Single Particle Studies for Drug Delivery Systems

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ABSTRACT

This paper provides a more fundamental investigation of the formation and dissolution of PLGA microspheres. A series of single microparticle studies that were conducted include: measurement and modeling of gas microbubbles and lipid coated gas microspheres in water; measurement and modeling of the dissolution of chloroform and ethyl acetate (EtAc) microdroplets in water; PLGA solidification from EtAc microdroplet and visualization of second phase water inclusions; and visualization of PLGA degradation (by convection using a blowing pipette). With the gas bubbles, chloroform, and ethyl acetate droplets, it was found that the Epstein-Plesset (EP) model does provide a good model for the dissolution curves. During the PLGA microsphere experiments, surprisingly, second phase inclusions of water were seen forming inside the microspheres. Further, in order to visualize degradation of the microspheres, a blowing pipette was set up to bathe the microsphere with 1 M NaOH, which sped up the hydrolysis reaction through a base catalyzed mechanism. Finally, it is proposed that vacuum coating technology be used to coat these microspheres with hydrophobic fluorine containing polymer films in order to slow down the overall degradation process prolonging the longevity of the microspheres in the body and also to achieve "inside-out" degradation, which may help quantify the second phase water inclusions seen inside the microspheres.

INTRODUCTION

The field of protein therapeutics is burgeoning, and industry experts estimate the demand for innovative protein therapeutics to grow at a rate of 30% per year [1]. However, with this increased focus on proteins and peptides as possible new therapeutics, the issue of drug delivery surfaces as a significant obstacle. Polymer microspheres, especially those made from poly(lactic-co-glycolic acid) (PLGA) and similar degradable or biodegradable polymers, have shown success in the delivery of more conventional drugs. Thus, such polymer microspheres are being further investigated for protein drug delivery [2].

Due to the relatively large size of proteins, they are marked by low transdermal bioavailabilities. Also, oral bioavailability is obviated by the presence of proteolytic enzymes in the gastrointestinal tract, which will degrade the protein. Similarly, ocular and nasal delivery is not preferred due to degradation by enzymes present in the eye tissues and nasal mucosa; frequent injections would be required in disease treatment. The overlying objective in optimizing controlled release protein injectables is to avoid such regular invasive and repeated doses for the patient, thereby catering to patient compliance and comfort [3].

Thus, subcutaneous injection and parenteral delivery of proteins in biodegradable polymer microspheres seems to be the most efficient mode of delivery. Advantages include localized delivery, stability, and sustained release of drugs in the body [4,5,6]. Moreover, polymer microspheres such as PLGA microspheres will undergo hydrolysis in the body producing the harmless metabolites glycolic and lactic acid, which are easily eliminated through natural body functions.

Although PLGA has been very well studied, and new protein and peptide drugs are being created and discovered, only one product that utilizes a microsphere loaded with protein is available—the endogenous growth hormone by Genentech and its formulation in Nutropin Depot® --the long-acting form of protein using Alkermes' ProLease® injectable extended-release drug delivery system [7]. Problems such as low encapsulation efficiency, protein and peptide inactivation during the encapsulation process, and difficulties in controlling the release have limited the use of other proteins or peptides [8].

Often, the only way to gauge success of a certain delivery system is to observe the finished product by SEM and/or carry out drug release profiles and physico/chemical characterizations after the fact [9,10]. It is impossible to obtain definitive *in situ* data on microdroplets and microspheres as they are forming, test theoretical models, or tailor experimental and process conditions in a rapid and effective manner. Improved basic scientific understanding of these systems is needed to increase the number and types of drugs that can be formulated as well as to achieve greater control over drug release from microspheres.

