REVIEW

Effect of Structural Relaxation on the Preparation and Drug Release Behavior of Poly(lactic-*co*-glycolic) acid Microparticle Drug Delivery Systems

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ABSTRACT: Control of burst release is a major challenge in the development of poly(lactide-*co*-glycolide) (PLGA) microparticle drug delivery systems. It has been well-documented in previous literature that formulation and processing variables determine particle morphology, which in turn, governs drug diffusivity and burst release. However, it is not generally appreciated that PLGA polymers used for microparticle systems are typically amorphous, and as such, undergo structural relaxation during processing and storage, characterized by enthalpy and volume reduction. Volume reduction due to structural relaxation can decrease drug diffusivity within microparticles and affect burst release. The magnitude of the driving force leading to structural relaxation is linked to the rate of particle hardening, and is affected by process parameters. Studies that directly address structural relaxation in PLGA microparticles indicate that the manufacturing process and residual solvent levels, as well as the nature of the interaction between drug and polymer affect the rate of structural relaxation. Therefore, the conditions chosen for particle fabrication may be a major source of variability in the burst release and may affect the stability of the drug release profile during storage. The potential effects of structural relaxation on drug release are likely to be formulation specific. Additional work is required to understand and control the relationship between microparticle processing, structural relaxation, and performance of PLGA microparticle drug delivery systems. J Pharm Sci 97:2022–2035, 2008

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INTRODUCTION

Drug delivery systems composed of biodegradable polymer microparticles promise improved safety and efficacy through sustained release. Amorphous copolymers of lactic and glycolic acid (PLGA) are often considered for these delivery systems, because of their proven safety record and programmable biodegradation rates that can range from a few days to many months. Several PLGA microparticle products are currently on the US market (Tab. 1). However, the number of products appears to be small compared to the intensive research into this technology that began in the 1970s.[1–3]

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Table 1. List of PLGA Microparticle Products Currently Approved for Use in the United States

Drug	Approval Date	Indication
Leuprolide acetate	1997	Prostate cancer
Octreotide	1998	GI symptoms associated with cancer, acromegaly
Minocycline HCl	2001	Periodontal disease
Risperidone	2003	Schizophrenia
Estradiol benzoate	2003	Cattle growth supplement
Naltrexone	2006	Alcohol dependence

A major reason for the limited success in product development over the years has been the difficulty in reproducibly manufacturing products with acceptable drug release profiles. Control over the release of drug occurring immediately upon exposure of microparticles to a release medium, that is, burst release, has been a major challenge. Excessive burst release can result in the acute exposure of patients to quantities of drug intended for months of treatment, with potentially harmful consequences. However, burst need not necessarily be eliminated. In some cases, burst release of a drug may be advantageous.[4] Nonetheless, the rate and extent of burst release must be reproducibly controlled. Even in the absence of toxicity, excessive or variable burst may adversely affect subsequent drug release. Excessive burst release may also have a negative economic impact on manufacturing, if higher quantities of active must be added to the formulation to ensure adequate delivery over the intended duration of the dose. The fact that control of burst release continues to be an impediment to product development indicates that common analytical methods do not adequately characterize the determinants of burst release.

Drugs may be incorporated into PLGA microparticles by a variety of techniques, including wet emulsification, coacervation, phase inversion, and spray drying. The methods used to form particles and remove solvent vary for each of these techniques. Wet emulsification methods have been most commonly used to encapsulate drugs and are specifically considered here, but the effects of processing (i.e., solvent removal/particle hardening) on particle structure and drug release performance are common to all methods. Techniques have been adapted to optimize microencapsulation of a wide variety of compounds, from hydrophobic small molecules[5] to large, water soluble proteins.[6] The encapsulation method determines how the drug is dispersed within the microparticle. The encapsulation method also determines the morphological features of the microparticle that govern drug diffusivity and burst release.

Process features common to all drug encapsulation techniques include dissolving or dispersing the drug into an organic solvent containing the polymer (oil phase), forming particles by dispersing the oil phase into a medium that is a nonsolvent for the polymer (often in the presence of an interfacial emulsion stabilizer), and removing the solvent to form hardened particles. The organic solvent, frequently dichloromethane or ethyl acetate, comprises most of the oil phase volume in order to decrease the viscosity to a range that will allow droplets to form in the desired size range. Solvent removal from the oil droplets may be achieved by extraction into the nonsolvent medium (usually water), by evaporation (e.g., spray drying), or a combination of the two processes (evaporation of a methylene chloride from a saturated solution in water). Solvent removal leads to gelation and precipitation of the polymer from solution. Viscosity of the polymer increases rapidly during gelation, trapping drug molecules within microparticles that were initially dissolved or dispersed in the oil phase. Further removal of the oil phase solvent and water from the gel state polymer phase leads to glass formation. Since many drugs tend to diffuse out of the oil phase and into the surrounding medium during processing, thereby reducing encapsulation efficiency, the time during which solvent removal, or quenching can take place to form a sufficiently viscous particle is critical, but can vary depending on the specific drug–polymer combination.

