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Release of gentamicin sulphate from biodegradable PLGA-implants produced by hot melt extrusion

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For a long term local treatment of osteomyelitis biodegradable poly(lactic-co-glycolic acid) (PLGA) implants loaded with gentamicin sulphate (GS) were prepared, analysed and compared to the marketed product Septopal® (Biomet, Darmstadt, Germany), which consists of polymethylmethacrylate (PMMA) beads loaded with the same active ingredient. The implants were manufactured by hot melt extrusion with a twin screw extruder. In order to decrease the processing temperature and to improve the drug release behaviour, polyethylene glycol 400 (PEG 400) was added as plasticizer in different concentrations. The glass transition temperature of PLGA measured by differential scanning calorimetry declined in the same manner as the extrusion temperature with increasing PEG 400 concentration. The extrudates of all batches exhibited good encapsulation efficiency between 85% and 115% of the specified content. The behaviour of the implants during exposure to a release medium were characterised by scanning electron microscopy, gravimetric analysis and finally *in vitro* drug release studies. The results suggest that drug liberation is not affected by the addition of PEG 400, and depends on the drug-PLGA ratio only. Extrudates with 25% GS showed a release pattern with an initially higher drug release followed by a zero order kinetic for about four weeks and showed release profiles equivalent to Septopal®.

1. Introduction

Exogenic osteomyelitis is an infection of the bone tissue caused by a post-traumatic or post-operative event and it can occur as acute and chronic disease. Especially the chronic form which can evolve from the acute one is difficult to treat and to eradicate (Lazzarini et al. 2004). Therefore, it takes a complicated procedure, often making a debridement of the infected bone material necessary, and always including long-lasting intensive antibiotic therapy. Systemic administration of broadspectrum antibiotics has several side effects, mainly generated through the high blood concentration which is necessary to provoke an adequate antibiotic concentration above the minimal inhibitory concentration (MIC) in the infected bone poorly supplied with blood (Lew and Waldvogel 2004). Nevertheless an optimal drug release pattern for the treatment of osteomyelitis after surgical debridement includes a local high initial release rate to annihilate remaining infections followed by a 3–5 weeks constant release period (Soundrapandian et al. 2007). In order to avoid the disadvantages of systemic administration various local antibiotic therapeutic systems like, e.g., loaded microparticles (Friess and Schlapp 2002; Ambrose et al. 2003), beads (Wang et al. 2004), implants (Wei et al. 1991; Castro et al. 2005) and discs (Yoo et al. 2004) consisting of different materials and antibiotics were developed. Unfortunately, these delivery systems have often complex manufacturing procedures like, e.g., compressing and sintering in molds (beads), solvent evaporation and solvent extraction process (microparticles) (Jain 2000) or unsatisfactory release profiles.

A product for long-term local antibiotic treatment of osteomyelitis is Septopal® (Biomet, Darmstadt, Germany) which consists of non-biodegradable polymethylmethacrylate (PMMA) beads loaded with gentamicin sulphate releasing its drug over 30–70 days (Wahlig et al. 1978; Flick et al. 1987). However, as matrix system, biodegradable polymers are preferable as a second surgery for the removal of non-biodegradable material is not required, which leads in turn to a less cost-intensive therapy and prevents the physiological stress of the removal (Ambrose et al. 2004; Lazzarini et al. 2004).

Poly(lactide-co-glycolide) (PLGA), a well known biodegradable polymer, was chosen as matrix system as it is known to be highly biocompatible (Anderson 1997; Bostman and Pihlajamäki 2000). The copolymer from glycolic acid and lactic acid degrades through hydrolysis of its ester linkages into its two components and in the body the monomers metabolize further to naturally occurring products of the carbohydrate metabolism causing excellent biocompatibility (Garvin and Feschuk 2005). The degradation time of PLGA is related to its molecular weight and the ratio of the monomers in the copolymer: the higher the content of glycolic acid (up to 50%), the shorter the degradation time and the faster the drug release (Miller et al. 1977). Release profiles from delivery systems based on PLGA are often characterized by a burst effect through initial dissolution of drug from the surface, followed by a period of slow release (lag-phase) that is attributed to degradation of the polymer and diffusion of the drug out of the dosage form and a third phase of increased drug liberation caused by solubilization and erosion of the matrix. Improvements in opti-