In the current study, a more fundamental approach is taken, utilizing a single particle technology platform for experimental and theoretical characterization of the formation and degradation of polymer microspheres. Several experiments have established the underlying foundation and methods for these single particle studies. By using a pipette filled with solution A and placed inside a chamber with solution B, one can apply

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pressure to the pipette through a syringe to form single droplets of solution A in solution B. These underlying experiments include measurement and modeling of gas microbubbles and lipid coated gas microspheres in water; measurement and modeling of the dissolution of chloroform and EtAc in water; PLGA solidification from EtAc and visualization of second phase water inclusions; and visualization of PLGA degradation (by convection using a blowing pipette).

METHODS

Micropipette Manipulation Technique

The glass micropipettes (0.75 mm x 0.4 mm x 6 in, A-M Systems, Inc., Everett, WA) were modified using a vertical pipette puller. The ends of the pipettes were forged, usually to an inner diameter of about 4-8 μm, by employing a heated molten glass bead. The pipettes were then mounted in a chuck. Tygon tubing was used to connect the pipette chuck sidearm to a pressure control system; the pressure was controlled by a 5 mL syringe.

The pipette was filled with the desired fluid by a frontfilling method, where the pipette is dipped into the fluid intended for droplet formation and a negative pressure is applied. Once the pipette was filled with a sufficient amount of fluid, the pipette was connected to the manipulator setup. The manipulator allowed precise three-dimensional control over the pipette through the use of a pneumatic joystick controller.

The actual manipulation chamber was constructed from standard glass microscope slides (25x75 mm) and glass coverslips (No. 1 22x30 mm) cut and joined together using optical cement and minimal silicone grease (vacuum grade). Typical single microchambers were made with a volume capacity of about 0.5 mL. Other microchambers were constructed by simply using a standard optical glass cuvette with a 2 mm path length.

The pipette was then inserted into the chamber using the manipulator apparatus. The overall experimental setup is shown in Figure 1. Microparticles of desired diameters, usually ranging from 50 to 100 μm, were created by gently pressing the plunger on the syringe. In addition to viewing the microparticles under an optical microscope, video recordings were taken. These clips were then analyzed using a video caliper system, which allowed one to obtain diameter readings of the microparticles over time.

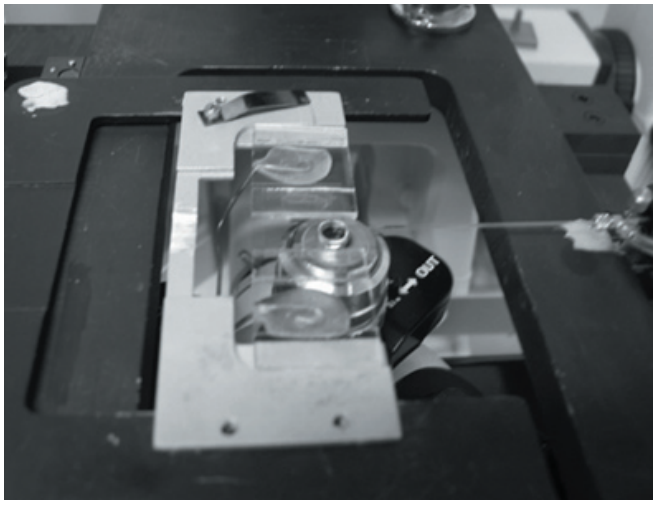

Figure 1: The overall experimental setup with micropipette, manipulator, and chamber.

EXPERIMENT

Measurement/Modeling of Gas Microbubbles and Lipid Coated Gas Microspheres in Water

In a recent paper [11], we tested an, as then, untested theory for gas bubble dissolution by Epstein and Plesset (EP) [12]. Two main factors determine the dissolution of gas from a free air bubble in water: the concentration (undersaturation) of dissolved gas in the aqueous phase and the surface tension of the gas bubble-water interface via a Laplace overpressure in the bubble. In order to test the effect of undersaturation, solid distearoylphosphocholine (DSPC) lipid was used to coat the gas microparticle with a waxy monolayer to attain zero tension in the surface. In order to study the effects of surface tension, single and double-chain surfactants were employed to control and define interfacial conditions of the microbubble in saturated solution.