Glassy polymers in general, are nonequilibrium, supercooled liquids. They are characterized by excess structural energy compared to the equilibrium liquid state. The quantity of excess energy in quenched polymers is directly related to the quench rate. Faster quenching of a polymer

block from the molten state, for example, will produce a glassy block with a higher excess energy compared to the equilibrium liquid state, than would a slow quenching process. The excess structural energy is directly related to polymer density, with fast quenching producing lower density (higher energy) structures. Molecular mobility in the glassy state allows the glass to relax toward the equilibrium liquid state in a process referred to as structural relaxation (also called annealing or aging), with the rate of relaxation dependent on temperature and the amount of excess structural energy in the system. The relaxation process in thermally quenched polymers is characterized by enthalpy and volume reduction (density increase). Structural relaxation in glassy PLGA microparticles may therefore affect diffusion-mediated release of drug molecules by density-dependent reduction of drug diffusivity in the polymer matrix.

Structural relaxation analysis has been useful in understanding and predicting stability in amorphous dispersions intended to increase the bioavailability of low solubility drugs.[7–12] Analysis of structural relaxation in PLGA microparticle formulations during preparation and storage may provide valuable insight into the problem of controlling burst in sustained release drug delivery systems. However, comparatively few studies have directly addressed possible effects of structural relaxation on drug release performance in extended release PLGA microparticle preparations. The purpose of this article is to review the link between microparticle morphology and burst release, to describe how processing may result in microparticles with varying levels of excess structural energy and subsequently different rates of structural relaxation in PLGA, and to examine the effects of structural relaxation on drug release from PLGA microparticles.

PARTICLE MANUFACTURING, STRUCTURE AND BURST RELEASE

Burst release from PLGA microparticles may be defined as the uncontrolled release of drug that takes place prior to the onset of polymer degradation. Duration of the burst release can vary, and is characterized as an initial spike in drug release rate profiles, or as an initial steep slope in cumulative release profiles. In vitro tests are often designed to measure burst release within an experimentally convenient time frame.

The physical mechanisms of burst release from PLGA microparticles and the relationship of burst release to processing factors that affect drug encapsulation have been reviewed elsewhere.[4,6] However, the effects of processing on the physical properties of particles that affect burst release also have an important impact on the dynamic behavior of the amorphous polymer during manufacturing and storage of the microparticle product. The dynamic behavior of the amorphous material is caused by the thermodynamic departure of the system from equilibrium, and can further affect particle structure and drug release performance.

Burst release from PLGA microparticles has been attributed to the dissolution of surface-associated drug, although burst has also been demonstrated in formulations where drug was exclusively present inside the microparticles.[13] Burst release may be attributed to an increased concentration of drug near the surface of the particle resulting from convective solvent flow during processing.[4] However, burst release has also been shown for particles containing a uniform distribution of drug.[14] Therefore we may conclude that both surface-associated and internally located drug contribute to burst release.[6,15]

Burst release is a diffusion mediated event.[16] The rate and degree of drug diffusion from microparticles that characterizes burst release is affected by intrinsic factors like drug molecular weight, particle size, and the partition coefficient of the drug between the polymer and release medium environments.[6,17–20] Diffusion also depends on the mode of drug dispersion within the particle, which is determined by solubility of the drug in the organic solvents commonly used for microparticle fabrication, such as methylene chloride or ethyl acetate. Small, hydrophobic drugs like steroids are typically soluble in these organic solvents and are distributed in microparticles as molecular dispersions within the amorphous polymer matrix. For this broad category of drug, the affinity of the drug for the polymer may affect release to varying degrees. For example, progesterone may be encapsulated as a molecular dispersion in PLGA, but has little affinity for the polymer, since it does not affect polymer properties, and tends to crystallize over time.[21,22] On the other hand, peptides, such as salmon calcitonin,[23,24] neurotensin analog,[25] and leuprolide,[26] are encapsulated using single-phase cosolvent techniques. Each of these peptides has a positive affinity for PLGA, as characterized by the

induction of concentration-dependent changes in polymer thermal properties. The second category of drugs encapsulated in PLGA consists of large, hydrophilic molecules, such as proteins or polynucleotides. These molecules are not soluble in the organic solvents typically used for microparticle fabrication. Encapsulation of these compounds usually requires dispersing the drug as an aqueous solution or in solid form into the polymer solution prior to droplet formation. In this case, drug molecules reside on the surface of internal voids within microparticles, rather than being dispersed through the polymer phase.[6,27–29]

Burst release of these two main types of drugs depends on the regulation of diffusivity by physical barriers with different dimensions within microparticles; the polymer matrix density and the particle porosity. The polymer matrix density is related to the nanometer-scale distance between polymer chains within the solid matrix.[6,30] Porosity within the particle is characterized by voids and channels through the solid polymer matrix that are often visible in micrographs.[6,18,31] The effect of these microparticle characteristics on burst release depends on the molecular weight of the drug and its disposition within the particle. Diffusion of drugs that are homogeneously dispersed in the amorphous polymer phase of microparticles is restricted by the density of the polymer matrix.[32] Variation in the density of the polymer matrix therefore affects the diffusivity of drugs dispersed in this manner, increasing or reducing burst release. Porosity serves to increase the surface area of the microparticle in this case, which itself can contribute to burst.[18,19] Burst release of high M_w compounds depends on microparticle porosity. Their disposition within internal voids, large size, and hydrophilic nature prevent diffusion through the polymer matrix, irrespective of matrix density.[6,18] Instead, the number and diameter of pores within the microparticle controls drug diffusion.[6,18,19]