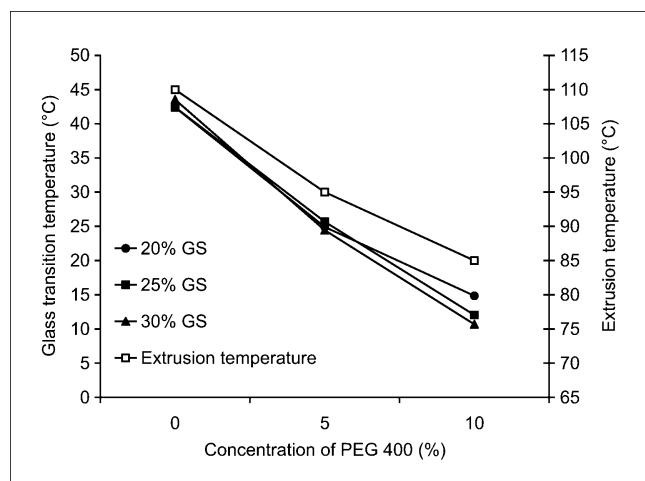


Fig. 1: Effect of the admixture of PEG 400 on the glass transition temperature of PLGA and on the extrusion temperature

mization of the release profiles have been achieved *inter alia* by changes of the size of the dosage forms (Siepmann et al. 2004), by addition of monomers of the polymer (Yoo et al. 2004), by variation of the molecular weight (Kanellakopoulou et al. 1999), by combining PLGA with other biodegradable polymers (Schmidt et al. 1995) and of course by modifying the drug content (Cevher et al. 2007). In this study, a plasticizer (PEG 400) is added in different concentration to reduce the lag-phase and to gain a prolonged constant drug release.

Gentamicin sulphate is an aminoglycoside broadspectrum antibiotic, which works by binding the 50S subunit of the bacterial ribosome and hence interrupting the protein synthesis. The antibiotic is very potent and has a high temperature stability (Wang, Liu et al. 2004). It is therefore often incorporated in polymer matrices via a melting process for extended drug release (Neut et al. 2001).

The production of the implants was performed by hot melt extrusion (HME), an old manufacturing process first used in the plastics industry. Pharmacists have increasing interests in the HME technique as an alternative production method (Crowley et al. 2007). The major advantages of this technique are that no solvents are involved in the process, only a few processing steps are needed, it is a continuous and well reproducible process, there are no requirements on the compressibility of the drug and the bioavailability of the active ingredient can be increased enormously if it is molecularly dispersed in the polymer (McGinity et al. 2007). Furthermore, hot melt extrusion allows producing the favoured release behaviour by varying the composition of the formulations or by different geometry of the formed dosage form. The main disadvantage of HME is the thermal stress the active ingredient and the polymer are exposed to during processing (Breitenbach 2002). In this study, biodegradable gentamicin sulphate loaded PLGA implants were produced via HME and PEG 400 was admixed as plasticizer to decrease the processing temperature and to affect the drug release behaviour beneficially.

2. Investigations and results

2.1. Plasticizer influence on glass transition and extrusion temperature

Gentamicin sulphate has no decreasing effect on the glass transition temperature of PLGA (Fig. 1). Therefore PEG 400 was added as plasticizer to produce the implants at lower extrusion temperatures. With increasing PEG 400-concentration up

