Phase inversion dynamics of PLGA solutions related to drug delivery

P.D. Graham, K.J. Brodbeck, A.J. McHugh*

Department of Chemical Engineering, University of Illinois, 600 S. Mathews, Urbana, IL 61801, USA

Accepted 31 August 1998

Abstract

Dark ground optical microscopy, electron microscopy, and high performance liquid chromatography (HPLC) have been used to quantify the effects of formulation changes on the phase inversion dynamics and in vitro drug release properties of a PLGA-based drug delivery system. Gel growth rates and water influx rates are determined from plots of the square of the respective front motion with time. Results show that additives that accelerate the solution gelation rate at constant morphology result in high initial release rates. Conversely, additives that slow the rate of gelation dramatically reduce the initial drug release rate and lead to a more dense sponge-like morphology. Moreover, the phase inversion dynamics and morphology are the same regardless of whether the solutions are quenched with water, a PBS buffer solution or horse serum.

Keywords: Phase inversion; Drug release; Morphology; Diffusion kinetics

1. Introduction

The recent development of several protein based-drug therapies has spurred renewed interest in the design of effective drug delivery devices [1]. The performance of these devices is evaluated primarily in terms of their release kinetics and overall ease of administration. Devices that release drug with zero-order kinetics (a time-independent rate) for an extended time period are usually considered optimal. As shown by others [2], such release properties are attainable with polymeric implants that have been formed into complex geometric shapes. The problem with these devices is that they must be surgically implanted, and in some cases explanted, which raises medical costs.

A recent series of patents by Dunn and co-workers [3,4] describes a novel approach which potentially can provide prolonged zero-order release along with much greater ease of administration than surgically implanted systems. In their approach, a water-insoluble polymer, biocompatible solvent, bioactive agent and possibly several additives are injected by syringe into the targeted organism. As the polymers are usually water-insoluble, contact with the aqueous-based physiologic surroundings causes the solution to undergo liquid–liquid phase separation to produce a two-phase, gelled implant. As we will show in this article, the exact way that the injected solution responds to its physiologic surroundings determines...
the final depot morphology and, consequently, its eventual drug release characteristics.

The dynamics of the interactions that take place between a polymer solution and one of its nonsolvents (or coagulants) have been studied quite extensively because of their critical role in polymer membrane formation by nonsolvent-induced phase inversion [5]. In the latter process, a thin film of a concentrated polymer solution is immersed into a coagulation bath containing a nonsolvent for the polymer. The ensuing solvent/nonsolvent counter-diffusion lowers the polymer solubility, eventually causing the solution to phase separate into a polymer-rich matrix surrounding dispersed polymer-lean droplets. The exact arrangement of the two-phase structure determines the final membrane morphology and thus its separation characteristics. Phase inversion membranes formed in this way have been used successfully in a variety of applications including microfiltration [6], ultrafiltration [7–11], gas separation [12,13] and biological reactors [14]. In each case, the phase inversion processing conditions (coagulation bath composition, polymer solution composition, additive composition, polymer type) are varied until a membrane with desirable performance characteristics results. In all of these more traditional applications of phase inversion, the membrane is not used until its entire fabrication is complete, and therefore it is only the final structure that is of import during membrane design.

Numerous studies have demonstrated that the dynamics of nonsolvent-induced phase inversion can be quantified in terms of the system ternary phase diagram (i.e., polymer, solvent and nonsolvent) coupled with models for the mass transfer dynamics during the quench period [15–18]. Generally speaking, conditions leading to rapid phase separation (e.g., increasing the affinity between the solvent and nonsolvent) also promote the formation of thin skins at the surface of the membrane and finger-like porous cavities in the body of the film. Conversely, changes leading to more delayed precipitation, promote thicker skins and more uniform, spongy sub-layers in the body of the film. In either case, the final morphology has a direct influence on the resulting membrane transport characteristics.

