Research article

Towards a better understanding of the release mechanisms of caffeine from PLGA microparticles

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Abstract:

Poly (lactic-co-glycolic acid) (PLGA)-based microparticles can be successfully used to control the release rate of a drug and optimize the therapeutic efficacy of a medical treatment. However, the underlying drug release mechanisms can be complex and are often not fully understood. This renders system optimization cumbersome. In this study, differently sized caffeine-loaded PLGA microparticles were prepared and the swelling and drug release behaviors of *single* microparticles were monitored upon exposure to phosphate buffer pH 7.4. *Ensembles* of microparticles were characterized by X-ray diffraction, DSC, SEM, GPC and optical microscopy. The observed tri-phasic drug release patterns could be explained as follows: The initial burst release can be attributed to the dissolution of tiny drug crystals with direct surface access. The subsequent 2nd drug release phase (with an about constant release rate) could be attributed to the release of drug crystals in regions, which undergo local swelling. The 3rd release phase (again rapid, leading to complete drug exhaust) could be explained by substantial polymer swelling throughout the systems: Once a critical polymer molecular weight is reached, the PLGA chains are sufficiently hydrophilic, insufficiently entangled and the osmotic pressure created by water soluble degradation products attracts high amounts of water into the system.

Keywords: PLGA; caffeine; microparticle; drug release mechanism; dissolution; diffusion; swelling.

1. Introduction

Poly (lactic-co-glycolic acid) (PLGA) is frequently used as a polymeric matrix former in controlled drug delivery systems, in particular microparticles,[1-6] scaffolds,[7,8] nanofibers[9] and implants.[10-14] This type of advanced drug products allows to pre-program the release rate of the active agent into the human body after injection or implantation. Flexible release periods can be provided, e.g. ranging from a few days up to several months.[15,16] Controlling the "entry" rate into the human body allows optimizing the therapeutic efficacy and minimizing the risk of toxic side effects: Each drug has a characteristic *minimal effective concentration*, below which no therapeutic effects occur, and a characteristic *minimal toxic concentration*, above which undesired side effects occur. The aim is to achieve drug concentrations at the site of action between these two concentrations: in the so-called "therapeutic window". Unfortunately, certain drugs have narrow therapeutic windows and severe toxic side effects. Controlled drug delivery systems can be of great interest in these cases. Generally, the basic idea is to trap the drug in a polymeric matrix. The presence of the latter avoids rapid drug dissolution upon administration into the human body (e.g. by sub-cutaneous injection or implantation). The drug "has to find its way" out of the dosage form to be released. Different types of physico-chemical processes can be involved in the control of the resulting drug release rate,[17] such as drug dissolution,[18] drug diffusion,[19] polymer degradation,[20-22] polymer swelling,[23-25] and osmotic effects[26] to mention just a few.

PLGA offers several major advantages as polymeric matrix former for injectable and implantable drug delivery systems, since it is biocompatible[27] and biodegradable.[28] Thus, upon drug exhaust, there is no need to remove empty remnants: a major benefit for the patient. Various types of PLGA-based controlled drug delivery systems have been described in the literature.[29-37] PLGA *microparticles* are often more easy to administer than PLGA *implants*, e.g. using relatively thin needles. Frequently, 3 drug release phases can be observed with PLGA

microparticles (their relative importance can very much depend on the type of drug and manufacturing procedure): At early time points (e.g. during the first day), the release rate is often high. This is also called "burst effect". The 2nd release phase is generally characterized by an about constant drug release rate and can last several days or weeks. The 3rd release phase is again rapid and leads to complete drug exhaust.

Despite the great practical importance of PLGA microparticles as advanced drug delivery systems, the underlying mass transport phenomena are often not fully understood. Various types of physical and chemical processes might be involved,[38-42] including for instance water penetration into the system, drug dissolution, drug diffusion through water-filled pores and/or the polymer matrix, hydrolytic polyester degradation, polymer swelling, the creation of osmotic pressure within the system due to the accumulation of water-soluble monomers and oligomers, drug – polymer interactions (e.g. plasticizing effects of certain drugs), the creation of acidic micro-environments (due to the generation of short chain acids as degradation products, especially at the center of the systems), and autocatalytic effects (since ester bond cleavage is catalyzed by protons). The relative importance of these phenomena in a particular type of PLGA microparticles likely depends on the type of drug, type of PLGA (e.g. type of end groups and average polymer molecular weight), composition of the system (e.g. presence of other excipients and drug loading) and the manufacturing procedure, which can affect the internal and external system structure (e.g. porosity). The resulting complexity makes it often difficult to reliably predict the effects of formulation and processing parameters on the resulting drug release kinetics. This renders the optimization of this type of advanced drug delivery systems cumbersome, e.g. being based on time-consuming and cost-intensive series of trial-and-error studies (with sometimes surprising tendencies).

Another particularly challenging aspect is the fact that PLGA microparticles are so called "multiple unit" dosage forms: Generally, numerous tiny microparticles (often less than

100 µm in diameter) are administered. In most cases, only such *ensembles* of microparticles are studied and characterized with respect to their drug release behavior. However, each microparticle is individual and might release the drug "in its own way", e.g. due to its unique internal structure. For this reason, it can be very helpful to monitor also the behavior of *single* microparticles. For example, the group of Anders Axelsson studied the release behavior of polymer coated "pellets" (little spherical beads, about 1 mm in diameter, which can be filled into hard gelatin capsules to control drug release). It was shown that the release behavior of the individual beads could be very different, but the use of hundreds of these beads at the same time could provide reproducible release profiles, which were different in shape compared to the individual bead release profiles.[43,44] For instance, if hundreds of *single* unit dosage forms release a drug in a "pulsatile manner" at randomly distributed time points, the overall release rate of the *ensemble* of dosage forms is about constant. Studying only the release of *ensembles* of dosage forms can, thus, be misleading.

The aim of this study was to prepare differently sized caffeine-loaded PLGA microparticles using an emulsion solvent extraction/evaporation method and to characterize the systems thoroughly before and after exposure to phosphate buffer pH 7.4 (a release medium, which is frequently used to simulate aqueous body fluids upon injection). X-ray diffraction, gel permeation chromatography, scanning electron microscopy, optical microscopy, differential scanning calorimetry and in vitro drug release studies were used to monitor the physical states of the drug and PLGA during drug release. Importantly, both, *single* microparticles as well as *ensembles* of microparticles were studied.

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); caffeine (BASF, Ludwigshafen, Germany); polyvinyl alcohol (Mowiol 4-88; Sigma-Aldrich, Steinheim, Germany); acetonitrile and dichloromethane (VWR, Fontenay-sous-Bois, France); tetrahydrofuran (HPLC grade; Fisher Scientific, Illkirch, France).

2.2. Microparticle preparation

Drug-loaded microparticles were prepared using an oil-in-water (O/W) emulsion solvent extraction/evaporation technique: Appropriate amounts of caffeine and PLGA were dissolved in a well-defined volume of dichloromethane (Table 1). “Small”, “medium-sized” and “large” microparticles were prepared, adapting the formulation and processing parameters accordingly (Table 1). The organic phase was emulsified into 2.5 L of an outer aqueous polyvinyl alcohol solution (0.25% w/w) under stirring (1000, 1500 and 2000 rpm, Eurostar power-b; Ika, Staufen, Germany) for 30 min. Upon solvent exchange, the PLGA precipitated, trapping the drug. The formed microparticles were hardened by adding 2.5 L of the same outer aqueous polyvinyl alcohol solution (0.25 %) and further stirring at 700 rpm (Eurostar power-b) for 4 h. The microparticles were separated by filtration (Nylon filter, 0.45 µm, 13 mm; GE Healthcare Life Sciences Whatman, Kent, UK), washed with de-mineralized water and subsequently freeze-dried (freezing at -45°C for 1 h 45 min, primary drying at -40 °C and 0.07 mbar for 35 h and secondary drying at +20 °C/0.0014 mbar for 35 h) (Christ Epsilon 2-4 LSC+; Martin Christ, Osterode, Germany).

