The mechanism behind the biphasic pulsatile drug release from physically mixed poly(DL-lactic-(co-glycolic) acid)-based compacts

Max Beugeling\textsuperscript{a,1}, Niels Grasmeijer\textsuperscript{b,1}, Philip A. Born\textsuperscript{b}, Merel van der Meulen\textsuperscript{b}, Renée S. van der Kooij\textsuperscript{a}, Kevin Schwengle\textsuperscript{b}, Lieven Baert\textsuperscript{b}, Katie Amssoms\textsuperscript{c}, Henderik W. Frijlink\textsuperscript{a}, Wouter L.J. Hinrichs\textsuperscript{a,⁎}

\textsuperscript{a}Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV, Groningen, the Netherlands
\textsuperscript{b}Jalima Pharma bvba, Josef Van Walleghemstraat 11, 8200 Brugge, Belgium
\textsuperscript{c}Infectious Diseases & Vaccines Therapeutic Area, Janssen Research & Development, A Division of Janssen Pharmaceutica NV, Turnhoutseweg 30, 2340 Beerse, Belgium

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A B S T R A C T

Successful immunization often requires a primer, and after a certain lag time, a booster administration of the antigen. To improve the vaccinees' comfort and compliance, a single-injection vaccine formulation with a biphasic pulsatile release would be preferable. Previous work has shown that such a release profile can be obtained with compacts prepared from physical mixtures of various poly(\textit{n}-lactic-(co-glycolic) acid) types (Murakami et al., 2000). However, the mechanism behind this release profile is not fully understood. In the present study, the mechanism that leads to this biphasic pulsatile release was investigated by studying the effect of the glass transition temperature ($T_g$) of the polymer, the temperature of compaction, the compression force, the temperature of the release medium, and the molecular weight of the incorporated drug on the release behavior. Compaction resulted in a porous compact. Once immersed into release medium with a temperature above the $T_g$ of the polymer, the drug was released by diffusion through the pores. Simultaneously, the polymer underwent a transition from the glassy state into the rubbery state. The pores were gradually closed by viscous flow of the polymer and further release was inhibited. After a certain period of time, the polymer matrix ruptured, possibly due to a build-up in osmotic pressure, resulting in a pulsatile release of the remaining amount of drug. The compression force and the molecular weight of the incorporated drug did not influence the release profile. Understanding this mechanism could contribute to further develop single-injection vaccines.

1. Introduction

Annually, vaccination prevents approximately 2–3 million deaths caused by more than thirty different infectious diseases (Kardani et al., 2016; WHO, 2018). Successful vaccination, i.e. providing protection by creating pools of long-term memory B- and T-cells, often requires a primary (primer) and after a certain lag time, a secondary (booster) administration of the vaccine (McHeyzer-Williams and McHeyzer-Williams, 2005; Siegrist, 2013). Hence, most traditional vaccines are administered using a multi-injection regime (McHugh et al., 2015). This multi-injection regime is discomforting for the vaccinee and might compromise compliance (Cleland, 1999; McHugh et al., 2015). Improved vaccinees' comfort and compliance could be achieved with an injectable device with a biphasic pulsatile release profile. Such an implant releases one part of the antigenic substance instantly (primer), while the remainder is released after a certain lag time (booster) (Cleland, 1999; McHugh et al., 2015). Ideally, this lag time can be tailored to the requirements of the specific antigen that is used.

Previous work on the development of such a single-injection vaccine has shown the use of various polymers (Cleland, 1999). Of these polymers, poly(\textit{n}-lactic-(co-glycolic) acid) (PL(G)A) is most widely investigated. PL(G)A is a biodegradable and biocompatible copolymer that has been used in many drug products approved by the Food and Drug Administration (Fredenberg et al., 2011a; Makadia and Siegel, 2011). The copolymer consists of lactic and glycolic acid monomers. By changing the lactic:glycolic acid ratio of the copolymer, physicochemical characteristics (e.g. glass transition temperature ($T_g$) and degradation rate) of the polymer can be tailored (Fredenberg et al., 2011a; Makadia and Siegel, 2011; Xu et al., 2017). Other ways to tailor the physicochemical characteristics of the polymer are using different

⁎ Corresponding author.
E-mail address: w.l.j.hinrichs@rug.nl (W.L.J. Hinrichs).

Both authors contributed equally to this work.

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molecular weights of the polymer and changing the end group of the polymer (Fredenberg et al., 2011a; Makadia and Siegel, 2011; Xu et al., 2017).