Where undersaturation was tested, the EP model on average overpredicted the dissolution time by 8.2%, with gas saturation levels ranging from 70% to 100%. In contrast, where the effect of surface tension was tested, the EP model on average underpredicted the dissolution time by 8.2%, with surface tensions ranging from 25 to 72 mN/m. Overall, it was confirmed that the gas from a free air microbubble readily dissolves in water in a way predicted by EP. Also, it was confirmed that the dissolution is modified by the presence of an impermeable boundary. Figure 2 shows the videomicrographs for the dissolution of a gas bubble, while Figure 3 shows the dissolution curves for a microbubble held with a micropipette in the center of the solution and one against an impermeable wall (with a modified EP model to correct for the wall effect).

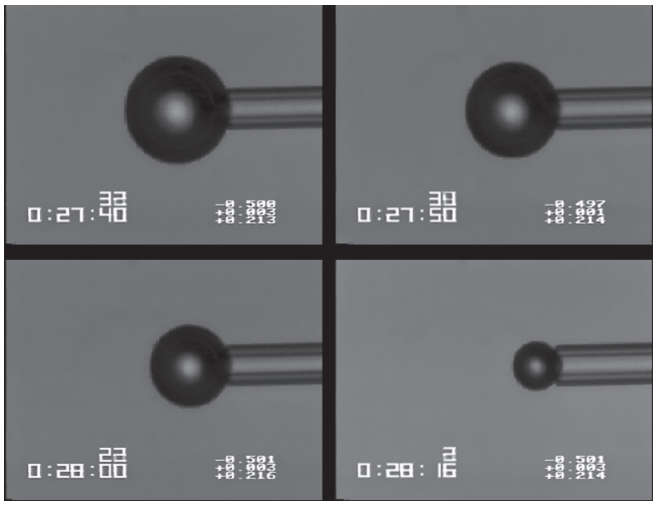

Figure 2: Videomicrographs of the dissolution of an air microbubble in 10 mM SDS solution held in the center of the chamber by a pipette with a suction pressure of 5 kdyn/cm^2 and solution temperature of 21.5°C. The air bubble is shown at 15.0, 13.5, 11.0, and 3.5 μm in radius after 0 (top left), 10 (top right), 20 (bottom left), and 36 (bottom right) seconds, respectively.

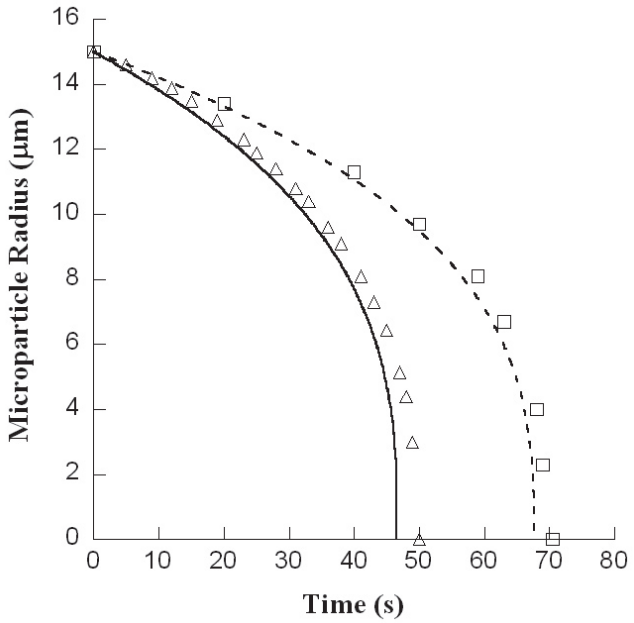

Figure 3: Graph of dissolution (radius vs time) and influence of an impermeable boundary. (Δ) microbubble held with a micropipette in center of solution; (□) microbubble against an impermeable wall. EP theoretical model; EP theory modified with empirical constant to correct for wall effect.

