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Research Paper

Towards More Realistic *In Vitro* Release Measurement Techniques for Biodegradable Microparticles

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Purpose. To better understand the importance of the environmental conditions for drug release from biodegradable microparticles allowing for the development of more appropriate *in vitro* release measurement techniques.
Methods. Propranolol HCl diffusion in various agarose gels was characterized by NMR and UV analysis. Fick's law was used to theoretically predict the mass transport kinetics. Drug release from PLGA-based microparticles in such agarose gels was compared to that measured in agitated bulk fluids ("standard" method).
Results. NMR analysis revealed that the drug diffusivity was almost independent of the hydrogel concentration, despite of the significant differences in the systems' mechanical properties. This is due to the small size of the drug molecules/ions with respect to the hydrogel mesh size. Interestingly, the theoretically predicted drug concentration-distance-profiles could be confirmed by *independent* experiments. Most important from a practical point of view, significant differences in the release rates from the same batch of PLGA-based microparticles into a well agitated bulk fluid *versus* a semi-solid agarose gel were observed.
Conclusion. Great care must be taken when defining the *in vitro* conditions for drug release measurements from biodegradable microparticles. The obtained new insight can help facilitating the development of more appropriate *in vitro* release testing procedures.

KEY WORDS: agarose gel; diffusion; microparticles; PLGA; release test.

INTRODUCTION

Poly(lactic-co-glycolic acid) (PLGA)-based microparticles are steadily gaining in importance as advanced parenteral drug delivery systems, because they offer various major advantages, including: (i) the possibility to accurately control the resulting drug release kinetics over periods of days to months (1,2); (ii) complete biodegradability (avoiding the removal of empty remnants upon drug exhaustion) (3,4); (iii) good biocompatibility (5,6), even if directly administered into brain tissue (intracranially) (7,8); and (iv) easy administration (compared to implants) using standard syringes and needles. Various products based on PLGA-based controlled release microparticles are commercially available, for instance Lupron Depot, containing the anticancer drug leuprolide acetate for the treatment of prostate cancer, and Risperdal Consta containing the antipsychotic drug risperidone for the treatment of schizophrenia.

Despite the considerable practical importance of PLGA-based microparticles, and of biodegradable microparticles in general as advanced drug delivery systems, no regulatory standards have been established for the experimental measurement of *in vitro* drug release from this type of dosage forms. Workshops sponsored by the FDA and USP (9) and by the EUFEPS (10) have clearly pointed out the need for such standards. They are not only required for quality assurance of the final product, but also during formulation development. Importantly, the type of experimental testing procedure used can significantly affect the measured drug release kinetics (11–15). Generally, the microparticles are directly exposed to a bulk fluid which is kept constant at 37°C. These fluids often differ in composition (e.g., with respect to their ionic strength, type and amount of ions). In addition, the ratio "amount of PLGA-based microparticles:bulk fluid volume" significantly differs between the various studies described in the literature. Furthermore, in some release testing procedures the bulk fluids are agitated, whereas in others they are not (16).

One major drawback of all drug release measurement techniques using bulk fluids is the fact that they generally do not realistically simulate the conditions the microparticles are exposed to upon *in vivo* administration. For instance, after subcutaneous or intramuscular injection, the microparticles are not in contact with a bulk fluid, but with living tissue. Thus, the environment surrounding the controlled drug

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delivery systems is completely different from that in commonly used *in vitro* release tests. Unfortunately, this difference can be expected to significantly affect the resulting drug release kinetics from various types of biodegradable microparticles, in particular from those based on PLGA, because the degradation of this polyester strongly depends on the pH: PLGA hydrolysis is catalyzed by bases and acids. It is well known that water penetration into PLGA-based microparticles is much faster than the subsequent polymer chain cleavage (17). Thus, upon *in vivo* administration, the entire devices are rather rapidly wetted and ester hydrolysis occurs throughout the systems (bulk-erosion). Consequently, shorter chains acids are generated within the entire microparticles. Due to concentration gradients these acids diffuse out into the surrounding environment, where they are neutralized. In addition, bases from the surrounding environment diffuse into the devices, neutralizing the generated acids. However, diffusional mass transport is generally slow and the rate at which the acids are generated can be higher than the rate at which they are neutralized, resulting in potential drops in the micro-pH (18–20). Obviously, the extent and velocity of the exchange of acids and bases with the surrounding environment strongly depends on the *nature of this environment*. Living human tissue exhibits very different mass transport phenomena compared to a bulk fluid within an agitated test tube. In the latter case, convection dominates and assures very rapid mass transfer, resulting in a homogeneous bulk fluid composition. This can have important consequences for the resulting drug release kinetics: If the acids generated upon polymer degradation are artificially rapidly neutralized *in vitro*, the pH around and within the microparticles does not as quickly and strongly decrease as under *in vivo* conditions. Consequently, the degradation of the polyester can be significantly slower. In the living tissue the pH might be much lower, resulting in potentially accelerated ester hydrolysis/autocatalytic effects (21). S.P. Schwendeman (22) clearly pointed out the importance of this phenomenon. Hence, the type of environment the microparticles are exposed to can strongly affect the rate at which the matrix forming polymer in PLGA-based microparticles is degraded. This is of fundamental importance for the mobility of encapsulated drug molecules: With decreasing polymer molecular weight, the mobility of the macromolecules increases, resulting in increasing free volumes available for drug diffusion. Consequently, the resulting drug release rate increases (23,24).