A common method to achieve a biphasic pulsatile release profile is to develop a core-shell device. In such a device, a water-soluble core containing the active component is encapsulated in a water-insoluble biodegradable polymer shell to enable a delayed pulsatile release. This device can be combined with an additional water-soluble outer layer containing the active component, or can simply be co-injected with a solution containing the active component to obtain the desired biphasic pulsatile release profile. A great disadvantage of core-shell devices is that they require complicated production methods, amongst which emulsification, coating, multiple-compaction processes, and more recently, a microfabrication production method named StampEd Assembly of polymer Layers (Cleland, 1999; De Geest et al., 2009; Guse et al., 2006; McHugh et al., 2017; Sunghongjeen et al., 2004; Tzeng et al., 2018).

However, in 2000, Murakami et al. (2000) reported a device consisting of a physical mixture of theophyline as a model drug and several types of PL(G)A, simply prepared by a single compaction procedure. This device exhibited the desired biphasic pulsatile release profile. The mechanism behind the biphasic pulsatile drug release from these PL(G)A-based compacts, however, is not fully understood. Furthermore, although the single compaction step seems a simple process, the authors used a complicated emulsion technique to prepare polymeric nanoparticles for compaction and it is unclear whether similar results can be obtained if larger polymer particles are used.

Based on literature, monolithic PL(G)A-based systems, such as the physically mixed compacts described by Murakami et al. (2000) seem less suitable for protein-based vaccines. An incomplete release of native protein from monolithic devices due to protein instability is often observed (Ghalanbor et al., 2012; Hines and Kaplan, 2013; van de Weert et al., 2000). Major reasons for protein instability within these devices are the formation of an acidic microclimate within the matrix and incompatibility of the polymer degradation products and proteins, leading to aggregation and inactivation of the protein (Estey et al., 2006; Kang and Schwendeman, 2002; Liu and Schwendeman, 2012; Stanković et al., 2015). To overcome these issues, several excipients (e.g. magnesium hydroxide and shellac) could potentially be incorporated to stabilize proteins within monolithic PL(G)A-based devices (Duque et al., 2018; Zhu and Schwendeman, 2000). However, polysaccharides, used as antigens in bacterial vaccines (e.g. pneumococcal polysaccharide vaccine) (Weintraub, 2003) are more stable and compatible with polymer degradation products. Therefore, a device based on a physical mixture might be more interesting for the biphasic delivery of a polysaccharide-based vaccine.

We hypothesize that compaction at a temperature below the $T_g$ of the polymer results in a porous compact, as the polymer is in the glassy state. Once immersed into release medium with a temperature above the $T_g$ of the polymer, the drug can diffuse through the pores of the compact, leading to a pulsatile burst release. However, at the same time, the polymer undergoes a transition from the glassy state into the rubbery state. Therefore, the pores of the compact are gradually closed by viscous flow of the polymer and further release is inhibited. After a certain period of time, the polymer matrix ruptures, resulting in a pulsatile boost release. To investigate this hypothesis, physically mixed compacts based on theophylline as a model drug and various types of PL(G)A were produced. The influence of the $T_g$ of the polymer, the temperature of compaction, the compression force, and the temperature of the release medium on the release of theophylline from physically mixed PL(G)A-based compacts were investigated. To investigate the influence of the molecular weight of the incorporated drug on the release, blue dextran (BD) with a molecular weight of either 70 kDa or 2000 kDa was incorporated in the physically mixed compact. As bacterial vaccines are often polysaccharide-based, BD was also used to mimic bacterial vaccines. Furthermore, core-shell compacts consisting of a theophylline containing core tablet and a nonporous and a porous shell composed of PLA with a high $T_g$ were used as a control. Understanding the mechanism behind the biphasic pulsatile release could contribute to the further development of an effective single-injection vaccine.

2. Materials and methods

2.1. Materials

Poly(3-lactic-co-glycolic acid) with a lactic:glycolic acid ratio of 50:50 and an intrinsic viscosity of 0.2 dL/g (PLGA5002), poly(3-lactic acid) with an intrinsic viscosity of 0.2 dL/g (PLA02), and 0.5 dL/g (PLA05) were purchased from Corbion Purac Biomaterials (Gorinchem, The Netherlands). Theophylline anhydrous was obtained from Boehringer Ingelheim (Ingelheim am Rhein, Germany). BD with molecular weights of 70 kDa and 2000 kDa were purchased from TdB Consultancy (Upsala, Sweden). Sodium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Merck (Darmstadt, Germany). Sodium azide was obtained from ACS Organics (Geel, Belgium). Inulin (4 kDa) with a degree of polymerization of 23 was a generous gift from Sensus (Roosendaal, The Netherlands). Sodium chloride was purchased from Fluka Chemie GmbH (Buchs, Switzerland). Mannitol was purchased from Roquette (Nord-Pas-de-Calais, France). All experiments were performed with MilliQ, type 1 water.