A feature that distinguishes injectable drug delivery systems is that a critical aspect of its performance (its initial drug release rate) occurs simultaneously with the formation of the device by phase inversion. Therefore, if one wishes to control the drug release characteristics of a delivery system, it is important to be able to quantify the dynamics of the in vivo phase separation process in terms of relevant system and physiologic variables. Fundamental parameters of the formation kinetics include the water influx rate and the gelation rate. The water influx rate refers to the diffusion of water from the physiologic surroundings and subsequent accumulation within the injected polymer solution. As many drugs of interest are water-soluble, the water influx rate is critical because it determines the rate at which the drugs dissolve and therefore how readily they are able to diffuse through the implant. The gelation rate is the rate at which the solution is transformed into a semi-solid, porous implant. It is important because, as will be shown, it determines the properties of the diffusional path that the drug molecules take as they leave the implant.

The goal of the studies to be presented in this paper is to demonstrate the link between the critical aspects of the in vitro gel formation kinetics and the resulting drug release properties of injectable implants. Our experimental approach is based on the use of a dark ground, video imaging technique recently developed in our laboratories that enables simultaneous visualization of the diffusion, liquid–liquid phase separation, and gel formation processes during solution quenching in a nonsolvent bath [19,20]. In this paper, we focus on quantifying the effects that formulation changes in the injected polymer solution and the physiologic surroundings have on the in vitro water influx rate, the gelation rate and the final depot morphology. Specific changes to the injected solution formulation include the addition of a high molecular weight hydrophilic polymer (polyvinylpyrrolidone, PVP), the use of a hydrophobic cosolvent (triacetin), and variations in the polymer and water concentration.

2. Experimental

2.1. Dark ground optics

Fig. 1 shows a sketch of the imaging system used
in our experiments along with an example of a typical dark ground image of a quenched polymer solution. A filtered laser beam is focused by several collimating lenses onto a quartz cell that contains the polymer solution. The cell containing the polymer solution is made from optical quality quartz and has a rectangular cavity 3 mm deep and an optical path length of 4 mm. The initially homogeneous solution is separated from the coagulation bath by a thin metal foil and the solution quench is activated by removal of the foil. The optics are set up to allow video imaging of the concentration gradient that is caused by water diffusion into the polymer solution. The region of striations in the dark ground image is interference fringes whose position is related to the distance water has penetrated into the solution. In addition to the diffusion fringes, reflected light is used to illuminate the portion of the solution nearer the bath interface that has undergone liquid–liquid phase separation and formed into a two-phase structure. With high molecular weight solutions, liquid–liquid phase separation and gel solidification occur simultaneously, while, with lower molecular weight systems, there is usually a time lag between liquid phase separation and gel solidification [21]. Thus, in the latter case the transformation characteristics are quantified in terms of the liquid–liquid phase separation front, while, in the former case the front motion reported is that of the solidified gel. With the exception of the solutions containing PVP additive, the PLGA system used in our study showed liquid–liquid phase separation followed by solidification. The positions of both the diffusion and liquid–liquid gelation fronts, relative to the initial bath-film interface are labelled as \( Y_{\text{Fringe}} \) and \( Y_{\text{Gel}} \) in Fig. 2. As shown, both the diffusion fringes and the liquid–liquid/gelation front move progressively deeper into the solution following exposure to nonsolvent. Squares of the positions of both fronts are plotted as a function of time following the quench and the
slopes of these lines are related to the water influx rate and the gelation rate, respectively. Detailed relations between the slopes of the two fronts and the appropriate system diffusivities have been derived and are described elsewhere [21].

3. Materials and methods

Poly(lactide-co-glycolide) (PLGA Resomer 502, MW 10000, Boehringer-Ingelheim) was the polymer used in the formulations. Polyvinylpyrrolidone (PVP, MW 1300000) from Aldrich was used as an additive. Horse serum was from a donor herd and obtained from Aldrich. Reagent grade 1-methyl-2-pyrrolidinone (NMP) and triacetin were used as solvents. Both solvents are of low toxicity and are recommended for use in injectable drug delivery devices [3]. For stability reasons, the protein used for the release rate studies was chicken egg lysozyme (Muramidase; mucopeptide N-acetylmuramoyl-hydrolase; EC 3.2.1.17, Sigma L 6876), triple crystallized, dialyzed and lyophilized to a 95% protein level with the balance primarily buffer salts (sodium acetate and sodium chloride). The activity of this material was approximately 50000 units per mg protein.

