Acid) and Poly(Lactic-co-glycolic Acid) Types

The rate of poly(α-hydroxy acid) breakdown is critically dependent on the polymer properties and greatly influences the release of encapsulated drug. Therefore, amorphous polymers of low-molecular weight were selected, since they exhibit rapid resorption in vivo within weeks or months as compared to crystalline materials (31,32). Both PLA homopolymers and faster degrading PLGA copolymers as well as different functionalities at the end of the polymer chain were considered. Polymerization of the regular Resomer types is terminated by addition of long chain alcohol, resulting in a hydrophobic ester endcap. As an alternative, the reaction can be stopped by admixture of lactic acid leading to hydrophilic free carboxyl groups at the terminal position (Resomer H-types), thus higher water uptake, stronger swelling, and consequently faster degradation of these uncapped polymers (33).

The microparticle manufacturing method has been optimized initially using RG 503. As the studies had indicated the critical role of the CH₂Cl₂ amount on the GS incorporation rate, 1 and 0.66 mL CH₂Cl₂ were tested. For all polymers, both drug incorporation and microparticle size were higher at 0.66 mL as compared to 1 mL organic phase

Table 3

Gentamicin Loading, Particle Size, and Viscosity of Polymer Solution for Microparticle Preparation from R 104, R 202, R 202H, RG 502H, RG 503, and 50/50 RG 502H/RG 503 Using 1 or 0.66 mL Methylene Chloride and Residual CH₂Cl₂ Content After 30 min Stirring, 60 min Stirring, and After the Final Lyophilization Using 0.66 mL Methylene Chloride

	R 104	R 202	R 202H	RG 502H	RG 503	50/50 RG 502H/RG 503
GM loading [%]						
1 mL CH ₂ Cl ₂	8.9 ± 2.3	9.8 ± 1.2	7.2 ± 1.1	10.1 ± 1.4	11.2 ± 1.6	—
0.66 mL CH ₂ Cl ₂	10.2 ± 2.0	15.7 ± 4.9	14.0 ± 4.2	12.8 ± 3.8	18.5 ± 1.5	15 ± 3
Diameter [μm]						
1 mL CH ₂ Cl ₂	111 ± 45	213 ± 106	257 ± 78	222 ± 91	267 ± 79	—
0.66 mL CH ₂ Cl ₂	155 ± 71	403 ± 118	486 ± 195	266 ± 100	553 ± 207	367 ± 130
Viscosity [mPa]						
1 mL CH ₂ Cl ₂	2.1 ± 0.1	6.9 ± 0.4	5.3 ± 0.3	5.7 ± 0.5	33.5 ± 1.0	—
0.66 mL CH ₂ Cl ₂	4.4 ± 0.5	13.2 ± 0.7	11.8 ± 0.9	27.4 ± 1.8	181 ± 14	70 ± 1
CH ₂ Cl ₂ [ppm]						
30 min stirring				170	282	290
60 min stirring				75	83	120
Dry particles				2	20	21

volume (Table 3). This effect could be attributed to accelerated hardening, decreased diffusivity through the organic phase, or reduced local demulsification of the primary oil-in-water emulsion (34–36). The GS loading was less influenced by the type of polymer used, ranging between 7 and 11% at 1 mL and between 10 and 20% at 0.66 mL CH_2Cl_2 , despite the marked difference in viscosity.

Further testing was performed on particles prepared using 0.66 mL CH_2Cl_2 because of their advantageous

higher GS incorporation rates. Release studies indicated that antibiotic liberation from R 104 particles was almost completed within 4 hr (Fig. 1a). Such rapid release could be caused by dissolution of drug bound at the surface (37). In case of R 104, the polymer is characterized by a low T_g of 27°C, which is even further reduced by the plasticizer effect of penetrating water. Upon incubation at 37°C, these particles collapsed and agglomeration of the particles to an unstructured mass