In addition, once the drug is released from the microparticles, it must cross living tissue to reach its target site. Various phenomena can be involved in this type of transport (25), which is not simulated when using *agitated bulk fluids* as release media. To provide more realistic conditions *in vitro* (e.g., minimizing convective transport), hydrogels have been proposed to mimic living tissue (26–29). Hydrogels are used for a large variety of applications, including chromatography techniques, as membranes, in controlled drug delivery systems (30) and for the simulation of human tissue. Agarose is a linear polysaccharide with a main chain consisting of 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose. Upon cooling a heated aqueous agarose solution, the polysaccharide molecules form double helices, which aggregate to form fibers (31). These microcrystalline fibers are connected and form a three-dimensional network. The pore size distribution generally ranges from 1–900 nm, depending on the agarose concentration (32,33). Agarose gels have been proposed to simulate vitreous humour (28), spinal cord (27) as well as brain tissue (29). Moreover, agarose gels have been proposed to simulate the release conditions in living tissue: Different concentrations of agarose were investigated. Chen *et al.* (29) concluded that 0.6% agarose gels best simulate pig brain tissue for intraparenchymal infusions. However, yet little is known on the transport of drugs in such gels and no studies have been reported on the importance of the respective environmental conditions for drug release from biodegradable controlled drug delivery systems.

The major aim of this study was to better understand drug transport in agarose gels, which can be used for *in vitro* drug release measurements, via NMR analysis and mechanistic realistic mathematical theories based on Fick's second law of diffusion. A thorough understanding of the importance of the various mass transport processes and chemical reactions involved can help to develop more reliable *in vitro* release testing procedures and to facilitate the optimization of biodegradable controlled drug delivery systems for parenteral administration.

MATERIALS AND METHODS

Materials

Poly (D,L lactic-co-glycolic acid) (PLGA; Resomer RG 504H; PLGA 50:50, containing 25% D-lactic units, 25% L-lactic units and 50% glycolic units; Boehringer Ingelheim, Ingelheim, Germany), agarose (GenAgarose LE; Genaxxon BioScience, Biberach, Germany), propranolol HCl (Salutas, Barleben, Germany), dichloromethane (VWR, Fontenoy-sous-Bois, France), polyvinyl alcohol (Mowiol 4-88; Kuraray Specialities Europe, Frankfurt, Germany), and deuterium oxide (D_2O; Euriso-Top, Saint-Aubin, France).

Preparation of Agarose Gels

Agarose gels of different concentrations (0.01 to 0.6% *w*/*w*) were prepared by dissolving the polysaccharide in boiling water and subsequent casting into Petri dishes (diameter = 12 cm). Upon cooling to room temperature the gels formed. For the NMR measurements, water was replaced by deuterium oxide (D_2O). Optionally, propranolol HCl (10 mM) was added.

NMR Analysis

Spectra were recorded at 300 MHz on a Bruker Avance-DPX 300 spectrometer (Bruker, Wissembourg, France) at 37° C. One dimensional (1D) spectra were collected into 32K computer data points, with a spectral width of 3,600 Hz, a 30° pulse and a relaxation delay of 2 s. The diffusivity of water and propranolol HCl in the gels was measured by a DOSY (Diffusion Ordered SpectroscopY) NMR technique, based on a stimulated echo sequence incorporating bipolar gradient pulses and longitudinal eddy current delay (BPP-LED). This technique is based on a diffusion delay, flanked by two pulse-

field gradients, where the magnetization fraction re-phased by the second pulse, is described by:

$$I = I_0 \exp\left[-D(2\pi\gamma G\,\delta)^2(\Delta - \delta/3)\right] \quad (1)$$

Here, I is the signal strength at gradient G [T/m], D the diffusion coefficient [m^2/s], γ the gyromagnetic ratio [rad/(T.s)], δ the gradient duration [s], and Δ the time between the start of the two gradient pulses [s]. The diffusion coefficient was determined based on the decrease in the resonance intensity.

Microparticle Preparation

Propranolol HCl-loaded, PLGA-based microparticles were prepared using a water-in-oil-in-water (W/O/W) solvent extraction/evaporation technique: Two g PLGA were dissolved in 18 g dichloromethane. Two milliliters aqueous propranolol HCl solution (25% *w*/*v*) were emulsified into this organic phase using an Ultra-Thurrax (90 s, 20,000 rpm; T25 basic, IKA, Staufen, Germany). This primary water-in-oil (W/O) emulsion was dispersed into 2.5 L of an outer aqueous polyvinyl alcohol solution (0.25% *w*/*w*) under stirring with a three-blade propeller for 30 min (2000 rpm). Upon contact with the outer aqueous phase, the organic solvent diffused into the water and the microparticles formed. The latter were hardened by adding 2.5 L further outer aqueous phase and 3 h additional stirring (700 rpm). The particles were separated by filtration and subsequently freeze–dried to minimize the residual solvents' content. Very small (<90 μm) and large (>160 μm) particles were excluded by sieving.

Microparticle Size, Initial Drug Loading and Morphology

The mean diameters and size distributions of the microparticles were determined by laser diffraction (Malvern Mastersizer S, Malvern, Orsay, France). The initial, practical drug loading was measured UV spectrophotometrically (λ = 290 nm; Anthelie Advanced; Secomam, Domont, France) upon dissolution of accurately weighed amounts (approximately 20 mg) of microparticles in 7 mL acetonitrile. The morphology of surfaces and cross-sections was monitored using a scanning electron microscope (S-4000; Hitachi High-Technologies Europe, Krefeld, Germany). Samples were covered under an argon atmosphere with a fine gold layer (10 nm; SCD 040; Bal-tec, Witten, Germany). Cross-sections of the microparticles were obtained after inclusion into water-based glue and cutting with a razor blade.