Solutions were prepared by mixing together appropriate amounts of polymer, solvent and additive in a small glass vial at room temperature. Generally, it took several hours for the polymer to dissolve completely and for all the bubbles to leave.

As mentioned, the diffusion fringes in the dark ground images are related to the refractive index that develops when water penetrates into the solution. In most ternary solutions, the overall solution refractive index changes when any of the three concentrations change, and it is, therefore, difficult to relate the refractive index gradient to a concentration gradient. However, from an optical standpoint, a desirable feature of the PLGA/NMP system is that the refractive indices of the PLGA and NMP are nearly matched which means that the refractive index of PLGA/NMP/Water solutions depend only on the water concentration. As a result, the diffusion fringes shown in Figs. 1 and 2 can be related directly to the water concentration gradient.

Morphologies of several hardened depots were examined using scanning electron microscopy. Samples were prepared by smearing the solution across an aluminum plate containing a 1.6-cm diameter circular depression with a depth of 500 µm. Samples were immediately quenched into a water bath and allowed to coagulate for at least 24 h. Films were fractured in liquid nitrogen and vacuum dried at room temperature for at least 24 h. Samples for microscopy were sputter coated with an Au/Pd mixture using an Emscope SC400 sputter coater. Micrographs of the depots were taken using an Hitachi S-530 scanning electron microscope.

Protein release experiments were conducted by placing approximately 0.5 g of depot formulation on a vertical polypropylene lattice in 10 ml of phosphate-buffered saline (PBS) heated to 37°C. The receptor solution was replaced every 1–4 days. Polypropylene vials were used to minimize protein
absorption. Protein concentration was determined using high performance liquid chromatography (HPLC) utilizing a reversed phase POROS® column with UV detection at 210 nm. Lysozyme concentrations were determined using a calibration curve of standards ranging from 100 to 0.5 µg/ml.

4. Results and discussion

4.1. Changes to the solution formulation

4.1.1. Part A: effect of the PVP additive

PVP is a hydrophilic polymer known to influence the morphology of phase inversion membranes in several ways [9,11,22,23], including inducing finger to sponge transitions [22] and causing changes to the permeability [9,11,23]. Our measurements show that small additions of PVP to PLGA/NMP solutions dramatically affect both the phase inversion dynamics and the in vitro protein release behavior. As an example, Fig. 3 shows that the addition of only 3 wt.% PVP results in a dramatic 8-fold increase in the liquid–liquid phase separation rate. However, as shown by Figs. 4 and 5, both the water influx rate (as measured by the slope of the first fringe position) and the overall morphology do not change significantly. Study of PVP addition is, therefore, especially illustrative because changes in the protein release
kinetics can be ascribed directly to a particular generic aspect of the phase inversion dynamics (the liquid–liquid phase separation rate). The data in Fig. 6 show that the drug release rate, especially during the initial gel formation period or burst phase (i.e., $t<1$ day), is much higher for the solution that contains the PVP additive. It is, therefore, clear that increasing the rate of formation of the gelled, two-phase structure results in a much higher initial protein release rate. These data also show that once the entire film is gelled ($t>1$ day) the release characteristics of the two systems are more or less identical. The fact that the PVP additive increases both the gelation rate and the protein release rate, but does not affect the water influx rate and the overall film morphology, suggests that drug release occurs primarily by diffusion through the interconnected polymer-lean phase that exists in the two-phase, gelled structure. This behavior is, in general, quite reasonable considering that diffusivities in the polymer-lean phase (ca. $10^{-5}$ cm$^2$/s) [24] can be up to two orders of magnitude higher than those in the polymer-rich phase (ca. $10^{-7}$ cm$^2$/s) [24,25]. As illustrated schematically in Fig. 7, during the initial stages of the quench period, drug molecules contained within a slowly gelling system must first diffuse through the rather viscous polymer solution before they can enter into the more mobile polymer-lean phase. Therefore, under most conditions, formulation changes that increase the rate of liquid–liquid phase separation should result in a higher initial burst. Conversely, changes that slow the rate of liquid–liquid phase

---

**Fig. 6.** Lysozyme release rate from formulations with PLGA/NMP/lysozyme/PVP weight ratios of: 35/55/10/0 (○); 32.3/55/10/2.62 (△).

