Research paper

Polymer degradation induced drug precipitation in PLGA implants – Why less is sometimes more

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ABSTRACT

Nifedipine and nicardipine loaded PLGA extrudates have a great potential to prevent cerebral vasospasms after subarachnoid hemorrhage or surgical clipping of aneurysm. A constant release over approx. two weeks is desired. Although *in vivo* studies on humans have been reported, there is limited knowledge about the release kinetics and the underlying mechanisms. Therefore, nifedipine and nicardipine loaded PLGA implants with different drug loads were manufactured by extrusion and investigated. In addition to the measurements of the release kinetics, GPC, DSC, X-ray diffraction and light microscopic investigations were performed for a detailed characterization. The water uptake and polymer erosion studies showed an initial lag phase of 5–7 days and an acceleration of both processes thereafter. With 5% loaded implants a higher drug release compared to 10% drug loaded polymers could be achieved and not only the relative amount of drug release (% of loaded drug), but surprisingly also the absolute amount of the released drug increased. The drugs were initially in an amorphous state. For nifedipine, formation of drug crystals with time has been observed by light microscopy and X-ray diffraction. The analysis of the drug content in the degrading polymer showed a very large increase from 10% to about 20% (nifedipine) and over 50% (nicardipine). In contrast, no or only a moderate increase of the drug content occurred for initially 5% loaded polymer implants. We postulate that water penetration and polymer degradation induced changes of the microenvironment lead to supersaturated systems. A supersaturated state is faster reached for polymers with higher drug load and therefore, drug precipitation takes place at earlier time points.

As a result, drug release might be incomplete for poorly soluble drugs and paradoxically, the total amount of drug release might be higher for systems with a lower drug load. Drug release is initially controlled by the PLGA matrix, but later by the dissolution kinetics of the precipitated drug which are very slow for poorly soluble drugs according to the Noyes-Whitney equation.

1. Introduction

Poly(lactic-co-glycolic acid) (PLGA) is one of the most extensively used polymers for medical applications like sutures, microparticles or implants. Several drug delivery systems containing PLGA are approved by FDA and EMA for human use and until now the polymer remains the gold standard for biodegradable parenteral controlled release due to its favorable properties like biodegradability and biocompatibility [1,2].

Further on, different types of PLGA allow a wide range of use because the dependency of degradation on e.g. molecular weight and monomer composition of the polymer [3,4] opens up the possibility of tunable release kinetics. Although PLGA-based drug delivery systems for hydrophilic [5] and hydrophobic drugs [6] as well as proteins [7] have been studied for several years, new clinical applications still emerge like treatment of inner ear diseases [8,9].

A promising application of PLGA-based drug delivery systems is the

delivery of dihydropyridines to the subarachnoid space in the brain with the aim to prevent cerebral vasospasms after subarachnoid hemorrhage or surgical clipping of aneurysm [10]. Systems called nicardipine prolonged release implants (NPRI) were developed by Kasuya et al [11]. They consist of a low molecular weight PLGA and should deliver the drug over at least two weeks. The NPRI have shown good clinical results [12,13] and might be possible candidates for market products in future. Nevertheless, the developed systems are in some cases suffering from low mechanical strength [14] due to the very low molecular weight of the polymer. Furthermore, important information about the release kinetics and mechanisms of drug release are not available. PLGA and PLA polymers often show complex polymer degradation and drug release behavior, where the contributions of diffusion, swelling and erosion processes change with time [15]. Important parameters include the water content, the microacidity and the glass transition temperature. It is well known, that PLA and PLGA polymers might undergo autocatalytic degradation processes and develop a highly acidic microenvironment with pH-values around 2 [16,17]. Besides the influence of the polymer properties like molecular weight, monomer composition and end groups [18,19], drug release has been shown to be dependent from various factors like device geometry [20], composition of the release medium [21,22], experimental conditions [23] and of course from the drug itself [24]. Particularly the influence of the drug type on polymer degradation rate should not be underestimated. Siegel et al [24] could show a dependency of PLGA degradation on the incorporated drug type but they were not able to correlate polymer degradation and drug release with drug characteristics like water solubility and hydroxyl group density. However, a relationship of the pK_a values of the drugs and matrix degradation was found [25] resulting in a higher polymer degradation due to the presence of basic drugs, which was confirmed by several groups [26,27,28,29]. This leads to the conclusion that new drug delivery systems need to be characterized intensively *in vitro* with respect to their individual degradation and release behavior. Nifedipine and nicardipine are basic dihydropyridines known to be susceptible to oxidation especially due to UV irradiation. Nifedipine already has been formulated in terms of parenteral PLGA-based DDS as microparticles [30] but not as implants, whereas the NPRI are to our knowledge the only PLGA-DDS containing nicardipine. Bege et al developed PLGA microparticles containing the related drug nimodipine for the same application [31] which were also tested in preclinical *in vivo* studies [32] and led to promising results. Now, these systems are in Phase 3 clinical trials for treatment after subarachnoid hemorrhage [33] where the microparticles are administered via an external ventrical drain.

Therefore, it was the aim of this study to investigate the properties and *in vitro* release of PLGA-based implants as alternative drug delivery systems containing nifedipine and nicardipine, two dihydropyridines with different physicochemical properties. The manufactured implants were characterized via GPC and DSC. Drug release was studied under sink conditions *in vitro*, critical parameters for polymer degradation e. g. water uptake and mass loss were measured with the aim to understand the polymer degradation behavior in relation to water penetration. Furthermore, drug characteristics like solid state properties and content should be investigated to evaluate the influence of the drug type as well as the drug load on release behavior. With the gained knowledge the release mechanism of these drug delivery systems should become clear leading to a rational and optimized design of this kind of implants as a reliable basis for upcoming *in vivo* studies.

2. Materials and methods

2.1. Materials

Nifedipine (> 98%) was purchased by TCI, Japan, and nicardipine hydrochloride (> 98%) was purchased by Alfa Aesar, Germany. PLGA Resomer® RG 502 H (lactic acid:glycolic acid 50:50, intrinsic viscosity 0.16–0.24 dl/l, molecular weight range 7,000–17,000) was kindly provided by Evonik Industries, Germany. All solvents were of HPLC grade and buffer salts of analytical grade.

2.2. Manufacturing of the implants

Nicardipine was obtained from nicardipine hydrochloride by dissolving the salt in water and pH adjustment to pH 7 with 1 M NaOH. The formed nicardipine free base was extracted with methylene chloride. Afterwards the solvent was removed by rotary evaporation and the drug was dried in vacuum. Nifedipine was used as received. With respect to the light sensitivity of dihydropyridines and especially nifedipine, all samples were protected from light by covering or wrapping.

PLGA and the drug (5 wt% or 10 wt% regarding to the polymer) were weighed precisely in a vial and dissolved in acetone at 25 wt%. The mixture was casted on PTFE slides and dried at room temperature. After approximately 30 min of solvent evaporation the organic solvent was removed under vacuum in a desiccator for at least six hours until no further weight variation occurred. Approximately 40 mg of the dried films were inserted in Teflon tubes with an inner diameter of 2 mm. The film was compressed between two punches in a custom built press according to Qian et al. [34] with a force of approx. 19 kg/cm^2 for 3 min when the press was placed in an oven at 100 °C. Afterwards the implants were removed out of the tube with a plunger.