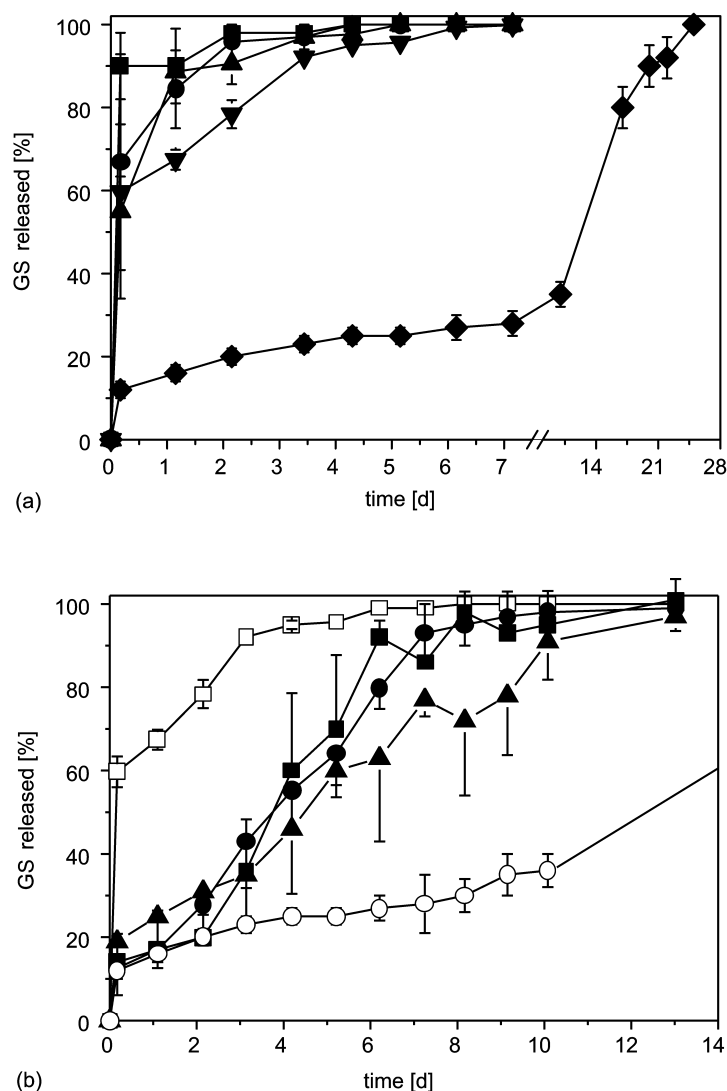


Figure 1. (a) The GS release from microparticles prepared from Resomer R 104 (■), R 202 (●), R 202H (▲), RG 502H (▼), RG 503 (◆). (b) The GS release from microparticles prepared from RG 502H/RG 503 blends 100/0 (□), 70/30 (■), 50/50 (●), 30/70 (▲), 0/100 (○). (c) The GS release from microparticles prepared from 50/50 RG 502H/RG 503 with different RG 503 lots [lot 34032 (■), lot 211794 (●), lot 211824 (▲), lot 223808 (▼)]. (d) The GS release from microparticles prepared from 50/50 RG 502H/RG 503 with RG 503 lot 34032 tested in different PBS formulations [buffer 1 (■), buffer 2 (●), buffer 3 (▲), buffer 4 (▼), buffer 5 (◆)].

