Expert Opinion





# Analysis of initial burst in PLGA microparticles

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*Background*: This review addresses recent advances in the understanding of the mechanisms that underlie burst release and strategies developed to control burst from poly(lactide-co-glycolide) (PLGA) microparticle formulations. While the initial burst release of drug is not always detrimental, excessive drug release in the burst phase may be toxic, and irregularity in the amount of drug released (e.g., from batch to batch) is not acceptable. Many drugs that are good candidates for sustained release treatments are not miscible in PLGA and common microparticle processing solvents, and, as a result, suffer from excessive initial burst release. *Objective*: The aim of this review is to provide an update on research to understand the mechanisms that underlie burst release of drugs from PLGA microparticles, and strategies developed to control burst. *Methods*: This review focuses on literature published since 2004. *Results*: Strategies to control burst release fall into two general categories. First are efforts to improve the miscibility of drug and polymer by altering the composition of the formulation, for example by altering the salt form of the drug. Secondly, processing methods may be altered (increasing the rate of solvent removal, for example) to prevent drug–polymer separation. The goal of most strategies is to reduce or eliminate burst release, so that the encapsulated drug may be maximally retained in the delivery system for long-term delivery.

**Keywords:** burst release, diffusion, drug encapsulation, microparticle, PLGA

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## 1. Introduction

The potential benefits of extended release microparticle drug delivery systems are well known in terms of improved safety, convenience, compliance and therapeutic outcomes. However, the development of robust microparticle formulations remains a difficult problem. This was highlighted by the 2006 approval of a sustained release naltrexone microparticle product, after the initial descriptions of efficient drug encapsulation in the 1970s [1,2]. A major problem encountered in microparticle product development has been the inability to reproducibly control the initial burst release of drug. Certainly with some drugs, such as insulin, high burst release can potentially be fatal. On the other hand, some drugs require a loading dose, and a microparticle formulation with a programmed burst may be an elegant solution in this situation. In any case, excessive burst is wasteful, in that drug lost in the burst phase is not available for later release. A candidate product with burst release levels that are not reproducible from batch to batch will not be approvable. Accordingly, the goal of most efforts to control burst release is to minimize or eliminate burst entirely.

The mechanisms that underlie the initial burst release, the link between microparticle processing and burst release, and strategies used to control or eliminate

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burst have been reviewed previously [3,4]. The purpose of this review is to provide an update on recent developments in mechanistic understanding and control strategies. The focus is primarily on poly(lactide-co-glycolide) (PLGA)-based systems due to the predominant use of this polymer. Also, a PLGA focus allows for continuity with earlier reviews on burst release. While most examples cited to illustrate strategies to control burst have involved PLGA, the general principles behind the strategies are applicable to other systems. These principles include improving the uniformity of drug distribution within the microparticle, controlling particle microstructure and increasing the miscibility between drug and polymer.

## 2. Mechanisms of burst release

The initial burst release phase of drug from a PLGA microparticle system can be defined as the quantity of drug that escapes from microparticles prior to the onset of polymer erosion-mediated drug release. Burst may be an inherent property of diffusion-controlled drug delivery devices. Burst involves the initial release of drug with easy access to the particle surface. Drug dissolution and diffusion contribute to the burst phenomenon, and are accounted for in different models. Analytical solutions to Fick's second law at early release times in a model zero-order device show that the rate and extent of burst release is dependent on the interplay between thermodynamic (drug solubility) and transport (diffusion) properties of the system [5]. The burst effect has been modeled as normal diffusion of drug located near the particle surface through short diffusion pathways [6]. While mathematical models predict a burst release phase, the extent to which burst is observed, or even absent, in experimental systems depends on intrinsic properties of the formulation components, as well as on extrinsic properties of the system induced by processing. Burst release via diffusion through the polymer matrix should correlate with particle surface area [7,8]. However, such correlations can be obviated by wide particle size distributions and by irregularities in particle structure, such as cracks and channels that serve to increase the effective surface area and/or decrease the resistance to drug diffusion, thereby affecting burst [7,9].

### 2.1 Contribution of drug and polymer properties to burst release

Drug solubility and the thermodynamic compatibility between drug and polymer affect burst release. These properties dictate the methods required to encapsulate the drug, the disposition of the drug within the microparticle, the mode of diffusion of the drug and thermodynamic barriers to dissolution in the release medium. Drugs that are thermodynamically compatible with the encapsulating polymer tend to be uniformly dissolved in the polymer matrix, while incompatible drugs are dispersed as a separate phase within the microparticle. Burst from dissolved and dispersed systems are treated by distinct mathematical models [10]. Qualitatively, the drug and polymer properties that affect burst release for dissolved and dispersed systems can be illustrated in the following examples.

Ivermectin is an example of a hydrophobic small molecule that is compatible with PLGA and can be considered a dissolved system. In this case, the drug and polymer are soluble in the same solvent, ethyl acetate, and microparticle formation is achieved by a single emulsion process. After processing, the drug remains dissolved in the polymer, as indicated by thermal analysis (D Allison, unpublished). Because the drug is molecularly dispersed in the polymer, its release, even from locations near the surface of the particle, is likely to be impeded by the polymer. Low diffusivity combined with the partition coefficient of ivermectin (log P = 3.2 [11]), result in a burst release phase *in vivo* that is a small fraction of the encapsulated drug, but lasts several days [12].