**Fig. 7.** Comparison of diffusional path between fast and slow gelling systems.

**Fig. 8.** Liquid–liquid phase separation rate for 40 wt.% PLGA/NMP solutions with: 0 wt.% water (○); 2.5 wt.% water (△); 5.0 wt.% water (△).
separation decrease the burst magnitude because the drug remains trapped in the less mobile polymer solution.

4.1.2. Part B: effect of water addition

Adding water to the polymer solution formulation is another change that leads to increases in both the phase separation rate and the magnitude of the initial burst. Figs. 8 and 9 show that the liquid–liquid phase separation rate increases and the water influx decreases as additional water is added to the formulation. Liquid–liquid phase separation occurs more rapidly because the overall solution composition is closer to the ternary miscibility gap, and therefore, less water influx is required to induce phase separation. At higher water compositions, a gelled layer rapidly forms at the surface and slows subsequent

Fig. 9. First diffusion fringe motion for 40 wt.% PLGA/NMP solutions with: 0 wt.% water (•); 2.5 wt.% water (○); 5.0 wt.% water (△).

Fig. 10. Morphologies of depots made by water quenching 50 wt.% PLGA/NMP solutions with: (a) 0 wt.%; (b) 1.25 wt.%; (c) 2.5 wt.%; (d) 4.5 wt.% water added to the formulation.
water permeation. However, the micrographs in Fig. 10 show that these changes in the phase inversion dynamics do not result in dramatic changes in the film morphology. In all cases, the system is characterized by a more-or-less uniform distribution of tear-shaped macrovoids throughout the film. The protein release data in Fig. 11 show that as water is added to the film (increasing the liquid–liquid phase separation rate), the magnitude of the initial burst generally increases, which is consistent with the trends observed in the PVP experiments. As the protein is water-soluble, the mere fact that additional water is present initially in the system may itself contribute to the higher bursts. Once completely formed, however, (i.e., \( t > 1 \) day), both depots display more or less the same protein release characteristics. Together with Figs. 5 and 10, these results clearly suggest that while initial protein transport characteristics are strongly influenced by the membrane formation rate, the longer term transport characteristics are more a function of the final membrane morphology.

4.1.3. Part C: effect of increasing polymer concentration

There are several additional changes to the formulation that can have converse effects. As shown by Fig. 12, increasing the polymer concentration slows the liquid–liquid phase separation rate. This reduc-

Fig. 11. Lysozyme release rate from formulations with PLGA/NMP/lysozyme/water weight ratios of: 35/55/10/0 (●); 35/54/10/1 (○); 35/52.8/10/2.2 (∙); 35/50.6/10/4.4 (□).

Fig. 12. Liquid–liquid phase separation rate for PLGA/NMP solutions with several PLGA concentrations: 40 wt.% (●); 45 wt.% (○); 50 wt.% (∙).

Fig. 13. First diffusion fringe motion for PLGA/NMP solutions with several PLGA concentrations: 40 wt.% (●); 45 wt.% (○); 50 wt.% (∙).
because increases in the polymer concentration result in a multitude of effects, including lower system diffusivities, thicker skins and increased solution hydrophobicity, all of which may contribute to the dynamics. Similar to most membrane forming systems [8, 27–30], increasing the polymer concentration results in a finger to sponge transition, as shown by Fig. 14. As expected, the in vitro drug release data of Yewey et al. [31] show that increases in the polymer concentration do slow the initial release rate. In this case, the slower phase separation rate certainly contributes to the slower release; however, both the slower water influx rate and the morphological transition to a less porous sponge-type structure are presumably additional contributing factors.

Fig. 14. Morphologies of depots made by water quenching PLGA/NMP solutions with several PLGA concentrations: (a) 40 wt.%; (b) 50 wt.%; (c) 60 wt.%.  

Fig. 15. First diffusion fringe motion for water quenched PLGA/NMP/triacetin solutions with weight ratios of: 40/60/10 (♦); 40/47.5/12.5 (¸); 40/33.5/26.5 (○); 40/15/45 (○).