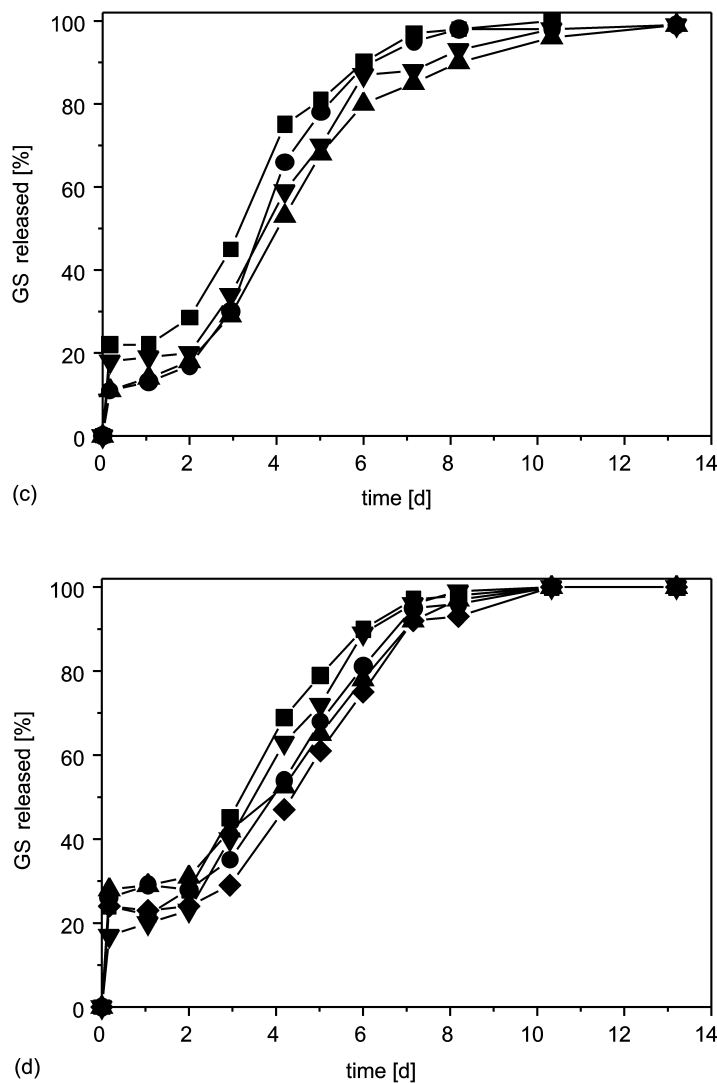


Figure 1. Continued.

was macroscopically observed after 4 hr. This led to a rapid release of the GS load. The burst of GS from R 202, R 202H, and RG 502H particles was less pronounced. The remaining GS was liberated within one day in case of R 202 and R 202H and approximately four days for RG 502H particles. Again, these particles collapsed in the course of the release study. In contrast, particles prepared from RG 503 released only 10% within 4 hr and until day 10 only another 10% GS was liberated. After this lag phase, substantial GS release set in and was completed after approximately 4 weeks. The S-shape profile reflects the bulk erosion process of the polymer. Only marginal diffusional drug liberation

occurs, until, with ongoing polymer hydrolysis, the molecular weight of the poly(α -hydroxy acid) reaches a critical value (38,39). At this point, erosion of the polymer manifests itself in substantial drug release. Hence, none of the microparticle formulations prepared from the five poly(α -hydroxy acid)s tested showed the targeted release profile of GS over one week.

Gentamicin Loaded Microparticles from RG 502H/RG 503 Blends

In order to fine tune drug release from microparticles, various approaches have been investigated, such as

preparation of coated particles (40,41), the use of copolymers (42,43), combinations of different PLA and PLGA types (44–46), or soluble additives (19,47). Since the desired release of GS over one week represents a profile between the time courses of antibiotic liberation achieved with RG 502H and RG 503 particles, blends of the two polymers were tested. Release studies of microparticles prepared from RG 502H/RG 503 blends at different polymer ratios demonstrated that admixing RG 503 to RG 502H reduced the initial drug release within 4 hr to approximately 10–15% (Fig. 1b). Microparticles prepared at 70/30, 50/50, and 30/70 ratios resulted in a lag phase of one to two days, followed by GS release until day 7 to 10. As described for other poly(α -hydroxy acid) mixtures, an intermediate release profile between the ones from the individual polymers was achieved (46,48). For microparticles prepared from different lots of RG 503 Scanning Electron Microscopy (SEM) demonstrated similar size and shape. All preparations presented an initial GS liberation between 10 and 25%, a plateau phase of one to two days, and sustained release of the remaining GS within 7–10 days (Fig. 1c). In addition, liberation of GS from microparticles was tested in different PBS formulations pH 7.4 with varying ionic strength and buffer capacity as compared to the standard PBS used throughout the article, since manifold setups and incubation media for release testing are used in the literature (49,50). Reducing ionic strength has been shown to increase the cell surface binding of GS (51) and to enhance binding to negatively charged molecules (52). However, pH does not critically affect GS solubility (30) as described to manipulate release of peptides or proteins from poly(α -hydroxy acid) microparticles (50,53), but the buffer capacity may influence the local pH inside the microparticles affecting the polymer degradation process. The study demonstrated similar release patterns (Fig. 1d) with differences of less than 20% in the amount of GS release at all time points, except for day 4 when the spread reached 35% at the maximum slope, which indicates only minor effects of ionic strength or buffer capacity and comparability of the liberation data to literature results.