Polymer matrix density and porosity in microparticles are determined by a number of formulation and processing variables. Because the bulk of the oil phase volume consists of organic solvent, removal of the solvent leads to decreasing particle volume.[33] At the same time, solvent loss also leads to a composition-dependent gelation and eventual vitrification of the polymer.[31] During solvent removal, particle structure is determined in part by the extent to which oil droplets shrink prior to hardening. Under slow solvent removal conditions, nascent particles can shrink completely as hardening takes place to form microparticles with a uniform, maximally dense polymer matrix structure.[33] Increasing the solvent removal rate will lead to particle hardening before complete shrinkage can occur, leading to a decrease in polymer matrix density. Very rapid solvent removal can result in localized polymer hardening at the surface of the particle to form a dense exterior shell with a less dense, sometimes hollow interior.[23,24]

Other factors related to formulation and processing can lead to pore and channel formation. Porosity may be introduced into the microparticle by the partial fusion of internal water droplets during processing with water-oil-water double emulsion techniques.[34,35] In addition, porosity may be caused by the efflux of oil phase cosolvents that are miscible with the extraction medium, such as alcohols or dimethyl sulfoxide.[17,23,25,36] Osmotic effects may lead to influx of aqueous extraction medium during processing, producing channels that connect internal regions of the particle with the surface.[15,24,37]

Shell and pore formation add complexity to the relationship between processing and microparticle structure as it pertains to burst release.[24,31] Changing processing variables, such as increasing the volume of extraction liquid relative to the oil phase volume, is expected to reduce the polymer matrix density by hardening a solvent-swollen oil droplet and result in increased burst release. In practice, this outcome may be realized if the particle hardening rate is adjusted to some value within a narrow range. However, if the extraction volume is increased too far, solvent will be removed from the surface of the oil droplet at a rate that exceeds the diffusion of solvent from the inside of the droplet to the surface. As a result, polymer hardening will occur at the surface of the particle, leading to the formation of a shell with a density high enough to prevent drug diffusion. The net result would be a decrease in observed burst release.[24] This situation may be further complicated by formulation conditions that induce pore formation, such as the presence of a water soluble drug, or water miscible cosolvent in the oil phase.[6,24,35] Processing conditions may also affect shell formation and porosity. For example, when temperature was used to facilitate solvent removal, the rate at which temperature was increased affected both the shell thickness and porosity of microparticles.[23,24,38] Thus, while the particular combination of matrix density and porosity within a microparticle formulation that leads to a

particular rate and extent of burst release may be difficult to predict, they are important determinants of drug diffusivity and burst release.

The phenomenological evidence presented above indicates that microparticle processing methods affect burst release by changing matrix density and porosity. Polymer matrix density and porosity combine to determine the specific volume of the microparticle. Density is also a dynamic property of amorphous materials that is affected by structural relaxation.

DETERMINANTS OF PLGA MOLECULAR MOBILITY

Dielectric studies of amorphous and semicrystalline poly(D,L-lactic acid) show that the polymer exhibits three modes of molecular motion. The alpha relaxation normal mode related to viscous flow is observed in PLGA at temperatures greater than the nominal T_g.[39] Molecular motion leading to the glass transition is assigned to the segmental mode of the alpha relaxation, as determined by NMR,[40] and dielectric spectroscopy.[39,41] The activation energy determined by dielectric spectroscopy for the alpha relaxation in polylactide, polyglycolide, and a 1:1 copolymer of lactide and glycolide is approximately 300 kJ/mol.[42] At temperatures far below T_g, the beta relaxation is observed as a result of low amplitude twisting motions of the polymer backbone.[40] Mobility may increase with increasing copolymer content, especially if the distribution of the more flexible glycolide monomers along the polymer chain tends to be nonrandom.[43]

In dilute solution, PLGA mobility is independent of concentration, but as concentration increases, that is, during solvent removal, polymer mobility decreases in response to two types of polymer chain interactions. First, mobility is impeded by friction between polymer chains.[30,44] The magnitude of the frictional effect increases with polymer molecular weight.[43] Second, molecular mobility decreases due to the formation of molecular entanglements. Viscoelastic behavior in PLGA is observed above a critical entanglement molecular weight of approximately 7000.[39,45] Frictional and entanglement effects are therefore partly responsible for the decrease in molecular mobility observed with increasing polymer molecular weight.

Segmental molecular mobility that gives rise to structural relaxation also depends on the polydispersity of the polymer.[36,45–47] PLGA polymers (50% lactide) treated by ultrafiltration to remove low molecular weight species decreased polydispersity and increased the average molecular weight of the material. Dielectric analysis of the polymer films formed from the filtered material showed a substantial decrease in mobility compared to that seen in the starting material. Thermal analysis showed an increase in T_g from 14 to 27°C, as well as increases in the thermal expansion coefficient and modulus at 37°C for the filtered polymer.[47] PLGA properties such as molecular weight, copolymer composition, and polydispersity, that are manipulated in PLGA polymers to achieve the desired biodegradation rate, are also properties that affect polymer structural relaxation. These polymer characteristics vary in different polymer preparations, and may contribute to structural relaxation-mediated effects on formulation stability.