On the other hand, a more water soluble drug, like 5-fluorouracil, is not sufficiently soluble in PLGA solvents like ethyl acetate or methylene chloride to achieve acceptable drug loading with single emulsion methods. Instead, this drug must be encapsulated by double emulsion processes that involve dispersion of the drug as a solid or aqueous solution in the polymer phase prior to the formation of microparticles. As a result, the drug molecules in the finished microparticles are dispersed as a separate physical phase that occupy voids in the polymer matrix [6]. In many cases, the drug-rich regions are visible by microscopic examination [6,13-15]. Because of its distribution within the microparticle and its water solubility, 5-FU is not expected to partition into the polymer matrix during release. Rather, diffusion is restricted to a network of channels that connect voids within the microparticle, and that may communicate with the microparticle surface. The structure of this network is determined by formulation and process conditions.

A pore network that is necessary for the diffusion of high molecular weight water soluble drugs out of polymer microparticles is also required for high molecular weight water soluble drugs, such as proteins [16], although accessible pore diameters must be larger. Studies of protein diffusion through PLGA membranes using a two-compartment diffusion cell showed limited reproducibility in determining the diffusion coefficient ($5.0 \times 10^{-13}$ $m^2/s$), probably because of variability in membrane swelling and degradation rates [17].

The two examples described above show that the miscibility between drug and polymer have a fundamental effect on the mode of drug deposition in microparticles, that is the drug is molecularly dispersed in the polymer, or occupies internal voids within the microparticle. The microenvironment surrounding drug molecules (dense polymer network or channels/pores) affects diffusion. Drug molecular weight and solubility in the release medium further affect drug release rate during the initial burst phase.

### 2.2 Contribution of microparticle processing to burst release

Polymers inherently contain a certain amount of free volume. Free volume in this sense consists of interstitial space between polymer chains (i.e., the polymer matrix density) and is uniformly distributed throughout the polymer phase. The scale of the interstitial space is around 2 nm [18]. Interstitial space within the polymer phase is considered microporous according to IUPAC terminology. [19] Micropores are not resolved in standard SEM images of PLGA microparticles but are similar in size to small drug molecules, such as steroids. Higher molecular weight polymers have increased free volume, which may contribute to higher burst release [20]. Drug that is dissolved in the polymer matrix occupies this space and increases the free volume of the matrix. Higher loading of drugs that are miscible with PLGA therefore increases the initial diffusion coefficient of encapsulated drug, resulting in increased burst [21].

It is reasonable to expect the pore size distribution in PLGA microparticles to increase continuously from the microporous range to the macroporous range (up to 1 – 5 μm [19]), depending on the method by which the microparticles are prepared. Microparticles formed by solvent removal techniques involve substantial droplet shrinkage during hardening, since solvent occupies the majority of the polymer phase. A high initial solvent volume is necessary to control polymer phase viscosity to allow particle formation in the desired small size range, using accessible dispersing technologies. As solvent is removed from the polymer solution, droplet volume decreases [22,23]. In addition to volume loss as solvent is removed, the polymer also solidifies in a composition-dependent manner [24]. The rate at which solvent is removed from the oil droplet determines the degree to which the droplet shrinks as the polymer hardens. The relative rates of oil droplet shrinkage and polymer hardening can lead to differences in the polymer matrix density in finished microparticles. For example, if the polymer remains in a mobile, rubbery state until the solvent is fully removed (i.e., by slow solvent removal at temperatures above the composition-dependent polymer $T_g$), the polymer can anneal more completely during processing. The resulting microparticles will have maximum density, with minimum process-induced free volume. Conversely, more rapid solvent extraction can lead to lower density polymer matrices and higher burst release because microparticles harden in a more solvent-swollen state. This is supported by the observation that burst release has been shown to correlate inversely with formulation bulk density [25].

In addition to changing the polymer matrix density, the encapsulation process can introduce larger scale irregularities into the structure of the microparticle. These include the formation of regions of varying polymer density, cracks and channels. These defects can alter the specific surface area or create void networks within microparticles that affect burst release.

Process conditions that affect the solvent extraction rate (e.g., temperature, continuous and dispersed phase volume ratios, pressure, etc) can lead to non-uniform polymer hardening. Removal of polymer solvent from the surface of microparticles at a rate that is faster than the rate of solvent diffusion within the polymer phase can lead to the formation of a high density shell at the particle surface [26]. Subsequent diffusion of solvent from the particle is retarded by the high density shell. The liquid internal polymer can adhere to the surface, creating structures with lower inner polymer density, or hollow particles [27]. Solvent efflux through the polymer matrix may cause pore formation [28]. In addition, solvent extraction and particle shrinkage creates an inward stress on the shell, which can sometimes be seen as triangular or Fibonacci number patterns on particle surfaces [29,30].

Other mechanisms during processing can lead to the formation of pore and channel networks within microparticles. Encapsulation of water soluble molecules using double emulsion techniques or single emulsion techniques involving the use of water miscible cosolvents (e.g., methanol in o/w peptide encapsulation systems [28]), can create osmotic gradients within particles that result in water influx, creating channels that affect subsequent drug release [31]. The degree of water influx and/or cosolvent efflux affects particle structure and drug distribution [32].