2.3. Microparticle characterization

2.3.1. Microparticle size

Microparticle sizes were determined by optical microscopy: Microscopic pictures were taken using an Axiovision Zeiss Scope-A1 microscope, equipped with an AxioCam ICc1 camera and the Axiovision Zeiss Software (Carl Zeiss, Jena, Germany). For *ensembles* of microparticles, each measurement included 200 particles. Mean values +/- standard deviations are reported.

2.3.2. Practical drug loading

The practical drug loading was determined by dissolving approximately 5 mg microparticles in 5 mL acetonitrile, followed by filtration (PVDF syringe filters, 0.45 µm; GE Healthcare, Kent, UK). The drug content was determined by HPLC analysis (Alliance, Separation Modules e2695, 2489, UV-Vis Detector; Waters, Milford, USA). A reversed phase column C18 (Gemini 5 µm; 110 A °; 150 x 4.6 mm; Phenomenex, Le Pecq, France) was used. The mobile phase was a mixture of acetonitrile: water (70:30, v:v). The detection wavelength was 254 nm and the flow rate 1 mL/min. Twenty µL samples were injected. The standard curve covered the range of 0.1 to 50 µg/mL. Each experiment was conducted in triplicate. Mean values +/- standard deviations are reported.

2.3.3. X ray powder diffraction

X ray powder diffraction analysis was performed using a Panalytical X'pert pro diffractometer (λ Cu K α=1.54 Å) and Lindemann glass capillaries (diameter 0.7 mm) (PANalytical, Almelo, The Netherlands). The measurements were conducted in transmission mode with an incident beam parabolic mirror and the X'celerator detector.

2.3.4. Differential scanning calorimetry (DSC)

DSC thermograms of raw materials (as received: caffeine and PLGA) and of microparticles were recorded with a DSC1 Star System (Mettler Toledo, Greifensee, Switzerland). Approximately 5 mg samples were heated in sealed aluminium pans from 10 to 120 °C, cooled to -70 °C and reheated to 120 °C at a rate of 10 °C/min. The indicated glass temperature (Tgs) were obtained from the second heating cycles. Each experiment was conducted in triplicate. Mean values +/- standard deviations are reported.

*2.3.5. Drug release measurements from **ensembles** of microparticles*

Ten mg of microparticles were placed into Eppendorf tubes (Safe-lock, 2.0 mL; Eppendorf, Hamburg, Germany), filled with 2 mL phosphate buffer pH 7.4 (USP 42). The tubes were placed into a horizontal shaker (37°C, 80 rpm, GFL 3033, Gesellschaft fuer Labortechnik, Burgwedel, Germany). At predetermined time points, 1.5 mL samples were withdrawn, filtered (PVDF syringe filters, 0.45 µm; GE Healthcare, Kent, UK) and analysed for their drug contents by HPLC analysis, as described above. To keep the volume of the release medium constant and to avoid the potential loss of microparticles due to sampling, 1.5 mL fresh release medium was injected into the Eppendorf tubes using the same syringe filters at each time point. Each experiment was conducted in triplicate. Mean values +/- standard deviations are reported. Sink conditions were provided throughout the experiments.

2.3.6. *Drug release measurements from **single** microparticles*

Caffeine release from *single* microparticles was monitored in 1 mL syringes (three-part single-use syringes; HSW Henke-Ject, Tuttlingen, Germany) as follows: One microparticle was introduced into a syringe, which was filled with 200 µL phosphate buffer pH 7.4 (USP 42) and

closed with a cap [BD Luer-Lok (TM) (caps with male/female protection); Dominique Dutscher, Brumath, France]. The syringes were placed into a horizontal shaker (37°C, 80 rpm, GFL 3033). At predetermined time points, 50 µL samples were withdrawn (replaced with fresh medium) using Hamilton syringes (Microlite/#710, 100 µL; Hamilton, Bonaduz, Switzerland) and analysed for their drug contents by HPLC, as described above (the standard curve covering the range of 0.025 to 5 µg/mL).

2.3.7. Swelling of *single* microparticles

The swelling of individual microparticles was monitored in 96-well standard microplates (Tissue culture plate 96 well; Carl Roth, Karlsruhe, Germany) as follows: One microparticle was introduced into each well, which was filled with 200 µL phosphate buffer pH 7.4 (USP 42). The well microplates were placed into a horizontal shaker (37°C, 80 rpm, GFL 3033). At pre-determined time points, pictures were taken using an Axiovision Zeiss Scope-A1 microscope and the Axiovision Zeiss Software (Carl Zeiss, Jena, Germany). Also, as for the drug release studies, 50 µL samples were withdrawn and replaced with fresh medium at each sampling time point.

2.3.8. Polymer degradation

Microparticles were treated as for the drug release studies. At predetermined time points, samples were withdrawn, freeze-dried for 3d (as described above) and the lyophilisates were dissolved in tetrahydrofuran (at a concentration for 3 mg/mL). The average polymer molecular weight (Mw) of the PLGA in the samples was determined by Gel Permeation Chromatography (GPC, Alliance, refractometer detector: 2414 RI, separation module e2695, Empower GPC software; Waters, Milford, USA), using a Phenogel 5 µm column (which was kept at 35°C, 7.8 × 300 mm; Phenomenex, Le Pecq, France). The injection volume was 50 µL.

Tetrahydrofuran was the mobile phase (flow rate: 1 mL/min). Polystyrene standards with molecular weights between 1480 and 70,950 Da (Polymer Laboratories, Varian, Les Ulis, France) were used to prepare the calibration curve. All experiments were conducted in triplicate. Mean values and ± standard deviations are reported.

2.3.9. Scanning Electron Microscopy (SEM)

The internal and external morphology of microparticles was studied using a JEOL Field Emission Scanning Electron Microscope (JSM-7800F, Tokyo, Japan). Samples were fixed with a ribbon carbon double-sided adhesive and covered with a fine chrome layer. Cross-sections were obtained after inclusion of microparticles into “OCT embedding medium” (“embedding medium” for frozen tissue specimen to ensure Optimal Cutting Temperature; VWR, Lutterworth, UK) and cutting with cryostat (Leica CM3050 S, Wetzlar, Germany).

3. Results and discussion

3.1. ***Ensembles*** *of microparticles*

To obtain differently sized PLGA microparticles loaded with caffeine using an emulsion oil-in-water (O/W) solvent extraction/evaporation method, the stirring speed and polymer concentration of the organic phase were varied, as shown in Table 1. A higher stirring speed and lower polymer concentration (resulting in a lower viscosity of this phase) led to smaller organic phase droplets and, hence, smaller microparticles. Under the given conditions, the mean particles sizes (+/- standard deviations) of "small", "medium-sized" and "large" microparticles were equal to 62 (+/- 19), 94 (+/- 31) and 287 (+/- 159) µm, respectively. In practice, most often microparticles with a diameter of less than 100 µm are used. However, they are difficult to study individually, for technical reasons. In this study, also larger microparticles were prepared and their behavior upon exposure to the release medium was monitored individually: This can provide very interesting information on the underlying drug release mechanisms, especially in the case of multiple unit dosage forms (as PLGA microparticles): Generally, only drug release from the *ensembles* of numerous microparticles are measured. However, these are only the sums of all the individual microparticle release profiles, which might substantially vary from particle to particle. The basic underlying assumption of this study is that the inner and outer structures of the prepared smaller and larger microparticles are similar. If this would not be the case, the underlying drug release mechanisms might be different. No evidence was observed in this study for any relevant differences in the internal or external structures of the investigated microparticles. There was a minor difference in the practical drug loading, which varied from 6 to 7 % (Table 2): The smaller particles had a slightly lower practical drug content. This can be explained by the smaller size of the droplets of the organic phase formed during microparticle preparation, resulting in higher drug loss into the outer aqueous phase (due to

shorter diffusion pathways). We believe that these differences have no major impact on the resulting drug release mechanisms.