Residual solvents and plasticizers increase polymer mobility by increasing the free volume surrounding polymer chains in a way similar to the presence of low molecular weight polymer species mentioned above.[43] The equilibrium solubility of water in PLGA (50% D,L-lactide, M_w 30,000, acid end group) is 2.6% (w/w), with a consequent decrease in T_g of 15°C.[48] Water solubility in PLGA is expected to vary depending on copolymer composition, end group treatment, and molecular weight.[49] Plasticizers, such as emulsion stabilizers that are partially miscible with the polymer also decrease T_g. The reduction in T_g can result in increased molecular mobility from density-restricted segmental motion to normal mode mobility related to viscous flow. Molecular mobility in PLGA may lead to structural changes, such as the closure of microscopic pores on the surface of particles that can affect burst release during aging. Microspheres prepared with a plasticizing emulsifier and aged under controlled humidity conditions for 2 weeks at room temperature led to the closure of surface pores and a reduction in burst release.[50] In addition, film formation at the surface of PLGA microspheres exposed to aqueous media led to surface pore closure and decreased drug release rate.[51] In both cases, mobility of PLGA at the particle surface was increased by water, or a combination of water and a plasticizing surfactant, leading to enhanced mobility that resulted in reduced surface porosity.

In addition to polymer characteristics and plasticizers, molecular mobility can vary in PLGA matrices as a result of variation in preparation

conditions that alter the density of the polymer matrix. Sasaki et al.,[30] manipulated freeze-drying parameters to examine the effect of polymer chain entanglement on PLGA molecular mobility. PLGA samples were freeze-dried from dioxane solution at initial concentrations that were substantially above or below the critical chain entanglement concentration. The samples were either rapidly frozen by adding the solution to liquid nitrogen, or slowly frozen on a −26°C shelf. Rapid freezing of very low concentration solutions formed hardened polymer blocks with minimal coalescence of polymer chains during processing. These samples had a lower glass transition temperature and broader glass transitions than did samples prepared by slow freezing or high initial polymer concentration. The decrease in T_g observed for the low concentration, rapidly frozen material was interpreted as an increase in mobility resulting from the formation of polymer matrices with lower density. The increased width of the transition seen in low concentration samples was interpreted as a wider distribution of mobility states. Furthermore, since T_g decreased progressively as freezing and drying temperatures were lowered during processing, it was concluded that relaxation toward the bulk polymer structure occurs to some extent during freeze-drying.[30]

POLYMER QUENCHING AND STRUCTURAL RELAXATION

Bulk PLGA polymers typically used for microparticle formulations have nominal T_gs in the range of approximately 25–50°C, depending on molecular weight and lactide:glycolide ratio. Microparticle formulations are often in the glassy state at temperatures encountered during processing, storage, and use. PLGA quenching by solvent removal is analogous to the thermal quenching of a pure amorphous polymer from the molten state.[43] The molten polymer has a temperature dependent relaxation time that allows the system to maintain equilibrium with changing temperature as described by the thermodynamic state functions, that is, entropy, enthalpy, and volume (Fig. 1, line AG). As the molten sample is cooled to temperatures near the glass transition, mobility decreases and the relaxation time of the polymer lengthens. At the glass transition, the temperature-dependent mobility decreases below a level that allows the equilibrium to be maintained with a further decrease in temperature. The liquid becomes kinetically trapped with a structural energy that is in excess of the equilibrium liquid value (compare the temperature dependent structural energy of the glass in line BC with the structural energy of the liquid line extrapolated below the glass transition, line BG in Fig. 1).

An important aspect of the thermal quenching process is that the amount of excess energy stored in the glassy polymer depends on the cooling rate. Rapid cooling leads to a faster reduction in the polymer relaxation time, with a higher energy structure trapped in the polymer. Slower cooling rates allow the system to maintain equilibrium for a longer time through the glass transition region, by progressing further along the equilibrium liquid line prior to vitrification. In this case, the system attains a lower energy structure prior to glass formation. Adjustment of the kinetics of thermal quenching therefore allows formation of glassy structures with different structural energies that can be measured calorimetrically.

While the classic example involves the change in mobility of a pure, amorphous polymer with decreasing temperature, PLGA glass formation by solvent quenching may be described by composition dependent mobility, as would be described by Figure 1 if temperature in the x-axis were replaced by solvent fraction. By analogy, solvent quenching (as opposed to thermal quenching) will lead to the formation of microparticles with varying levels of excess structural energy, depending on the solvent removal rate. Furthermore, because the bulk of the oil droplet volume consists of solvent, the potential exists to generate glassy polymer matrices with very high levels of excess structural energy. Spray drying of dilute polymer solutions (where solvent may be extracted within seconds) is an example of a process that can lead to microparticles with very high structural energy, whereas slow extraction of solvent from a high concentration polymer solution would be expected to be more fully annealed during processing, and thus have a much lower structural energy.