Fig. 16. Liquid–liquid phase separation rate for water quenched PLGA/NMP/triacetin solutions with weight ratios of: 40/60/10 (♦); 40/47.5/12.5 (¸); 40/33.5/26.5 (○).
4.1.4. Part D: effect of triacetin addition

Changing the solvent quality can have a dramatic effect on the phase inversion dynamics and the final morphology [8,23,27,30,32]. Because triacetin (a short chain triglyceride) is only sparingly miscible with water, its addition decreases the affinity between the water and the polymer solution. As shown in Fig. 15, this results in a corresponding decrease in the water influx rate. As shown by Fig. 16, the lower water influx rate results in a lower rate of liquid-liquid phase separation. Most interesting, however, is the morphological transition that occurs. This is depicted in Fig. 17. The addition of 10 wt.% triacetin causes a transition from a finger-type morphology to a sponge type morphology. Based on the fact that drug release occurs primarily through the polymer-lean phase, the long, columnar fingers should be especially efficient passageways and their removal should contribute to lower initial release rates. As even more triacetin is added to the formulation, the sponge structure appears to become less porous, thereby removing some of the polymer-lean regions through which drug diffusion occurs. One of the remarkable features of triacetin addition is that the lower water influx rate, the lower gelation rate and the decreasing structure porosity all contribute to lower drug release rates. It is for this reason that, as shown in Fig. 18, triacetin addition dramatically lowers the initial protein release rates. Shah et al. [33] noted a similar decrease in the drug release rate when they added a medium chain triglyceride to the formulation.

---

Fig. 17. Morphologies of depots made by quenching solution with PLGA/NMP/triacetin mass ratios of: (a) 40/60/0; (b) 40/50/10; (c) 40/0/60.

Fig. 18. Lysozyme release rate from formulations with PLGA/NMP/triacetin/lysozyme weight ratios of: 35/55/0/10 (♦); 35/43.4/11.5/10 (♦); 35/31.9/23.1/10 (♦); 35/0/55/10 (♦).
5. Changes to the physiologic surroundings

Owing to the complexity of most biological systems, variations in the properties of the physiologic medium surrounding the injected polymer solution are expected. In this section, we describe the influence such changes have on the phase inversion dynamics of PLGA solutions.

Figs. 19 and 20 show that the liquid–liquid phase separation and water influx rates are identical when 50 wt.% PLGA/DMSO solutions are quenched with water, phosphate-buffered saline solution, or horse serum. Moreover, both the overall morphology, Fig.

![Fig. 19](image-url)

**Fig. 19.** Liquid–liquid phase separation rates for 50 wt.% PLGA/DMSO solutions quenched with: pure water (○); PBS buffer solution (△); horse serum (●).

![Fig. 20](image-url)

**Fig. 20.** First diffusion fringe motion for 50 wt.% PLGA/DMSO solutions quenched with: pure water (●); PBS buffer solution (△); horse serum (○).

![Fig. 21](image-url)

**Fig. 21.** Morphologies of depots made by quenching 50 wt.% PLGA/DMSO solutions with: (a) pure water; (b) PBS buffer solution; (c) horse serum.
21, and the skin structure, Fig. 22, are nearly indistinguishable. Considering the similarity of the phase inversion dynamics for the three quench media, it is somewhat surprising that the protein release data of Yewey et al. [31] show that switching the quench medium from a buffered solution to horse serum lead to fairly large increases in the release rates. Apparently, horse serum (and other such physiologic media) contains chemical species that can change the bath-side mass transfer characteristics and thereby influence drug release behavior.

6. Conclusion

In this study, dark ground optical microscopy, electron microscopy, and HPLC have been used to quantify the effects that formulation changes have on the phase inversion dynamics and in vitro drug release properties of a PLGA based drug delivery system. Our results enable insights into the mechanisms of drug release and suggest ways to control drug burst for these injectable systems. Specifically, additives that accelerate the solution gelation rate at constant morphology result in high initial release rates, implying that drug diffusion through a tortuous two-phase gelled structure is faster than through a one-phase polymer solution. Conversely, additives that slow the rate of gelation dramatically reduce the initial drug release rate and lead to a more dense sponge like morphology. Moreover, the phase inversion dynamics and morphology are the same regardless of whether the solutions are quenched with water, a PBS solution or horse serum.

Acknowledgements

These studies have been partially supported under grants from the National Science Foundation, CTS 94-21580 and 97-31509.

References