Measurement and Modeling of Dissolution of Chloroform and EtAc in Water

Also, a series of studies that measure the same parameters (dissolution time, radius-time profile) have been carried out on chloroform and EtAc. The dissolution profiles once again were modeled using the same EP theory. The chloroform droplet with similar diffusion coefficient D and solubility Cs in water as air dissolves in a similar time and profile, well modeled by the diffusion-solubility model of EP. The EtAc droplets, also well modeled by the EP model, dissolved very fast, and the dissolution did not go to completion, which hints at some impurity present in the initial EtAc solution. Both were also dissolved in 10 mM SDS solution, and no changes in dissolution times were seen. The dissolution profiles along with the EP models for chloroform and EtAc are shown in Figures 4 and 5 respectively.

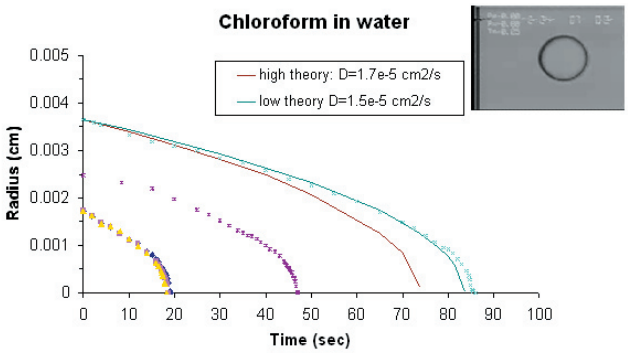

Figure 4: Dissolution time of chloroform microdroplets in water as radius of the droplet versus time. Solid lines for 35 μm droplet is EP theory.

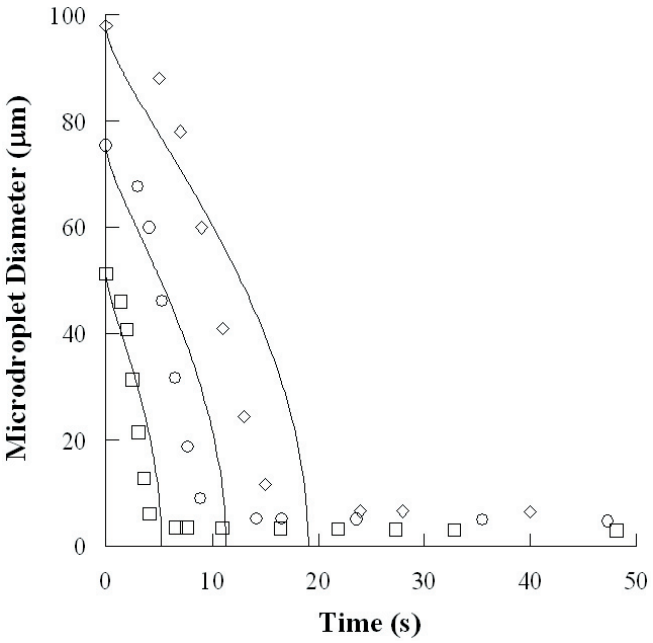

Figure 5: Dissolution of ethyl acetate droplet in water with initial droplet diameter of (◊) 97.9, (○) 75.3, and (□) 51.2 μm. The solid lines represent the non steady state theoretical model that neglects interfacial tension.