2.2. Powder formulations for physically mixed compacts

For theophylline containing compacts, an aqueous solution containing 5 mg/ml theophylline and 50 mg/ml inulin was prepared. Of this solution, amounts of 2 ml were transferred to 20 ml vials. The solutions were freeze-dried using a Christ Epsilon 2-4 LSC freeze-dryer (Salm & Kipp, Breukelen, The Netherlands) at 0.220 mBar and a shelf temperature of $35 \pm 5 \degree C$ for 24 h, after which the pressure was reduced to 0.050 mBar while the shelf temperature was increased to 25 $\degree C$ over a period of 24 h. The obtained freeze-dried powder was then ground and mixed with mannitol in a smooth agate mortar. This powder mixture was then physically mixed with PLGA5002 or PLA02 in a smooth agate mortar. Prior to mixing, PLGA5002 and PLA02 were ground with an AR100 mill (Moulinex, Écully, France) and subsequently sieved with a 150 $\mu$m sieve. The resulting powder blends were used for the production of the physically mixed PL(G)A-based compacts. The final powder formulation consisted of 4 wt-% freeze-dried theophylline and inulin (in a 1:10 w/w ratio), 5.1 wt-% mannitol, and 90.9 wt-% polymer.

For BD containing compacts, an aqueous solution containing 5 mg/ml BD with either a molecular weight of 70 kDa or 2000 kDa and 6.4 mg/ml mannitol was prepared. Solutions were freeze-dried as described above. The obtained freeze-dried powder was then physically mixed with PLGA5002 with a particle size of $\leq 150 \mu$m in a smooth agate mortar. The final powder formulation consisted of 4 wt-% BD (with a molecular weight of either 70 kDa or 2000 kDa), 5.1 wt-% mannitol, and 90.9 wt-% polymer.

2.3. Production of physically mixed PL(G)A-based compacts

The powder blends containing theophylline were compacted using a hydraulic press (Hydro Mooi, Appingedam, The Netherlands) and an oblong tablet die (6 x 2 mm). Approximately 26 mg of the powder formulation described above was compressed at room temperature (RT) with a compaction load of 7 kN and a compaction rate of 0.7 kN/s, or a compaction load of 2.8 kN and a compaction rate of 0.28 kN/s. In both cases the hold time was 10 s. To investigate the influence of temperature, the compact containing tablet die compressed at a compaction load of 2.8 kN was stored in an oven for 1 h at 48 $\degree C$ to allow viscous flow of the polymer. After removal from the oven, the compact was
immediately re-compressed using the same settings as described above. For BD containing compacts, approximately 27.5 mg of the powder formulation was compressed at RT with a compaction load of either 3 kN and a compaction rate of 0.3 kN/s, or a compaction load of 9 kN and a compaction rate of 0.9 kN/s. In both cases the hold time was 10 s. These compaction loads were chosen to further investigate the influence of compression force on the release profile. Note that with the specific oblong tablet die, three tablets were simultaneously prepared, resulting in a compaction force per tablet that was three times lower than the total compaction force.

2.4. Production of PLA core-shell compacts

First, PLA05 pellets were milled with a Pulverisette 14 (Fritsch GmbH, Idar-Oberstein, Germany) at 6000–10,000 rpm and sieved with a 200 μm sieve. To produce the core tablet, approximately 25 mg of a physical mixture consisting of 44 wt-% freeze-dried theophylline and inulin (in a 1:10 w/w ratio) and 56 wt-% mannitol was compressed in a 7 mm diameter tablet die at a compaction load of 5 kN, a compaction rate of 0.5 kN/s, and a hold time of 10 s. Subsequently, the core was inserted into a 9 mm diameter tablet die, in which ground PLA02 or PLA05 was added as top layer (23.5 mg) and as bottom layer (93 mg). The PLA02 or PLA05 shell was then compressed at a compaction load of 12.5 kN, a compaction rate of 1.25 kN/s, and a hold time of 10 s. To investigate the effect of heating on the release characteristics of PLA05 core-shell compacts, the same procedure was used except that compression was at a compaction load of 5 kN, a compaction rate of 0.5 kN/s, and a hold time of 10 s. Thereafter, the core-shell compact containing tablet die was stored in an oven for 1 h at 80 °C to allow for sufficient viscous flow of the polymer. After removal from the oven, the core-shell compact was immediately re-compressed using the same settings as described above. The resulting core-shell compact had a shell thickness of approximately 300 μm on top and 1000 μm at the bottom and the sides.