The glass transition temperatures (Tgs, determined by DSC analysis) were equal to about 44 °C in all cases (Table 2). Please note that this indicates that the PLGA is in the glassy state at 37 °C body temperature. However, it is well known that upon contact with aqueous fluids, limited amounts of water rather rapidly penetrate into the entire system (often within hours or up to 1 d). Although these amounts are low, they effectively decrease the Tg of the PLGA by about 10 °C[45,46] (and start polyester hydrolysis throughout the system: "bulk erosion"). Thus, it can be expected that the polymer undergoes a transition from the glassy to the rubbery state rather rapidly upon administration into the human body.

Figure 1 shows the experimentally measured caffeine release kinetics from *ensembles* of PLGA microparticles, differing in size: The mean particle diameters (+/- standard deviations) are indicated in the diagram. As it can be seen, classical "tri-phasic" drug release profiles were observed (although the 1st release phase was not very pronounced), irrespective of the microparticle size:

(i) At early time points (during the first day), the drug release rate was high. This is also called the "burst effect".

(ii) Then, the release rate remains about constant during several days. This is generally called the "2nd release phase". Please note that the slope of the release curve was higher for the smaller microparticles in this phase.

(iii) At a later time point (here, after about 1 week), a final rapid drug release phase set on, leading to complete drug exhaust. This phase is often referred to as the "3rd release phase".

The optical microscopy pictures in Table 2 and the SEM pictures at the top of Figure 2 show that the microparticles were spherical in shape and exhibited a rather smooth, non-porous

surface before exposure to the release medium. The SEM pictures at the bottom of Figure 2 show cross-sections of the differently sized microparticles. As it can be seen, small pores were distributed throughout the systems, irrespective of the microparticle size. Importantly, very small crystals (1 µm or less in size) were visible in the different cross-sections at higher magnification (bottom row in Figure 2). These crystals are likely caffeine crystals, since X-Ray diffraction revealed sharp Bragg peaks in the different microparticle batches at the same angles as observed with the caffeine raw material (as received) (Figure 3). This is important information for the underlying drug release mechanisms. The investigated microparticles are dispersions of very small drug crystals in a PLGA matrix. Please note that the caffeine was *dissolved* in the organic phase during microparticle preparation. However, at least parts of the drug recrystallized upon solvent evaporation. This is consistent with the fact that the glass transition temperature (Tg) of the PLGA raw material (as received) was equal to 47 +/- 0.2 °C, as compared to Tg values around 44 °C in the case of the PLGA microparticles loaded with 6-7 % caffeine (Table 2). The slight decrease in Tg (by about 3 °C) can serve as an indication that parts of the drug are likely *dissolved* in the PLGA and act as a plasticizer for this polymer. But the solubility of caffeine in PLGA is likely limited: The decrease in Tg is limited and crystals are visible in cross-sections of particles loaded with 6-7 % drug.

To better understand why the different release phases were observed from the investigated caffeine-loaded PLGA microparticles (and why there was a moderate difference in the release rate during the 2nd release phase), the behavior of *single* microparticles was studied, in particular their swelling and drug release kinetics upon exposure to an aqueous phase simulating body fluids at the administration site.

3.2. ***Single*** *microparticles*

Figure 4 shows optical microscopy pictures of differently sized PLGA microparticles loaded with caffeine upon exposure to phosphate buffer pH 7.4 at 37 °C. As it can be seen, the size of the particles remained about constant during the first few days, but after about 1 week substantial microparticle swelling set on. The dynamic changes in the diameters of the *single* microparticles are plotted as a function of exposure time to the release medium in Figure 5. The initial particle sizes are indicated at the top of the diagrams. A superposition of the different curves is shown in the diagram at the bottom of Figure 5 on the right hand side. Clearly, microparticle swelling was very much limited during the first week, but then substantial swelling set on. This phenomenon has recently been explained as followed, in the context of macroscopic cylindrical PLGA implants:[47] Initially, the PLGA chains are rather hydrophobic and intensively entangled. This effectively limits the amounts of water, which can penetrate into the system upon contact with aqueous fluids. However, the limited amounts of water that enter the microparticles start cleaving the ester bonds of the PLGA throughout the system ("bulk erosion").[48] This has at least 3 major consequences: (i) The polymer chains become more and more hydrophilic, since new –OH and –COOH end groups are created upon ester bond hydrolysis. (ii) The polymer chains become less entangled, because their molecular weights decrease. This affects the "mechanical stability" of the polymeric matrix. (iii) Water soluble monomers and oligomers are generated, creating a steadily increasing osmotic pressure within the system. As soon as a certain, critical threshold value is reached, the polymer matrix is sufficiently hydrophilic and mechanically instable, so that high amounts of water are effectively attracted by the osmotic pressure built up within the microparticles: Substantial swelling of the entire system sets on. The presence of high amounts of water within the microparticles allows for the complete dissolution of the caffeine crystals and results in relatively high mobilities of

the dissolved drug molecules in the PLGA gels. Both effects lead to an increase in the resulting drug release rate: The final, rapid drug release phase (= 3rd release phase) starts.

This type of drug release mechanism is likely also of importance in the investigated caffeine-loaded PLGA microparticles: As it can be seen in Figure 1, after about 1 week the final rapid drug release phase set on, irrespective of the microparticle size. Also, the drug release profiles observed with *single* microparticles confirm this theory: Figure 6 shows the release of caffeine from individual PLGA microparticles in phosphate buffer pH 7.4 at 37 °C (body temperature). The diagram on the right hand side at the bottom of Figure 6 shows the superposition of the different curves. As it can be seen, a final rapid drug release phase was observed in all cases (marked in green). Please note that there is some variability in the onset time of this 3rd release phase. Often, the onset is slightly delayed with respect to the onset of the substantial swelling of the entire microparticles (Figure 5). This might be due to inter-particle variability (e.g. only a few microparticles have been studied, *ensembles* of microparticles consist of numerous *single* particles), and/or it might take some time for the drug to diffuse out upon polymer swelling.

Interestingly, the onset of substantial microparticle swelling was observed after about 1 week in this study, which corresponds to a polymer molecular weight of about 20 kDa: Figure 7 shows the decrease in the average polymer molecular weight (Mw) of the PLGA in the investigated microparticles as a function of the exposure time to the release medium at 37 °C. The degradation kinetics were similar for the differently sized microparticles. A threshold value of about 20 kDa was also observed by Gamsi et al.,[23,24] studying dexamethasone- as well as prilocaine-loaded PLGA microparticles. In contrast, a threshold value of about only 8 kDa was reported to coincide with the onset of substantial PLGA *implant* swelling by Bode et al.[47] Those implants were based on Resomer RG 502H, which is a shorter chain polymer compared to the one used in this study. But the most likely reason for the

difference in the threshold value might be the difference in the dimensions of the systems: microparticles versus macroscopic implants: One pre-requisite for substantial swelling of the entire drug delivery system is the absence of a stable core. As long as such a stable core exists, it mechanically restricts the swelling of the other regions. Once also the core of the device starts to swell, the entire system can rather easily expand. In the case of macroscopic implants this takes more time than in much smaller microparticles. But this is only a hypothesis, and it would be interesting to study this aspect in more detail in the future.