In Vitro Drug Release in Agitated Test Tubes

Fifty mg microparticles were placed into 10 mL glass tubes filled with 10 mL phosphate buffer pH 7.4 (USP 30). The latter were horizontally shaken at 37°C (80 rpm; GFL 3033; Gesellschaft für Labortechnik, Burgwedel, Germany). At pre-determined time intervals, 1 mL samples were withdrawn (replaced with fresh medium) and analyzed UV-spectrophotometrically (λ = 290 nm; Anthelie Advanced). For the calculation of the cumulative absolute and relative drug release rates, also the drug present in all withdrawn samples was considered. Each experiment was conducted in triplicate.

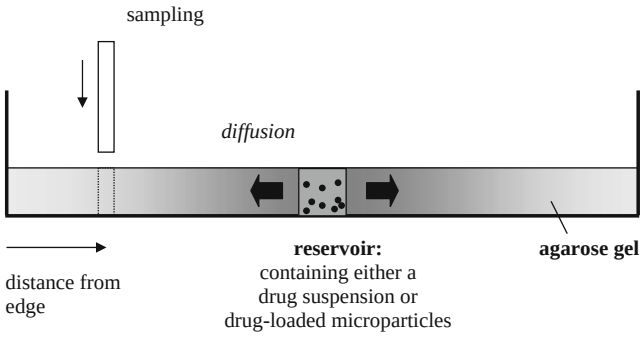

Fig. 1. Schematic illustration of the testing method used to more realistically simulate drug transport in living tissue upon parenteral administration. Petri dishes were filled with agarose gels and kept constant at 37°C. In the middle of the disc-shaped gels, a drug reservoir was deposited (either a drug suspension or drug-loaded microparticles). At pre-determined time points, gel samples were withdrawn at different distances from the edge of the Petri dish and analyzed for drug content.

Monitoring Drug Transport Within Agarose Gels

To more realistically simulate the conditions for drug transport in living tissue upon parenteral administration, the experimental testing method illustrated in Fig. 1 was used. Agarose gels of different concentrations were prepared as described in the "Preparation of Agarose Gels" section and kept constant at 37°C. At the center of these gels (diameter = 12 cm), cylindrical holes (diameter = 1.3 cm) were created and filled with either a drug suspension (in water, 150 mg/mL) or propranolol HCl-loaded microparticles (suspended in water, 110 mg), serving as a drug reservoir. The Petri dishes were placed into water-filled desiccators in an oven (ED115, Binder, Tutlingen, Germany) to prevent water evaporation during the experiments. To assure the maintenance of a saturated drug solution in the reservoir in the case of drug suspensions, further drug powder was added every 12 h. These "constant activity source" conditions allowed for a significant simplification of the respective mathematical treatment. At pre-determined time points, cylindrical gel samples (diameter = 0.5 cm) were removed at different distances from the edge of the Petri dishes, weighed, and analyzed for their drug content (UV-spectrophotometrically, λ = 290 nm; Anthelie Advanced). Due to the given symmetries it is assumed that the drug concentration in the gel at a certain distance from the drug source is similar throughout the system. Thus, the drug concentration measured in a particular sample withdrawn at a specific distance from the drug source allows calculating the amount of drug present in the corresponding concentric ring described by rotation of the gel sample around the drug source. Summing up the drug amounts in all concentric rings allowed for the calculation of the cumulative absolute and relative drug release rates.

Theory

Drug transport into the initially drug-free agarose gels from saturated propranolol HCl solutions at the center of the

Petri dishes was quantitatively described using the following analytical solution of Fick's law of diffusion (considering the given initial and boundary conditions and simplifying the geometry to that of a plane sheet, Fig. 1) (34):

$$\frac{c_{x,t}}{c_s} = 1 - \frac{4}{\pi} \cdot \sum_{n=0}^{\infty} \frac{(-1)^n}{2 \cdot n + 1} \cdot exp\left\{ -\frac{(2 \cdot n + 1)^2}{4 \cdot L^2} \cdot D \cdot t \cdot \pi^2 \right\} \cdot cos\frac{(2 \cdot n + 1) \cdot \pi \cdot x}{2 \cdot L} \quad (2)$$

where $c_{x,t}$ denotes the concentration of the drug at position x and time t; c_s is the solubility of propranolol HCl at 37°C, and L the distance from the reservoir.

Importantly, the apparent diffusion coefficient, D, of propranolol HCl within the agarose gels could be *independently* determined by NMR measurements as described in the "NMR Analysis" section. Thus, all variables on the right hand side of Equation 2 were known, and the resulting drug concentration-distance-profiles within the different gels could be quantitatively predicted at different time points.

RESULTS AND DISCUSSION

NMR Analysis of Propranolol HCl in Agarose Gels

To determine the diffusion coefficient of propranolol HCl in agarose gels of different concentrations (0.01% to 0.6% *w/w*), NMR spectra of drug-D_2O-agarose and drug-D_2O systems were recorded. Fig. 2a and b show exemplarily the ^{1}H-NMR spectra of a 10 mM propranolol HCl solution in D_2O, and of a 10 mM propranolol HCl solution in D_2O containing 0.3% (*w/w*) agarose, respectively. Clearly, the spectra are very similar: Only a few additional peaks appear due to the presence of agarose in Fig. 2b. Importantly, there are no drug-agarose interferences and no peak shifts occur when adding the polysaccharide to the drug solution. Thus, propranolol HCl can be analyzed in the presence of agarose by ^{1}H-NMR analysis.