2.5. Differential scanning calorimetry (DSC)

DSC measurements of PLGA5002, PLA02, and PLA05 were done with a Q2000 differential scanning calorimeter (TA Instruments, New Castle, DE, United States). Dry samples were weighed in open Tzero pans at ambient conditions. The samples were preheated to a temperature of 120 °C prior to scanning at a rate of 20 °C/min and a temperature range of −20 °C to 90 °C. The same samples were used to determine the Tg when moisturized. To achieve this, 40 μL of water was added to the sample and the sample was moisturized over a period of 30 min. The excess of water was subsequently removed. Finally, the pan was hermetically sealed, after which the sample was cooled to −50 °C and then heated at a rate of 20 °C/min to a temperature of 90 °C. Each sample was measured directly and 2, 5, and 7.5 h after moisturizing. To gain insight in the degradation process of PLG(A), DSC measurements were conducted on physically mixed theophylline containing PLGA5002 compacts after different exposure times to the release medium. At predetermined time points, compacts exposed to the release medium at 37 °C were removed from the release medium and cut to pieces using a scalpel. The samples were weighed in open Tzero pans at ambient conditions and were first preheated for 10 min at a temperature of 120 °C. After this preheating step, the samples were cooled to −20 °C and then heated with a rate of 20 °C/min to 90 °C. The Tg was defined as the onset of the transition.

2.6. In vitro release of theophylline and blue dextran from physically mixed PLG(A)-based compacts and PLA core-shell compacts

The in vitro release tests were performed in 20 ml (for physically mixed theophylline containing compacts) or 50 ml (for core-shell compacts) 100 mM phosphate buffered saline (PBS) (pH 7.4) supplemented with 0.02% (w/v) sodium azide. The temperature of the release medium in the standard procedure was 37 °C. However, to investigate the influence of release medium temperature, physically mixed compacts were immersed into release medium of either 4 °C, RT, or 37 °C. The release medium was first preheated or precooled to the temperature at which the release was studied. The release studies were performed in a shaking water bath (80 rpm) to allow refreshment of the release medium at the surface of the compacts. Theophylline release was measured with a UV–visible spectrophotometer (Thermo Spectronic Unicam UV-540, Waltham, MA, United States) at λ = 272 nm and at λ = 325 nm (reference wavelength). Sampling was performed by taking 2.5–3 ml of the release medium through a flow-through cuvette (L = 10 mm) and returning the sample back to the release medium. The in vitro release tests with BD containing compacts were performed in 1 ml release medium at a temperature of 37 °C. At predetermined time points, samples of 0.9 ml were taken and 0.9 ml of fresh preheated (37 °C) release medium was added to the vial to keep the volume constant. The samples were centrifuged for 15 min at 14,800 rpm and the supernatant was used to measure BD release at λ = 616 nm using a flow-through cuvette (L = 50 mm).

2.7. Scanning electron microscopy (SEM)

SEM images of physically mixed compacts were taken with a JEOL 6460 microscope (JEOL, Tokyo, Japan). To investigate the influence of the compression force on surface porosity, SEM images of PLA02 compacts compressed at 2.8 kN and 7 kN were taken prior to release. Theophylline containing PLA02 compacts were imaged after 3 days of release at 4 °C, RT, or 37 °C. In addition, physically mixed PLA02 compacts were imaged after 4, 8, and 72 h of release at 37 °C. To gain insight in the boost release, theophylline containing PLGA5002 compacts were imaged after 18 days of exposure to the release medium. Prior to imaging, incubated compacts were freeze-dried using the same program as described above. The dry compacts were stuck on top of double-sided adhesive carbon tape on aluminum disks and coated with a 17 nm layer of gold in a JFC-1300 sputtering device fitted with an MTM-20 thickness controller system (JEOL, Tokyo, Japan). An acceleration voltage of 10 kV, a spot size of 25, and a Z-distance of 15 mm was used for all recordings.

2.8. Comparison of the release profiles

The release profiles were compared by using the similarity factor (f2), which is calculated using the following equation (Shah et al., 1998; Srinarong et al., 2009):

$$f_2 = 50\log[\left(1 + \frac{1}{n} \sum_{i=1}^{n} \left(\frac{T_i - R_i}{T_i + R_i}\right)^2\right)] + 100$$

where $n$ is the number of release sampling times, and $R_i$ and $T_i$ are the average percentage drug released at each time point from the reference formulation and the test formulation, respectively. The time point $t = 0$ was excluded and only one point after more than 85% drug release was included, as recommended by Shah et al. (1998). If the $f_2 > 50$, the release profiles can be considered similar.