The proposed drug release mechanism for the 3rd drug release phase is also illustrated in the scheme at the bottom of Figure 8. The rectangles represent caffeine *crystals* (which cannot diffuse), the crosses represent caffeine *molecules*, which can diffuse. Prior to the onset of substantial microparticle swelling, the amounts of water in the systems are limited and insufficient to dissolve major portions of the drug. However, once substantial microparticle swelling starts, the drug crystals can dissolve and the dissolved caffeine molecules are rather mobile in the swollen PLGA gel.

The burst release (= 1st release phase) from the investigated PLGA microparticles can probably be explained by the presence of caffeine crystals, which are located close to or at the surface of the systems, with immediate direct access to the surrounding bulk fluid (or obtaining such access shortly after exposure to the release medium). As illustrated in the scheme at the top of Figure 8, water can immediately dissolve these drug crystals. If the drug has to diffuse through a tiny pore to be released, this process might take some time. However, this type of "early drug release" is very much limited in the investigated microparticles (Figure 1). This is consistent with the drug release profiles observed with *single* microparticles, shown in Figure 6. The particle with an initial size of 297 µm exhibits a burst release of about 10 % of its loading, but the other particles show much less caffeine release within the first day. This is in contrast to recently reported PLGA microparticles loaded with *diprophylline* crystals, which exhibited

burst releases of up to more than 50 %.[49] Importantly, in that study, the drug crystals were much larger than in the present case. If a large drug crystal rapidly dissolves during the first day, the impact on the relative drug release rate is much higher than if a small drug crystal dissolves (containing much less drug).

The scheme in the middle of Figure 8 illustrates the root cause for the 2nd drug release phase (which has recently been proposed for diprophylline-loaded PLGA microparticles): Upon contact with aqueous media, the hydrophobicity and mechanical stability of the systems initially limit the amounts of water that can penetrate into the microparticles. As discussed above, a certain lag time (here about 1 week) is observed prior to substantial swelling of the entire systems. However, already during the first few days, the microparticles become less spherical and the surfaces of the systems become more and more (locally) deformed. This can serve as an indication for the fact that *locally*, especially in surface near regions, parts of the system start swelling. Some kind of "swelling front" might be observed, as illustrated in the scheme in the middle of Figure 8, but caution should be paid: In reality, no clear "swelling front" might exist, it might be a more or less random swelling of certain microparticle regions (with a higher likelihood of swelling in surface near regions). If a drug crystal is located in such a region, it will get into contact with important amounts of water, dissolve and the dissolved caffeine molecules will subsequently rather rapidly diffuse out through the swollen PLGA.

In the case of the recently reported diprophylline-loaded microparticles,[49] this led to "step-like" release profiles from single microparticles, such as observed in this study with the 273 µm particle shown in Figure 6: After about 7 d, within a short period of time about 25 % of the drug was released. This likely corresponds to a high number of caffeine crystals in this case, which might be interconnected via tiny pores or be in direct contact with each other. Importantly, such "steep drug release steps" were not observed with the other *single* microparticles in this study (Figure 6). This is consistent with the very small drug crystal size

(probably less than 1 µm, please see above). Once such a small caffeine crystal dissolves and is released, only a "small step" can be observed, and the 2nd phases of the release profiles in Figure 6 can be attributed to the release of various small caffeine crystals at random time points. The fact that the "swelling front" more or less homogeneously moves inwards can likely explain that the release rate remains about constant in this phase (changes in the surface area with time are likely of minor importance, since substantial swelling of the entire system sets on after about 1 week).

Please note that the slope of the release curve in the 2nd release phase of *ensembles* of microparticles was higher for the smaller systems (red versus black curve in Figure 1). This is consistent with the hypothesized release mechanism: If the inner microparticle structure is similar, the number of surface near crystals is higher in an *ensemble* of smaller microparticles compared to an *ensemble* of larger microparticles.

3.3. Drug release mechanisms

Based on the above described experimental findings and discussion, the following drug release mechanisms are suggested for the control of caffeine release from the investigated microparticles (as illustrated in Figure 8):

The burst release (= 1st release phase) is caused by the rapid dissolution of caffeine crystals with immediate direct surface access. This phenomenon is very much limited in the present study, e.g. due to the very small size of the drug crystals.

The 2nd drug release phase with an about constant release rate is caused by the local swelling of certain PLGA regions (e.g. visible as deformations of the spheres' surfaces during the first few days): Drug crystals located in these regions dissolve and the dissolved drug molecules rather rapidly diffuse through the swollen PLGA gel.

The 3rd drug release phase (= final, again rapid drug release phase) can be attributed to substantial swelling of the *entire* microparticles, which starts as soon as the polymer chains are sufficiently hydrophilic and less intense entangled, driven by the osmotic pressure generated by the water soluble PLGA degradation products. The presence of high amounts of water dissolves the drug crystals throughout the system, and the dissolved drug molecules rather rapidly diffuse through the swollen PLGA gel. This leads to complete drug exhaust.

Please note that a certain portion of the caffeine is likely also *dissolved* in the PLGA matrix: For instance, the glass transition temperature of the PLGA decreased from about 47 to 44 °C. This might indicate that some of the drug might have a possibility to diffuse also through the non-swollen PLGA matrix (which is likely in the rubbery state, as discussed above). However, the importance of such a contribution is difficult to estimate. The observed release profiles from *single* microparticles (Figure 6) suggest that it might not be of major impact: Otherwise the shape of the release curves prior to the onset of substantial swelling of the entire system should be different: the release rate would be expected to monotonically *de*crease with time, due to the increasing length of the diffusion pathways. It would be interesting to study this aspect in more detail in the future, especially also for other types of drugs, which can dissolve to important extents in PLGA and act as efficient plasticizers for this polymer.

Furthermore, please note that the swollen PLGA gel structures are likely not homogeneous (e.g. Figure 4). Their density probably varies at the micro/nano-scale. Regions with very high water contents might be considered as “pores”. However, this term should not be misunderstood: Such “pores” are likely not free of PLGA, at least not at later time points. It would be interesting to study this aspect in more detail in the future. Also, the dissolution and release of drug crystals likely contributes to the creation of such “polymer-poor regions”.

4. Conclusion

The obtained new knowledge of the underlying drug release mechanisms in caffeine-loaded PLGA microparticles can probably be helpful to understand the drug release mechanisms also in other types of PLGA microparticles, and even macroscopic implants. These types of advanced drug delivery systems offer many interesting advantages and are of increasing practical importance. But device optimization is often challenging, due to the complexity of the involved mass transport mechanisms. Rather surprising effects can be observed when varying formulation and processing parameters. A better understanding of how these systems work can facilitate their optimization. Also, “worst case scenarios” can be considered in a more realistic manner, rendering the respective medical treatments safer.

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Table. 1:

Composition of the inner organic phase and stirring speed used for the preparation of “small”, “medium-sized” and “large” PLGA microparticles loaded with caffeine.

Microparticle size	CH_2Cl_2, mL	PLGA, mg	Drug, mg	Stirring speed, rpm
″Small″	10	903.1	97.9	2000
″Medium-sized″	6	900.7	104.6	1500
″Large″	4	902.4	104.0	1000

TABLE 2:

Practical drug loading, mean practical size, glass transition temperature (Tgs) and morphology of “small”, “medium-sized” and “large” PLGA microparticles loaded with caffeine.

