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CHANGES IN LIPID-ENCAPSULATED MICROBUBBLE POPULATION DURING CONTINUOUS INFUSION AND METHODS TO MAINTAIN CONSISTENCY

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Abstract

Stabilized microbubbles are utilized as ultrasound contrast agents. These micron-sized gas capsules are injected into the bloodstream to provide contrast enhancement during ultrasound imaging. Some contrast imaging strategies, such as destruction-reperfusion, require a continuous injection of microbubbles over several minutes. Most quantitative imaging strategies rely on the ability to administer a consistent dose of contrast agent. Because of the buoyancy of these gas-filled agents, their spatial distribution within a syringe changes over time. The population of microbubbles that is pumped from a horizontal syringe outlet differs from initial population as the microbubbles float to the syringe top. In this manuscript, we study the changes in the population of a contrast agent that is pumped from a syringe due to microbubble floatation. Results are presented in terms of change in concentration and change in mean diameter, as a function of time, suspension medium, and syringe diameter. Data illustrate that the distribution of contrast agents injected from a syringe changes in both concentration and mean diameter over several minutes without mixing. We discuss the application of a mixing system and viscosity agents to keep the contrast solution more evenly distributed in a syringe. These results are significant for researchers utilizing microbubble contrast agents in continuous-infusion applications where it is important to maintain consistent contrast agent delivery rate, or in situations where the injection syringe cannot be mixed immediately prior to administration.

Keywords

Microbubbles; Contrast Agent; Ultrasound; Size Distribution; Concentration; Mixing; Floatation; Rotating Syringe

INTRODUCTION

Lipid encapsulated microbubbles are used clinically and pre-clinically as contrast agents for ultrasound imaging. These microbubbles have diameters of the order of 1–10 microns, and are stabilized with a thin shell such as a lipid, protein, or polymer (Doinikov et. al 2009). Although some contrast agents are filled with air, typically the core is filled with a high-molecular-weight

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gas such as a perfluorocarbon, which dissolves into the surrounding medium more slowly than air and thus prolongs contrast agent circulation time (Klibanov 2006).

Due to the density and compressibility of their gas core, the microbubbles scatter ultrasound waves much better than the surrounding tissues or red blood cells. Additionally, these compressible spheres oscillate nonlinearly in an acoustic field, allowing for the use of detection strategies which can separate microbubble signals from those of tissue (Burns and Wilson 2006, Dayton and Rychak 2007, Sboros 2008)

In addition to imaging applications, microbubbles have demonstrated significant potential in a wide range of therapeutic applications such as targeted drug delivery, gene therapy, vascular permeability enhancement, and thrombolysis (Bekeredjian et. al 2005, Bekeredjian et. al 2006, Rubiera et. al 2008, Shortencarier et. al 2004, Stieger et. al 2007)

Contrast imaging studies, such as destruction-reperfusion imaging, may utilize a continuous infusion of contrast agents over several minutes, and rely on the assumption of a constant concentration of contrast agents in the blood pool in order to make quantifiable measurements (Broumas et. al 2005, Henao et. al 2006, Wei et. al 2001, Xie et. al 2007). Although injected dose varies widely depending on protocol and animal weight, Broumas et al. and Pollard et al. reported administration of $\sim 10^9$ bubbles/mL at 10 mL/h for several minutes for destruction-reperfusion imaging in rats (Broumas et. al 2005, Pollard et. al 2002).

Similarly, for drug delivery using microbubble contrast agents, the number and size of microbubbles determine the drug dose that would be delivered to the target regions (Unger et. al 2004). However, the concentration and size distribution of microbubbles injected into a patient's blood pool can be different from the concentration and size distribution of microbubbles in the syringe. Due to their thin shell and gas core, microbubble contrast agents are substantially less dense than water, and thus float towards the top of the syringe. The rate of floatation is primarily determined by the microbubble size, density and viscosity of liquid in the syringe, and density of the gas that is within the microbubbles (Feshitan et. al 2009, Kvale et. al 1996)

Recent studies by Talu et al. have evaluated the microbubble distribution injected from a syringe as a function of syringe diameter, needle diameter, injection rate, and microbubble parameters (Talu et. al 2008). Barrack and Stride have extended this work, examining syringe diameter, infusion rate, and the effect of the microbubble suspension's fluid properties such as viscosity and density (Barrack and Stride 2009). In both of these studies it was reported that microbubbles are sensitive to changes in hydrostatic pressure and shear stress in the syringe, however, the change in microbubble population due to buoyancy as the microbubbles are pumped from the syringe was not considered in these cases. In 2004, Lohmaier et al. considered the effect of bubble mixing within a rotating syringe as measured by ultrasound video intensity as contrast agents pumped through a phantom over time (Lohmaier et. al 2004). In this study, we extend the work of Lohmaier et al. with the use of an optical particle sizer, and we present data describing changes in the diameter and concentration of microbubbles pumped from a horizontal syringe as a function of microbubble size, microbubble suspension viscosity, and syringe diameter. Additionally, we present a model which simulates the distribution of microbubbles in a syringe over time, with and without mixing. Although Lohmaier et al described the use of a prototype commercial device to keep ultrasound contrast agents mixed, and Bracco Research (Geneva, Switzerland) has reported development of a commercial syringe mixer (VueJect), we were unable to obtain such a device in the United States, and hence our studies utilize a custom syringe-pump with integrated mixing device.