Importantly, the apparent diffusion coefficients of D_2O and propranolol HCl in the agarose gels of different concentrations could be determined by fitting Eq. 1 to the experimentally measured "intensity-field gradient" data points. Fig. 3 shows an example of such a fitting: The curve shows the theoretical values, the symbols the experimentally measured intensities obtained with propranolol HCl in 0.1% *w/w* agarose gels. Clearly, good agreement between theory and experiment was obtained. This was the case for all types

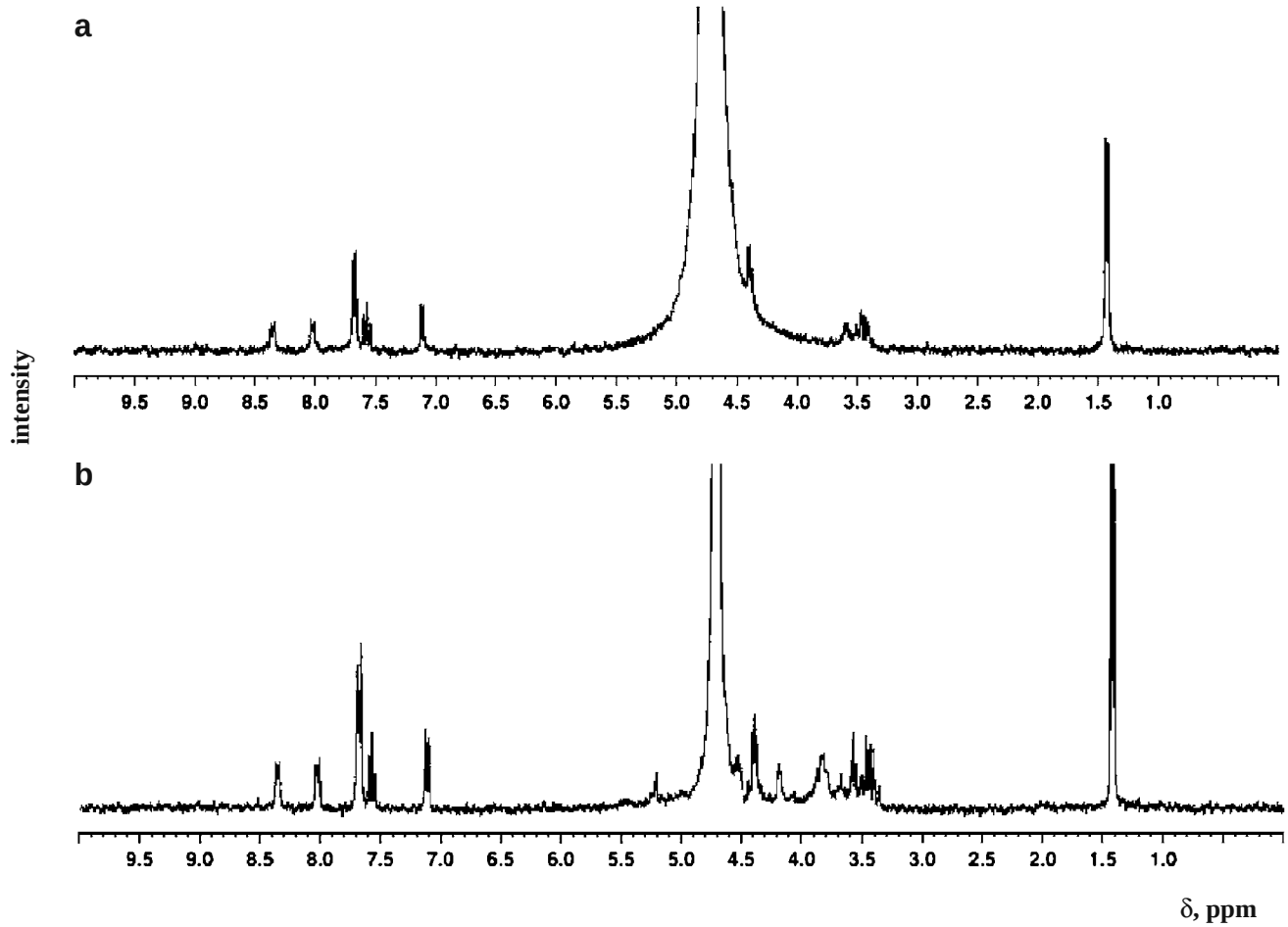

Fig. 2. ^{1}H-NMR spectra of propranolol HCl (10 mM) in: **a** D_2O, or **b** 0.3% (*w/w*) agarose gel (with D_2O as liquid phase). In both cases the temperature is 37°C.