PLGA Solidification from EtAc and Second Phase Inclusions

As shown in Figure 6, a 50 μm PLGA solution microdroplet was formed on the end of a 5 μm diameter micropipette and then released. The microdroplet lost EtAc into the aqueous phase, and the polymer solidified and formed a 5 μm microsphere. The experimental results showed that the polymer microsphere volumes were set by the initial PLGA concentration, size of droplet, and saturation level of EtAc in the aqueous phase. Surprisingly, it was also seen that there were small inclusions in the formed microspheres. These appear to be trapped water that dissolved and reached its own saturation limit as the EtAc dissolved out, and remained as nanodroplets in the viscous polymer solution as it finally solidified. This is in contrast to the relatively homogeneous sphere that is present for the whole dissolution of pure EtAc. The difference between the microdroplets is shown in Figure 7. In this experiment, we also saw that the Brownian motion of the water inclusions slowed dramatically, indicating the viscosity change during the final stages of hardening. These second phase inclusions may affect the hydrolysis rate of the microspheres and subsequently the release rate of the drug.

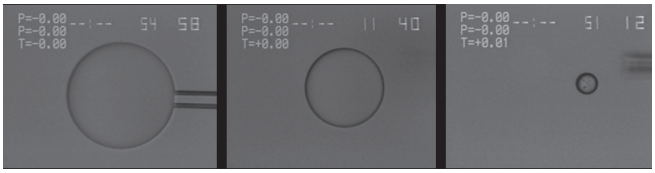

Figure 6: PLGA-EtOAc solution microdroplet (50 μm diameter) formed from a micropipette in water (left), dissolving (center) and forming PLGA microsphere (right).

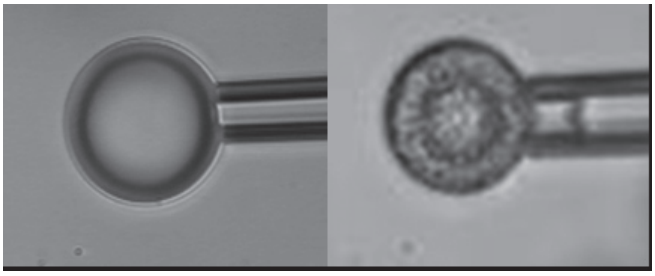

Figure 7: Comparison between EtAc microdroplet (left) in process of dissolution and EtAc/PLGA solution microdroplet (right) showing multiple inclusions which form as it solidifies.

In order to view the actual degradation of these microspheres, an experiment was set up where a blowing pipette delivered controlled rates of flow of bathing solution, providing controlled convective transport during the dissolution phase, as shown in Figure 8. The microdroplet was seen to decrease in size several percent faster than under diffusion controlled conditions when the bathing solution was 1 M NaOH. The blowing technique speeds up the hydrolysis rate through a base catalyzed mechanism.

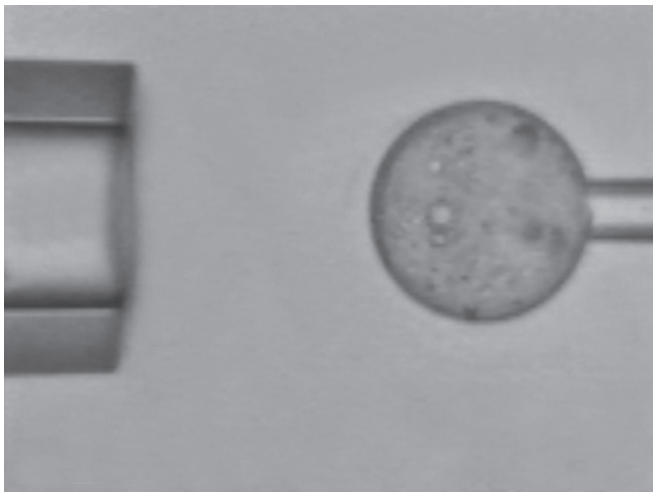

Figure 8: Blowing pipette delivering controlled rates of flow of bathing solution to an EtAc/PLGA microdroplet, providing controlled convective transport to the microdroplet during the dissolution phase.

DISCUSSION

Many of these experiments establish the foundation and methods for further single particle studies. Vacuum coating technology can be applied in these experiments to obtain a more solid understanding of the fundamentals behind particle dissolution and formation, which will ultimately help to create more efficient and useful drug delivery systems.