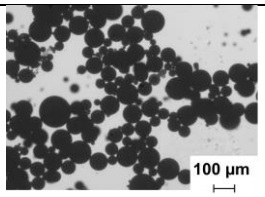
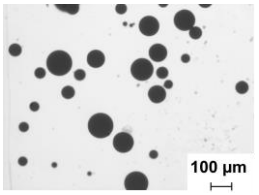
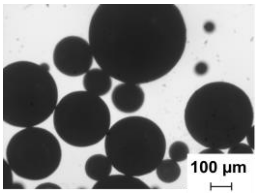
	Practical loading, %	Mean size, µm	Tg, °C	Optical microscopy
″Small″	5.9 ± 0.5	61.8 ± 19.4	44.6 ± 0.2	
″Medium-sized″	5.5 ± 0.2	94.1 ± 31.4	44.3 ± 0.1	
″Large″	7.1 ± 1.0	286.9 ± 158.9	43.5 ± 0.4	

FIGURE CAPTIONS

Fig. 1: Caffeine release from *ensembles* of PLGA microparticles in phosphate buffer pH 7.4: Impact of the mean particle size (indicated in the diagram +/- standard deviation). The release profiles are tri-phasic (although the first phase is not very pronounced): an initial (limited) burst release (= 1st phase) is followed by a period with an about constant drug release rate (= 2nd phase) and a final (again) rapid drug release phase leading to complete drug exhaust (= 3rd phase). The cartoons at the bottom indicate the hypothesized drug release mechanisms (details are given in the text).

Fig. 2: SEM pictures of surfaces (lower and higher magnification) and cross-sections (lower and higher magnification) of caffeine-loaded microparticles before exposure to the release medium.

Fig. 3: X-ray diffraction patterns of *ensembles* of caffeine-loaded PLGA microparticles (mean diameters +/- standard deviations are indicated), and of caffeine raw material (as received) for reasons of comparison.

Fig. 4: Optical microscopy pictures of *single* caffeine-loaded PLGA microparticles before and after exposure to phosphate buffer pH 7.4 for different time periods (indicated at the top). The initial particle size is given on the left hand side.

Fig. 5: Swelling kinetics of *single* PLGA microparticles upon exposure to phosphate buffer pH 7.4 (monitored by optical microscopy). The initial microparticle sizes are indicated at the top of each diagram. The diagram at the right hand side at the bottom shows the superposition of all individual curves.

Fig. 6: Caffeine release from *single* PLGA microparticles in phosphate buffer pH 7.4. The initial microparticle size is indicated at the top of each diagram. The diagram on the right hand side at the bottom shows the superposition of all individual curves. The green region indicates the 3rd release phase (= final, rapid drug release phase).

Fig. 7: Polymer degradation kinetics upon exposure to phosphate buffer pH 7.4 for differently sized microparticle batches (the mean diameters +/- standard deviations are indicated in the diagram). The polymer molecular weight (Mw) was determined by GPC analysis.

Fig. 8: Schematic presentation of the involved mass transport phenomena controlling caffeine release from the investigated PLGA microparticles during the 1st, 2nd and 3rd release phases. Only slightly hydrated (non-swollen) PLGA is marked in dark grey, swollen PLGA in light grey. Details are given in the text. Please note that the schemes are simplifications, e.g. with respect to the homogeneity of polymer swelling and the presence of “pores” (which are neglected in the cartoons for reasons of simplicity). Also, each microparticle has a specific, individual inner structure (e.g. location of the trapped drug crystals) and might or might not contribute to more than 1 release phase observed with an *ensemble* of microparticles.

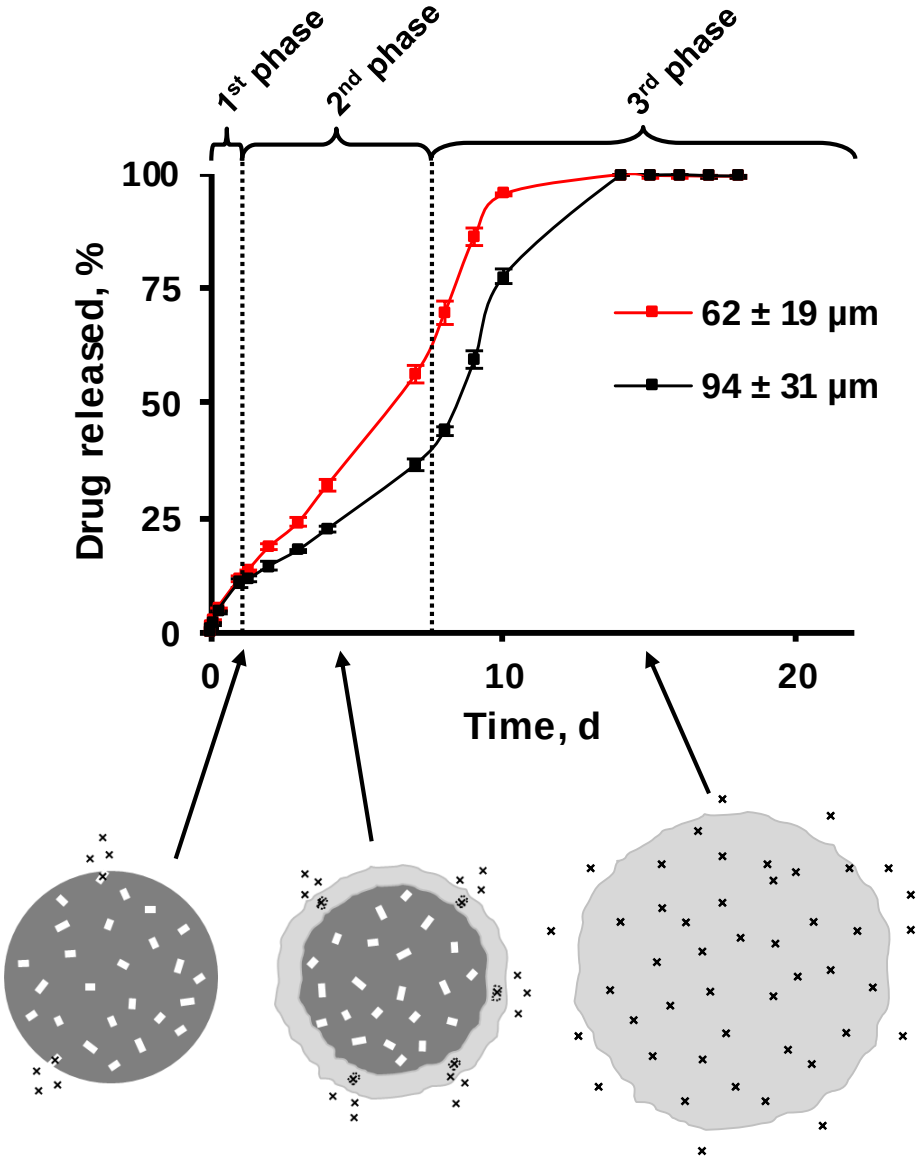

1st phase
2nd phase
3rd phase
100
75
50
25
0
Drug released, %
0
10
20
Time, d
62 ± 19 µm
94 ± 31 µm