The thermodynamic and structural properties of glassy PLGA microparticles will relax toward the equilibrium liquid state as a result of molecular mobility within the system. The process is referred to equivalently as physical aging, annealing, or structural relaxation. A number of classic[52,53] and more recent[54–56] reviews have been published on the dynamics of structural relaxation, and the reader is referred to these

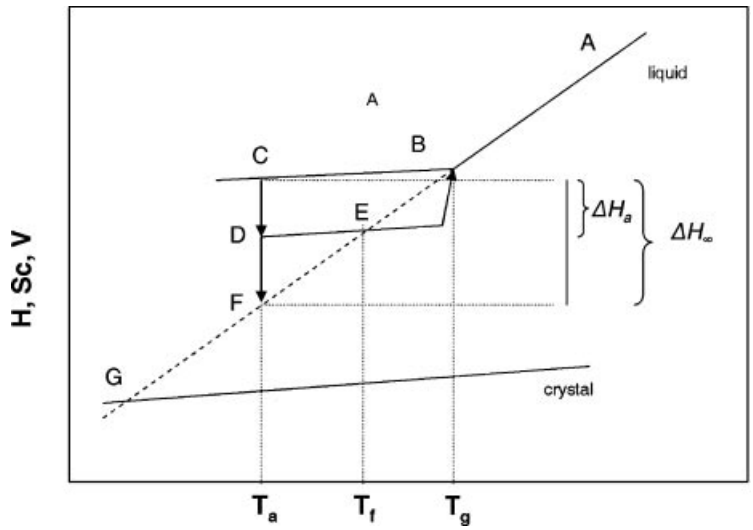

Figure 1. Schematic representation of excess structural energy, measurable as configurational entropy, enthalpy, or volume versus temperature in a glass-forming liquid.

works for more detailed explanations of the process.

The driving forces for structural relaxation are temperature and the excess structural energy of the material.[56] At constant temperature, the rate at which the system relaxes toward equilibrium depends in a nonlinear way on the excess structural energy imparted to the system during quenching. Lower density structures have a higher free volume surrounding polymer chains, so there is relatively little resistance to relaxation. However, as density increases during aging, an increased number of polymer units must move in concert to relax, reducing mobility which decreases the rate of progression toward equilibrium.[52] Thus, structural relaxation rate depends on the extent to which the PLGA matrix has relaxed toward the equilibrium state.

Hypothetically, formulations prepared under conditions that favor a high degree of annealing during processing may be expected to undergo little structural relaxation during the lifetime of the finished product because of the low potential created by the relatively small amount of excess structural energy in the system. Burst release of drug from a highly annealed microparticle formulation would be expected to remain at a consistent level in samples taken from the batch over time. However, in many instances, formulations are prepared under conditions designed to rapidly harden particles in order to maximize encapsulation efficiency. These rapidly quenched preparations are characterized by increased excess structural energy and a corresponding high potential to undergo extensive structural relaxation when exposed to favorable temperature conditions (i.e., temperatures close to but still below T_g). Because structural relaxation is characterized by an increase in density, rapidly quenched preparations would therefore be more susceptible to a decrease in burst release rates during storage. In this way, slight differences in excess structural energy imparted to replicate formulations by fluctuations in processing conditions may lead (through structural relaxation) to the unacceptable variability in burst release rates that have impeded the development of PLGA microparticle products. This hypothesis has not been adequately addressed in the literature.

QUANTIFYING STRUCTURAL RELAXATION IN PLGA

Structural relaxation is characterized by enthalpy reduction. Differential scanning calorimetry is commonly used to analyze this process. As an aged sample is heated through the glass transition (line DEB in Fig. 1) the enthalpy lost as a result of structural relaxation is recovered as an endothermic event superimposed on the heat capacity increase due to the glass transition. Average molecular mobility, expressed as a relaxation correlation time, τ, which represents the most probable value of the relaxation time, can be determined by application of the empirical Kohlrausch Williams

Watts (KWW) kinetic model to measurements of relaxation enthalpy

$$\phi(t_a) = \exp\left(\left(-\frac{t_a}{\tau}\right)^{\beta}\right) \quad (1)$$

where $\phi(t_a)$ being the relaxation function, with $(1-\phi(t_a))$ being the extent of aging relative to the equilibrium state, t_a is the aging time, and β is a parameter that takes a value from 0 to 1.[54] The average relaxation time and β parameters are determined from enthalpy relaxation data by regression analysis. The value of β has no formal physical meaning in the KWW model, but has been taken to indicate the distribution of individual relaxation processes, or the degree of cooperativity.[57] Values of β closer to 0 indicate a relatively large number of individual processes or a high degree of cooperativity in the relaxation process.[58]

The extent of aging for enthalpy relaxation is given by the Cowie–Ferguson equation,[59]

$$\frac{\Delta H(t_a)}{\Delta H_\infty} = 1 - \phi(t_a) \quad (2)$$

where the extent of aging is equal to the ratio of the relaxation enthalpy at the aging time to the maximum relaxation enthalpy, ΔH_∞ (the ratio of CD/CF in Fig. 1). The maximum relaxation enthalpy may be taken from experimental data as the asymptotic enthalpy relaxation value actually observed after long aging times, or estimated by[8]

$$\Delta H_\infty = (T_g - T_a)\Delta C_p \quad (3)$$

Molecular mobility measured from below T_g by enthalpy relaxation using the KWW model has been shown to correlate well with mobility measured from above T_g by dielectric relaxation spectroscopy,[60] supporting the validity of the empirical model.