3. Results

3.1. Glass transition temperatures of the polymers

The Tg’s of PLGA5002, PLA02, and PLA05 were measured with DSC. The average onset of the transition of two measurements were 31.6 °C, 33.5 °C, and 44.3 °C, respectively. Furthermore, moisturizing the polymers with water reduced the Tg to 19.1 °C, 24.3 °C, and 37.1 °C, respectively. The latter values were measured 5 h after moisturizing, this was when the Tg reached a plateau value.
3.2. In vitro release of theophylline from physically mixed PLGA5002- and PLA02-based compacts

Fig. 1 shows the release at 37 °C of theophylline from compacts with either a PLGA5002 or a PLA02 polymer matrix prepared at a compaction load of 7 kN and RT. The physically mixed compacts released $56.6 \pm 3.7\%$ and $49.3 \pm 2.9\%$ of the total theophylline content during the initial burst release, respectively. The remaining content was released as a pulse after a lag phase. The lag phase was substantially shorter for PLGA5002-based compacts (approximately 18 days) than for PLA02-based compacts (approximately 50 days).

3.3. Effect of compression force and heating on theophylline release from PLA02-based compacts

Physically mixed compacts with PLA02 were compressed at 2.8 kN, instead of 7 kN to study the effect of a possibly more porous compact on the burst release. Furthermore, compacts compressed at 2.8 kN were heated in an oven set at 48 °C for 1 h in between two compaction procedures to study the effect of heating. A lower compression force resulted in a visibly more porous surface of the compact (Fig. 2A and B), but only in a minor increase in burst release (Fig. 2C). The burst release was $55.8 \pm 0.9\%$ and $49.3 \pm 2.9\%$ for compacts compressed at 2.8 kN and 7 kN, respectively. However, based on the $f_2$, which was 63.3, the two release profiles were found to be similar. Heating of the compact resulted in a reduction of the burst release of theophylline from $55.8 \pm 0.9\%$ to $22.6 \pm 5.8\%$ (Fig. 3). The lag phase duration was unaffected, and although a small fraction appeared to release during the lag phase, most of the remaining theophylline was released as a pulse after the lag phase. Based on the $f_2$, which was 27.4, the two release profiles were not similar.

3.4. Effect of release medium temperature on theophylline release from PLA02-based compacts

Physically mixed PLA02-based compacts compressed at a compaction load of 7 kN and RT were set to release at 4 °C, RT, and 37 °C (Fig. 4). At RT and below, all theophylline was released during the burst in the first 3 days, whereas at 37 °C, $51.1 \pm 3.1\%$ of theophylline was released. In addition, based on the $f_2$, which was 26.9, the release profiles at 4 °C and RT were different. SEM images from physically mixed PLA02-based compacts containing theophylline directly after compaction (Fig. 2B) and after 3 days of release at 4 °C, RT, and 37 °C showed a noticeable difference in the surface structure of the compacts (Fig. 5A–C). Directly after compaction, a rough, porous structure was observed. After 3 days of release at 4 °C and RT, the compacts still showed a rough, porous structure. However, after 3 days of release at 37 °C, the surface appeared to be completely smooth and nonporous. Fig. 6A–C shows SEM images of PLA02 compacts after 4, 8, and 72 h of release at 37 °C. After 4 and 8 h of release at 37 °C, a few pores can still be seen on the surface of the compact. However, after 72 h, the compact showed a smooth, nonporous surface.

3.5. Degradation of PLGA: DSC measurements after different exposure times to the release medium and boost release

Fig. 7A shows thermograms of theophylline containing PLGA5002-based compacts compressed at 7 kN and RT after different exposure times to the release medium at 37 °C. The results clearly show that the
Tg of the polymer decreased over time. Prior to exposure to the release medium, the Tg was 32.8 °C. After 18 days of release, when the pulse was observed for theophylline containing PLGA5002-based compacts (Fig. 1), the Tg was found to be 27.0 °C. After 24 days of release at 37 °C, the Tg further decreased to 24.1 °C. Fig. 7B shows a SEM image of a PLGA5002-based compact after 18 days of release at 37 °C. The image clearly shows a ruptured compact.