In double emulsion systems, the stability of the primary dispersion of drug in the polymer phase affects microparticle structure and burst. For double emulsion systems with poorly stabilized primary emulsions, the emulsion will tend to phase separate. This can result in the fusion of inner water droplets during processing, reducing the tortuosity of diffusion pathways for dispersed drug molecules, and can increase the potential for unacceptably high burst release [33].

### 2.3 Microparticle porosity and percolation models of burst release

As described above, formulation and process variables affect microparticle porosity, which can affect burst. Descriptions of microparticle porosity vary in the literature, and are often based on the appearance of particles in SEM or confocal micrographs. However, pores and channels can exist within microparticles with diameters that are below the resolution of typical SEM observation, and these channels can contribute significantly to drug release [34]. Low molecular weight drugs, for example, can access micropores with diameters less than 40 Å. Nitrogen porosimetry or NMR techniques are being used with increasing frequency to quantify porosity in microparticle drug delivery systems [9,34-37].

Messaritaki *et al.* (2005) showed that the release rates of 5-fluorouracil from PLGA microparticles prepared by water-in-oil-in-water (w/o/w)-solvent extraction was heterogeneous at different locations within a microparticle, and thus

cannot be modeled as purely a diffusive process [38]. One approach that has been used with some success to model this type of behavior is percolation theory [38-40]. Percolation theory provides a statistical description of the morphology and transport properties of drug-loaded microparticles. Release rates are defined in terms of relative exposure of drug to isolated or surface-accessible porous pathways. Pore diameter and network tortuosity inhibit drug diffusion. Accordingly, manipulation of initial conditions in percolation models of drug release to simulate increased drug loading, or to increase the number or diameter of surface-accessible pores, leads to a predicted increase in burst release [39]. A percolation model was used to show that drug loading below a critical value would result in non-Fickian release kinetics [41]. A combinatorial method has been developed to determine the structure of a hydrogel that will give any desired release profile [42].

Recent studies in which microparticle porosity was manipulated to affect burst release supports the percolation models. For example, release of propaphenone from PEG–PLA nanoparticles correlated with pore size distribution [36]. Polymer properties can influence microparticle porosity. Composition of PEG–PLA block copolymers (i.e., with PEG chains grafted to PLA or as multiblock copolymers) dictates internal pore structure and burst release from nanoparticles formed by a single emulsion process [35].

### 2.4 Polymer molecular mobility and burst release

There is a growing recognition in the recent literature that polymer mobility plays a key role in drug release from microparticle formulations [43]. Diffusion of a fluorescent probe in PLGA microparticles was shown to vary dramatically as the temperature increased over a 20 degree range [44]. Drug diffusion in PLGA–PEG was found to depend primarily on polymer mobility in molecular dynamics simulations [45]. Experimentally, Messaritaki *et al.* [38] studied the release of 5-fluorouracil from microspheres prepared by double emulsion, using a combination of pulsed field gradient NMR and confocal microscopy to monitor water penetration and drug diffusion. They observed that the diffusion coefficient of the drug measured by confocal microscopy was orders of magnitude slower than the penetration of water into microparticles, as measured by NMR. Their interpretation was that microparticle swelling, rather than diffusion of water or drug in microparticle cavities, was rate controlling [38].

The process by which burst release occurs appears to involve more than water influx and drug diffusion, but may also require pore and channel opening. This mechanism is consistent with a variation of the percolation model of drug release in which polymer mobility affects the interconversion of internal cavities and channels between isolated and surface-continuous states. The hydrophilicity and content of a specific drug encapsulated in microparticles can affect water penetration and polymer swelling [46].

For hydrophobic drugs encapsulated in PLGA using water immiscible solvents and single emulsion processes, water influx is expected to be a relatively minor contributor to particle microstructure. The solubility of water in PLGA composed of 50% lactide and 50% glycolide is less than 3% [47] and increases with glycolide content [48]. Water solubility in methylene chloride is approximately 0.2% [49], and approximately 3% for ethyl acetate [50]. Thus, the encapsulation of non-water soluble drug molecules that are miscible with the polymer phase would not be expected to lead to substantial water penetration and subsequent meso- or macropore formation, and this is often observed [51,52]. Therefore, the kinetics of polymer swelling and burst release may be expected to vary with the properties of the individual formulation components.

While the depletion of drug molecules with easy surface accessibility and creation of longer diffusion pathways contribute to the termination of burst release, evidence is mounting for another mechanism related to the formation of diffusion barriers. Wang *et al.* (2002) [43] demonstrated for the first time that termination of the burst release phase of a model peptide encapsulated by a double emulsion process was seen to coincide with the formation and thickening of an outer 'skin' layer during exposure to an aqueous release medium. The collapse of process-induced pores and channels at the surface of PLGA microparticles in physiological environments was confirmed by the reduced penetration of a fluorescent dye after immersion in an aqueous buffer. The paper also documented the contribution of pores initially present on the microparticle surface as well as the creation of new pores to the burst release of the peptide in the first hours of release [43]. More recently, it has been shown that microparticle porosity visible in SEM cross-sectional images of PLGA microparticles decreased during the diffusion phase of drug release [53]. The rate of pore closure increases with temperature [54]. In addition, closure of surface macropores, as measured by atomic force microscopy, during the exposure of PLGA microparticles to high humidity conditions, led to reduced burst release of a protein drug [55]. Taken together, these results indicate that polymer annealing in the first hours after exposure to a release medium is at least partly responsible for the termination of the burst phase of drug release from polymer microparticles.