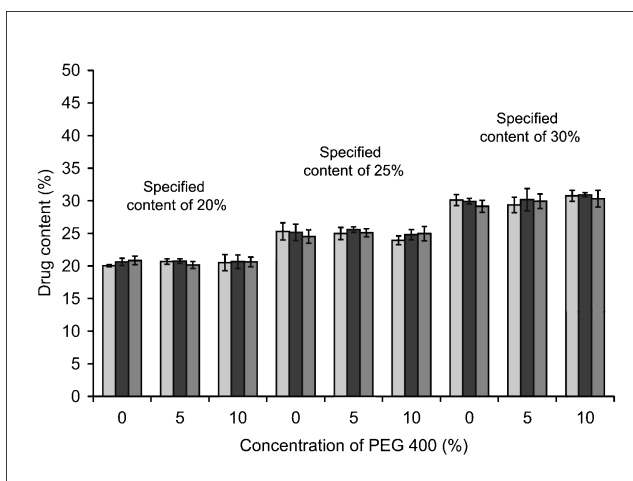


Fig. 2: Drug content of the batches of all formulations

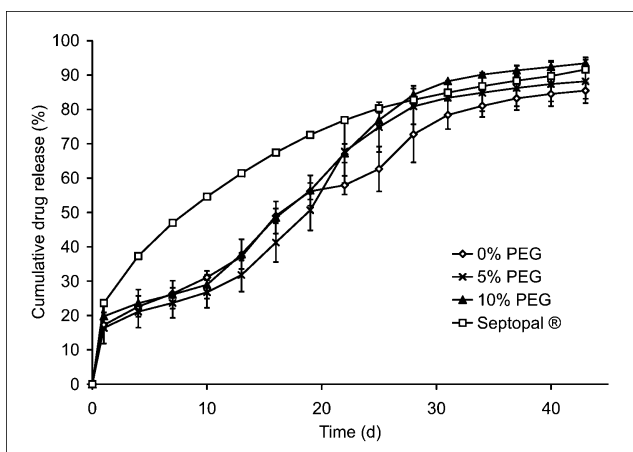


Fig. 3: Cumulative drug release of the 20% GS batches with increasing PEG 400 concentration compared to Septopal®

to 10% the glass transition temperature decreases linearly from 43 °C to about 12 °C. Accordingly, it was possible to reduce the extrusion temperature from 110 °C to 85 °C and consequently minimize the temperature impact on the active ingredient. Nevertheless, the intense decrease of the glass transition temperature up to 12 °C can eventually lead to stability problems during storage.

2.2. Drug content of the extrudates

As shown in Fig. 2 the measured drug contents reach well the specified contents with 95% confidence intervals below 1.3% and each single extrudate exhibited an encapsulation efficiency of 85% to 115%. The p-values between the batches of the formulations exceeded 0.1 which indicates that there were no significant differences between the batches. Two exceptions were between 0.05 (>0.05 indicates a significant difference) and 0.1, due to small confidence intervals. Thus, it can be stated that the manufacturing process led to reproducible implants.

2.3. Release of gentamicin sulphate from melt extruded implants

Fig. 3 shows the cumulative drug release of the 20% gentamicin sulphate batches with increasing concentration of PEG 400 versus one Septopal®-sphere. After a burst effect, where about 20% of the drug amount is released due to the dissolving of

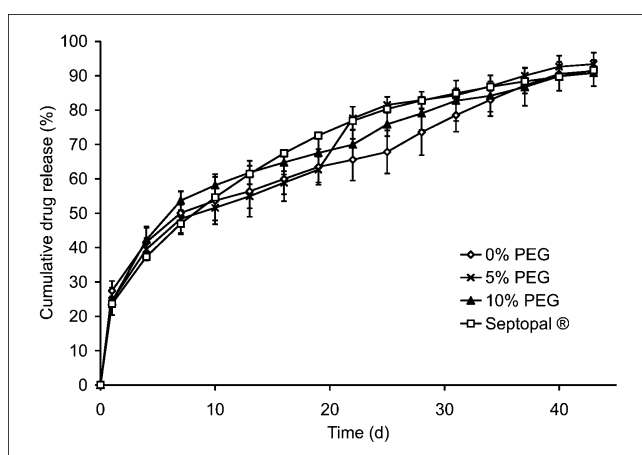


Fig. 4: Cumulative drug release of the 25% GS batches with increasing PEG 400 concentration compared to Septopal®