MATERIALS AND METHODS

Microbubble Preparation

Lipid-encapsulated microbubbles were prepared as described previously (Borden et. al 2005). The lipids 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000) were purchased from Avanti Polar Lipids (Alabaster, AL). The lipid composition consisted of 1.5 mg/mL DSPC with a 9:1 mol:mol ratio of DSPC to DSPE-PEG2000. The buffer solution for the lipids was prepared using Tris (Hydroxymethyl) Aminomethane purchased from EMD Chemicals (Gibbstown, NJ). The pH of the buffer solution was adjusted to 7.4 using hydrochloric acid. Viscosity control was achieved using 10 vol. % glycerin (Fisher Scientific, Pittsburgh, PA), 10 vol. % propylene glycol (Sigma-Aldrich, St. Louis, MO), and 80 vol. % deionized water. The lipids dissolved in chloroform were dried under nitrogen to evaporate the solvent. Once the lipids were dry, the buffer solution was added to the lipids in a vial. The lipid solution was sonicated in an ultrasonic bath (Branson, Danbury, CT) at 60°C for 20 minutes until a transparent and homogeneous mixture was achieved. Vials of 1.5 mL of the lipid solution were degassed for 5 minutes, followed by overpressurization with 5 psi of perfluorobutane gas (Synquest, Alachua, FL) for 2 minutes. Microbubble solutions were activated by shaking vials for 45 seconds in a Vialmix shaker (Bristol-Myers Squibb Medical Imaging Inc., North Billerica, MA). Concentration and size distribution of microbubble suspensions was determined with an optical particle counter with a 0.5 μ m diameter lower detection limit (Accusizer 780; Particle Sizing Systems, Santa Barbara, CA).

Measurement & Experimentation

Microbubble solutions were prepared by diluting an aliquot of microbubbles into one of three buffer solutions. Buffer solutions consisted of either phosphate buffered saline (PBS), Tris + 10% Glycerol, 10% Propylene Glycol, or Tris + 20% Glycerol, 10% Propylene Glycol. Studies were performed using both 3 mL and 10 mL syringes. Unless otherwise noted, all microbubble dilutions were made with a 1:10 dilution in buffer. The syringe filled with contrast agents was mixed thoroughly before initiating the experiment. An initial 200 μ L sample of the microbubble solution was taken immediately as the zero time point, after which the syringe was placed on the syringe pump (Genie Plus; Kent Scientific, Torrington, CT). The syringe pump was set to inject at a rate of 6 mL/hour, and the droplets which were pumped through the needle outlet were collected in 1.5 mL conical sample tubes (Eppendorf Tube; Fisher Scientific, Rockville, MD). Talu et al. (2008) observed that this injection rate was relatively nondestructive to microbubbles when injected through a 27 gauge needle. Each sample was mixed prior to analysis, and 25 μ L from the total sample was injected into the particle sizer for analysis. Mean diameter and concentration of particles were recorded during each measurement. All experiments were repeated three times. Microbubble concentration and size information are expressed as mean $\pm$ standard deviation. Error bars on bar graphs indicate standard deviation of the difference in values with and without mixing.

Mixing Apparatus Design

The syringe pump was modified for mixing during injection by incorporation of a motor drive and worm gear mechanism (Figure 1). A polycarbonate fitting was designed to integrate with the syringe head, and a 40 tooth gear mounted to the fitting allowed the entire syringe to be rotated by the gear motion. A Teflon sheath reduced the friction of the syringe rotation when clamped in the syringe pump. A DC motor was mated to the syringe fitting through a worm gear. The rotating syringe/gear fitting contained a small neodymium magnet which triggered a magnetic reed switch in the syringe pump body each time the syringe rotated by approximately 330 degrees. The resulting system rotated the syringe back and forth in 330

degree increments at approximately 15 rpm. Because the syringe never rotated more than a full revolution, a catheter could be used with the system without the tube becoming knotted.

Model Formulation

A mathematical model was used to simulate the changes in the microbubble concentration and size distribution in the syringe. The model considers syringe mixing rate, microbubble suspension fluid density and viscosity, microbubble diameter, density of filling gas, and microbubble wall thickness.