Analysis of particle size, GS loading, and viscosity of the polymer solution showed that the values for the 50/50 blend represented intermediate stages between the results for the individual polymers as well (Table 3). In addition, the residual CH_2Cl_2 content was quantified as CH_2Cl_2 and represents a class II solvent limited to 600 ppm in pharmaceutical preparations if the daily dose is not known or fixed (54). Application of an appropriate drying procedure as well as extended times of stirring and increased temperature during microparticle manu-

facturing affecting the permeability of the air/liquid interface contribute to stay within the solvent limit (55–58). The amount of residual CH_2Cl_2 decreased with hardening time reaching 170–290 ppm after 30 min and was reduced by another 50–70% after 60 min (Table 3). Post lyophilization only 2–21 ppm were found in the microparticles. At all stages, the lowest CH_2Cl_2 levels were detected for RG 502H microparticles, which show the smallest diameter and consequently largest surface area as well as higher diffusivity in the polymer phase due to the lowest molecular weight, viscosity, and T_g . For RG 503 and 50/50 RG 502H/RG 503 similar levels were obtained. All types of microparticles did meet the pharmacopoeial requirements with respect to residual CH_2Cl_2 on the small laboratory manufacturing scale.

Water Uptake

Drug release from poly(α -hydroxy acid) systems can be related to initial dissolution from the surface, diffusion through pores, through the polymer, or the swollen polymer phase as well as to liberation in the course of polymer erosion. In order to elucidate the importance of these different aspects on GS delivery, at first, water uptake by the microparticles upon incubation in PBS was investigated. Penetration of incubation medium leads to diffusion of drug through the swollen hydrated polymer structures and creates the basis for bulk hydrolysis of the polymer. The delayed GS release from RG 503 microparticles was connected to limited water uptake by the polymer (59). During the first hours (release of 10% of the GS load), these spheres incorporated approximately 20% water (Fig. 2), which stayed consistent until day 6. After 10 days, substantial antibiotic release set in (Fig. 1b) and at the same time additional liquid penetrated into the microparticles (Fig. 2). The water content increased to 50% in the second week and up to 120 and 150% after three and four weeks, respectively. At this time point, the particles collapsed and the glass transition temperature markedly decreased (60). In contrast, approximately 150% incubation medium penetrated into particles prepared from RG 502H within the first day. This polymer is more hydrophilic due to its uncapped chains and in addition, water uptake and swelling become more pronounced with decreasing molecular weight of PLGA (48,61). During this same period, 60–70% of the GS load was released (Fig. 1b) and the particles show progressing deformation as the initial glass transition temperature is below 27°C in the hydrated state (60). The water content remained at that high level until day 3 (when 95% of the

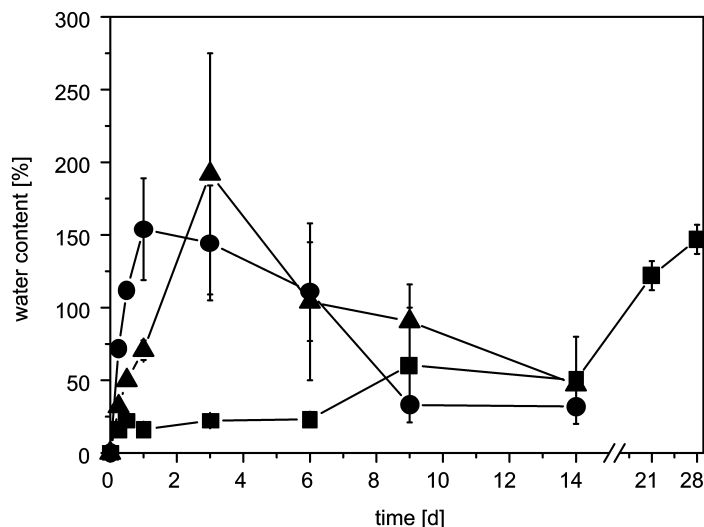


Figure 2. Water content of microparticles prepared from RG 503 (■), RG 502H (●), and 50/50 RG 502H/RG 503 (▲) during incubation in PBS.

GS load was released) and subsequently decreased to 30% at day 9 as the particles form an unstructured mass. Water uptake into particles prepared from the polymer blend steadily increased up to 190% at day 3. At that time point also the substantial liberation of GS set in (Fig. 1b). For one week, the water content stayed above 100% and during this period the antibiotic was almost completely released. Thus, RG 502H and RG 503 exhibited substantial differences in their water penetration and swelling behavior. The phase of high-water content correlated with the time window of GS release from the microparticles, which suggested that drug liberation occurred for the most part from the hydrated polymer structures.