gentamicin sulphate from the outer surface, water penetrates slowly into the extrudates and lets them swell. The drug that is further dissolved by intruding water diffuses into the release medium and thus generates pores which in turn accelerate the process of water penetration and drug liberation. In addition, the steady degradation of the polymer enhances drug release. PLGA hydrolytically decomposed into different products with chain lengths with free carboxyl groups at the end, which cannot penetrate out of the extrudate due to their size. Therefore the mass changes are only attributed to the loss in drug mass. The oligomers create an acidic microclimate inside the extrudate and hence accelerate the autocatalytic degradation process of PLGA (Shenderova et al. 1999). In contrary the emerged fragments can diffuse away from the surface, so that the acidity here is neutralized by the buffer and the degradation of the surface is not autocatalysed. So, the outer layer is stable for a longer time. When the pores on the surface reach a size, where they allow an evacuation of the polymer fragments from the inside or when the oligomers degrade to dimers and monomers so that they can penetrate outside, a critical threshold is passed and the mass loss and the erosion of the extrudates starts (McGinity and O'Donnell 1997). At this point the surface of the extrudates can collapse and release an enlarged drug amount as can be seen at the 20% gentamicin sulphate batch without plasticizer after approximately 20 days. By the end of the gentamicin sulphate release the liberation slows down until the drug is released almost completely after four to five weeks. The increasing polyethylene glycol concentration does not significantly affect the release behaviour of the implants. In comparison to the release profile of the Septopal®-sphere, which releases gentamicin sulphate by diffusion from a non-biodegradable matrix system (polymethylmethacrylate) with release kinetics according to Higuchi the 20% drug batches show more sigmoidal release curves. Although these batches release well above the minimal inhibitory concentration of *Staphylococcus aureus*, the main pathogen of osteomyelitis (Brady et al. 2006), even over the first two weeks (data not shown), a higher drug release especially at the beginning of the antibiotic therapy would be preferable.

The batches with 25% gentamicin sulphate and increasing polyethylene glycol concentration, shown in Fig. 4, exhibit a higher initial rate of release. After seven days about 50% of the theoretical drug amount is liberated caused by the higher drug concentration yielding more pores. However, it is followed by a constant release over approximately four weeks. During this steady drug release the swelling of the extrudate and the liberation of the active ingredient equilibrate resulting in zero order

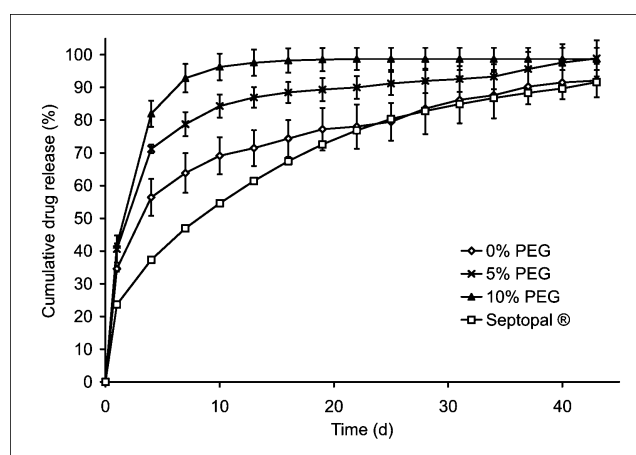


Fig. 5: Cumulative drug release of the 30% GS batches with increasing PEG 400 concentration compared to Septopal®

kinetics with a coefficient of determination of 0.99 (formulation 4 and 6). The 25% gentamicin sulphate batches, which again do not differ significantly with increasing plasticizer content, have release profiles equivalent to the Septopal®-sphere. Three extrudates with 25% gentamicin sulphate are pharmaceutically equivalent to one Septopal®-sphere (containing 7.5 mg GS) and bioequivalence is anticipated by these release studies although this has to be verified *in vivo*.

The release behaviour from 30% drug content batches is shown in Fig. 5. Basically, gentamicin sulphate is liberated quite fast, about 55%, 70% and 80% drug respectively is set free depending on the plasticizer content after four days. In consideration of the fact that polyethylene glycol had no impact on the release rates of the batches with lower drug content, it can be assumed that the ebbing retarding effect of the batches is caused by the decreased PLGA-concentration. Although the 30% gentamicin sulphate formulations combined with 0% and 5% plasticizer have the same PLGA-concentration as the 25% drug batches combined with 5% and 10% PEG 400, they show a less retarded drug release rate. This can be explained by the higher content of gentamicin sulphate, generating more pores and therefore triggering faster drug liberation.