Figure 1

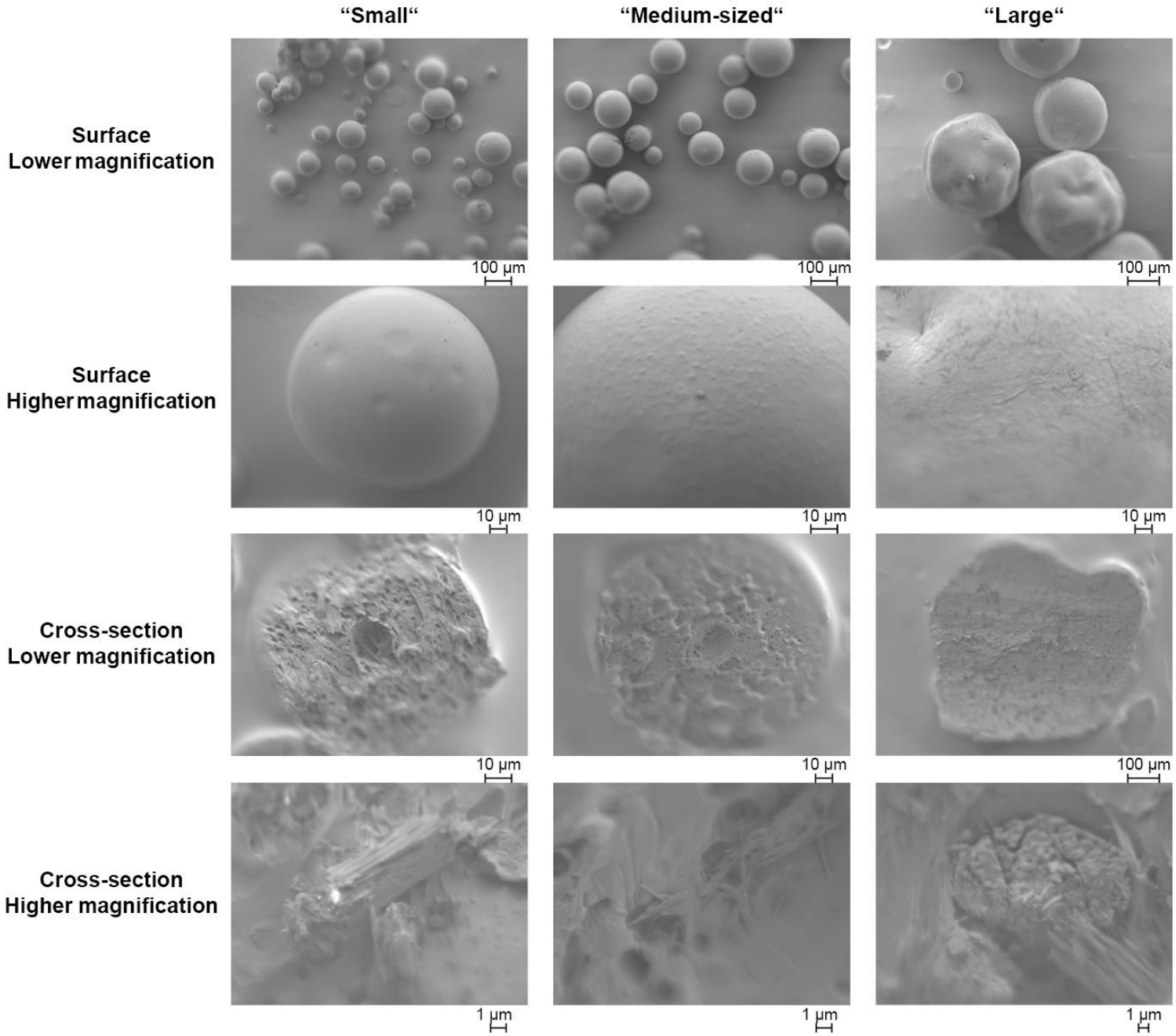

“Small“
“Medium-sized“
“Large“
Surface
Lower magnification
Surface
Higher magnification
Cross-section
Lower magnification
Cross-section
Higher magnification
100 µm
100 µm
100 µm
10 µm
10 µm
10 µm
10 µm
10 µm
100 µm
1 µm
1 µm
1 µm