3.6. In vitro release of BD from physically mixed PLGA5002-based compacts

Fig. 8A and B shows the release at 37 °C of BD with a molecular weight of 70 kDa (Fig. 8A) and 2000 kDa (Fig. 8B) from compacts with a PLGA5002 polymer matrix prepared at RT and a compression force of 3 kN or 9 kN. A larger difference between the two compression forces than previously (2.8 kN and 7 kN) was chosen to further investigate the influence of compression force on the release profile. Physically mixed compacts compressed at 3 kN and 9 kN containing BD with a molecular weight of 70 kDa released 58.4 ± 9.8% and 54.3 ± 6.1% of the total BD content during the initial pulsatile burst release, respectively. Based on the f2, which was 69.9, the two release profiles were similar. Physically mixed compacts compressed at 3 kN and 9 kN containing BD with a molecular weight of 2000 kDa released 48.3 ± 4.1% and 53.8 ± 1.8% of the total BD content during the initial pulsatile burst release, respectively. Based on the f2, which was 55.0, the release profiles were similar. The release profiles of the different BD molecular weights (70 kDa and 2000 kDa) compressed at either 3 kN or 9 kN were also similar, with f2 values of 51.7 and 78.8, respectively. The remaining content was released as a pulse after a lag phase. The lag phase was approximately 21 days for both molecular weights of BD.

3.7. Effect of a nonporous shell on the burst release

Three different core-shell compacts were prepared by either omitting the heating step with PLA02 or PLA05 as shell material and compressing with a compaction load of 12.5 kN, or by heating at 80 °C for 1 h during compaction and compressing at a compaction load of 5 kN with PLA05 as shell material. The non-heated PLA02 core-shell compact exhibited a delayed release profile without any initial burst release. The lag time was approximately 40 days. All the theophylline was released...
or PLA02 and theophylline exhibited a biphasic pulsatile release profile without any initial burst release was exhibited. The lag time was within 2 days from the non-heated PLA05 core-shell compact, however, if the PLA05 core-shell compact was heated at 80 °C for 1 h, a delayed release without any initial burst release was exhibited. The lag time was approximately 100 days (Fig. 9).

4. Discussion

The compacts prepared at RT from a physical mixture of PLGA5002 or PLA02 and theophylline exhibited a biphasic pulsatile release profile at 37 °C, similar to the previous findings (Murakami et al., 2000) (Fig. 1). Furthermore, the lag time could be adjusted by changing the polymer composition, which is also in line with the previous study (Murakami et al., 2000). These results imply that the release mechanism may be independent of the polymer particle size, as in the study of Murakami et al. (2000) nanoparticles were used, while in the present study more coarse particles were used. However, head-to-head experiments should be performed to confirm this. Not all compacts exhibited a biphasic pulsatile release profile. It was found that the release profile was greatly dependent on the Tg of the polymer, the temperature during compaction, and the temperature of the release medium. If the temperature during compaction was below and the temperature during release was above the Tg of the polymer, a biphasic pulsatile release profile was obtained with a 50–60% burst release and a 40–50% boost release. According to the results shown in this study, this biphasic pulsatile release profile can be attributed to the transition from the glassy state into the rubbery state of the polymer, as will be made clear in the following discussion.

As described by Fredenberg et al. (2011a) four release mechanisms should be considered when investigating drug release from a PL(G)A-based device, namely: diffusion through water-filled pores, diffusion through the polymer, osmotic pumping, and polymer erosion. During compaction at RT, a porous matrix was formed, as confirmed by SEM for PLA02-based compacts (Figs. 2A and B). This porous structure is typical for a glassy polymer and is caused by the relatively high Tg of dry PLGA5002 and PLA02 of 31.6 °C and 33.5 °C, respectively. When the compact is set to release at 37 °C, the polymer will heat up and absorb water. As the Tg of the moisturized polymer is below environmental temperatures, i.e. 19.1 °C for PLGA5002 and 24.3 °C for PLA02, a transition from the glassy state into the rubbery state will occur. This causes the polymer chains to become mobile and viscous flow will close the pores that were originally present after compaction (Fig. 5C). The phenomenon of pore closure has been described in several other studies (Fredenberg et al., 2011b; Kang and Schwendeman, 2007; Wang et al., 2002; Yamaguchi et al., 2002). However, different PL(G)A-based devices were studied and therefore the mechanisms of pore closure may be different. Because pore closure of the physically mixed compacts was not instantly (Fig. 6A–C), part of the drug could diffuse through the pores of the compact before the pores were sealed, resulting in a pulsatile burst release. The diffusion through pores has also been described as a possible drug release mechanism from various other types of PL(G)A-based devices in literature (Fredenberg et al., 2011a; Gao et al., 2007; Yushu and Venkatraman, 2006; Zidan et al., 2006). This release mechanism is further supported by the fact that the burst release was reduced from 55.8 ± 0.9% to 22.6 ± 5.8% when the PLA02-based compacts were heated for 1 h at 48 °C (a temperature above the Tg of dry PLA02) prior to starting the release experiment (Fig. 3). A small amount of burst release can be expected, as some of the dispersed drug will be at or near the surface of the compact. The burst release was followed by a lag phase, since the hydrophilic drug molecules were not able to diffuse through the nonporous polymer matrix of the physically mixed compacts.