While use of the empirical stretched exponential kinetic model produces relaxation times that extrapolate to values commonly accepted for T_g, experimentally determined KWW τ values represent an average value for the relaxation process that is skewed. Relaxation times increase (potentially by orders of magnitude) during the course of the aging experiment, resulting in measurements of τ that are too large and β that are too small.[61] The experimentally determined average relaxation time could then depend on the annealing time.[62]

Annealing in samples during the course of experiments undertaken to determine τ and β values adds uncertainty to the significance of the apparent difference in molecular mobility between two samples given by the KWW parameters. The relaxation enthalpy of molded semicrystalline poly(L-lactide), M_n 69,000 g mol^{-1}, with a nominal T_g between 50 and 55°C was measured by differential scanning calorimetry at a scan rate of 10°C/min during aging at 37°C. As expected, the rate of enthalpy relaxation slowed with aging time. The KWW relaxation parameters measured for this sample were $\tau = 1600$ min and $\beta = 0.37$.[63] In another sample of semicrystalline PLA, the KWW β value was 0.35 ± 0.006.[64,65] In semicrystalline polymers, populations within a sample are known to exist that differ with respect to their association with rigid crystalline spherulites.[41] The presence of polymer chains within the sample with differing degrees of molecular mobility are characterized by KWW β values substantially less than 1, as expected. However, the KWW β value found for PLGA samples that were amorphous, as opposed to semicrystalline, and in addition, were not annealed prior to analysis was 0.41 ± 0.01.[41,65] For the amorphous PLGA samples, there was no reason to expect any particular β value. However, because of the experimental conditions, that required aging to determine the KWW parameters, the amorphous, un-annealed properties of the polymer samples would be likely to change dramatically during the course of the analysis. As a result, no real significance can be placed on the apparent close agreement between β values for samples with widely different physical properties and thermal histories.

Measurement of bulk properties (i.e., enthalpy) in microparticles in order to analyze structural relaxation may overlook relaxation processes that could conceivably take place at different rates in different regions of the microparticle. For example, density differences between the particle surface and the interior (which are commonly seen in PLGA microparticles) could lead to different rates of structural relaxation in the two regions. Structural relaxation analysis by thermal and mechanical methods using KWW kinetic modeling has been shown to generate different values for τ and β. For the same M_n 69,000 PLA blocks described in the preceding paragraph, mechanical analysis of structural relaxation by microhardness testing returned a twofold longer relaxation time and a lower β parameter value of 0.28 compared to 0.37.[63] Two explanations were proposed to account for the discrepancy between the two sets of relaxation parameters. First, it

may have been possible that the mechanical indentation method was sampling a surface-specific environment that differed from the bulk sample. Second, the relaxation of mechanical properties may actually have been slower than enthalpy during aging.[63] The large differences in τ and β determined by the two methods are likely to be significant, given that the thermal history of the sample was the same during the experiment. However, no corroborating evidence (micrographs showing increased density at the surface of the polymer sample, for instance) was presented. It seems reasonable to expect structural relaxation in PLGA microparticles to proceed at different rates at internal compared to external regions, given the variation in density observable in cross sections. More research is required to demonstrate possible regional differences in structural relaxation in PLGA microparticle systems.

An accurate measure of the characteristic relaxation time and distribution of subpopulations with differing mobility within the sample is required to understand and predict the causes and effects of structural relaxation.[66] Higher resolution analytical methods need to be applied to structural relaxation in PLGA microparticles in order to allow real differences to be resolved between samples. In order to validate the resolving power of calorimetric or mechanical measurements of structural relaxation, these measurements would be related to changes in tangible physical properties of the microparticles, such as density and porosity. An alternative approach using Vogel Tamman Fulcher kinetics may be taken to evaluate the time dependence of τ.[67–70] This method provides a "snapshot" of τ at any given time during annealing, and may allow higher resolution of differences in structural relaxation between samples. However, this type of analysis has not been applied to the study of structural relaxation in PLGA microparticle formulations.

STRUCTURAL RELAXATION IN PLGA MICROPARTICLE FORMULATIONS

Much of the preceding discussion has focused on a tendency for processing methods to form microparticles with low density polymer matrices as a result of the need to maximize drug encapsulation efficiency. Such low density formulations may be more likely to suffer increased degrees of structural relaxation and subsequent effects on drug release. However, processing can also create particles with decreased rates of structural relaxation relative to the rate of structural relaxation in the raw polymer prior to fabrication. For example, processing methods that use organic solvents to extract the polymer solvent and may be stronger plasticizers for PLGA than water may form particles with a low potential to undergo structural relaxation. PLGA nanoparticles were prepared by a phase inversion method, in which particles are formed spontaneously as the polymer solvent is rapidly displaced by a liquid which is a nonsolvent for the polymer, but is miscible with the polymer solvent.[71] Enthalpy relaxation studies on the nanoparticles during storage at multiple temperatures below T_g using the Cowie–Ferguson KWW method showed approximately threefold longer average relaxation times compared to bulk polymer, with little difference observed between the two samples in β.[11] One possible explanation for the reduction in molecular mobility may be restriction of PLGA molecular mobility at the surface of particles magnified by the higher surface to volume ratio of the nanometer scale particles. The effects of the processing method and aging on drug release were not studied. These results combined with those of the freeze-dried PLGA described above, suggest that reorganization of polymer chains from the bulk raw material into hardened particles by different processing methods can lead to particle formulations with higher or lower molecular mobility than the bulk polymer.

