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Research paper

Importance of PLGA microparticle swelling for the control of prilocaine release

H. Gasmi [a, b], J.-F. Willart [c], F. Danede [c], M.C. Hamoudi [a, b], J. Siepmann [a, b, *], F. Siepmann [a, b]

[a] Univ. Lille, College of Pharmacy, 3 Rue du Prof. Laguesse, 59006 Lille, France
[b] INSERM U1008, Controlled Drug Delivery Systems and Biomaterials, 3 Rue du Prof. Laguesse, 59006 Lille, France
[c] Univ. Lille, USTL UMET (Unité Matériaux et Transformations) UMR CNRS 8207, 59650 Villeneuve d'Ascq, France

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ABSTRACT

It has recently been reported that system swelling is likely to control the onset of the third release phase from *ketoprofen*-loaded PLGA microparticles: However, yet it is unclear whether this type of release mechanism is also valid for other types of drugs. In this study, PLGA microparticles were loaded with different amounts of the free base prilocaine, keeping the microparticle size constant. The systems were characterized using GPC, DSC, SEM, X-ray powder diffraction, drug release measurements and the monitoring of *single* microparticle swelling. At lower drug loadings, tri-phasic release patterns were observed: An initial burst was followed by a period with an about constant release rate, which was followed by a third, again rapid release phase. Interestingly, the beginning of this final rapid drug release phase coincided with the onset of substantial microparticle swelling. GPC analysis revealed that the PLGA molecular weight was about 18–19 k Da at these "onset time points". Thus, it seems that as soon as a critical polymer molecular weight is reached, important amounts of water penetrate into the system, leading to significantly increased polymer and drug mobility. Hence, microparticle swelling seems to cause the onset of the final release phase of different types of drugs.

1. Introduction

Poly(lactic-co-glycolic acid) (PLGA) offers major advantages as matrix former for parenteral controlled drug delivery systems, because: (i) It is biodegradable [1]. (ii) It is biocompatible [2]. (iii) Drug release can be controlled over broad ranges of time periods [3–8]. Often, PLGA *microparticles* are used as delivery systems, since they can be rather easily administered (e.g., s.c. or i.m.). Since many years a variety of controlled release drug products based on PLGA microparticles is available on the market, in particular for cancer treatments. It has to be pointed out that the observed drug release kinetics might depend on the surrounding environment [9,10]. Generally, drug release from PLGA microparticles is mono-, bi-, or tri-phasic [9,11,12]. In the latter case, an initial burst release is followed by a period with an about constant release rate and a final (again rapid) drug release phase.

Interestingly, yet relatively little knowledge is available on the exact underlying mass transport mechanisms controlling drug release from PLGA microparticles. This can be attributed to the complexity of the involved physico-chemical phenomena [13–17]: Upon contact with aqueous body fluids, water penetrates into the system, leading to drug dissolution (if the latter is not already dissolved in the polymer) and PLGA degradation (ester bond cleavage). Once dissolved, the drug can diffuse out of the system (through water-filled channels and/or intact polymeric networks). In addition, shorter chain PLGA degradation products can diffuse out of the system, while molecules and ions of the surrounding bulk fluid can penetrate into the microparticles. It has been shown that water penetration into PLGA microparticles is generally much more rapid than the subsequent ester hydrolysis [18]. Consequently, the systems are soon completely wetted and polymer degradation occurs throughout the microparticles (leading to "bulk erosion"). Depending on various factors (including for instance the system size and porosity), the generation of shorter chain acids can be faster than the diffusion of these acidic degradation products out of the microparticles (and the diffusion of bases from the environment into the system). Consequently, acids can accumulate and the

* Corresponding author. Univ. Lille, College of Pharmacy, INSERM U1008, 3 Rue du Prof. Laguesse, 59006 Lille, France.
E-mail address: juergen.siepmann@univ-lille2.fr (J. Siepmann).

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micro-pH within the particles might significantly drop (especially in large and non-porous systems) [19,20]. Since ester hydrolysis is catalyzed by protons, this can lead to autocatalytic effects and accelerated polymer degradation and drug release [21,22]. Furthermore, the initial presence of tiny pores at the microparticles' surface during the first hours might explain the often observed "burst release" from these systems. Upon exposure to the release medium, these pores might be closed, resulting in decreased drug mobility [23–25].

Concerning the exact reasons for the onset of the *third* (and again rapid) drug release phase from PLGA microparticles, very little is known up to now. Recently, it has been reported that in the case of PLGA microparticles loaded with the acidic drug ketoprofen, significant particle swelling coincided with the onset of this third release phase [26]: The monitoring of *single* particle swelling (by optical microscopy) allowed correlating swelling and drug release kinetics of different types of particles. However, yet, it is unclear whether this correlation between particle swelling and drug release was eventually only a coincidence by hazard, or whether it is only observed in the case of acidic drugs, or whether PLGA microparticle swelling is generally the cause for the onset of the final rapid drug release phase from this type of advanced drug delivery systems.