Figure 2

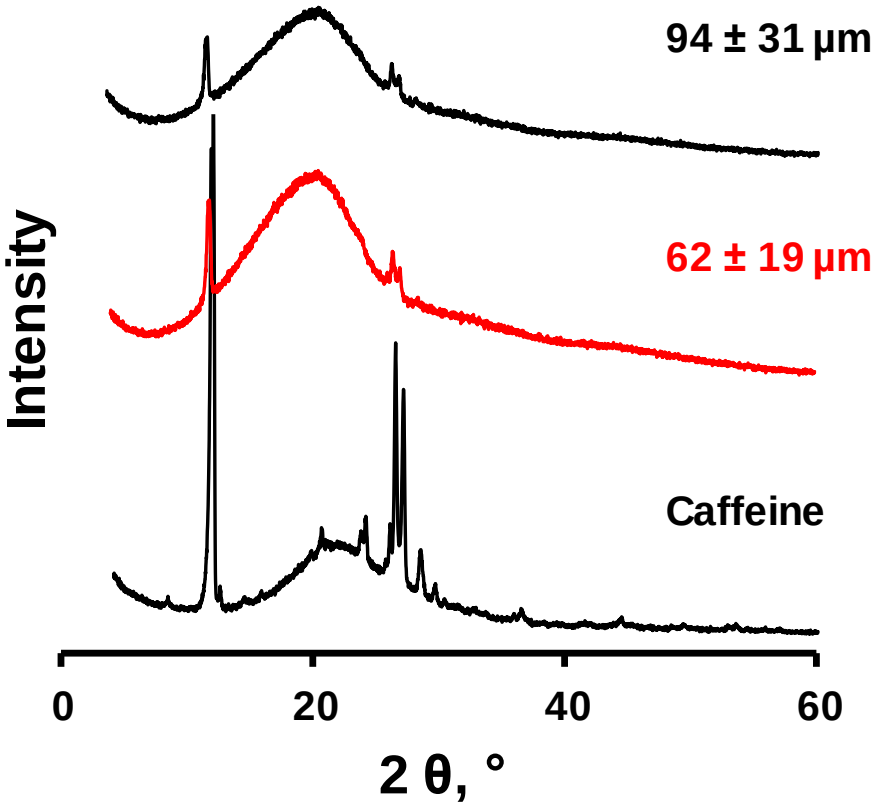


Figure 3

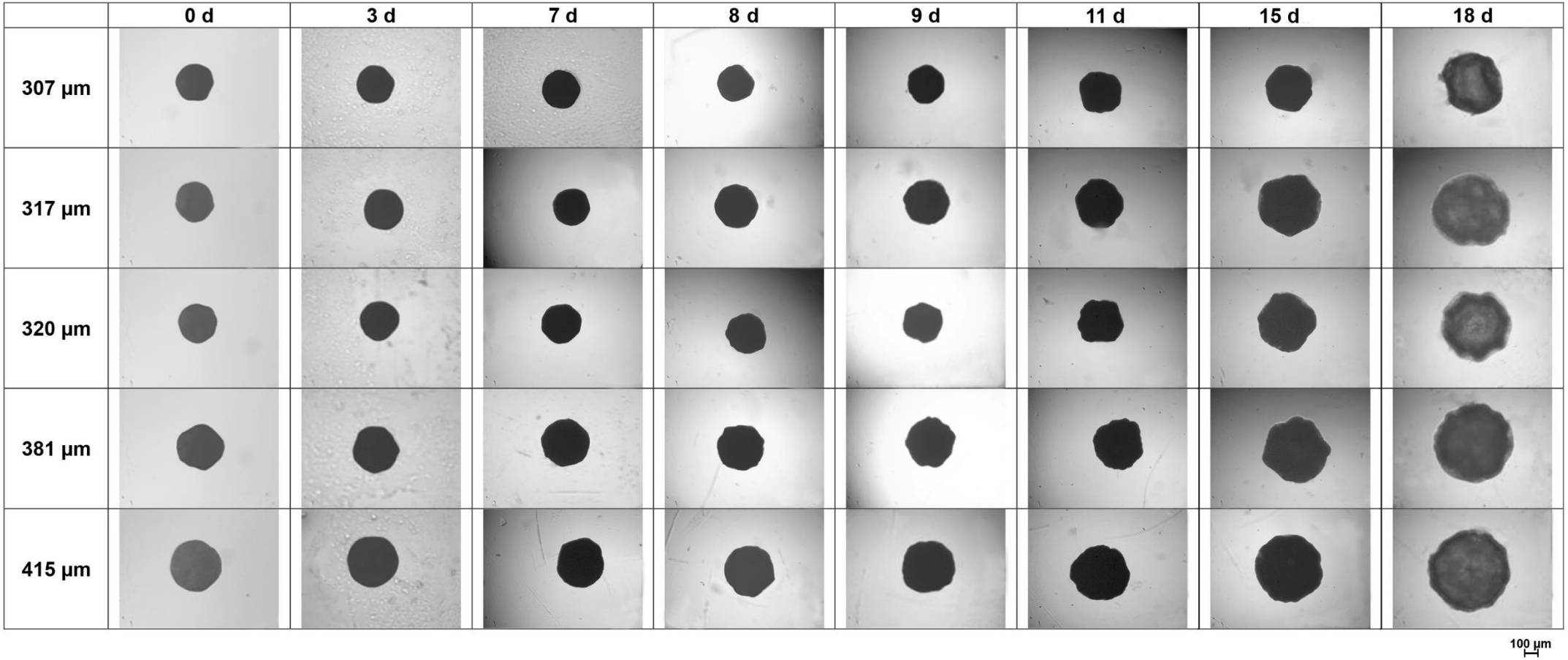


Figure 4

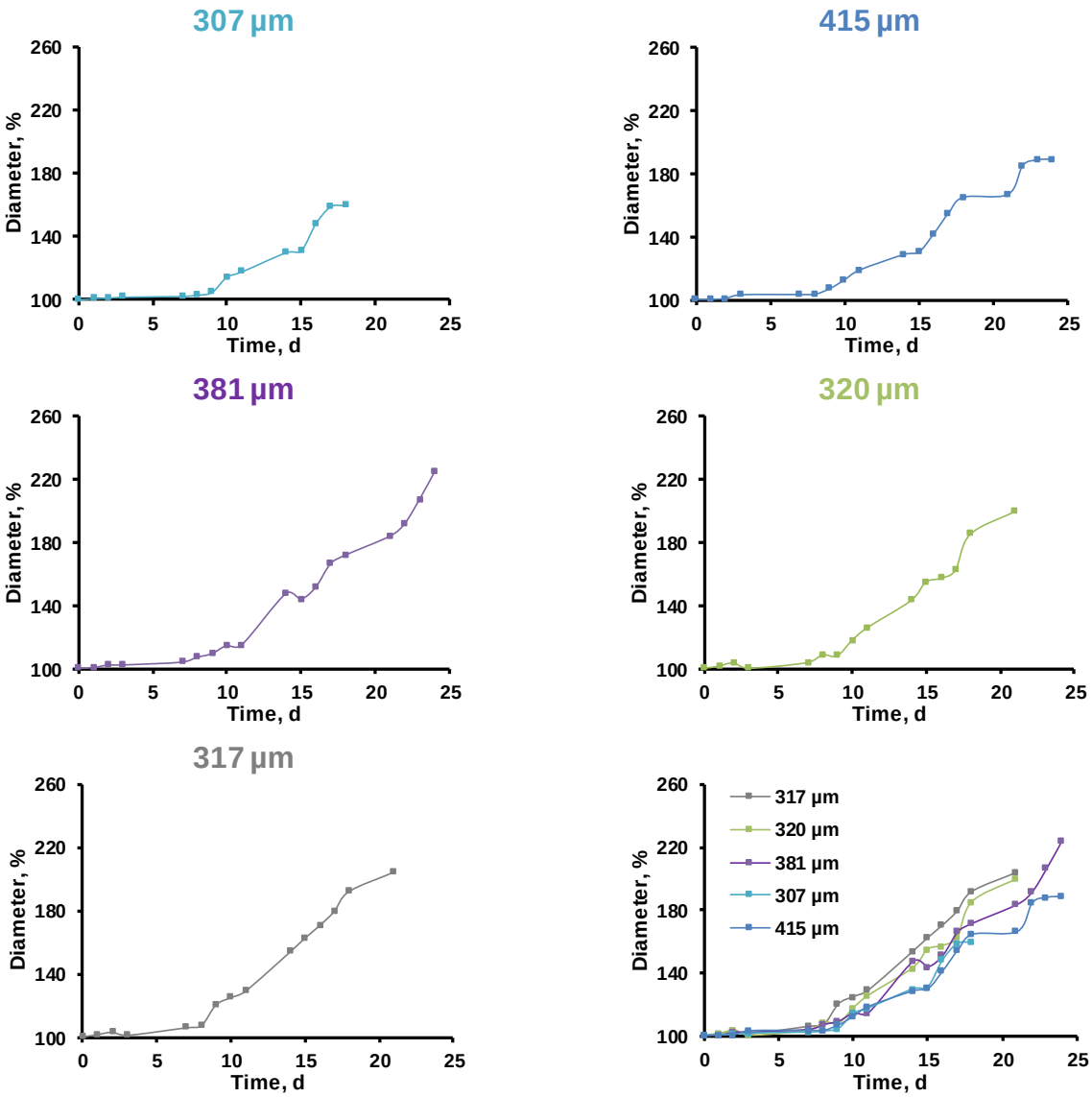

307 µm
415 µm
381 µm
320 µm
317 µm
Diameter, %
Time, d
260
220
180
140
100
0
5
10
15
20
25
317 µm
320 µm
381 µm
307 µm
415 µm