2.3. Gel permeation chromatography

To obtain information about molecular weight changes during manufacturing, GPC was performed with a Viscotek GPCmax VE 2001 (Malvern Instruments, UK) equipped with a RI detector (Viscotek VE 3580) with the mobile phase DMF containing 10 mM lithium bromide at 60 °C and a flow rate of 1 ml/min. A GRAM LUX analytical column (8 × 300 mm, 1000 Å, 10 μm particle size, PSS, Germany) was used. Samples were dissolved in DMF at concentrations of 2–3 mg/ml. Molecular weight was determined by use of PMMA standard curves. Each measurement was run in triplicate and data was processed by OmniSEC software.

2.4. Differential scanning calorimetry

DSC measurements were performed with a DSC 200 (Netzsch, Germany). Approx. 3 mg of the pure polymer, the drugs or the implants respectively were measured in a sealed aluminum pan against an empty pan as reference. Temperature ranges differed by the samples, the heating rate was 5 K/min and each measurement was performed at least in duplicate. Glass transition temperature was defined as the midpoint of the tangent of inflection and melting peaks were determined with Netzsch TA Analysis software.

2.5. HPLC analysis – Determination of drug load during release

An exactly weighed amount (Sartorius M2P microbalance, Sartorius, Germany) of the implant was dissolved in acetone. The polymer was precipitated with methanol and the suspension was centrifuged to obtain the drug containing supernatant which was diluted appropriately for HPLC analysis.

Nifedipine and nicardipine were analyzed by HPLC (Jasco, Japan) and data were processed with Jasco Borwin and Jasco Chrompass Software, respectively. For both drugs their concentration was determined from recorded standard curves.

The chromatographic conditions for each drug are displayed in Table 1.

Table 1

HPLC methods for determination of nifedipine and nicardipine content.

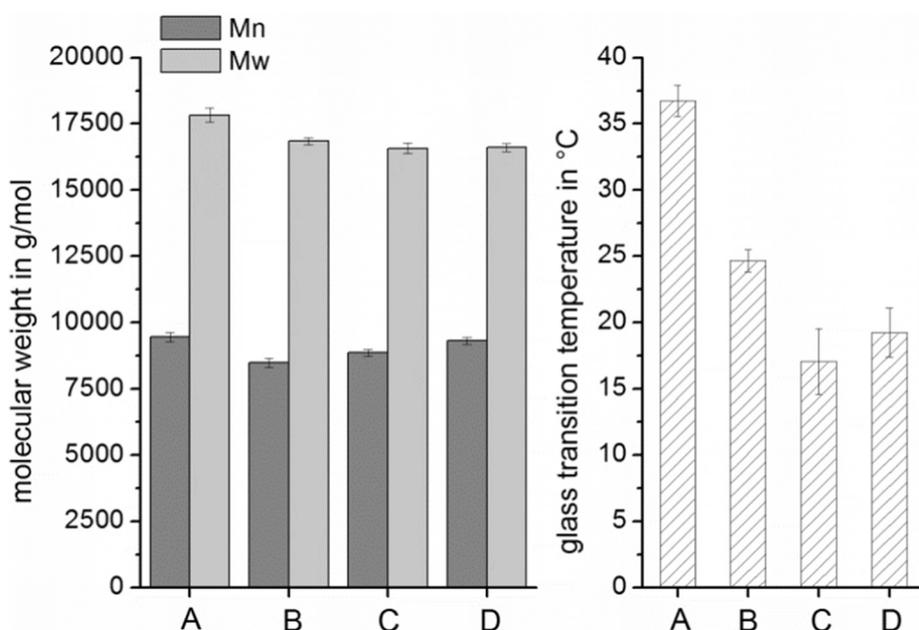|	Nifedipine	Nicardipine
Column	LiChroSpher 100 RP-18e LiChroCART 125 × 4 mm 5 μm (Merck, Germany)	Purospher STAR RP-18e, LiChroCART 125 × 4 mm 5 μm (Merck, Germany)
Column temperature	Ambient	40 °C
Mobile phase	methanol:water 50:50	methanol:buffer 65:35 (buffer pH 4.9 consisting of 0.045 vol-% triethylamine and 0.03 vol-% phosphoric acid, preserved with 0.02% sodium azide)
Flow mobile phase	1 ml/min	0.7 ml/min
Wavelength UV detector	235 nm	237 nm
Injection volume	20 μl	10 μl

Fig. 1. Influence of the thermal molding and drug incorporation on the molecular weight(left) and the glass transition temperature (right) of the polymer: A) unprocessed PLGA polymer, B) PLGA implants, C) PLGA implants containing 5% nifedipine, D) PLGA implants containing 5% nicardipine. Data are shown as mean ± SD, n = 3.

2.6. Release studies and erosion profile

A precisely weighted implant (mass = m_{ini}) of approx. 20 mg was inserted in a tied regenerated cellulose based dialysis bag (Spectra/Por®, MWCO 6,000–8,000, Spectrum Laboratories, Canada) containing the release medium. To ensure perfect sink conditions, four bags were immersed in a flask containing 250 ml isotonic PBS pH 7.4 (preserved with 0.02% sodium azide) and the medium was changed every two days. Drug release was studied over 21 days at 37 °C in a shaking water bath with 40 strokes per minute.

After certain time points the implants were taken out of the dialysis bags, blotted with tissue, weighed (mass = m_{wet}) and afterwards dried extensively in a desiccator by applying vacuum and weighed again (mass = m_{dry}).

The released drug per day was determined by Eq. (1), where av $[c_{drug}]_{ini}$ is the mean initial drug content of the implants and $[c_{drug}]_{act}$ is the actual drug load of each implant after a certain release time.

$$released \ drug \ [\%] = \frac{m_{ini} * av \, [c_{drug}]_{ini} - m_{dry} * [c_{drug}]_{act}}{m_{ini} * av \, [c_{drug}]_{ini}} * 100 \quad (1)$$

The water uptake factor was calculated by Eq. (2) and the part of the remaining polymer by eq. (3).

$$water \ uptake \ factor = \frac{m_{wet}}{m_{dry}} \quad (2)$$

$$remaining \ polymer \ matrix \ [\%]$$
$$= \frac{m_{ini} * (1 - av \, [c_{drug}]_{ini}) - [m_{dry} - (m_{dry} * av \, [c_{drug}]_{act})]}{m_{ini} * (1 - av \, [c_{drug}]_{ini})} * 100 \quad (3)$$

2.7. Microscopy

Light microscopy was performed with the transmitted light microscope Zeiss Axiolab (Zeiss, Germany) and the incident light microscope Olympus SZX9 (Olympus, Japan). Photographs were taken by an Olympus UC 30 camera and processed by Stream Motion software.

2.8. PXRD

To obtain information about the crystalline or amorphous state of the drug inside of the implants, powder x-ray was performed with a Stadi 4P powder diffractometer (STOE, Germany) in transmission mode in a scanning range between $5 - 35°$ with a copper Co Kα (0.1789 nm) X-ray source at 40 kV and 30 mA. Data was processed by STOE WinXPOW software.