In addition to processing, affinity between drug and polymer may affect structural relaxation in PLGA microparticles. For example, encapsulation of a neurotensin analog peptide into poly(D,L-lactic acid) $M_w = 2000$ by a single emulsion process showed an antiplasticizing effect of the peptide on the polymer. A single glass transition that increased in temperature with increasing peptide content was observed for the product, indicating complex formation between drug in the polymer.[25] The energy of activation for the glass transition was determined calorimetrically by the dependence of the glass transition temperature on the thermal scan rate[72] and found to be higher for the drug loaded microspheres. This result suggests that attractive interactions between peptide and polymer restrict mobility in the amorphous phase, retarding the structural relaxation process.

Like the neurotensin analog peptide, other classes of drugs, such as steroids, can form molecular dispersions when encapsulated in PLGA

microparticles. But unlike that peptide, steroids do not influence the relaxation behavior of the polymer when the encapsulated steroid is in the amorphous state. Encapsulation of progesterone in poly(D,L-lactic acid) or two different PLGA polymers (60% D,L-lactide, or 85% D,L-lactide, M_w 70,000), at levels less than approximately 30% by weight, resulted in the formation of a metastable dispersion of amorphous drug in the polymer. Unlike the higher affinity neurotensin analog—PLA interaction, progesterone does not appear to interact with the polymer.[21,22,73] Amorphous progesterone had no effect on the rate of structural relaxation in microparticles. However, the formation of crystalline progesterone by annealing above T_g led to the slowing of structural relaxation in microspheres.[73] This observation is consistent with other examples of reduced mobility in PLGA resulting from interaction between the polymer and rigid surfaces like PLA polymer crystals in semicrystalline PLA.[41] Reduced polymer mobility is also seen more generally in confined polymers.[74]

An excellent example of the influence of the physical state of the encapsulated drug on structural relaxation was published by Rosilio et al.[73] Aging in the progesterone microparticles was characterized by changes in T_g that depended on the annealing temperature. Samples containing amorphous progesterone aged at 4°C (approximately 40°C below the nominal T_g of the formulation) showed an initial rapid reduction in T_g of approximately 10°C, followed by a return over several days to the value measured immediately after microsphere preparation. The time course of T_g change was much faster (on the order of hours) when samples were stored at 20°C. The effect was not observed in samples containing crystalline progesterone.[73]

In that study, the initial decrease in T_g seen early in the aging process may have been due to reduction in structural energy from a relatively high initial state that allowed the system to migrate along the equilibrium liquid line toward a lower energy equilibrium state (BE in Fig. 1). The initial decrease in T_g may be explained in terms of the fictive temperature (T_f) of the system. T_f is defined as the temperature at which an equilibrium glass has the same energy as the real glass at a given temperature, and can therefore be used to estimate the excess energy of a glassy system during aging.[62,68,75] In the liquid state (i.e., along line BE), the fictive temperature is equal to the temperature of the sample. As the liquid is cooled through the glass transition, T_f is equal to T_g. For samples measured immediately after quenching, T_g and T_f are indicative of the level of excess energy trapped in the glass, which is represents the maximum for that sample.[62] As a consequence, T_g measured immediately after quenching is the highest expected value for that sample. During aging at a temperature below T_g (e.g., T_a in Fig. 1), T_f will decrease toward T_a as energy is lost from the system. T_f is both temperature and time dependent. Shortly after quenching (particularly in cases where the sample was rapidly quenched to trap very high levels of excess energy in the glass), when the density of the glass is lowest and mobility is high (small τ), energy loss may occur via mechanical rearrangement of polymer chains. The glassy system may therefore attain a lower equilibrium energy state, essentially lengthening the equilibrium liquid line AB toward point G in Figure 1. As a result, both T_f and T_g decrease. Later in the aging process, as τ increases, reduced mobility in the system prevents larger mechanical rearrangement of polymer chains and T_f and enthalpy decrease. As T_f decreases toward T_a, or alternatively, as enthalpy decreases from point C toward point F in Figure 1, additional thermal energy must be added to the system during the thermal scan to overcome the energy lost during aging, and T_g increases in proportion to the difference between the enthalpy of the aged sample and the equilibrium enthalpy at the storage temperature (CD/CF in Fig. 1).[76] The magnitude and time course of the changes in T_g were reduced as storage temperatures increased toward T_g because increased molecular mobility allowed faster equilibration of the system. It was also observed that the degree to which T_g changed depended on the degree of progesterone crystallinity. Formulations that contained a higher percentage of crystalline drug experienced a smaller change in T_g during aging. Thus, encapsulated drug in the crystal state reduces the rate of structural relaxation in PLGA microparticles.