Figure 5

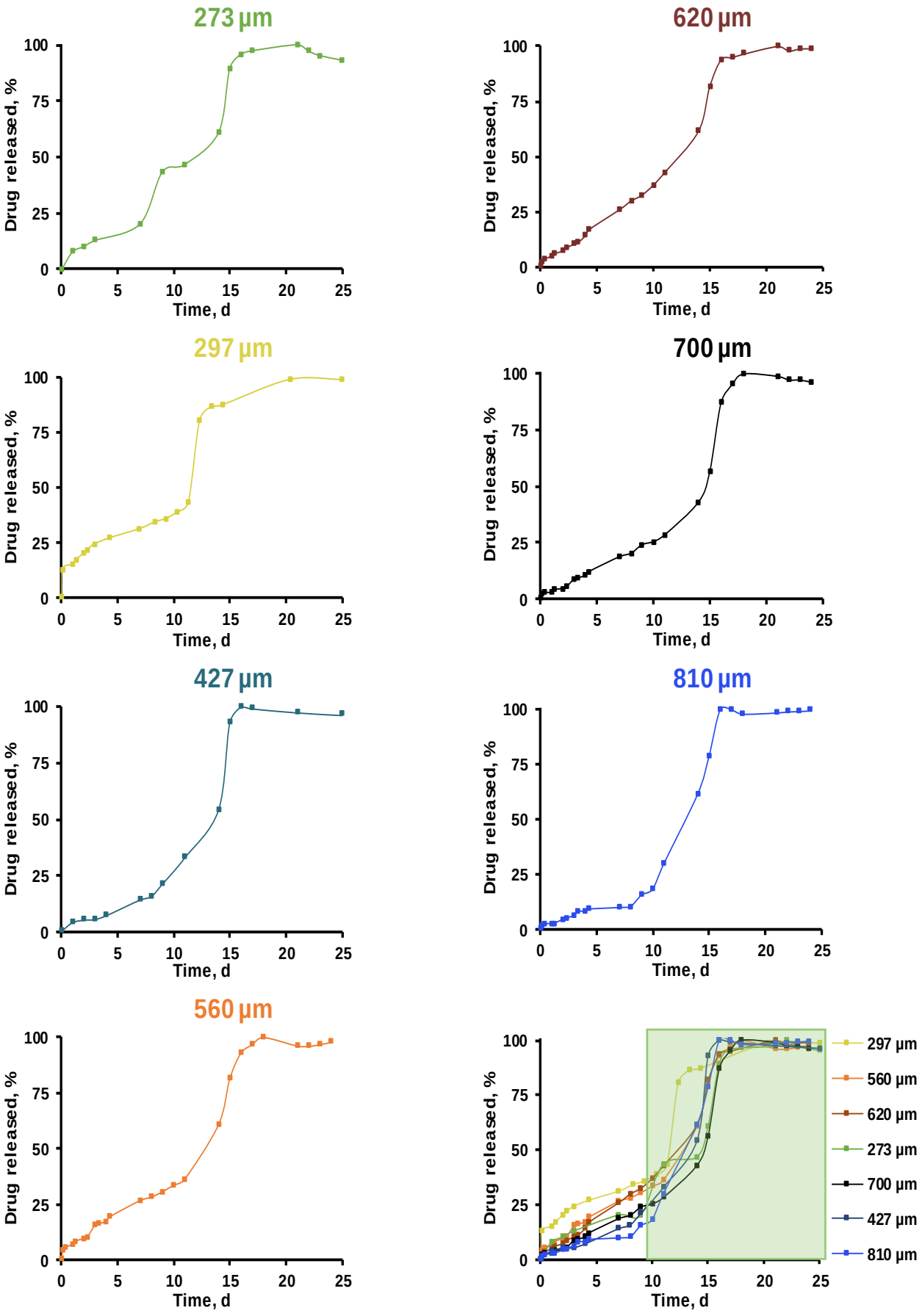

273 µm
620 µm
297 µm
700 µm
427 µm
810 µm
560 µm
Drug released, %
Time, d
0
25
50
75
100
0
5
10
15
20
25
297 µm
560 µm
620 µm
273 µm
700 µm
427 µm
810 µm

Figure 6

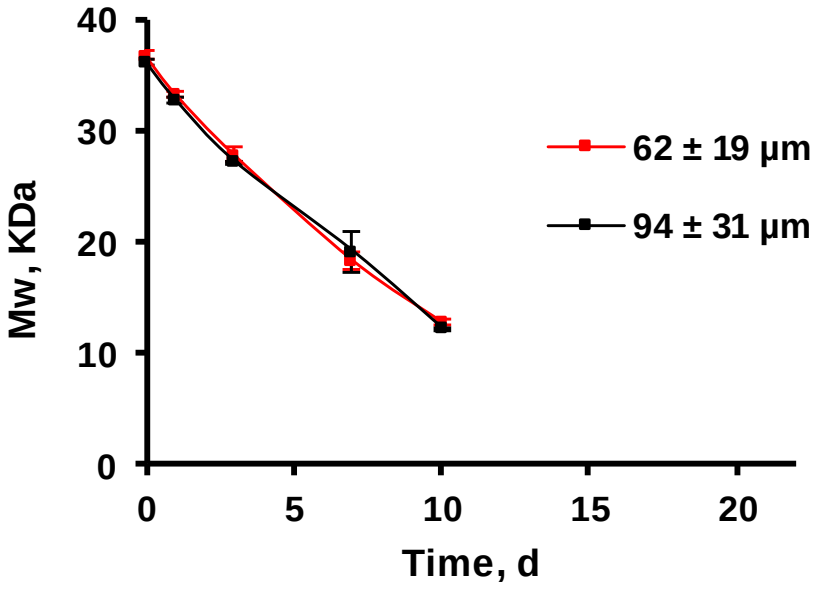


Figure 7

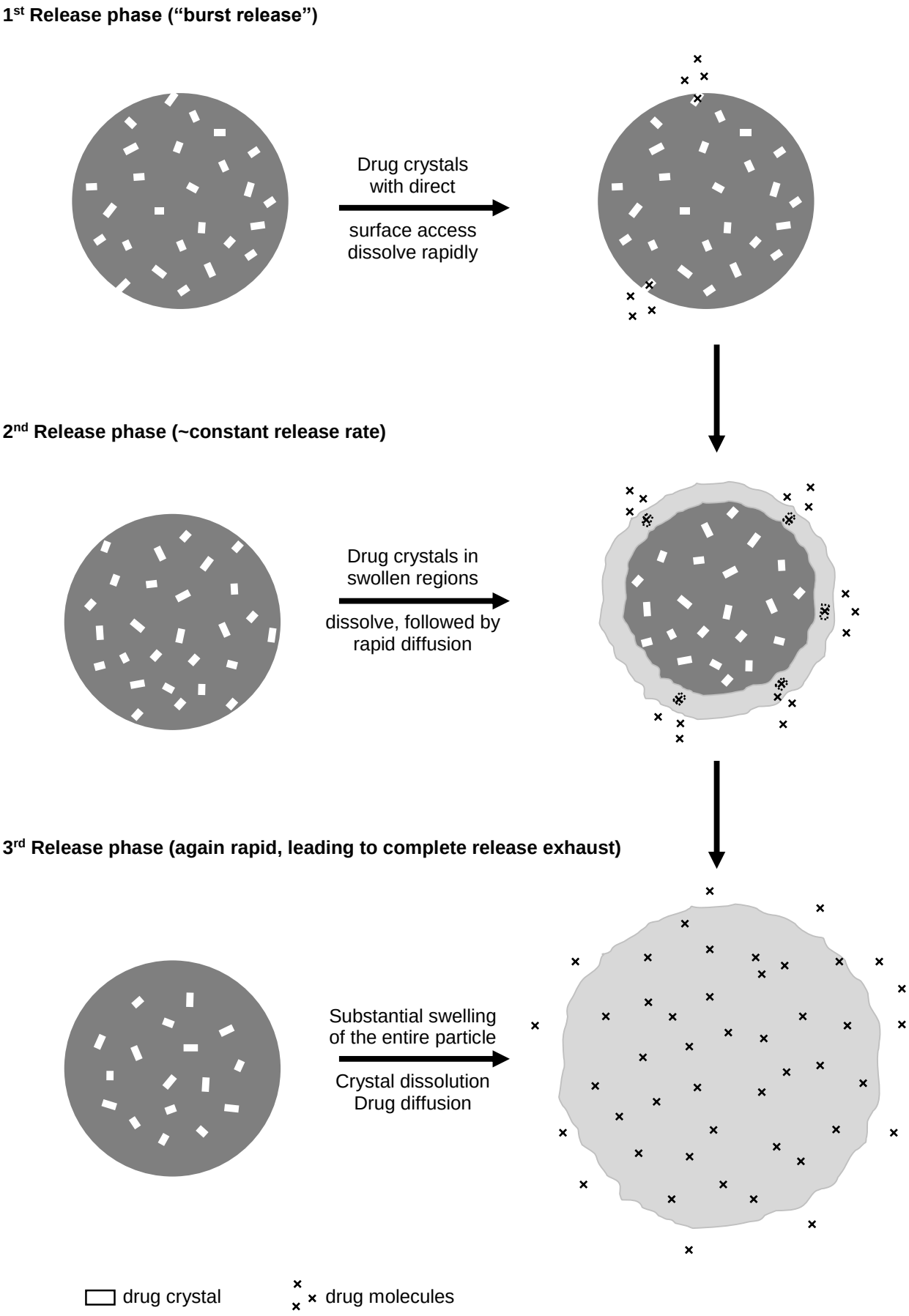

1st Release phase ("burst release")
Drug crystals with direct
surface access dissolve rapidly
2nd Release phase (~constant release rate)
Drug crystals in swollen regions
dissolve, followed by rapid diffusion
3rd Release phase (again rapid, leading to complete release exhaust)
Substantial swelling of the entire particle
Crystal dissolution Drug diffusion
drug crystal
drug molecules

Figure 8