3. Results and discussion

3.1. Manufacturing of the implants

The initial molecular weight of PLGA is one of the most important factors that determine the degradation kinetics [3]. The selected PLGA

Table 2
Nominal versus actual drug load of the implants in the release study (mean $\pm$ SD, n = 6).

	Nifedipine actual drug load	Nicardipine actual drug load
Theoretical drug load 5%	4.855 $\pm$ 0.16%	4.897 $\pm$ 0.046%
Theoretical drug load 10%	8.513 $\pm$ 0.077%	9.635 $\pm$ 0.121%

type Resomer® RG 502H is recommended for release times shorter than three months [35] due to its low molecular weight, the lactic/glycolic-acid ratio of 50:50 and the free carboxylic acid function which increases the hydrophilicity of the polymer. To determine the influence of the manufacturing process on the implant properties, DSC and GPC measurements were performed (Fig. 1). A molecular weight range of 7,000–17,000 for Resomer® RG 502H is given by the manufacturer (measured via intrinsic viscosity measurements). We used GPC analysis and obtained molecular weight values of M_w. 17,800 Da and M_n 9,450 Da, which are in agreement with the expected values.

The heat compression technique for manufacturing led to a small decrease of the molecular weight. Compared to the unprocessed polymer, thermal molding caused a drop of 5.5% M_w and a drop of approx. 10% M_n. This indicates an increase in low molecular weight compounds due to thermal processing. Drug containing rods showed similar results, the M_w decreased significantly (p = 0.05) by approx. 7% compared to the unprocessed polymer and also lower values for M_n were found. The results of the drug containing rods were comparable to the drug-free rods, similar GPC elution profiles with a shift to longer elution times are shown in the supplementary data (Fig. Suppl. 1).

All in all the molecular weight of PLGA is slightly decreased after manufacturing and more oligomers are present in the implants which should be kept in mind for the interpretation of release experiments. Nevertheless, the drug containing formulations seem to be comparable regarding the molecular weight.

The glass transition temperature of a polymer is also dependent from the molecular weight but not only. Manufacturing of implants as well as loading the implants with drugs led to a decrease of the glass transition temperature (Fig. 1). The unprocessed amorphous polymer shows a glass transition at approx. 37 °C which is slightly lower than the manufacturer's specification. A reduction of the glass transition temperature to 24.6 °C after manufacturing of a drug-free implant is likely due to partial degradation of the polyester which was shown with GPC analysis, because the formed degradation products (e.g. oligomers and monomers) will lead to a lower T_g. In addition, possible traces of residual solvents from the manufacturing step as well as moisture from environmental humidity will also lower the T_g.

The clear optical appearance of the extrudates is an indication for an amorphous system. By DSC analysis of all drug containing implants no melting peak of the drug was observed (Fig. Suppl. 2) indicating an amorphously embedded drug within the amorphous polymer after production which is supported by the absence of peaks in PXRD analysis (Fig. 6). Further on, no second possible glass transition temperature of the drug (determined at 45–50 °C, data not shown) could be detected by DSC, therefore we draw the conclusion that drug and polymer form a unitary solid dispersion. This is likely by virtue of the production method applied in this study, the previous dissolution of the components should provide homogeneously dispersed components. Similar results were described by Miyajima et al. [25] who prepared the implants in a comparable way. However, the coexistence of microdomains with two amorphous phases (PLGA and amorphous drug) cannot be ruled out totally.

The glass transition temperature of the drug containing implants is lower compared to drug-free implants, whereas the molecular weight does not differ. The incorporated drug acts as a plasticizer and interacts with the polymer matrix. This assumption can be supported by the fact that 5% nicardipine loaded implants show a higher glass transition temperature than the nifedipine containing implants (18.4 °C vs.

16.4 °C) and just a dependency on the type of drug and not on the drug concentration could be found. For interpretation of the degradation and erosion properties of the implants it should be mentioned that the T_g is lower than 37 °C and will decrease further after water penetration and polymer degradation.

Homogeneous distribution of the drug was proven by the HPLC analysis of the drug content of randomly selected pieces from three different implants (each 10 mg, two different locations for each implant). The results are shown in Table 2. The low standard deviation indicates a good homogeneity of the drug within the implants.

3.2. Monitoring of implant erosion and water uptake

The aliphatic polyester PLGA is mainly degraded by hydrolysis in the release medium or body fluids, nanosized PLGA dispersions are also digested by lipase [36]. By cleavage of the ester bonds the polymer is degraded to oligomers, which are getting water soluble at a certain molecular weight [3]. Degradation starts immediately when the polymer gets in contact with water. In contrast, polymer erosion, which is expressed in mass loss of the device, starts when the degradation is already ongoing and the first oligomers are able to diffuse out of the device. Water uptake is therefore an important parameter to monitor because polymer degradation requires prior water penetration into the polymer. In addition, drug release is also dependent on water penetration when the drug is diffusing out of the polymer and is dissolved in the surrounding medium. Water uptake and the hydrolysis induced formation of oligomers and monomers will plasticize the polymer and decrease the T_g [37]. Fig. 2A and C show the water uptake profiles of all formulations during the release studies.

We were able to calculate the mass of the drug within the implants from the HPLC results and subtract this mass from the total implant mass of each sample (see eq. (3)). This approach leads to the remaining polymer matrix as erosion parameter during the release studies (Fig. 2B and D).

For nifedipine, a water uptake factor (WUF) of 1 can be measured even after one day of incubation for both formulations and the implants become turbid upon water uptake. After a slow water uptake for one week a considerable acceleration of the water uptake factor to values up to the 11-fold for the 5% drug load and 9-fold for the 10% nifedipine formulation can be detected (Fig. 2A). This is most likely due to intensive degradation of the polymer resulting in emerging oligomers which will lead to a higher hydrophilicity of the system. Also osmotic effects of the acidic degradation products contribute to further water uptake. After two weeks a plateau is reached indicating a constant hydration level of the implant. This behavior was also described by Frank et al. for PLGA pellets of similar composition but higher molecular weight [38]. For the 5% nifedipine loaded formulation higher WUF values are obtained suggesting an influence of the drug load because the incorporated hydrophobic drug will decrease the hydrophilicity of the formulation. The WUF is an easy accessible parameter and describes the behavior of the whole formulation well. To exclude the influence of the drug load, the water polymer ratio (WPR) can be calculated. The application of this method gives a similar pattern, and yields only slightly higher values for the systems with 5% drug load (Suppl. Eq. (1), Suppl. Fig. 3A).