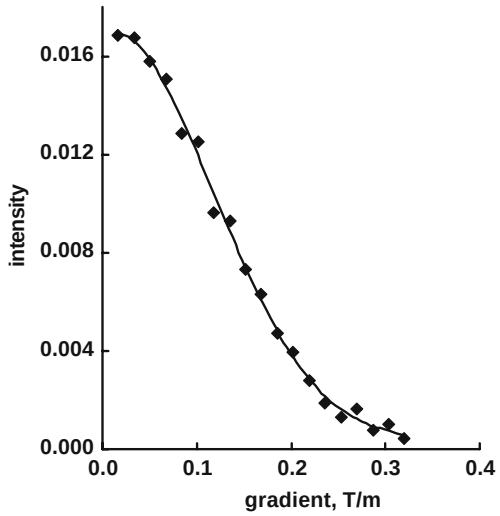

Fig. 3. Determination of the diffusion coefficient of propranolol HCl in an agarose gel (0.1% *w*/*w*) by NMR measurements: Fitting of Eq. 1 (curve) to the experimentally measured intensity (*symbols*) at different field gradients.

of gels, irrespective of the agarose concentration and type of diffusing species (data not shown). Based on these calculations, the apparent diffusion coefficients of D_2O and propranolol HCl could be determined for the various agarose concentrations (Fig. 4). Importantly, the agarose content had almost no effect on the mobility of the two species within the hydrogels. This is in contrast to the fundamental changes in the *mechanical* properties of the gels when increasing the polymer concentration within the investigated range: At 0.01% (*w*/*w*) agarose the system is *liquid*, whereas at 0.6% (*w*/*w*) polysaccharide it is *semi-solid*. At intermediate polymer concentrations, gels are formed upon standing, which are easily liquefied upon shaking. As it can be seen in Fig. 4, the diffusion coefficients determined by NMR analysis in these mechanically very different gels were found to vary only in the following ranges: 1.9–2.6 × 10^{-5} cm^2/s for D_2O, and 6.6–7.8 × 10^{-6} cm^2/s for the drug. This phenomenon can be attributed to the fact that D_2O as well as propranolol HCl molecules/ions are much smaller than the average mesh-size of the polymeric networks. For instance, Pluen *et al.* (33) reported a pore size of 100–200 nm in 2% (*w*/*w*) agarose gels. Consequently, the hindrance of D_2O and propranolol HCl diffusion in the investigated hydrogels due to sterical hindrance by the polymer networks can be expected to be minor. This explanation could be confirmed by the diffusion coefficients measured in the *absence of agarose*: D = 2.5 × 10^{-5} cm^2/s and 7.4 × 10^{-6} cm^2/s were determined for D_2O and the drug, respectively. Importantly, these values are similar to those measured within the agarose gels. Thus, the presence of the polymer immobilizes the aqueous phase and hinders convective mass transport, but does not significantly hinder the diffusion of *small* molecules. The measured diffusivities are also in good agreement with those reported in the literature for other types of small drugs in agarose gels. For instance, Sjoeberg *et al.* (35) determined the diffusion coefficient of lidocaine HCl (7.7 × 10^{-6} cm^2/s) and chlorpromazine HCl (6.9 × 10^{-6} cm^2/s) in 1% (*w*/*w*) agarose gels based on drug diffusion measurements into initially drug free, cylindrical gels. An appropriate analytical solution of Fick's law was fitted to experimentally determined "drug concentration—distance profiles" (obtained upon slicing the cylinders at pre-determined time points and subsequent UV measurements).

Modeling of Drug Transport within Agarose Gels

Once the apparent diffusion coefficient of the drug in the agarose gel is known, the resulting mass transport rates can be predicted using Fick's law of diffusion for different types of gel geometries and dimensions. To better understand the underlying mass transport phenomena for drugs in the investigated aqueous agarose gels serving as *in vitro* release model for biodegradable microparticles upon parenteral administration, the diffusion coefficients determined by NMR analysis were used to quantitatively predict the resulting drug concentration-time-profiles within the cylindrical gels at different time points and polysaccharide concen-

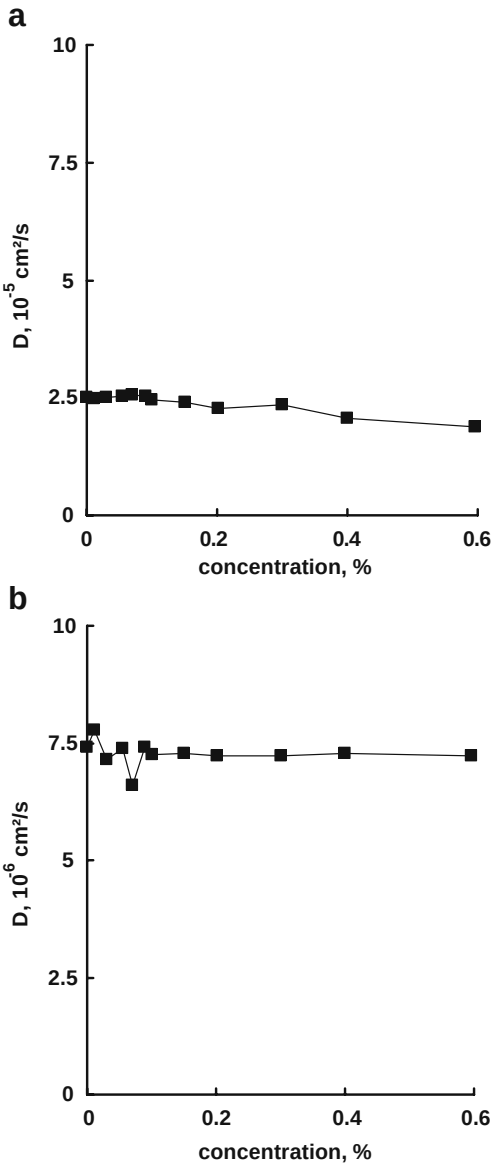

Fig. 4. Diffusion coefficients of: **a** D_2O and **b** propranolol–HCl in agarose gels of different concentrations determined by NMR analysis.