2.4. Water uptake and mass loss

The water uptake of all batches, ranked according to the PLGA-concentration, is illustrated in Fig. 6. It becomes apparent that the water uptake corresponds mainly to the PLGA-content independent of further formulation ingredients. With a lower polymer

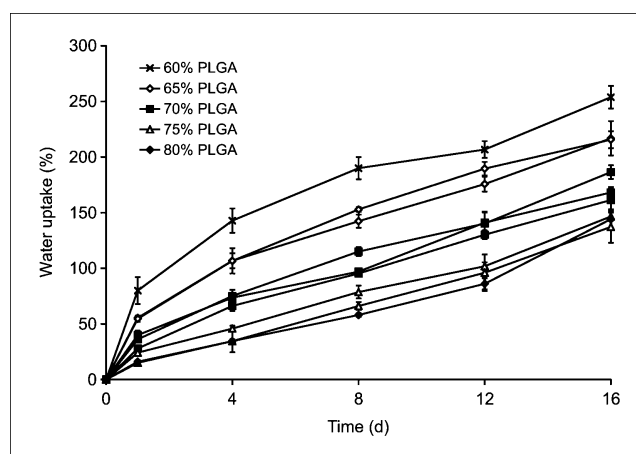


Fig. 6: Effect of the PLGA concentration on the water uptake of the extrudates

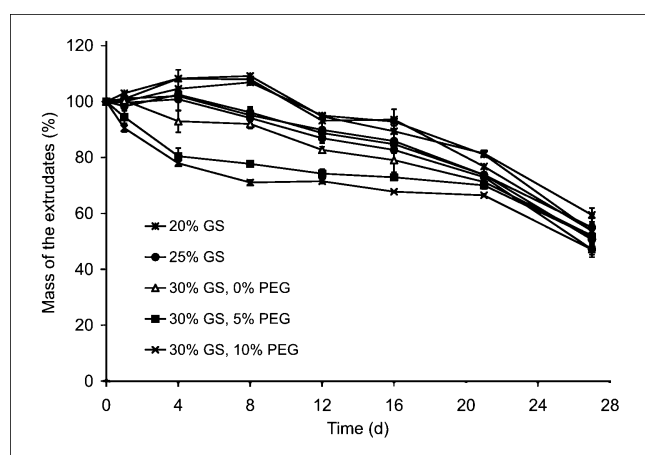


Fig. 7: Influence of the drug concentration and PEG 400 concentration on the mass loss of the extrudates

content a less tighter matrix system can be build up and water can more easily infiltrate the extrudate, whereby it is unimportant if gentamicin sulphate or polyethylene glycol are embedded between the polymer chains and spacing them apart. The smaller the PLGA-concentration is, the faster and the more water is taken up.

While the water uptake was observed to be dependent on the polymer weight fraction, the mass loss is dependent on the drug amount (Fig. 7). The 20% gentamicin sulphate batches initially

show a mass increase, probably generated by not completely freeze dried samples. Apart from that the other formulations exhibit a mass loss according to the released amount of drug. For example the 25% drug batches have a mass loss of approximately 16.5% after 16 days which correlates properly to the drug amount released (approx. 62% of 25% drug amount equals 15.5% mass loss). The mass loss data display the dependency of the 30% batches on the plasticizer content or rather on the remaining PLGA-concentration as well as the release curves. According to the faster drug liberation a higher mass loss is visible with decreasing PLGA-concentration. The erosion of the polymer starts slowly after 16 days and intensifies after approx. 20 days, which can also be observed in the increasing drug release of the 20% gentamicin sulphate batches.