Applying Vacuum Coating Technology

Vacuum coating technology has several potential applications to the current studies, especially in the form of polymer films. Many researchers have begun to use vacuum coating to deposit organic and inorganic polymer films. One can form these films by first condensing the monomer, and then employing E-beam or UV curing to polymerize the monomer. Because fluorine-containing films will form hydrophobic surfaces, one can coat the microparticles in the current studies with such a film.

One potential benefit of coating the PLGA microspheres with a hydrophobic surface would be to slow down the degradation process. Such a coating may prolong the time these microspheres remain microspheres in the body, and therefore deliver drugs to the patient over a longer period of time.

Furthermore, with a hydrophobic coating, one may see a greater extent of "inside-out" degradation, as the surface would be less prone to water passing through, while the second phase inclusions of water on the inside would continue to cleave the ester bonds of PLGA through hydrolysis. After characterizing this "inside-out" degradation, one could quantify the amount of water trapped inside the microspheres, assuming the degradation occurring from the inside is much more significant than the degradation from the surface. We believe that such factors

as PLGA concentration, the ratio of glycolic to lactic acid, the surfactant used, and the initial diameter of the sphere will govern the amount of water inclusions. This information will be very valuable, as the amount of trapped water will certainly affect the overall degradation rate of the microspheres.

CONCLUSION

A more solid understanding of the fundamentals behind drug delivery systems using polymer microspheres is needed to generate more effective protein therapeutics, and the single particle experiments in the current study provide just that. Furthermore, manipulating these experiments by employing vacuum coating technology will allow one to alter such processes as the manner by which the microspheres degrade and ultimately how drugs are released.

REFERENCES

1. E.B.D. Group, I., "Protein Therapeutics Course." 2004. p. http://www.ebdgroup.com/protein/overview.htm.

2. Banga, A.J., "Delivery of Protein Therapeutics", in *Business Briefing: Pharmatech 2004*. 2004, *Pharmatech*. p. 198-201.

3. Sinha, V.R. and A. Trehan, "Biodegradable microspheres for protein delivery." *J Control Release*, 2003. 90(3): p. 261-80.

4. Langer, R., "Drug delivery and targeting". *Nature*, 1998. 392(6679 Suppl): p. 5-10.

5. Gombotz, W.R. and D.K. Pettit, "Biodegradable polymers for protein and peptide drug delivery." *Bioconjug Chem*, 1995. 6(4): p. 332-51.

6. Sinha, V.R. and L. Khosla," Bioabsorbable polymers for implantable therapeutic systems." *Drug Dev Ind Pharm*, 1998. 24(12): p. 1129-38.

7. Banga, A.J., "Delivery of Protein Therapeutics," in *Business Briefing: Pharmatech 2004*. 2004, *Pharmatech*. p. 198-201.

8. Perez, C., et al., "Recent Trends In Stabilizing Protein Structure Upon Encapsulation and Release From Bioerodible Polymers." J *Pharm Pharmacol*, 2002. 54(3): p. 301-13.

9. Gombotz, W.R. and D.K. Pettit, "Biodegradable polymers for protein and peptide drug delivery." *Bioconjug Chem*, 1995. **6**(4): p. 332-51.

10. Maa, Y.F. and C.C. Hsu, "Effect of primary emulsions on microsphere size and protein-loading in the double emulsion process." *J Microencapsul*, 1997. 14(2): p. 225-41.

11. Duncan, P.B. and D. Needham," Test of the Epstein-Plesset Model for Gas Microparticle Dissolution in Aqueous Media: Effect of Surface Tension and Gas Undersaturation in Solution." *Langmuir*, 2004. 20(7): p. 2567-2578.

12. Epstein, P.S. and M.S. Plesset, "On the stability of gas bubbles in liquid-gas solutions." *Journal of Chemical Physics*, 1950. 18(11): p. 1505-1509.