While processing methods and the interactions between the encapsulated drug and polymer affect structural relaxation, finished formulations contain varying amounts of residual processing solvents and emulsion stabilizers that can also affect structural relaxation. The combined effects of humidity and plasticization by emulsion stabilizers on aging and protein release from PLGA microspheres was tested by Bouissou et al.[50] Formulations prepared with poly(vinyl alcohol)

(PVA) or Triton X100 had antiplasticizing and plasticizing effects on the mechanical stability of the respective microparticles. The plasticizing effect of Triton X100 was demonstrated by a reduction and broadening of the glass transition of the microparticles prepared with this emulsion stabilizer. Water uptake at 75% relative humidity was facilitated in formulations containing Triton X100 but not in formulations prepared with PVA. Storage of the microparticles prepared with Triton X100 for 24 h at ambient humidity near the T_g of the formulation resulted in an increase in T_g with the appearance of a small relaxation enthalpy endotherm (similar to the change in T_g observed for microparticles containing amorphous progesterone, described above). Under these conditions, burst release was unchanged compared to the control formulation prepared without any emulsion stabilizer. However, storage of the sample prepared with Triton X100 at high humidity for 24 h resulted in a dramatic reduction in burst release from 85 to 30%. The increase in T_g was not as large as that observed for the ambient storage condition, suggesting that structural relaxation occurred to a lesser degree at high humidity. Analysis of microparticle surfaces by atomic force microscopy showed evidence of pore closure after high humidity incubation that was not observed at ambient humidity. It was concluded that the combined plasticizing effects of the surfactant and water enabled large molecular rearrangements akin to viscous flow which resulted in microparticle surface remodeling.[50] These elegant studies connected a reduction in microparticle surface porosity and subsequent reduction in drug release with large scale rearrangements of PLGA due to the effects of plasticization.

While plasticization allowed larger scale motion in the polymer to close surface pores and dramatically reduce burst release, aging in the absence of particle surface reorganization was also accompanied by a reduction in burst release. Unlike microparticles prepared with Triton X100, T_g of the formulation prepared with PVA was comparable to that measured for a formulation prepared without any emulsion stabilizer. The glass transition temperature was higher in both the PVA formulations and the control formulation prepared without emulsifier compared to formulations prepared with Triton X100, which resulted in the aging experiment being conducted approximately 10°C below T_g (i.e., in the glassy state). A higher degree of enthalpy relaxation was observed in the control and PVA formulations compared to the Triton X100 formulation after storage at both ambient and high relative humidity. No change in the surface of either formulation was observed by atomic force microscopy. Nonetheless, compared to the formulation prepared without emulsifier and stored at ambient humidity, burst release was reduced from 85 to approximately 70% after aging at high relative humidity in the sample prepared without emulsifier. Comparative reductions in burst release were also observed in the formulation containing PVA for both storage conditions.[50] Thus, even in the absence of apparent morphological change, structural relaxation in the glassy state led to a decrease in burst release from PLGA microparticles. Taken together, these results show that different modes of molecular mobility can affect microparticle characteristics that influence drug diffusion. Viscous flow of PLGA in the rubbery state can reduce micron-scale porosity, and dramatically reduce drug release. In addition, segmental molecular mobility in the glassy state can significantly decrease burst release in the absence of observable changes to the surface porosity of PLGA microparticles.

In addition to affecting burst release, structural relaxation may affect later phases of drug release mediated by polymer degradation. Biodegradation of PLGA may occur via hydrolytic or enzymatic mechanisms. It is generally not recognized that both processes may be affected by structural relaxation. Biodegradation of lactide polymers can be affected by structural relaxation. In amorphous samples, the rate of enzymatic degradation of poly(D,L-lactide) decreased after aging the sample at a temperature approximately 35°C below T_g.[77] A decrease in the rate of enzymatic degradation of PLA by proteinase K was also observed for polymer blocks where the polymer chain ordering was increased by mechanical stretching. Reduced degradation rates were seen in the ordered polymer sample despite the formation of voids in the polymer matrix caused by the stretching process that may have increased exposure of the polymer to the aqueous medium.[77] Reduced enzyme access to polymer chains may be explained by a reduction of free volume caused by aging or by stretching. These observations are consistent with increased rates of hydrolytic degradation seen in lower density in PLGA microparticles.[38] Together, these results imply that structural relaxation occurring at temperatures substantially below T_g may affect the release performance of PLGA microparticle formulations during the polymer erosion-mediated release phase as well as the burst.

CONCLUSIONS

Structural relaxation in amorphous PLGA microparticles can decrease burst release of drug molecules by increasing particle density and reducing porosity. The rate and extent of structural relaxation, and thus, the magnitude of the effect on drug release, that can take place in a particular formulation depends on many factors. Polymer properties (e.g., molecular weight), fabrication methods, drug–polymer interactions, residual solvents, and storage conditions, all contribute to structural relaxation in PLGA microparticles. These factors may be responsible for the variability in burst release that impedes the development of products using this drug delivery technology. These issues must be understood in order to manufacture formulations with reproducible drug release profiles, and thereby more rationally exploit the benefits of sustained release. Structural relaxation analysis may allow an increased understanding of the relationship between these factors and their effect on the dynamics of PLGA microparticle formulations, and may allow prediction of stability for a given formulation. However, the validity of the analytical methods used to evaluate structural relaxation specifically in PLGA microparticles has yet to be determined. Certainly more work is required to understand this largely overlooked cause of variability in drug release.

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