The mass loss (erosion) of nifedipine implants is initially low (Fig. 2B). A slight mass loss of approx. 5% was observed in the first week of incubation. This finding is in good agreement with the low
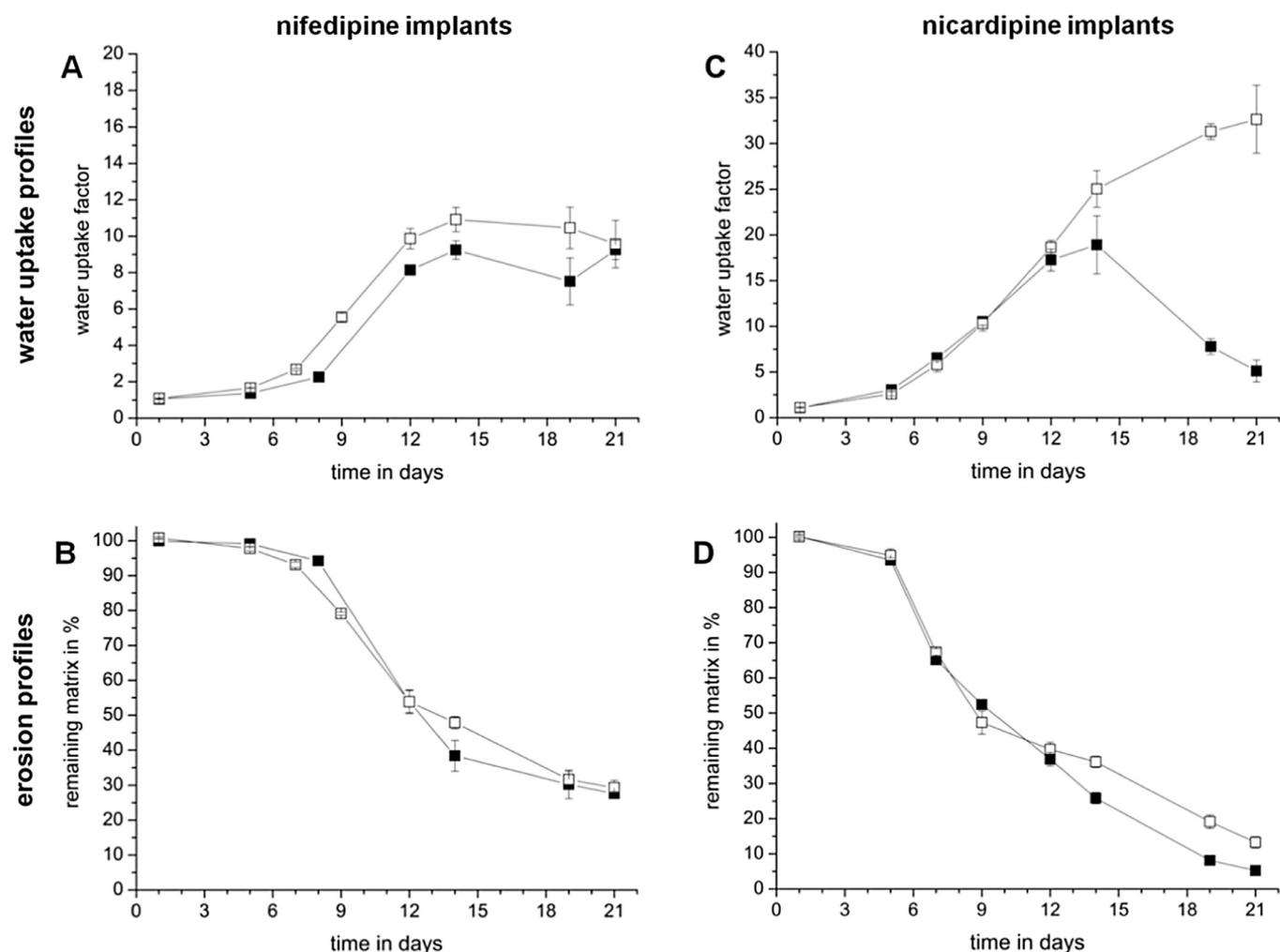
Fig. 2. Progress of water uptake factor (A, C) and erosion profiles (B, D) of PLGA implants with nifedipine (left) and nicardipine (right). Filled squares indicate 10% drug load and open squares 5% drug load, respectively (mean ± SD, n = 4). Please note the different scaling of the axis for water uptake factor.

initial water uptake, because mass loss demands prior degradation of the polymer after hydration leading to a possible transport of water soluble PLGA degradation products out of the device [3]. Erosion accelerates in the second week, probably due to autocatalysis of polyester hydrolysis by acidic degradation products [18]. After 14 days, nifedipine implants with 5% drug load lose 52.2% ± 1.8% of their polymer matrix and the implants with 10% drug load even 61.6% ± 4.4%, respectively. After 21 days of incubation a polymer mass of 29.3% ± 2.1% of the 5% formulation is remaining and 27.6% ± 0.6% of the 10% nifedipine formulation. The progress of mass loss is similar for both formulations and emphasizes the link between water uptake and the subsequent processes of degradation and erosion.

The water uptake profiles of the nicardipine loaded PLGA implants (Fig. 2C) deviate from the profiles of nifedipine implants. Besides the data for day one with a WUF of approx. 1 as well, nicardipine containing devices show a notable higher water uptake compared to nifedipine implants. After one week the formulation with less drug showed a WUF of 5.8 ± 0.8 and the formulation with 10% nicardipine a value of 6.5 ± 0.4. The progress of water uptake is quite the same for both drug loads until day 12 with approximately the 18-fold of the dry mass. Therefore, selection of drug seems to play a crucial role for implant degradation. Nicardipine containing PLGA devices bind more water than the nifedipine containing ones. The key properties (obtained from SciFinder) of both drugs are given in Table 1 of the supplement. At the neutral pH of 7, nicardipine is less soluble than nifedipine (2.6 mg/l vs 12 mg/l). Due to its low pKa value of 2.69, the solubility of nifedipine is

unaffected until the pH value of 4. In contrast, due to its higher pKa value of 7.3, the solubility of nicardipine is already changing in a neutral to slightly acidic environment. The low solubility of 2.6 mg/l at pH 7 increases to values of 18 mg/l (pH 6); 150 mg/l (pH 5); 720 mg/l (pH 4) and 1400 mg/l (pH 3). The higher water uptake of the nicardipine implants is seems to be not in alignment with higher log P-value (4.9 vs. 3.6). However, because PLGA hydrolysis will cause the formation of an acidic microenvironment, a significant part of nicardipine (in contrast to nifedipine) will be protonated and therefore more hydrophilic. This might cause an increased water uptake.

After two weeks of incubation a large difference between the two nicardipine drug loads becomes visible. The WUF value continues to increasing for the 5% nicardipine implants up to a value of 32.7 ± 3.7 after 3 weeks. A gel-like appearance of the implants is observed after this time. In contrast, the WUF value of the 10% loaded implants is decreasing after two weeks to a value of 5.1 ± 1.2 at day 21. The calculation of the water polymer ratio WPR (Suppl. Fig. 3B) gives a similar pattern. Both formulations are similar in the first days and very different after 14 days.

The erosion behavior of the nicardipine implants (Fig. 2D) is in good agreement to the water uptake and shows also a sigmoidal profile. The higher water uptake leads to higher degradation and, therefore, to faster and more intensive hydrolytic degradation compared to the nifedipine implants. The erosion of 50% requires 12 days for nifedipine and only 9 days for nicardipine. The drug load of nicardipine implants has no impact on the erosion rates within the first days. However,

similar to the water uptake, differences were observed at later time points. After 14 days, approximately 25% of the polymer mass remained for implants with 10% drug load. A slower erosion (36% remaining) was measured for 5% loaded nicardipine implants. The small amount of remaining polymer might be the reason for the drop of water uptake after 2 weeks of release, because the swollen polymer has already eroded.