2.5. SEM-pictures of the extrudates

The morphological changes during drug release are illustrated in the scanning electron pictures. The cutting zones of the extrudates, shown in Fig. 8, reveal the included spherical gentamicin sulphate particles in a nonporous and homogeneous matrix leaving little holes after exposure to the release medium (Figs. 9 and 10). In Fig. 10 the more structural roughness of the surface compared to Fig. 9 denote the incorporated polyethylene glycol in the polymer. The surface of the extrudates changes over time to less smooth and more porous. This results from the degradation and after 21 days erosion of the polymer becomes visible.

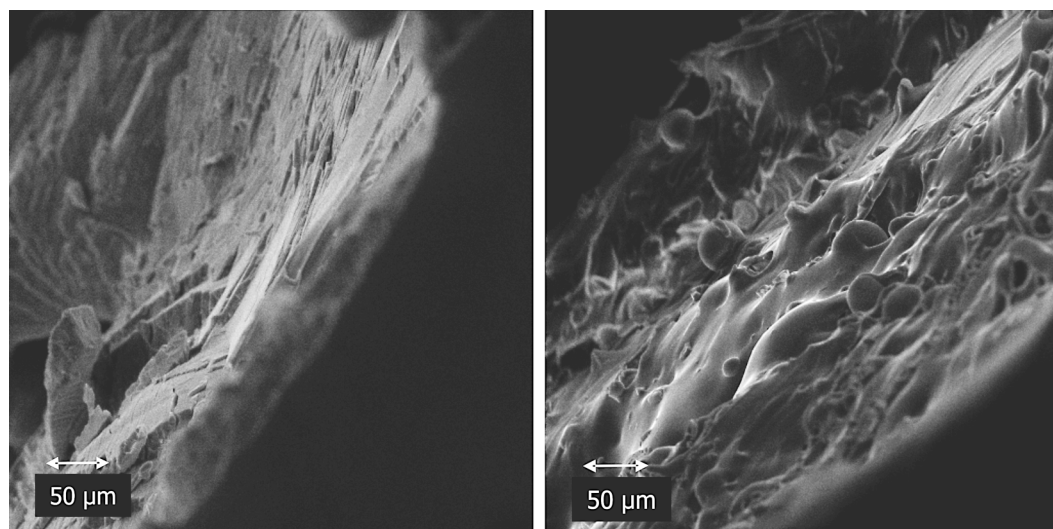


Fig. 8: SEM-pictures of the cutting zones of a pure PLGA-extrudate (left) and a gentamicin sulphate loaded extrudate (right)

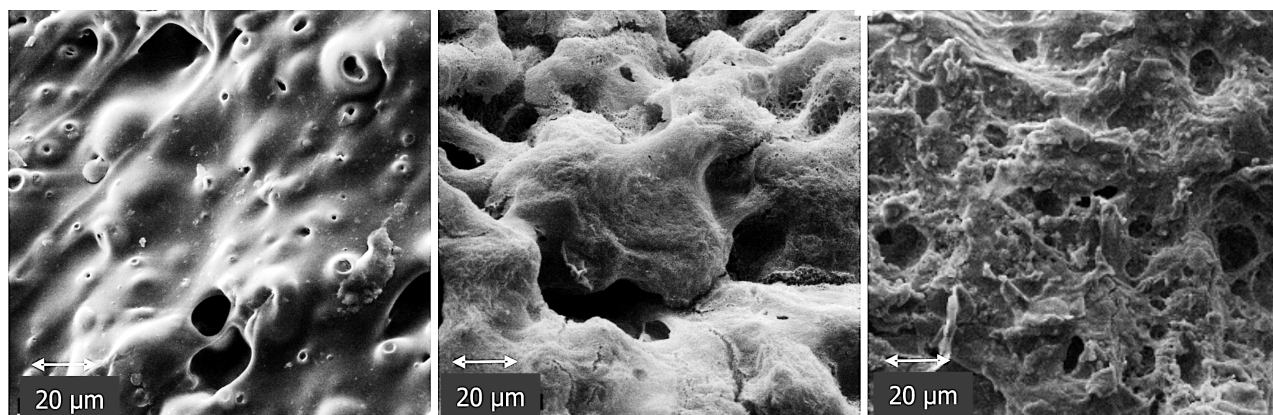


Fig. 9: SEM-pictures of the surfaces of extrudates with 25% gentamicin sulphate and 0% PEG 400 after 1, 16 and 21 days (left to right) exposure to phosphate buffer pH 7.4