Mechanistically, upon exposure of microparticles to water, $T_g$ of the polymer drops below body temperature, and the state of the polymer is transformed from the glassy to rubbery state. The increased molecular mobility of polymer in the rubbery state allows the polymer matrix structure to relax to its lowest energy state, with the net result being that voids in the polymer matrix collapse. This explanation is consistent with the absence of burst during *in vitro* release experiments done at high temperatures [56]. However, it has been generally observed that polymer annealing to form the diffusion barrier occurs at the surface, rather than uniformly

throughout the particle. One reason for this may be that surface polymer is plasticized by hydrated residual emulsion stabilizers adsorbed to the particle surface [55].

## 3. Strategies to control burst release

Burst release depends on a number of factors related to the physical properties of the microsphere system components and also on the conditions encountered during processing. Recent developments in understanding the mechanisms that underlie burst release have allowed the development of strategies to control burst. In general, the methods fall into several general categories: improving the miscibility of drug in the polymer phase, increasing the resistance to diffusion (coatings) and manipulation of processing methods to control or reduce microparticle porosity.

### 3.1 Drug–polymer compatibility

One strategy to reduce burst release is to increase the miscibility of drug in the polymer phase. As mentioned previously, drugs that are not soluble in common processing solvents (e.g., methylene chloride or ethyl acetate) and are not miscible with hydrophobic PLGA, are dispersed in voids within the polymer matrix, rather than in the polymer interstitial space. Modification of the drug or polymer to allow the drug to partition into the polymer matrix tends to reduce burst release, likely by reducing water solubility. Methods to achieve this involve alteration of the drug molecule, including changes to the salt form of the drug, esterification and complex formation. In some cases, cosolvent systems can be used to increase the solubility of certain drugs in polymer solvents. In addition, the polymer itself can be chemically modified to improve compatibility with encapsulated drugs.

Burst release from dispersed systems tends to be higher than that from dissolved systems, particularly with regard to PLGA systems where dispersed drugs are hydrophilic. One reason for this in hydrophobic polymer systems is the tendency of dispersed drugs to have higher water solubility. The burst of three model drugs with different equilibrium water solubilities from hydrophobic polyanhydride microparticles was proportional to the water solubility of the drug [57]. Thus, modifications designed to improve compatibility between drug and polymer often reduce burst. Detailed strategies are reviewed in the following sections.

#### 3.1.1 *Modification of the drug*

The salt form of certain drugs may be changed to reduce burst release. Doxorubicin in the hydrophobic free base form may be incorporated into PLGA matrices by single emulsion. Conversely, encapsulation of the water soluble salt form requires the double emulsion method, which reduces drug loading and encapsulation efficiency compared to formulations prepared using the single emulsion technique [58]. *In vitro* release profiles showed a higher burst from the double emulsion formulation, despite having a lower drug load [58]. Higher burst release of doxorubicin HCl compared to the free base was also seen in electrospun PLLA fibers containing the drug [59]. In both studies, encapsulation of the water insoluble free base form of doxorubicin resulted in lower burst release because in the free base form, the drug was more intimately mixed with the polymer. The deposition of the water soluble form of the drug in macroporous voids in the double emulsion formulations offered less resistance to drug release that presumably took place through surface-connected channels. By comparison, the drug in the single emulsion formulations was likely to be molecularly dispersed in the polymer. The observed slower release may have been due to the much smaller channel diameters in the polymer phase, in addition to reduced water solubility. A further example of the effect of miscibility between drug and polymer is that burst release of lidocaine salt was greater than that of lidocaine base from lactide polymer films [60]. In these examples, lower water solubility of the free base form of the encapsulated drug may have contributed to the reduction in burst.

In some cases it is possible to improve the miscibility between drug and polymer by covalent modification of the drug with appropriate compounds. Burst release of the more lipophilic ganciclovir monobutyrate ester was reduced compared to that of the parent compound after encapsulation using the same non-aqueous method [61]. Conversely, hydrophilic adducts increase burst. For instance, esterification of hydrocortisone to form the more water soluble acetate led to the necessity of encapsulation using double emulsion and increased burst release [62].

Proteins may be covalently modified with poly(ethylene glycol) to increase their solubility in organic solvents. Specific PEGylation at the amino terminus of the B chain of human insulin with a 5000 Da polymer allowed the complex to dissolve in dichloromethane and be encapsulated in PLGA microparticles using a single o/w emulsion–solvent extraction technique [52]. Burst release from this formulation was less than 1% of the encapsulated protein and biological activity was retained [52]. While insulin PEGylation simplifies encapsulation of the drug into PLGA microparticles, the mechanism by which burst is reduced was not investigated.