Fredenberg et al [39] investigated the water uptake and mass loss of different PLGA-types under several conditions with the focus on pore formation and closure. When using the same polymer like in our experiments they found comparable erosion profiles at pH 7.4 and also experienced highly swollen systems. Following their explanation, the fast emerging acidic degradation products of this low-molecular weight polymer are mostly charged at pH 7.4 leading to higher water uptake and partial pore closure. As seen in this study, both water uptake and polymer erosion are also dependent on the incorporated drug and the drug content. The differences might be explained by the properties of the dihydropyridines. Basic drugs are known to influence the degradation of PLGA. Both enhanced degradation via basic catalysis by the drug [40] and also decelerated degradation due to neutralization of the polymer terminal carboxyl groups have been reported [41]. Incorporated drugs will impact several important properties of the polymer microenvironment (e.g. hydrophilicity, osmotic pressure, microacidity). The complexity of drug release processes and interference between the polymer, the drug and the environment has been nicely reviewed by Fredenberg et al. [15]. The different pKa values and the different pH-dependent solubilities are key factors for the observed differences. With a pK$_a$ of 7.3 (SciFinder) or 7.4 [42] nicardipine is stronger basic than nifedipine (pK$_a$ 2.6) [42], causing catalytically higher degradation rates and therefore higher water uptake. For nicardipine, the impact of drug load on water uptake and erosion becomes evident after 14 days. At his time point, more that 60% of the polymer mass has already been eroded.

3.3. Drug release studies

The in-vitro release of implants containing 5 or 10% dihydropyridines was observed over 21 days (Fig. 3). Sink conditions and light protection were maintained all the time.

For nifedipine, the implants loaded with 10% drug released just the very small amount of less than 5% of the drug during the first week. This corresponds to the initially small values of water uptake and polymer erosion. In the following days the cumulative release accelerated and reached 25.8 ± 9.4% after 14 days. Thereafter, only a

slight increase was observed and only 30.2 ± 1.9% release were achieved after 21 days. The implants with 5% nifedipine load showed higher release rates. Already 23.3 ± 4.6% were released after one week, which is 5 times more compared to implants with 10% drug load. Much higher values were also observed at later time points: 68.5 ± 5.6% nifedipine were released after 14 days and 84.5 ± 5.1% after 21 days. The observed initial burst release of 14.2 ± 5.3% was unexpected for this formulation. No burst release was detected for any other implants. For this reason, the burst release of the 5% nifedipine formulation might be explained by dissolution of superficially present drug molecules. Both the 10% nifedipine formulation as well as the nicardipine implants might show a higher hydrophobicity due to a higher drug load and a higher logP, respectively and therefore maybe less contact with 37 °C water at the initial stages. Nevertheless, for nifedipine implants, a strong impact of the drug load on the release kinetics can be concluded.

The release of nicardipine from PLGA implants with drug loads of 5 and 10% was compared to investigate the impact of the drug load on the release kinetics as well. The cumulative release curves show a very similar exponential release profiles during the first week but differ thereafter. Also for nicardipine, the formulation with lower drug load released a higher percentage compared to implants with higher drug concentration. This behavior was observed until the end of the release study after three weeks. The slow release of Nicardipine is not controlled the PLGA polymer, at least at later time points, because polymer erosion exceeds drug release.

The effects of drug type depend from the absolute amount of the drug. A higher relative release rate from implants with a lower drug might still mean a lower absolute amount of the drug which has been released. A conclusive picture is only obtained if both parameters are considered. A comparison of the absolute drug release (Table 3) leads to the surprising fact, that a lower drug load causes in the case of nicardipine comparable release to the higher drug load and in the case of nifedipine even an improved release is detectable. In theory, the amount of the released drug from implants with 10% drug should be twice the amount of the mass which is released from the 5% implants. This scenario is only observed at early time points of nicardipine release. For nifedipine loaded polymers, implants with 5% drug load release a higher amount of drug in [mg] despite the lower drug content at all time points.

Sink conditions were maintained all time, so the release and dissolution of the drug were not limited by saturation effects in the medium. The initial lag phase of release is explained by the slow onset of water penetration into the hydrophobic polymer matrix. This
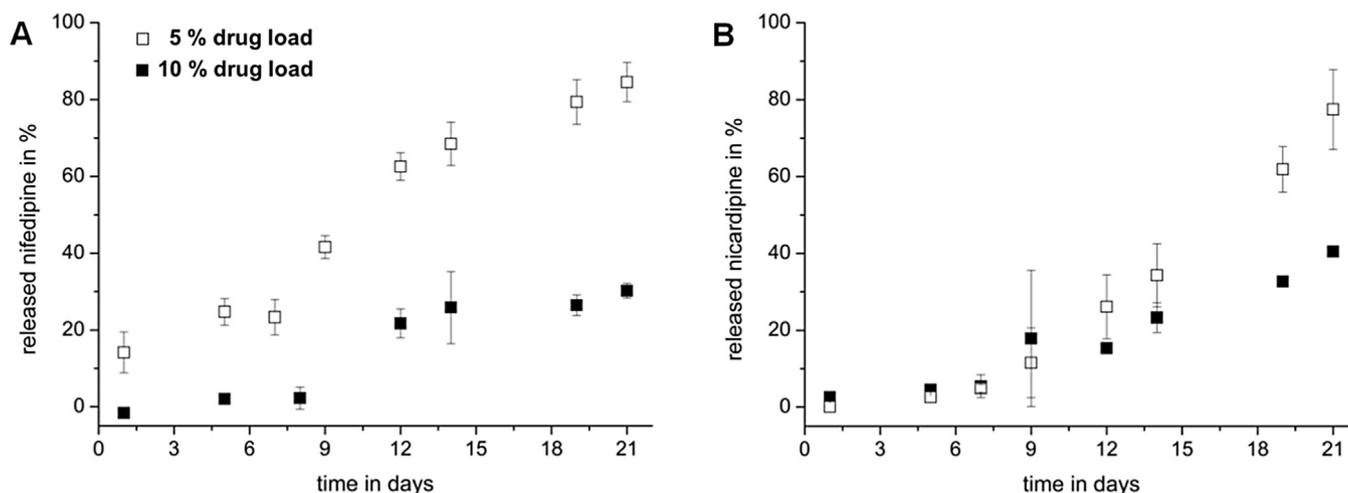

Fig. 3. Cumulative release of (A) nifedipine and (B) nicardipine at different drug loads. Open squares show 5% drug load, filled squares 10% drug load respectively. Data are presented as mean ± SD n = 3.

Table 3
Absolute amount [mg] of drug release from PLGA implants, data are presented as mean $\pm$ SD n = 3.