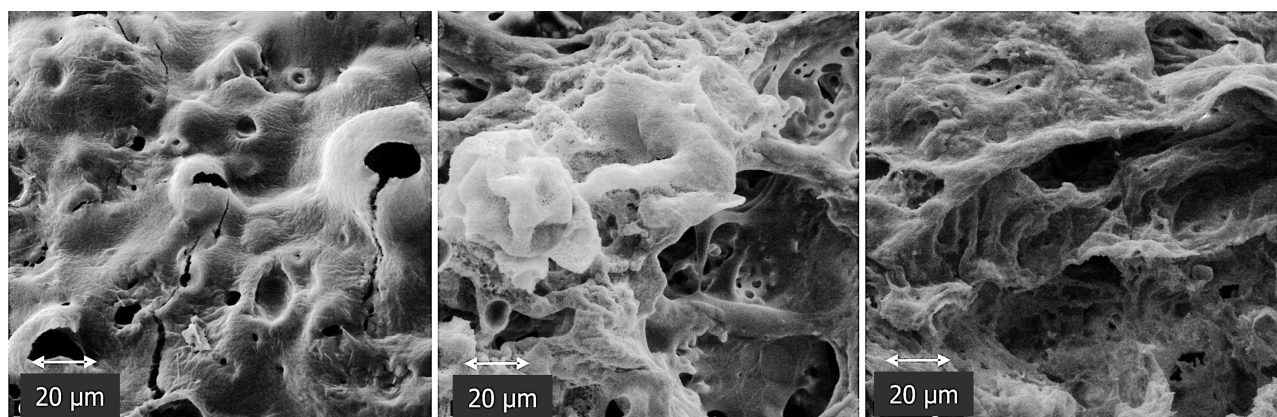


Fig. 10: SEM-pictures of the surfaces of extrudates with 25% gentamicin sulphate and 10% PEG 400 after 1, 16 and 21 days (left to right) exposure to phosphate buffer pH 7.4

Table: Composition of the formulations

Batch no.	GS (%)	PEG 400 (%)	PLGA (%)
1	20	0	80
2	20	5	75
3	20	10	70
4	25	0	75
5	25	5	70
6	25	10	65
7	30	0	70
8	30	0	70
9	30	10	60

3. Discussion

Although the admixture of plasticizer decreases the glass transition temperature of PLGA with increasing concentration and therefore the extrusion temperature of the blends, drug liberation is not affected up to a specific remaining PLGA-content, which is necessary to provoke a prolonged drug release. The release profiles of the extrudates differ depending on the drug amount from sigmoidal to exponential release curves, whereby 25% gentamicin sulphate amount lead to an initial high drug release followed by zero order kinetics over about four weeks. These formulations are consequently a good alternative to Septopal® without the disadvantage of a second surgery, although further *in vivo* investigations have to be done.

4. Experimental

4.1. Materials

As biodegradable polymer Resomer® RG 503 H (Boehringer Ingelheim, Ingelheim, Germany) was used. Resomer® RG 503 H is a poly(D,L-lactic-co-glycolic acid) with a ratio of 50:50, an intrinsic viscosity of 0.32–0.44 dL/g and a free carboxylic acid as end group. The antibiotic gentamicin sulphate purchased from Caelo (Hilden, Germany) was used as active ingredient. The plasticizer polyethylene glycol 400 was obtained by BASF (Ludwigshafen, Germany). *O*-Phthaldialdehyde thioglycolic acid (Merck, Darmstadt, Germany) and sodium 1-heptane-sulfonate (Sigma Aldrich, Taufkirchen, Germany) were used for the analytical methods.