Molecular Weight

Drug release and morphological changes of poly(α -hydroxy acid) carriers correlate with the molecular weight of the polymer, with 15,000 Da representing a critical value for PLGA material at which erosion initiates, as the threshold level of water solubility of the oligomeric breakdown products is reached (62,63). As shown in Fig. 3a and d, after a lag time of three days, the M_w of RG 503, initially 36,200 Da, decreased almost linearly over time (1150 Da per day). There were no signs in the polydispersity index for development of a bimodal distribution, which can occur for semi-crystalline poly(α -hydroxy acids), as crystalline regions degrade slower (64) and for amorphous PLA and

PLGA polymers when hydrolysis in the core of a device is accelerated by autocatalysis due to accumulation of carboxylic groups resulting in an acidic local environment (65). A M_w of 15,000 Da was reached between day 14 and day 21, the time when the particles collapsed. At that point, high quantities of water could penetrate and GS was released. Microparticles prepared from RG 502H exhibited an initial molecular weight of 13,500 Da, which is already below the critical point (Fig. 3b and d). Therefore, as the uncapped polymer was rapidly hydrated, the polymeric structures started to cave in and eroded almost instantaneously, which coincided with release of GS. Within the first two weeks, the change in molecular weight agreed with the concept of degradation being a process of first order or pseudo first order kinetics [$t_{1/2}$: 5.1 days; $t_{1/2} \sim 6$ days described for RG 502H pellets in Ref. (61)] (63,66). The molecular weight of the 50/50 RG 502H/RG 503 blend started out at 27,000 Da (Fig. 3c). As water penetration is more pronounced, degradation and reduction in polydispersity were faster as compared to RG 503. At day 3, a M_w of 15,000 Da was measured. This time point correlated with the maximum in water content, onset of GS release, as well as erosion and collapse identified by SEM (60). Within the first two weeks, the same degradation half-life of the 50/50 RG 502H/RG 503 blend of 5.0 days as for RG 502H was calculated. Hydrolysis can be considered as a bimolecular reaction and the reactivity can be increased by raising the concentration of either reaction partner (67). The high water uptake into particles prepared from the

mixture caused by the uncapped polymer fraction resulted in enhanced hydrolysis of the total poly(α -hydroxy acid) blend. In contrast, the zero-order degradation kinetic of RG 503 microparticles suggested a limitation of water molecules present on the hydrolysis reaction mixture. Thus, combination of uncapped and endcapped PLGA types provide a new quality of adjusting polymer degradation kinetics. The resulting initial mechanical stability of the microparticles in combination with the rapid degradation kinetics renders the slow release of GS during the first two days followed by antibiotic liberation within one week.

CONCLUSIONS

The presented study could demonstrate that GS release can be adapted to the desired release over one week by blending two different PLGA polymers. The liberation profile from the PLGA microparticles prepared from a 50:50 mixture of RG 502H and RG 503 during incubation at 37°C could be explained by analysis of water uptake and hydrolytic decrease in molecular weight. The GS release coincided with structural collapse and bulk erosion when the critical molecular weight of approximately 15,000 Da was reached. The degradation rate of the endcapped RG