	Absolute values of cumulative drug release [mg] from PLGA implants			
day	5% nifedipine	10% nifedipine	5% nicardipine	10% nicardipine
7[*]/8[**]	0.233 $\pm$ 0.046[*]	0.045 $\pm$ 0.058[**]	0.048 $\pm$ 0.013[*]	0.107 $\pm$ 0.061[*]
14	0.685 $\pm$ 0.056	0.517 $\pm$ 0.187	0.348 $\pm$ 0.075	0.453 $\pm$ 0.059
21	0.845 $\pm$ 0.051	0.604 $\pm$ 0.038	0.798 $\pm$ 0.138	0.832 $\pm$ 0.016

phenomenon is often observed for PLGA and PLA polymers. Release of the drug in this time period is mainly due to dissolution from the implant's surface or diffusion from the outer layers. After one week, the drug release accelerates, which coincides with increased water uptake and erosion. A prerequisite for polymer erosion is the formation of water soluble oligomers and monomers and therefore, polymer degradation. Diffusion, degradation and erosion processes do contribute simultaneously to the accelerated release phase which starts after one week. The higher release rates of nicardipine compared to the equivalent nifedipine formulation are in consistency with higher water uptake and also higher polymer erosion.

The phenomenon of increased drug release as a result of lower drug load needed to be further investigated. In addition, we observed especially for the 10% drug load an incomplete release that is not congruent with the polymer erosion which is nearly complete after three weeks for nicardipine implants (see Fig. 2D). This observation is in contradiction to an erosion, diffusion or swelling controlled drug release. For diffusion and swelling controlled drug release, drug release will precede polymer erosion. For erosion controlled release, both kinetics should be comparable. The biodegradability of PLGA is linked to polymer hydrolysis. By hydrolysis, the hydrophobic polymer matrix is converted to more hydrophilic chain segments and finally to the water soluble and highly acidic monomers lactic and glycolic acid. The hydrolysis will cause a change of the polymer microenvironment which might impact the capacity of the polymer to solubilize drug molecules. An increase of hydrophilicity caused by polymer degradation will decrease the solubility of hydrophobic molecules. Therefore, drug precipitation might occur. In order to investigate whether or not drug precipitation occurs, further measurements were conducted.

3.4. Determination of drug precipitation

To examine the state of the drugs within the implants during release several methods were applied. Incident light microscopy allows a detailed view of the whole size and the surface of the implant. Fig. 4 shows all implant types of this study before release and after 7, 14 and 21 days of release, respectively. The microscopic analysis has shown that the implants have initially a smooth surface but contain some air bubbles.

After one day of incubation the implants already get turbid upon water penetration. After one week swelling of the matrix leads to a change in shape. At this time, no significant differences in the optical appearance between the formulations could be observed, although the nicardipine formulations incorporated more water (Fig. 2). With increasing water uptake in the second week the implants are getting more and more transparent and soft which is probably due to the degradation progress. The mass loss becomes evident by the size reduction of the implants. At this time the difference in the release patterns of the formulations can easily be explained by detailed observation of the formulations. In case of nifedipine the 5% formulation becomes totally transparent with some small yellow drug crystals. Therefore, we conclude drug precipitation upon high water penetration due to low water solubility of the drug. For the 10% nifedipine implants this behavior is even more expressed resulting in a bright yellow drug crystal core which grows until the end of the release study time. Nifedipine crystals were also accessible by polarized light microscopy (Fig. 5) proving the

presence of crystalline drug. These findings indicate high local concentration of the drug resulting from the high solubility of the amorphous drug. This concentration exceeds the solubility of the drug resulting in drug precipitation. Similar results were obtained by Van Drooge et al. [43] when investigating the release of the poorly soluble drug diazepam from solid dispersions with different sugar carriers. In their study no controlled release by the carrier was intended and the carrier was already soluble in the medium though the whole drug was in direct contact with the medium after a short time. Nevertheless, similar observations were made: in case the carrier dissolves very fast the drug dissolves from the solid dispersion, too but at the same time a local oversaturation of the drug occurs leading to drug precipitation. The authors also described that a lower drug content could avoid the oversaturation like it is seen in our study.

In order to prove the physical state of the precipitated nifedipine with a second method, PXRD was applied. Fig. 6 shows the x-ray patterns of the amorphous polymer and the crystalline drug in comparison to the ground dried implants of the 10% nifedipine formulation after specified days of release. The measurement after production of the implants (day 0) supports the assumption that the drug is embedded in an amorphous form in the polymer because just a pattern comparable to the amorphous PLGA is visible. The PXRD diffractograms of buffer exposed polymers show small signals which are consistent with the position of the signals of the pure crystalline nifedipine.