To further support our hypothesis, a release experiment was performed in which the release temperature was lowered to refrigerated conditions (4 °C) or RT (Fig. 4). At these temperatures, the Tg of PLA02 is above the environmental temperature during release, and the polymer does not transition into the mobile rubbery state. Consequently, no pore closure occurred, as confirmed by SEM (Fig. 5A and B). Therefore, all the theophylline was released during the burst. At RT, the burst release was faster than at refrigerated conditions, this is due to a higher diffusion rate of theophylline through the porous compact to the release medium at higher temperatures. These results show that the temperature of the release medium affects pore closure.

Although decreasing the compression force from 7 kN to 2.8 kN resulted in a visibly more porous surface of the compact (Figs. 2A and B), the release profiles from both compacts were similar (Fig. 2C). Apparently, the initial porosity of the surface of the compact did not influence the burst release. This may be explained by the fact that the water soluble mannitol in the formulation acts as a pore former, creating a more porous compact when immersed into the release medium. As pore closure was not instantly (Fig. 6A–C), this led to a rapid initial burst release, which was independent of the initial porosity of the compact. It is known from literature that additives may act as pore formers and affect the structure of various PL(G)A-based devices (Fredenberg et al., 2011a).

The Tg of PLGA-based compacts clearly decreased over time.
was greatly in decrease in molecular weight of the polymer (Fox and Flory, 1950; release at 37 °C, the compact showed a ruptured surface (Fig. 7B), phylline containing compacts, the compression force did not in into the physically mixed compacts showed similar results as the matrices has been described in literature (Fredenberg et al., 2011a; Duque, L., Körber, M., Bodmeier, R., 2018. Improving release completeness from PLGA-based implants for the acid-labile model protein ovalbumin. Int. J. Pharm. 538, 139–146. https://doi.org/10.1016/j.ijpharm.2018.01.026., McHugh, K.J., Guarecuco, R., Langer, R., Jaklenec, A., 2015. Single-injection vaccines: acidic condition: a model for protein instability during release from PLGA delivery systems. J. Pharm. Sci. 95, 1626–1639. https://doi.org/10.1016/j.jpqs.2006.02.052., Fox, T.G., Flory, P.J., 1950. Second-order transition temperatures and related properties of polystyrene. I. Influence of molecular weight. J. Appl. Phys. 21, 581–591. https://doi.org/10.1063/1.1699711., Fredenberg, S., Wahlgren, M., Reslow, M., Axelsson, A., 2011a. The mechanisms of drug release in poly(lactic-co-glycolic acid)-based drug delivery systems—A review. Int. J. Pharm. 415, 34–52. https://doi.org/10.1016/j.ijpharm.2011.05.049., Fredenberg, S., Wahlgren, M., Reslow, M., Axelsson, A., 2011b. Pore formation and pore closure in poly(D, L-lacto-co-glycolide) films. J. Control. Release 150, 142–149. https://doi.org/10.1016/j.jconrel.2010.11.020., Gao, H., Gu, Y., Ping, P., 2007. The implantable 5-fluorouracil-loaded poly(lactic acid) fibers prepared by wet-spinning from suspension. J. Control. Release 118, 325–332. https://doi.org/10.1016/j.jconrel.2006.12.028., Ghahraman, Z., Körber, M., Bodmeier, R., 2012. Protein release from poly(lactic-co-glycolide) implants prepared by hot-melt extrusion: thioester formation as a reason for incomplete release. Int. J. Pharm. 438, 302–306. https://doi.org/10.1016/j.ijpharm.2012.09.015., Guse, C., Könnings, S., Blunk, T., Siepmann, J., Guse, C., Koennings, S., Blunk, T., Siepmann, J., Goepferich, A., 2006. Programmable implants—from pulsatile to controlled release. Int. J. Pharm. 314, 161–169. https://doi.org/10.1016/j.ijpharm.2005.12.051., Hines, D.J., Kaplan, D.L., 2013. Poly(lactic-co-glycolic) acid-controlled-release systems: experimental and modeling insights. Crit. Rev. Ther. Drug Carrier Syst. 30, 257–276. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.v30i3.135, 268. https://doi.org/10.1002/jps.20625., Kang, J., Schwendeman, S.P., 2007. Pore closing and opening in biodegradable polymers and their effect on the controlled release of proteins. Mol. Pharm. 4, 104–118. https://doi.org/10.1021/mp060411n., Kang, J., Schwendeman, S.P., 2002. Comparison of the effects of Mg(OH)2 and sucrose on the stability of bovine serum albumin encapsulated in injectable poly(d, l-lacto-co-glycolide) implants. Biomaterials 23, 239–245. https://doi.org/10.1016/S0142-9612(01)00101-6., Kardani, K., Bolbasani, A., Shahbazi, S., 2016. Prime-boost vaccine strategy against viral infections: Mechanisms and benefits. Vaccine 34, 413–423. https://doi.org/10.1016/j.vaccine.2015.11.062., Liu, Y., Schwendeman, S.P., 2012. Mapping microclimate pH distribution inside protein-encapsulated PLGA microspheres using confocal laser scanning microscopy. Mol. Pharm. 9, 1342–1350. https://doi.org/10.1021/mp200608y., Makadia, H.K., Siegel, S.J., 2011. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers (Basel) 3, 1377–1397. https://doi.org/10.3390/polym3121377., McHeyzer-Williams, L.J., McHeyzer-Williams, M.G., 2005. Antigen-specific memory B cell development. Ann. Rev. Immunol. 23, 487–513. https://doi.org/10.1146/annurev.immunol.23.021304.102752., McHugh, K.J., Guarecuco, R., Langer, R., Jaklenec, A., 2015. Single-injection vaccines: progress, challenges, and opportunities. J. Control. Release 219, 596–609. https://doi.org/10.1016/j.jconrel.2015.07.029., McHugh, K.J., Nguyen, T.D., Linehan, A.R., Yang, D., Behrens, A.M., Rose, S., Tochka, Z.L., Tzeng, S.Y., Norman, J.J., Anselmo, A.C., Xu, X., Tomasic, S., Taylor, M.A., Lu, J., Guarecuco, R., Langer, R., Jaklenec, A., 2017. Fabrication of fillable microparticles and other complex 3D microstructures. Science 357, 1138–1142. https://doi.org/10.1002/jps.20625."