#### 3.1.2 *Cosolvent systems*

The use of cosolvent systems, such as methanol in methylene chloride, to allow the dissolution of peptides like leuprolide or octreotide in the polymer phase has long been known [63]. This strategy can allow drug encapsulation using single emulsion techniques. Another cosolvent system, consisting of dimethyl sulfoxide (DMSO) and methylene chloride was used to co-dissolve PLGA and recombinant human G-CSF. Nanoparticles formed from this mixture using a single emulsion process showed higher burst than a formulation prepared by double emulsion, however, complete release of the encapsulated protein (although some of the protein was aggregated)

from the cosolvent nanoparticles was observed in the cosolvent formulation [64].

### 3.1.3 Complex formation

Complexation of drug molecules with counter ions or other molecules that improve compatibility between drug and polymer has been a strategy that has received more attention recently. Reasons for this include the fact that complexation is applicable to a wider range of drugs and does not involve chemical modification of the drug, where stability issues may appear.

Charge-carrying drug molecules may be neutralized and solubility in organic processing solvents may be increased by hydrophobic ion pairing. The formation of leuprolide–oleate ion pairs prior to encapsulation led to the reduction of burst release from PLGA microparticles [65]. Hydrophobic ion pairing of leuprolide with sodium docusate was also used to modify release of the peptide from oligosaccharide ester derivative microparticles produced by spray drying [66].

Hydrophobic ion pairing has also been used to improve encapsulation efficiency and burst release of larger proteins from microparticles. Fu *et al.* (2003) [67] showed that the hydrophobicity of a glial cell line-derived neurotrophic factor was increased by complexation with ionic surfactants to allow solubilization in a single-phase aqueous–organic solvent system. This allowed encapsulation of the protein in PLGA microparticles using a single emulsion technique that showed a lower burst release than that of a microparticle formulation consisting of the protein encapsulated by a double emulsion procedure [67].

Hydrophobic ion pairing has the added benefit of improving the stability of proteins during encapsulation. Native lysozyme conformation was retained to a higher degree when the protein was ion paired with sodium oleate prior to encapsulation in PLGA nanoparticles compared to uncomplexed lysozyme [68].

For proteins, biological activity depends on maintenance of native conformation. Exposure to organic solvents subjects proteins to denaturation and aggregation during encapsulation. Duncan *et al.* (2005) [69] showed that for a range of proteins, stability during encapsulation by emulsification could not be predicted by conformational stability measured by chemical denaturation. Furthermore, it was shown that protein denatured by emulsification remains insoluble and affects the amount of protein released in the burst phase [69]. Incorporating solid-state proteins into preformed inner water-in-oil (w/o) emulsions containing viscous stabilizers improves the encapsulation efficiency and reduces burst [70].

Complexes between hydrophilic drugs and other agents, such as polypeptides or polysaccharides, may be used to improve the burst release characteristics of microparticle formulations. Complexation of erythropoietin with HSA or polylysine prior to encapsulation in PLGA microparticles by double emulsion improved protein integrity and release compared to uncomplexed protein [71]. As with the covalent modification of drug molecules, properties of the modifier can affect burst release. Complexation of α-chymotrypsin with methyl-β-cyclodextrin (soluble in methylene chloride) by lyophilization allowed encapsulation using a single emulsion technique and resulted in improved preservation of biological activity and reduced burst release of the protein from PLGA microparticles compared to complexation with hydroxypropyl-β-cyclodextrin [72]. Likewise, bovine serum albumin encapsulation and release properties from microparticles prepared by a double emulsion process was improved by complexation with PEG-polyhistidine [73].

Complexation of chromosomal DNA with polycations, like polyethyleneimine (PEI), at high nitrogen to phosphate ratios prior to encapsulation led to reduced burst release from microparticles prepared by double emulsion or spray drying [74]. However, complexation may have unintended adverse effects on burst release. For example, Gomez dos Santos *et al.* (2006) [75] found that oligodeoxynucleotides complexed to PEI and encapsulated in PLGA microparticles by double emulsion showed a higher burst release than the burst from the uncomplexed drug. This result appeared to be caused by osmotic effects related to the presence of the PEI. The osmotic effect was mitigated by adding NaCl to the external aqueous phase at the appropriate concentration [75]. Thus, complexation can improve burst characteristics of a microparticle formulation, however, care must be taken to account for the unintended effects of adding more material to a formulation.

### 3.1.4 Other excipients used to modify the release of drugs from microparticles

Compounds that do not associate specifically with drug or polymer molecules may be added to microparticles during processing, or after microparticles have been formed to reduce burst release. Excipients may be added to increase the affinity between drug and polymer, to promote the uniform dispersion of drug through the polymer matrix, or to alter the structure of the polymer matrix. Alternatively, some excipients may be used to coat particles containing encapsulated drug in order to act as an additional barrier to diffusion.

Kang *et al.* (2007) [76] showed that drug molecules can partition differently in PEG–PLGA blends to affect release properties, depending on the PEG:PLGA ratio. Using a Raman microscopy method, paclitaxel was shown to partition preferentially into either the PEG- or PLGA-rich phases of cast films. Burst release of the drug from the films after immersion in water varied depending on the partitioning behavior of the drug, being higher when occupying the water soluble PEG phase [76].