In contrast to nifedipine, no crystals could be found for nicardipine by DSC, polarized light microscopy and PXRD, although accumulation of a yellowish compound is visible by microscopy. We hypothesize a comparable progress to the nifedipine implants, after the dissolution of the previous amorphously embedded drug supersaturation occurs leading to precipitation of the drug. In the case of nifedipine, the drug precipitates in it's crystalline form. In contrast, the nicardipine free base seems not to form a crystalline state like nicardipine hydrochloride and remains in a solid amorphous form or as supercooled melt in the further degrading implant. This hypothesis is supported by repeating the DSC, polarized light microscopy and PXRD measurements with the freshly extracted nicardipine free base, which were also providing no evidence for a crystalline state. We experienced the nicardipine free base as a glassy substance after excessive drying which tends to become quickly tacky after contact with environmental humidity. These observations are in accordance with the findings of Miyajima et al. [25] who described the nicardipine free base as an oily compound. Very interesting data about nicardipine have been published recently by Indulkar et al. [44]. The group clearly showed that water exposure might cause a transformation of the crystalline nicardipine base into an amorphous material. Within this publication, also theoretical and experimental data have been published on the pH-dependent solubility of amorphous nicardipine. They describe a liquid-liquid phase separation into a drug-enriched and a drug-lean compartment at a concentration approx. equal to the amorphous solubility. Moreno-Calvo et al. [45] describe different polymorphs and hydrates of nicardipine hydrochloride and nicardipine, respectively. A crystalline nicardipine hydrochloride bishydrate with a melting point around 410 K has been published by this group. The lack of nicardipine crystals in our study are in line with the observations of Ikeda's [25] and Taylor's group [44]. Water penetration and polymer degradation will lead to supersaturation of the amorphous drug. It is likely that the drug phase separates as a supercooled melt (oily liquid)
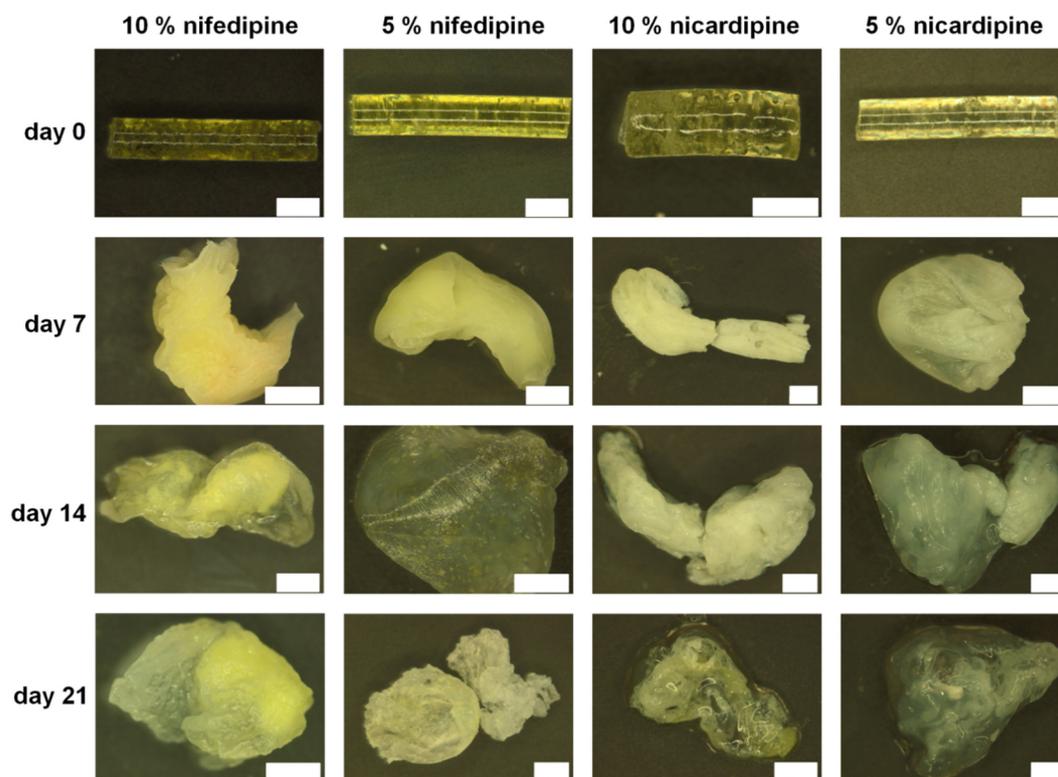
Fig. 4. Microscopic images of implants during selected times of release. The rod shaped implants lose their form quickly after exposure to the release medium. Due to load with dihydropyridines the implants appear yellow and over the time, the yellow colored drug crystals are getting more and more visible whereas the PLGA matrix degrades and becomes transparent. White scale bars indicate 2 mm.
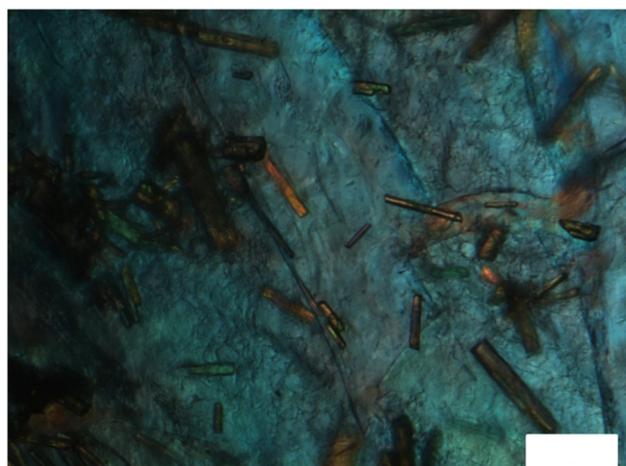

Fig. 5. Microscopic image of an implant containing initial 5% nifedipine after 14 days of release; rod-shaped large nifedipine crystals become visible with polarization microscopy, the amorphous polymer has already a rough surface and seems to be interspersed with pores. The white scale bar indicates 100 μm.

Fig. 6. PXRD pattern of the pure polymer (Resomer® RG 502H), nifedipine and dried implants initially loaded with 10% nifedipine after specified days of release.

as described by Indulkar et al. [44]. In principle, also the formation of nicardipine lactide or glycolide might take place.

To get further information on the drug release processes and the extend of drug precipitation, the drug content of the implants during release was quantified. It should decrease or remain constant for diffusion and erosion controlled release and should increase in the case of drug precipitation. The results can be seen in Fig. 7. The 5% nifedipine implants show a nearly constant drug content which is slightly decreasing until $2.57 \pm 0.96\%$ after 21 days when approx. 85% of the drug were released. For polymers with 10% initial nifedipine load a constant value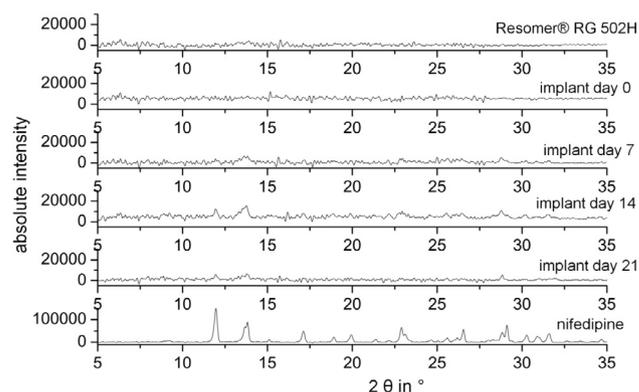 was observed for approx. 9 days. Thereafter, the drug content increased steadily and reached approx. 20 wt% after three weeks due to the drug separation. This increase starts at the same time when the polymer accelerates its water uptake and erosion (see Fig. 2A and B), proving that the drug becomes supersaturated in the degrading polymer matrix environment.

A similar pattern is observed for nicardipine formulations (Fig. 7B). The implants with 10% nicardipine maintain a constant drug load only within the first week. Thereafter, the drug content increases continuously to reach over 50% after three weeks. The implants with less drug load show just a small increase in drug content to $8.68 \pm 0.48\%$ after 14 days and $8.32 \pm 3.2\%$ after 21 days. It indicates that already at 5% drug load, slow drug dissolution might contribute to the overall release kinetics.

Taken all the investigations together (water uptake, erosion, drug release rate, microscopy and X-ray-experiments), the following picture
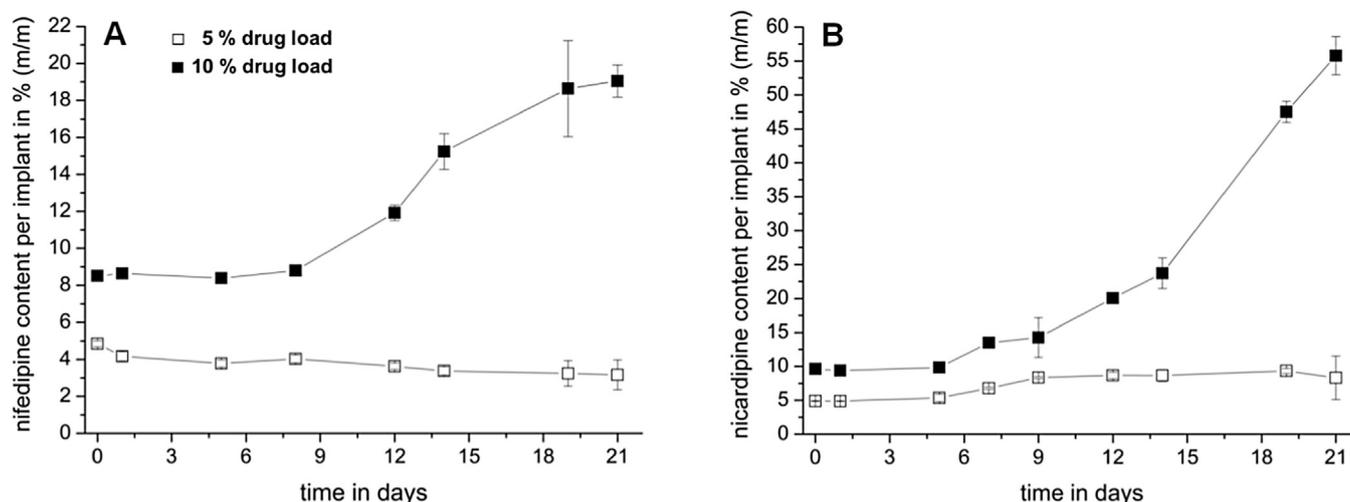
Fig. 7. Time dependence of the drug content during release for Nifedipine (A) and Nicardipine (B) formulations (mean ± SD, n = 3).