trations. The mathematical modeling considers drug diffusion in one dimension from a constant activity source (a saturated drug solution with a large excess of drug) at the center of the gels (Fig. 1). Taking into account time-independent drug diffusion coefficients and initially (t = 0) drug-free agarose gels, Equation 2 can be derived allowing for the calculation of the resulting drug concentration-distance-profiles (34). The theoretical results obtained are illustrated in Fig. 5a–d (curves = theory) for hydrogels with an agarose content of 0.2–0.6% (*w*/*w*). Clearly, the concentration-distance-profiles were similar for all polysaccharide concentrations. This is due to the fact that the polymer network does not significantly hinder drug diffusion within the investigated concentration range, as observed by NMR analysis. At early time points, the concentration gradients are very steep. With increasing time, their steepness decreases and the drug concentration even at the edges of the Petri dishes significantly increases.

To evaluate the validity of these theoretical predictions based on Fick's second law and the diffusion coefficients determined by NMR analysis, the drug concentration-distance-profiles within the agarose gels of different concentrations were also experimentally determined after 24, 48, 72 and 96 h. As it can be seen in Fig. 5a–d, good agreement was obtained between these *independent* experiments (symbols) and the theoretical predictions (curves), irrespective of the hydrogel concentration and time point. This clearly indicates that diffusion with constant diffusivities dominates propranolol HCl transport within the investigated agarose gels, irrespective of the polysaccharide concentration and mechanical strength of the systems.

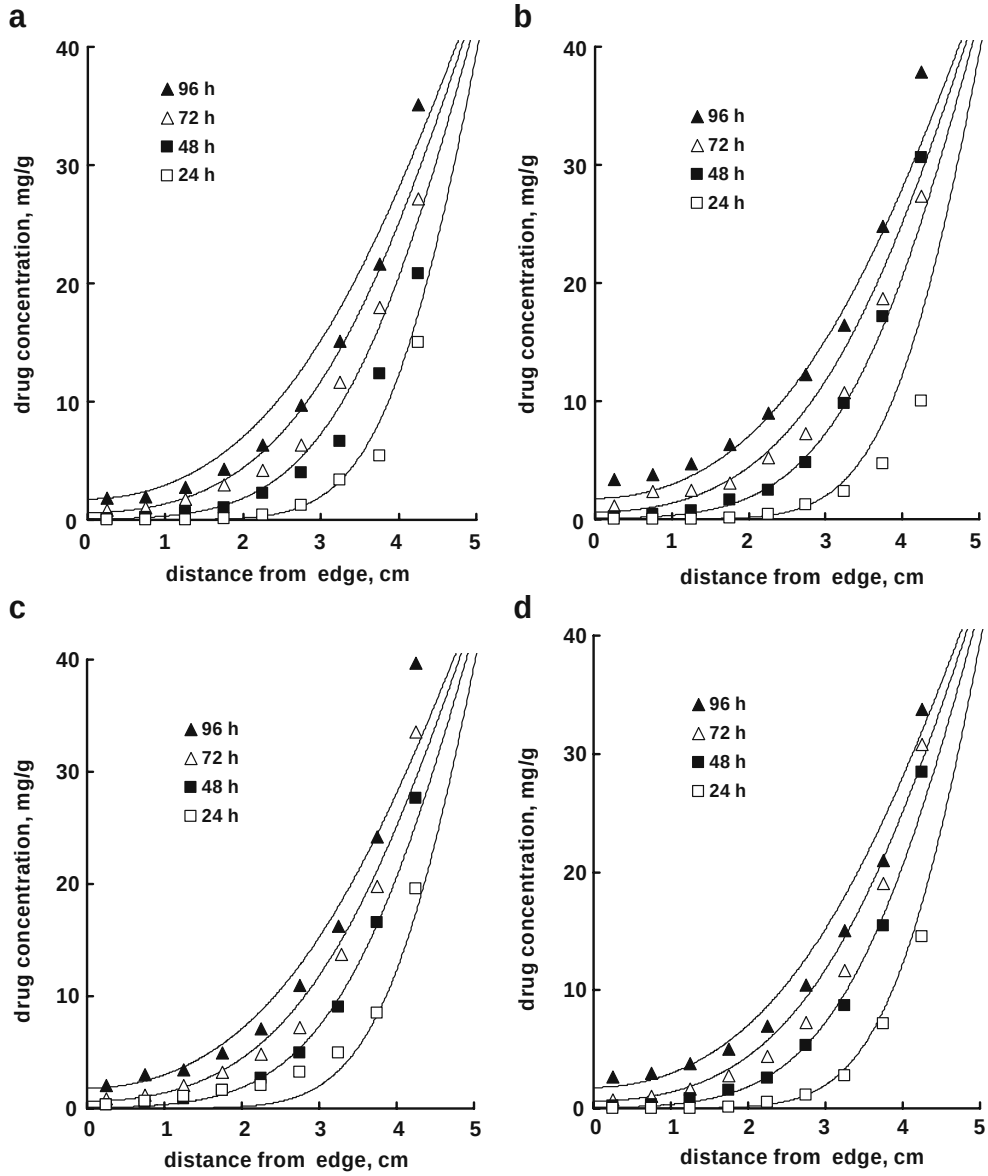

Fig. 5. Theoretical predictions and *independent* experiments: Propranolol HCl concentration-distance-profiles within agarose gels of different concentrations: **a** 0.2%, **b** 0.3%, **c** 0.4%, and **d** 0.6% (*w*/*w*) at various time points (indicated in the figures). The reservoir was filled with a drug suspension. The *curves* were calculated using Eq. 2 and the diffusion coefficients determined by NMR analysis. The experimental results (*symbols*) were measured using the testing method illustrated in Fig. 1 and UV measurements.