Excipients that are miscible with the polymer environment may be used to promote drug–polymer compatibility and/or to act as a plasticizers to reduce particle porosity, thereby reducing burst release. A novel biodegradable polymer

surfactant consisting of a polyethylene oxide mono-oleate-PLA copolymer was blended with PLA and found to reduce the burst release of irinotecan hydrochloride compared to burst from microparticles consisting of PLA alone [77]. However, the addition of a plasticizer may not always have this effect, since the addition of glycolide monomer, or glycolic acid-hydroxycarboxylic acid copolymers to the PLGA matrix acts as a porosigen which can lead to increased burst release [78]. A recent example of this strategy is the use of glycolide monomer in PLGA microparticles to control the release of gentamicin sulfate [79]. Furthermore, adding porosigenic excipients can qualitatively alter the release profile of a formulation. The addition of porosigens can eliminate burst release and shift drug release from microparticle formulations toward a zero-order profile. Coencapsulation of medium chain triglycerides with leuprolide using a methanol cosolvent method increased microparticle porosity without affecting the thermal properties of the polymer, allowing the linear release of the drug [80].

The addition of excipients that are not miscible with the polymer phase but partition with the drug during double emulsion processing, may also be used to reduce burst release. Encapsulation efficiency of the model protein drug BSA was improved and burst was reduced using a novel encapsulation method involving the suspension of protein in thermoreversible pluronic F127 gels prior to dispersion in a water miscible acetone – PLGA solution [81]. As with the addition of complexing agents, osmotic gradients can be generated by the addition of any excipient, with potential adverse effects on burst release. The addition of water soluble excipients can affect drug release in a concentration-dependent manner. Small amounts of glucose (0.2%) co-encapsulated along with octreotide by double emulsion decreased burst release of the peptide by affecting pore closing, while increasing glucose concentration in the inner water phase to 1% led to increased burst, presumably due to increased particle porosity resulting from osmotic effects [82].

In certain applications, for example, for lung delivery of microparticles, large, porous particles are desired in order to both allow delivery of particles to the deep lung and to prevent particle uptake by lung macrophages. To achieve the desired particle architecture, fabrication is performed using porosigens such as cyclodextrin derivatives [83], or poloxamers [84,85]. These water soluble compounds increase porosity by osmotic or leaching mechanisms. However, increased porosity correlates with high burst release. In order to reduce burst release from such systems, Lee *et al.* encapsulated positively charged hyaluronate–protein complexes that were present along with the porosigen in the internal water phase [83].

Several recent examples of particle coating strategies to reduce burst have been reported. One strategy used to reduce burst release from PLGA microparticles is to coat the microparticles with an additional layer of PLGA [86]. Coating microparticles with gelatin [87] or other polymers (e.g., pluronic F127 [88]) can reduce burst release by increasing the distance through which drugs must diffuse to exit the device. Burst reduced from PVA hydrogel containing water soluble drug, pentamidine, by encapsulating hydrogel particles with PLGA [89]. Inner water droplets were stabilized with alginate and calcium chloride during encapsulation of a model protein drug to reduce burst [90]. Burst release of baclofen was reduced from nanoparticles after suspension in pluronic F127 [91]. Burst release was eliminated in heparin-conjugated PLGA nanoparticles loaded with bFGF by suspending the particles in a fibrin gel [92]. Embedding TGF-β1-loaded PLGA microspheres in PEG-based biodegradable hydrogels reduced the burst release of the protein [93].

### 3.1.5 *Polymer modification*

As a complement to the modification of drug molecules, polymers may be modified to improve compatibility with the encapsulated drug. Miscibility between polymer and encapsulated biological molecules may be improved by modification with other polymer types, such as polyethylene oxide, dextran, lactone, or chitosan [94]. Amine modified polyesters were used to form complexes with insulin as part of a nanoparticle encapsulation strategy to improve the loading and release properties compared to a control formulation prepared using uncomplexed insulin [95]. Polymer modification can improve compatibility between drug and polymer. However, a potential drawback to using covalently modified polymers is the formation of microparticles with increased porosity and burst release. For example, nearly all of the dexamethasone or rapamycin was released within hours from nanoparticles composed of PEO–PEG block copolymers [87]. This effect may depend on the structure of the modified polymer. Sant *et al.* (2008) [35] showed that porosity and burst were increased in microparticles prepared from polymers consisting of PEG grafted to PLA as compared to the performance of a formulation prepared from a linear PEG–PLA block copolymer.

While increasing the miscibility between biological molecules and PLGA can result in decreased initial burst, drug–polymer interactions can adversely affect the drug. Peptides have been shown to be acylated by PLGA *in vitro* [96-98] and *in vivo* [99]. Covalent interaction between peptides and PLGA may result in loss of biological activity.

A review of factors affecting the mechanisms of burst release indicate that drug–polymer miscibility affects the distribution of drug within the microparticle, thereby affecting possible routes of escape during the burst phase of drug release. Numerous strategies to improve miscibility between drug and polymer have been described in the literature. The large number and variety of strategies that have been developed reflects the wide range of physical properties possessed by the large number of drugs in which injectable long-term release formulations are desired.