4.2. Methods

4.2.1. Manufacture of the implants

The manufacture of the antibiotic loaded implants was performed by hot melt extrusion. Nine formulations (Table) with a drug content of 20, 25 and 30% gentamicin sulphate and plasticizer content of 0, 5 and 10% were produced. The polymer and the active ingredient were mixed with a turbula-blender (Willy A. Bachofen AG, Basel, Switzerland) for 15 min and subsequently manually granulated with PEG 400 for at least 10 min. These blends were hot melt extruded by a twin screw extruder (Minilab II, Thermo Fisher Sci-

entific, Karlsruhe, Germany) with a die diameter of 2 mm. The temperature was set as low as possible between 85 °C and 110 °C depending on the plasticizer content. After cooling down, extrudates of 9.8 to 10.2 mg weight were produced by cutting.

4.2.2. Drug content

To determine the encapsulation efficiency, the extrudates were individually dissolved in dichloromethane. After addition of 4 mL water the samples were mixed to let the active ingredient migrate to the aqueous phase for at least one hour. The gentamicin sulphate content of the aqueous phase was analysed by HPLC.

For determination of gentamicin sulphate by HPLC the drug has to be derivatised as it does not possess UV absorbing chromophores. As derivatisation agent for pre-column derivatisation with UV detection *o*-phthaldialdehyde (OPA) was used. The *o*-phthaldialdehyd reagent was prepared as described in the USP XXXI. For derivatisation 1.0 mL of the sample was mixed with 0.4 mL of *o*-phthaldialdehyd solution and 1.1 mL of isopropanol, incubated at 60 °C for 15 min and in a final step cooled down to room temperature. The analysis of the drug content of the prepared samples was carried out using reversed phase HPLC with a HPLC system by the Agilent Series 1100 (Agilent Technologies, Santa Clara, USA). As stationary phase a Merck LiChroCart 125–4 mm column, filled with LiChrospher 100 RP18, 5 µm and a pre column LiChroChart 4–4, LiChrospher 100, 5 mm (all Merck KGaA, Darmstadt, Germany) were used. The mobile phase was composed of 700 mL methanol, 25 mL water, 50 mL glacial acetic acid (all obtained from Merck KGaA, Darmstadt, Germany) and 5 g sodium 1-heptane-sulfonate. The flow rate was fixed at 1.0 mL per min, the column temperature was 25 °C, the injection volume was 50 µL and the detection wavelength was set to 330 nm.

4.2.3. Differential scanning calorimetry

The thermal properties of the loaded implants were analysed with a differential scanning calorimeter from Perkin Elmer, Waltham, Massachusetts, USA (DSC 7). Samples of approximately 7 mg were weighed into aluminium pans, sealed hermetically and analysed under a nitrogen atmosphere with a heating procedure from 10 °C to 80 °C at a scan rate of 10 °C/min related to an empty reference pan.

4.2.4. Scanning electron microscopy

To analyse surface morphology SEM pictures were made with a Philips XL20 (Philips B.V., Eindhoven, The Netherlands) scanning electron microscope. The samples were fixed on a carbon fibre film and sputtered with gold in an argon atmosphere at a sputter current of 50 mA for 180 s using a SCD 005 Sputter coater (BalTec, Balzers, Liechtenstein).

4.2.5. In vitro release

Five gentamicin sulphate loaded extrudates of approximately 10 mg weight of each batch or one Septopal®-sphere (containing 7.5 mg (w/w) gentamicin sulphate) were placed into 3 mL phosphate buffer pH 7.4 (containing 250.0 mL 0.2 M monobasic potassium phosphate solution with 393.4 mL 0.1 M sodium hydroxide solution) as dissolution medium. The extrudates were incubated at 37 °C and horizontally shaken. At a time interval of 72 h starting at day one the whole dissolution medium was collected for analyzing via HPLC and replaced by fresh buffer. This procedure was repeated until no more gentamicin sulphate could be detected.

4.2.6. Water uptake and mass loss

To determine the swelling and the erosion of the extrudates water uptake and mass loss were analysed. Therefore implants were weighed exactly and placed in release medium over specific time periods. The implants were weighed wet after removing the water from the surface using soft wipes. After freeze drying (freeze dryer Alpha 1-4 LDC-1 M, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) over two days the extrudates were weighed again to investigate the mass loss. The measurement of the water uptake and mass loss was only possible as long as the extrudates stayed in shape.

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