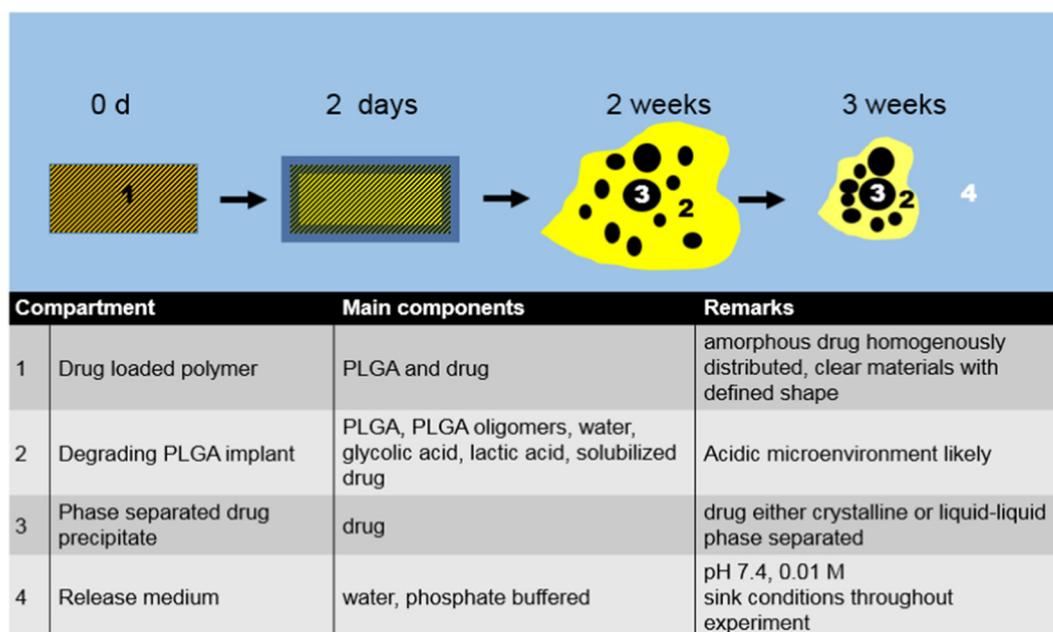| Compartment		Main components	Remarks
1	Drug loaded polymer	PLGA and drug	amorphous drug homogenously distributed, clear materials with defined shape
2	Degrading PLGA implant	PLGA, PLGA oligomers, water, glycolic acid, lactic acid, solubilized drug	Acidic microenvironment likely
3	Phase separated drug precipitate	drug	drug either crystalline or liquid-liquid phase separated
4	Release medium	water, phosphate buffered	pH 7.4, 0.01 M sink conditions throughout experiment

Fig. 8. Scheme buffer exposure induced changes with characterization of microcompartments.

emerges for all drug loaded implants and is displayed in Fig. 8.

Initially, the drug loaded polymer extrudate is clear and has a defined geometry. The water uptake is low with the first week and accelerates thereafter. After the first week, increased water uptake, swelling and loss of a defined geometry can be observed. Water penetration and polymer degradation change the drug microenvironment and cause supersaturation. A hydrophilic and most likely acidic microenvironment (compartment 2) is formed within the degrading polymer. Supersaturation causes phase separation (formation of compartment 3). In the case of nifedipine, crystallization occurs and drug crystals can be found by light microscopy and X-ray diffraction. Phase separation is also likely to occur the case of nicardipine. However, instead of forming crystals, an amorphous or liquid phase is very likely. For both drugs, the erosion of the PLGA polymer occurs faster than the release of the drug and the drug content in the polymer (in %) increases with time. At later time points (e.g. 2 or 3 weeks), drug molecules are present in at least three different environments (see Fig. 8): Released drug molecules will be localized in the phosphate buffer at pH 7.4, where sink conditions were maintained all the time. The second location will be the drug enriched phase either as crystal (nifedipine) or as

amorphous or liquid material (nicardipine). The third phase will be the hydrophilic and acidic environment within the degrading polymer, where – in addition to water - oligomers and lactic and glycolic acid are key components. An acidic microenvironment will increase the solubility of nicardipine due to the protonation of the tertiary amino group, for nifedipine, a pH-independent solubility is expected. At neutral pH, only about 5 µg Nicardipine are soluble in 1 ml, but more than 300 µg Nicardipine are solubilized at pH 5.5 [44]. It has also to be considered, that not only the pH, but also the counterion itself influences the solubility of nicardipine [46]. For nifedipine, slow dissolution kinetics from the crystal can limit the overall release kinetics. Because of the absence of crystallization, this cannot be the case for nicardipine. It is expected that the equilibrium processes will occur much faster due to the supercooled state of the phase separated nicardipine phase. Therefore, the slow release is probably only due to the low saturation concentration of nicardipine at neutral pH-values.

4. Conclusion

Drug release from PLGA matrices is very complex and dependent

from many parameters. An ideal formulation would release the drug in the desired time and degrade in a comparable period. The PLGA (RG 502 H) extrudates show a sigmoidal erosion behavior which is strongly influenced by the type of drug. After three weeks just a small amount of polymer remains which is quite favorable for the application after subarachnoid hemorrhage. Drug release should occur between day 4–10 after implantation [47]. Therefore, a small initial lag time can be tolerated, but the release should not exceed three weeks. Although the polymer erosion profiles did largely match with the desired time profile, the drug release rates did not. Drug release was found to be too slow and incomplete.

By having a closer look to the implants we could follow the stages of drug release. Once exposed to buffer, water penetrates into the implants and the drug can diffuse to the medium, but this diffusion control leads just to a very small amount of released drug. By time, the implants swell upon water uptake and lose their shape, the physicochemical properties of the polymer matrix change due to polymer degradation into more hydrophilic oligomers and monomers and a high water content of the implants due to osmotic effects of this degradation products. This alteration leads to an environment which is not favorable for solubilization of the investigated dihydropyridines. Although the amorphous state of the drugs should lead to an increased solubility, local supersaturation might occur followed by drug precipitation in the case of nifedipine and phase separation for nicardipine containing implants. As a result, drug release becomes mainly controlled by slow dissolution of the crystallized drug or drug rich separated phase. A lower drug load caused improved release rates, because it retarded the oversaturation. As a result, implants with a lower drug content showed a higher release not only with regard to the relative percentage, but also in the absolute amount [mg] of drug release.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2019.03.016.

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