### 3.2 Microparticle processing

In many instances, microparticle structure, which has an important effect on burst, has been the unintended consequence of formulation processing strategies primarily designed to maximize drug encapsulation efficiency. Structural irregularities that increase microparticle porosity and lead to unacceptably high burst release are favored by rapid solvent removal and particle hardening [27,100]. Process conditions that reduce solvent removal rates may lead to lower porosity but drug loading efficiency can suffer. Formulation development using emulsion-based methods requires the adjustment of process variables to maximize loading and minimize burst. The following summarizes recent reports of strategies for optimization.

Several formulation variables (conditions related to the amounts and concentrations of microparticle components) affect particle structure and burst release. The adjustment of formulation variables to optimize drug loading and burst characteristics has been reviewed previously [3,4]. Varying the polymer concentration in the oil phase can affect drug distribution in the microparticle, thereby also affecting burst. Increasing the polymer concentration effectively reduces the droplet volume that is occupied by solvent. Viscosity during processing is increased, reducing the tendency of drug to diffuse out of nascent microparticles. In addition, the density of the polymer matrix is higher during the solvent removal and particle hardening process. Both of these effects can reduce the tendency of drug to be carried toward the particle surface by solvent convection [3], resulting in more uniform drug distribution within microparticles, and lower burst. A recent example of this strategy is the observation that burst release of melittin encapsulated in PLGA microparticles by double emulsion decreased with increasing initial polymer concentration [101]. Encapsulation efficiency increased and burst was reduced because of the higher initial viscosity, more rapid particle hardening and increased density/reduced porosity of the polymer matrix.

Altering process variables (conditions related to the treatment of formulation components during microparticle formulation, e.g., temperature, mixing energy, etc) in an attempt to optimize microparticle formulations has been shown to alter the structure of PLGA microparticles, which, in turn, can change the burst release of a formulation [26]. Many process variables have multiple effects, often making it difficult to predict the outcome of any particular adjustment. For example, an increase in temperature will increase mass transport rates but will also reduce viscosity and may reduce the capacity of an extraction liquid for the organic solvent. Mao *et al.* (2007) [53] used a systematic approach to test the effect of a comprehensive array of process variables on the internal morphology and burst release of a hydrophilic fluorescent model drug. They found that process changes that led to a more homogeneous dispersion of drug through the microparticle (e.g., by increasing homogenization speed to decrease the size of internal water droplets in the primary emulsion) led to increased encapsulation efficiency and reduced burst [53]. In a study of the effect of multiple process variable changes on burst in peptide microparticles prepared using a cosolvent method, Luan *et al.* (2006) [28] showed that a combined approach to control both parameters affecting polymer precipitation kinetics and osmotic pressure can be beneficial in reducing burst release while maintaining encapsulation efficiency. Importantly, burst release characteristics of scaled-up formulations were maintained when using optimized process parameters [28].

Because of the large number of formulation and process variables that could potentially interact to affect burst release, it is important to be able to clearly identify variables that interact to affect the performance of a formulation. A systematic approach is time-consuming, labor-intensive and may not identify interacting process variables. Statistical approaches have been taken to optimize process parameters, and have successfully identified some process variables that interact to affect burst release. Zaghloul *et al.* (2006) [102] used statistical design to evaluate the interaction of formulation variables (drug/polymer ratio, emulsifier concentration and deaggregating agent concentration) that affected the encapsulation efficiency, burst release and microparticle yield of estradiol encapsulated in PLGA using a single emulsion method. They found that each formulation variable affected the outcome individually, but were also able to identify interactions between formulation variables that had a detrimental effect on drug loading and burst release [102]. These studies provide evidence that formulation and process variables can interact in unexpected ways, and show the potential of the statistical design approach in optimizing microparticle formulations.

### 3.3 Reduction of drug diffusion into the solvent extraction liquid

Diffusion of drug out of the oil phase and into the solvent extraction liquid can lead to reduced encapsulation efficiency. Diffusion also leads to higher drug concentrations near the surface of microparticles, resulting in higher burst. This problem is especially prevalent for water soluble drugs encapsulated in PLGA using aqueous solvent extraction media. In these cases, burst may be reduced by substituting the outer continuous phase in the second o/w emulsion from water to a liquid that is a nonsolvent for both the polymer and drug. Liquid paraffin [103] and silicone oil [77,104] have been used successfully for this purpose. Drug loading and burst characteristics are improved because the non-aqueous solvent extraction liquid is a nonsolvent for the drug.

Encapsulation efficiency may be increased and burst release may be reduced by stabilizing the drug dispersion in the polymer phase in formulations prepared by double emulsion processes. Methods used to accomplish this goal include the use of increased mixing energy to more uniformly disperse the inner aqueous phase by

probe sonication [105] or high pressure homogenization [106]. Loss of encapsulated drug may be minimized by decreasing the size of the internal water droplets. Smaller internal voids may hypothetically increase pore tortuosity, reducing the potential for high burst.

The primary w/o emulsion may be stabilized using certain surfactants. In situations involving the dispersion of solid drug particles in the organic polymer phase, an analogous strategy to increasing the mixing energy during emulsification, is to minimize the size of the suspended drug particles. Like more conventional aqueous dispersion methods, smaller particle size, achieved by optimized protein spraying methods, led to improved drug loading and reductions in burst release [107,108]. Minimizing the size of internal particles in solid/oil/water protein encapsulation systems decreases the fraction of encapsulated material that has access to the microparticle surface [107].