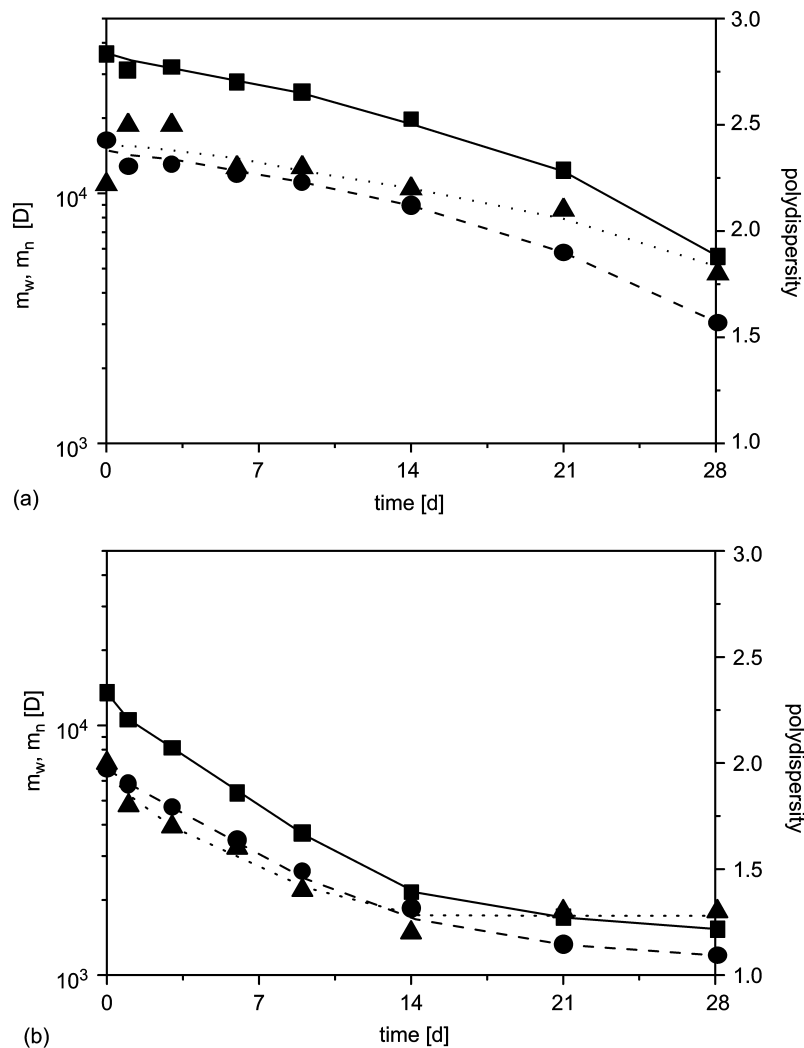


Figure 3. Molecular weight M_w (—■—) and M_n (---●---) and polydispersity index (···▲···) of microparticles prepared from RG 503 (a), RG 502H (b), and 50/50 RG 502H/RG 503 (c) and relative molecular weight [RG 503 (■), RG 502H (●), 50/50 RG 502H/RG 503 (▲)] (d) during incubation in PBS.

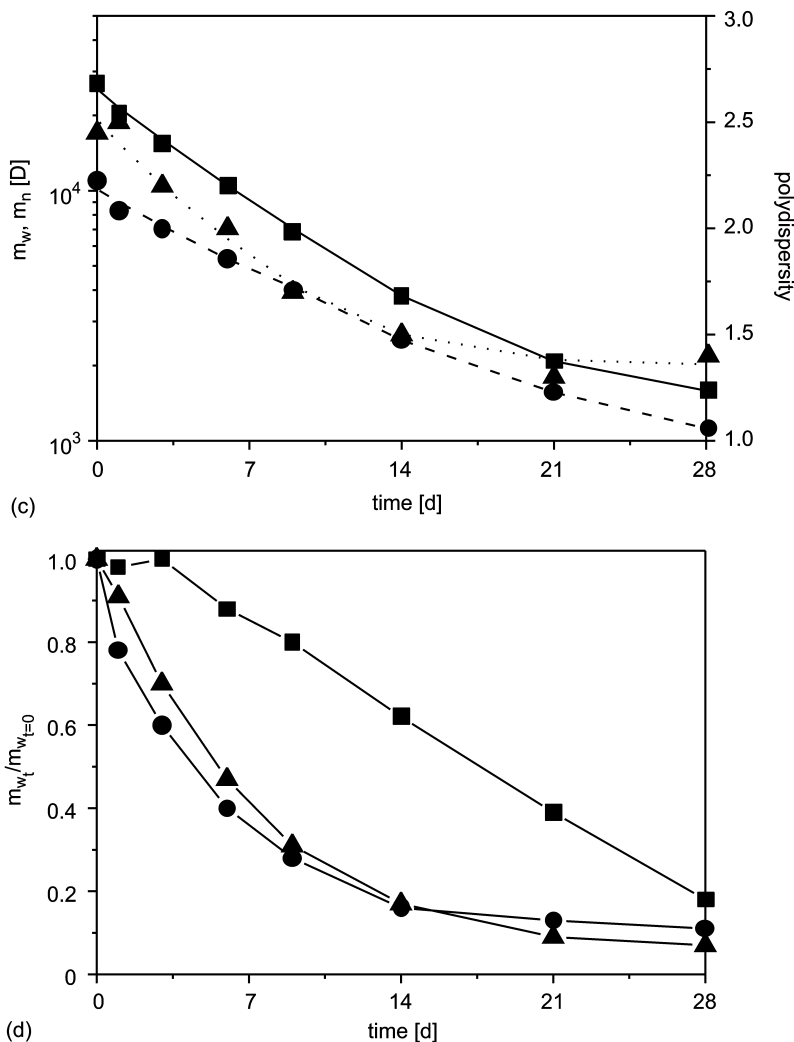


Figure 3. Continued.

503 could be enhanced to the rate of the uncapped RG 502H with admixture of the uncapped more hydrophilic polymer fraction as the water content is increased in the combination.

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