The aim of the present study was to prepare different types of PLGA microparticles loaded with the free base prilocaine: The initial drug loading was varied from 2 to 35% (w:w). Importantly, the mean particle size was kept about constant in order to minimize microparticle size effects [21,22]. Gel permeation chromatography (GPC), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), X-ray powder diffraction, drug release measurements and the monitoring of *single* microparticle swelling (by optical microscopy) were used to characterize the systems before and after exposure to phosphate buffer pH 7.4.

2. Materials and methods

2.1. Materials

Poly(D,L lactic-co-glycolic acid) (PLGA; Resomer RG 504H; 50:50 lactic acid:glycolic acid; Evonik, Darmstadt; Germany); prilocaine (free base) and polyvinyl alcohol (Mowiol 4–88) (Sigma–Aldrich, Steinheim, Germany); acetonitrile and dichloromethane (VWR, Fontenoy-sous-Bois, France); tetrahydrofurane (HPLC Grade; Fisher Scientific, Illkirch, France).

2.2. Preparation of PLGA microparticles

Prilocaine (free base)-loaded PLGA microparticles were prepared using an oil-in-water (O/W) solvent extraction/evaporation technique: Depending on the theoretical drug loading [which was varied from 3 to 50% (w:w)], 31.5–527.2 mg drug and 518.8–1015.1 mg PLGA were dissolved in 4.1–8.0 mL dichloromethane (Table 1) (the volume of the organic solvent was adapted to keep the mean microparticle diameter in the range of 80–90 μm in all cases). This organic phase was emulsified within 2.5 L of an outer aqueous polyvinyl alcohol solution (0.25%, w/w) for 30 min under stirring with a three-blade propeller (2000 rpm), inducing microparticle formation. The particles were hardened by adding 2.5 L of the same outer aqueous polyvinyl alcohol solution and further stirring at 700 rpm during 4 h. The microparticles were subsequently separated by filtration and freeze-dried (Christ Epsilon 2–4 LSC, Martin Christ, Osterode, Germany).

2.3. Microparticle characterization

2.3.1. Microparticle size

Particles sizes were determined by optical microscopy: Pictures were taken using an Axiovision Zeiss Scope-A1 microscope (Carl Zeiss Microimaging, Goettingen, Germany), equipped with an AxioCam ICc1 camera and Axiovision Zeiss Software (Carl Zeiss, Jena, Germany). Each measurement included 200 microparticles.

2.3.2. Practical drug loading

Accurately weighed amounts of microparticles (approximately 20 mg) were dissolved in 10 mL acetonitrile, followed by filtering (PTFE syringe filters, 0.45 μm) and determination of the prilocaine content by HPLC analysis (Prostar 210 pump, 410 autosampler, 335 Photodiode array Detector, Galaxy Software; Varian, Les Ulis, France). A reversed phase column C18 (Gemini 5 μm, 110 A; 150 mm × 4.6 mm; Phenomenex, Le Pecq, France) was used. The mobile phase was a mixture of acetonitrile:phosphate buffer pH 8 (Ph. Eur. 7) (40:60 v:v). The flow rate was 1 mL/min, the detection wavelength 260 nm (linear concentration range from 1 to 200 μg/mL). Twenty microliter samples were injected. Each experiment was conducted in triplicate.

2.3.3. In vitro drug release

Fifty milligrams of prilocaine-loaded microparticles were placed in 12 mL glass tubes, filled with 10 mL phosphate buffer pH 7.4 (USP 35). The tubes were horizontally shaken at 80 rpm at 37 °C (GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). At predetermined time points, 2 mL samples were withdrawn and replaced with fresh medium. The samples were filtered using PTFE syringe filters (0.45 μm, VWR, Fontenoy-sous-Bois, France) and their drug content was analyzed by HPLC analysis, as described above (but injecting 50 instead of 20 μL samples). Each experiment was conducted in triplicate. Sink conditions were provided throughout the experiments.

2.3.4. Gel permeation chromatography (GPC)

The decrease in polymer molecular weight (Mw) of PLGA in the microparticles during drug release was measured by GPC analysis

Table 1
Composition of the organic phases used for the preparation of PLGA microparticles and thin films, loaded with the free base prilocaine (using an O/W solvent extraction/evaporation technique and film casting method, respectively).

Theoretical loading, % (w:w)	3.0	6.7	9.3	15	23	33	40	50
Microparticles								
CH_2Cl_2, mL	8.0	7.7	7.6	7.0	6.4	5.5	5.0	4.1
PLGA, mg	1015.1	978.5	951.6	877.1	804.6	698.9	625.5	518.8
drug, mg	31.5	70.1	97.3	155.3	242.5	351.6	420.5	527.2
Films								
CH_2Cl_2, mL	8.4	8.4	8.2	7.6	7.0	6.0	5.5	5.0
PLGA, mg	3926.2	3820.8	3945.6	3439.1	3237.2	2832.6	2427.6	2023.0
Drug, mg	123.1	273.2	404.6	606.9	970.5	1420.8	1630.4	2057.2