### 3.4 Particle drying conditions

Late processing steps – particle isolation and drying – can also affect burst. Kim and Park (2004) [109] showed that burst release was higher in formulations that were freeze-dried compared to those that were vacuum dried or air dried at elevated temperature. The difference was explained as being due to the crystallization of water within internal voids in microparticles that led to increased porosity [109]. However, there is an alternative explanation for their observation that is related to polymer annealing. At the beginning of the drying process, microparticles contain substantial amounts of plasticizing residual water and organic solvent. At room temperature, where PLGA in freshly isolated microparticles is likely to be in the rubbery state, the polymer phase will continue the shrinking process, leading to an increase in polymer matrix density and a reduction in porosity. In contrast, microparticles dried at lower temperatures are less likely to possess sufficient mobility at a given polymer–solvent composition. As a result, microparticles dried at lower temperatures will not anneal to the same degree. Lower polymer matrix density and increased porosity are more likely to be maintained, favoring increased burst. Physical ageing of PLGA microparticles containing protein or DNA does occur during the freeze-drying process [110]. This explanation is consistent with the relative increase in free volume and porosity seen in PLGA freeze-dried at lower temperature conditions [111]. Higher porosity would be maintained in microparticles where polymer mobility is reduced by drying at lower temperatures, resulting in higher burst release.

Annealing PLGA microparticle formulations by heating particle suspensions to temperatures above the polymer $T_g$ is a patented strategy to reduce burst release [112]. Other methods have also been used to reduce burst from high porosity formulations by annealing. Treatment with plasticizers, for example a surfactant that is miscible with the polymer [55] or an organic solvent vapour [84], can also be used to seal the surface of microparticles, thereby reducing burst. As a further strategy to reduce burst, microparticle surfaces may be sealed by chemical crosslinking [113]. Process parameters adjusted to alter the drying kinetics or to seal the surfaces of microparticle formulations have in common the generation of diffusion barriers.

## 4. Conclusion

Recent advances in the understanding of burst release mechanisms have included refinements in the role of porosity and polymer mobility. The link between microparticle processing conditions, particle structure and burst is becoming more predictable with the increased application of statistical approaches to formulation development. The requirement for polymer motion in the release of drug molecules and in the formation of diffusion barriers in microparticles that is linked to the termination of the burst phase is becoming increasingly recognized as a key factor in controlling burst. Numerous strategies have been developed to improve the compatibility between PLGA and numerous drugs with a wide variety of physical and chemical properties, in order to minimize burst. Likewise, methods to control processing conditions in order to manipulate particle structure and the distribution of drug within microparticles are improving. Advances in these areas permit a more rational approach to microparticle formulation design, and will allow products to be brought to market more rapidly than in the past.

## 5. Expert opinion

Substantial gains have been made in recent years in the understanding the role of polymer molecular mobility in the mechanism of burst release from PLGA microparticles. One of the main problems in preventing the more widespread use of PLGA microparticle technology is the difficulty in creating robust manufacturing conditions to achieve reproducible burst release. Microparticles are formed by quenching processes where the formulation composition is initially dominated by organic solvent. Solvent removal results in volume reduction in the polymer phase, and the rate of solvent removal is one of the main factors responsible for the formation of microparticle structural features, such as internal channels or dense surface layers that have an impact on burst release. Processing conditions can be adjusted to alter microparticle structure and burst release, and *in situ* annealing of microparticles is an effective strategy to limit burst release.

It is very interesting to observe that the dynamic structural changes in microparticles that can lead to annealing and the formation of diffusion barriers in the polymer matrix during solvent removal resume after re-exposure of the microparticles to an aqueous environment. The annealing process involves the motion of polymer

molecules in plasticizing environments. Annealing is a response to reduce the excess structural energy in the microparticle polymer matrix resulting from free volume that was once occupied by solvent. Voids in the microparticle polymer matrix are filled and density increases.

This process continues to a more limited extent in the dried state between processing and release, as enthalpy is lost from microparticles stored in the glassy state. Of course, annealing of polymers in the glassy state depends on time and temperature. The rate of polymer ageing/annealing in the glassy state also depends on the amount of potential energy (free volume) present in the system [114]. This potential energy is substantial, given the differences in bulk density of typical PLGA microparticle formulations (0.3 – 0.7 g/cm$^3$) (S Dean Allison, unpublished observations) relative to that of the bulk polymer (~ 1.3 – 1.5 g/cm$^3$). Annealing during storage may therefore be one source of variability in burst release from otherwise replicate formulations.

Furthermore, rates of annealing in the dry state may be affected by process conditions via the initial free volume at each phase. Most process variables (e.g., temperature, volume ratios, mixing rates, etc) act on the overall solvent removal rate. Process variables can therefore interact to affect the amount of drug released in the burst, either directly, by affecting particle porosity, or indirectly, by affecting the potential energy available to drive annealing.

The hypothetical role of processing on particle structure and polymer molecular mobility as a source of burst variability has yet to be demonstrated. However, there is an increasing recognition of the link between microparticle porosity and density and burst, and that polymer mobility changes these characteristics. Increased understanding of the effect of interacting process variables on this relationship will allow a more rational approach to the design and control of burst release in microparticle formulations.

## Declaration of interest

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