Incorporation of BD with a molecular weight of 70 kDa or 2000 kDa into the physically mixed compacts showed similar results as the theophylline containing compacts (Fig. 8A and B). As with the theophylline containing compacts, the compression force did not influence the release profile. However, the lag phase for BD containing physically mixed compacts was approximately 3 days longer than with theophylline containing compacts. This difference in lag time might be explained by the difference in sampling procedure. These results indicate that molecular weight does not influence the release. The independence of molecular weight supports the fact that the compact ruptures, possibly due to a build-up in osmotic pressure. Finally, using PLA05, a polymer with a higher Tg (onset at 37.1 °C when moisturized), resulted in all of the theophylline being released during the initial burst (Fig. 9). This even occurred despite the fact that a core-shell configuration was used, resulting in a thicker polymer layer for the theophylline to diffuse through. This is due to the fact that the Tg of moisturized PLA05 is slightly above the environmental temperature during release. However, heating the PLA05 core-shell compact for 1 h at 80 °C (a temperature far above the Tg) prior to release reduced the initial burst release to zero. This is because there was no theophylline at or near the surface and the shell surrounding the core becomes nonporous upon the heating step. Non-heated PLA02 core-shell compacts exhibited a delayed release without any initial burst release. This can be explained by the fact that the Tg of moisturized PLA02 (24.3 °C) is below the environmental temperature during release. Understanding the mechanism behind the biphasic pulsatile release from physically mixed compacts could contribute to further develop single-injection vaccines. The release profile can be tailored to specific therapies, opening up new ways to optimize the protection against various pathogens. Specifically, a device based on a physical mixture might be interesting for the development of a single-injection polysaccharide-based vaccine, as it is possible to successfully incorporate polysaccharides into the compact. Because the release profile is not influenced by the molecular weight of the incorporated drug, multiple antigens with different molecular weights can be incorporated into the same device.

5. Conclusion

As described by Murakami et al. (2000), a biphasic pulsatile release could be obtained from a physically mixed PLGA-based compact. However, the mechanism behind this biphasic pulsatile release profile was previously not fully understood. We found that the pulsatile release was greatly influenced by the Tg of the polymer. After compaction, a porous compact was formed from which one part of the active component released instantly by diffusion through the pores of the compact. However, if a polymer with a Tg below the environmental temperature was used, the pores closed due to viscous flow of the polymer and release of the active component was inhibited, since the hydrophilic drug is not able to diffuse through the nonporous polymer matrix. Instead, the burst release was followed by a lag time and a second pulsatile release (booster) was obtained. This pulsatile boost release was not influenced by the molecular weight of the incorporated drug, as it was caused by rupturing of the compact, possibly due to a build-up of osmotic pressure. Furthermore, the initial porosity of the compact did not influence the release profile. The physically mixed compact prototype might be interesting for the development of a single-injection polysaccharide-based vaccine, as BD could successfully be incorporated into the compact.

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