Thus, the propranolol HCl diffusivities determined by NMR analysis could successfully be used to theoretically predict the macroscopic drug transport within agarose gels in a quantitative way for arbitrary time periods. This can significantly facilitate the device design of novel controlled drug delivery systems, because it can help to improve the predictability of the performance of new devices more realistically. Based on such calculations, time- and cost-intensive "trial-and-error" studies can be reduced and product development accelerated.

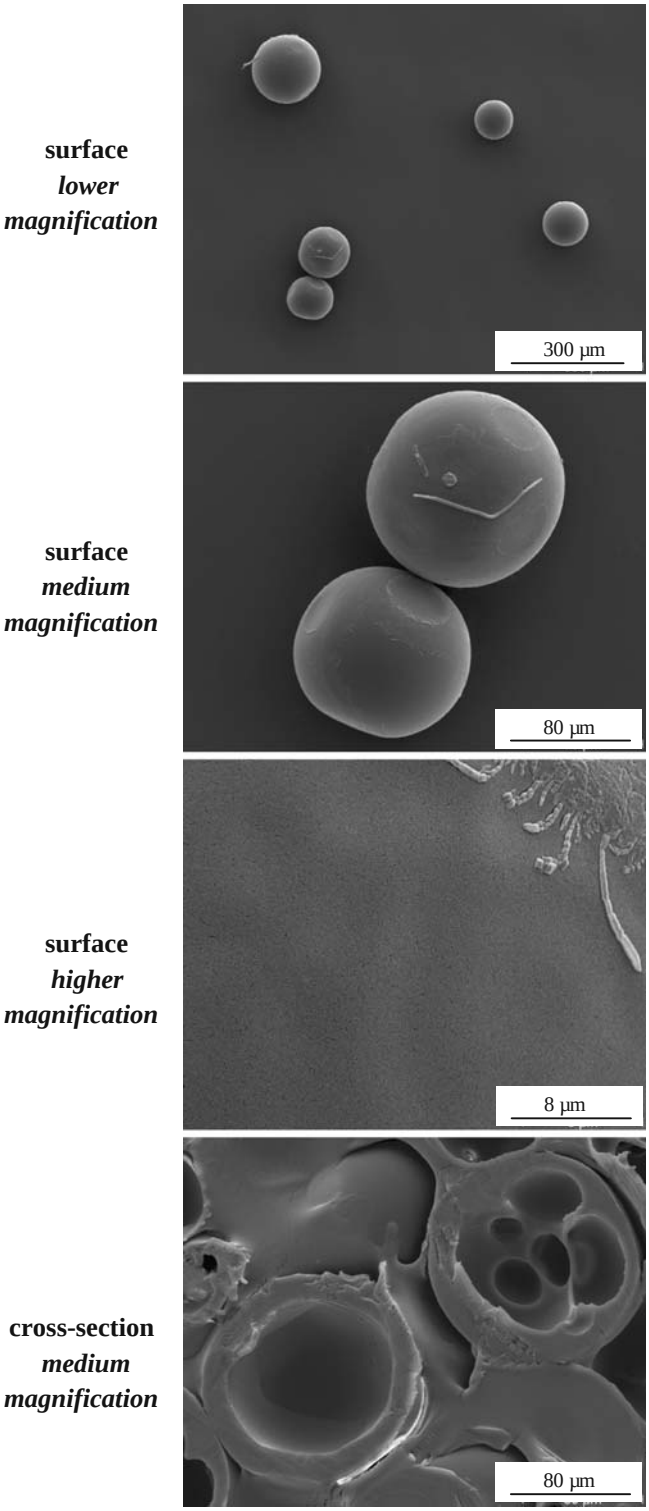

Fig. 6. Scanning electron microscopy pictures of propranolol HCl-loaded, PLGA-based microparticles before exposure to the release media (t=0): Surfaces and cross-sections at different magnifications (please note the different scaling).

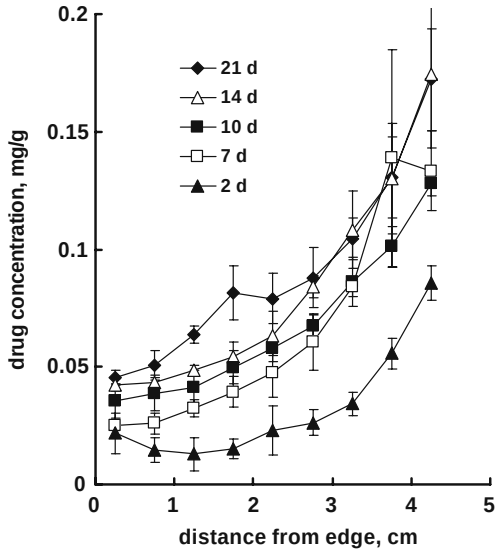

Fig. 7. Experimentally measured propranolol HCl concentration-distance-profiles in 0.6% (w/w) agarose gels at various time points (indicated in the figure). The testing method illustrated in Fig. 1 was used, the reservoir being filled with drug-loaded, PLGA-based microparticles.

In Vitro Drug Release from PLGA-Based Microparticles

Scanning electron microscopy revealed that the investigated propranolol HCl-loaded, poly(lactic-co-glycolic acid) (PLGA)-based microparticles were spherical in shape and exhibited a smooth, non-porous surface before exposure to

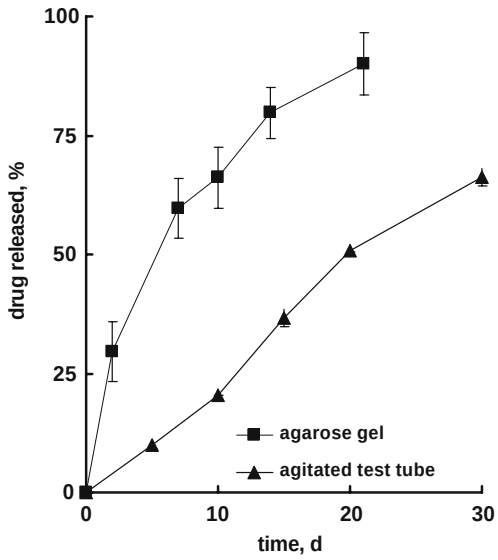

Fig. 8. Importance of the experimental testing method used to measure *in vitro* drug release from propranolol HCl-loaded, PLGA-based microparticles. "Standard" conditions (agitated test tubes filled with phosphate buffer pH 7.4) *versus* agarose gels.

the release media (Fig. 6). In contrast, the inner structure was highly porous, due to the type of preparation technique (W/O/W solvent extraction/evaporation method). The mean particle size was 140 μm (laser diffraction) and the initial drug loading 6% (*w*/*w*). To monitor drug diffusion from these devices into agarose gels, an aqueous microparticle dispersion was placed at the center of the Petri dishes filled with initially drug-free agarose gels (0.6%, providing the best mechanical stability of the investigated systems) (Fig. 1). At predetermined time points, gel samples were withdrawn at different distances from the drug source, weighed, and analyzed for their drug content. Fig. 7 shows the drug concentration-distance-profiles measured after 2, 7, 10, 14 and 21 d. The drug concentration increased with increasing exposure time, irrespective of the position, indicating the continuous release of drug from the microparticles throughout the observation period. Importantly, much lower propranolol HCl concentrations were obtained compared to the experiments with saturated drug solutions at the center of the agarose gels (Fig. 5), irrespective of the time point and position. This is due to the much lower concentration of dissolved drug at the center of the Petri dishes, only dissolved drug being available for diffusion.

The *cumulative* amount of drug released from the investigated microparticles into a 0.6% (*w*/*w*) agarose gel is illustrated in Fig. 8 as a function of time (squares). Clearly, the relative release rate monotonically decreased with time and propranolol HCl release was almost complete after 3 weeks. Importantly, drug release from the same batch of microparticles was much slower when using a standard *in vitro* release measurement technique (agitated test tubes filled with phosphate buffer pH 7.4, triangles in Fig. 8): For instance, only 51% (*vs*. 88% in gel) propranolol HCl were released after 20 d. This significant difference in the release rate might at least partially be explained as follows: When using the agitated tube method, acids generated by the hydrolysis of the polyester PLGA are likely to be more rapidly neutralized in the well-stirred phosphate buffer surrounding the microparticles than in the case of the agarose gel technique, in which rapid *convective* mass transport is effectively suppressed. Consequently, the pH of the release medium directly surrounding the microparticles can be expected to be artificially higher within the agitated test tubes. This is likely to result in the minimization/suppression of autocatalytic effects (and accelerated polymer degradation). Hence, the drug mobility within the microparticles can be expected to increase less rapidly when using the agitated test tube method than when using the agarose gel technique. Importantly, sink conditions were maintained throughout the experiments with both types of measurement techniques. The fact that drug release is more rapid when using the gel method instead of the agitated test tube technique is also in good agreement with the differences reported for the release rate of drugs *in vivo versus in vitro* (using standard test methods) from PLGA- and PLA-based microparticles (36–40) as well as from PLGA-based foams (41) and cylinders/implants (42, 43). Drug release *in vivo* has been reported to be more rapid than with the standard *in vitro* release tests. Furthermore, the hypothesis that *PLGA degradation* is faster when using the agarose gel technique compared to the agitated test tube method is in good agreement with the following visual observation: After only *4 d*, the microparticles started to stick together and formed a cake at the center of the Petri dishes, whereas visible lump formation of microparticles started only after *4 weeks* in the agitated bulk fluids.

These are important differences, which should not be neglected when characterizing this type of advanced drug delivery systems. Please note that the proposed agarose gel technique still significantly simplifies the highly complex conditions *in vivo*. For instance, a gel-free drug reservoir is located at the center of the Petri dish. The microparticles are, thus, not all in direct contact with the gel (which is intended to mimic living tissue). The intensity of this contact is likely to be different *in vivo*. Obviously, it is not possible to mimic the entire complexity of real *in vivo* conditions. However, efforts should be undertaken to minimize *crucial* differences, such as the conditions for diffusive and convective mass transport. Also, it should always be kept in mind that the *in vivo* and *in vitro* release patterns might differ in both: shape and slope.

CONCLUSION

Great care has to be taken when measuring drug release *in vitro* from biodegradable microparticles, because the underlying drug release mechanisms are complex and can be significantly altered by the test conditions. Aqueous agarose gels are much more realistically simulating the surrounding environment after subcutaneous, intramuscular or intracranial administration than agitated bulk fluids. Up to now there is a lack of regulatory standards for the *in vitro* drug release measurements from this type of advanced drug delivery systems, despite of the latter's steadily increasing practical importance.

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