Without mixing, we consider buoyant and drag forces on the floating bubbles. With mixing, we also consider the movement of the bubble with the fluid in the syringe. For simplification, it was assumed that the fluid does not slip within the syringe, and hence the displacement of the bubble within the syringe was only a rotational component around the syringe axis, and was the same as the syringe rotation velocity (Figure 2).

Kvale et al. (1996) simulated bubble rise using Stokes' equation that described the rise velocity of a buoyant particle relative to the bulk fluid under creeping flow conditions, and Feshitan et al (2009) have more recently used this formulation to simulate floatation rates of microbubble contrast agents under differential centrifugation. The formulation is as follows:

$$u_i = \frac{2(\rho - \rho_{pi})g}{9\mu} r_i^2 \quad (1)$$

where u_i is the relative velocity between the microbubbles and the suspension fluid and i indicates the microbubble size class

ρ = Density of microbubble suspension fluid (kg/m^3)

μ = Viscosity of microbubble suspension fluid (kg/(m.s))

g = Gravitational acceleration (9.8 m/s^2)

r = Microbubble radius (m)

ρ_{pi} is the density of microbubble density given by:

$$\rho_{pi} = \frac{(r_i^3 - (r_i - \delta r)^3) \rho_b + (r_i - \delta r)^3 \rho_g}{r_i^3} \quad (2)$$

where δr is the thickness of the microbubble wall ($2.7 \times 10^{-9} \text{ m}$), ρ_b is the density of the lipid solution and ρ_g is the density of perfluorobutane gas (1517 kg/m^3). The effective viscosity of the microbubble suspension, μ^* , was calculated using Batchelor and Greene's (1972) correlation which is an extension of Einstein's seminal theory:

$$\frac{\mu^*}{\mu} = 1 + 2.5\Phi + 7.6\Phi^2 \quad (3)$$

$$\Phi = \sum_{i=1}^{N_d} \varphi_i = \sum_{i=1}^{N_d} n_i \frac{\pi d_i^3}{6} \quad (4)$$

Where Φ is the total microbubble volume fraction for N_d size classes and d is the diameter of microbubble.

Because of the small diameter of the microbubbles, it was assumed that the velocity of the microbubbles due to buoyancy was constant (Leighton 1994). The syringe was rotated clockwise at 15 rotations per minute (rpm) followed by 330 degrees counterclockwise rotation with the same rotation rate. Rotation of the syringe was modeled by dividing each revolution into 10 discrete steps (smaller steps were initially used yielding similar results, however the program became computationally tedious). For each increment of syringe rotation, the distance a microbubble traveled along the y-axis included rise due to the floatation of the microbubble plus the y-component of the displacement vector of the bubble due to the syringe rotation. Displacement of a microbubble in the x-axis was due only to syringe rotation.

We analyzed the change in the concentration of microbubbles within a $0.2 \text{ mm} \times 0.2 \text{ mm}$ (0.04 mm^2) square cross section at the center of the syringe, which we have chosen to roughly approximate the volume of fluid at the center of the syringe immediately prior to being pumped out of the syringe into the catheter with a 27 gauge needle fitting (0.2 mm inner diameter).

With each run of the model, it was assumed there were 10^4 microbubbles randomly distributed throughout the syringe. A Gaussian distribution was utilized to approximate the diameters of the microbubble population. Results were obtained by running the model 10 times with different distributions within the syringe, and the results were averaged for any given microbubble size, type of suspension fluid, and mixing rate. The model was implemented in MATLAB (The Mathworks Inc., MA, USA).

SIMULATION RESULTS

Effect of Bubble Size

For the first part of the simulations, the influence of the microbubble size on the rate of floatation without mixing was investigated. The percent change in the microbubble population with respect to the initial values at the center of a 3 mL syringe (8.6 mm diameter) and in Tris + 10% Glycerol, 10% Propylene Glycol solution was evaluated over 16 minutes, which was the time range considered for our in-vitro experiments. Four different microbubble populations with mean diameters of 1, 2, 3 and 5 μm were compared. As microbubble diameter increased, floatation had a more dramatic effect on concentration. The concentration at the center of the syringe for populations with mean diameters of 1, 2, 3 and 5 μm was estimated to be $96 \pm 5\%$, $78 \pm 8\%$, $48 \pm 12\%$, and $25 \pm 9\%$ of the initial value over 4 minutes and $77 \pm 7\%$, $33 \pm 9\%$, $19 \pm 6\%$, and $12 \pm 7\%$ of the initial value over 16 minutes, respectively (Fig. 3a). The change in the mean diameter of the microbubble populations illustrated a similar pattern. The mean diameter of microbubbles with a size distribution of 1, 2, 3 and 5 μm within the cross-section at the center of a 3 mL syringe was predicted to be $99 \pm 1\%$, $89 \pm 3\%$, $75 \pm 7\%$, and $62 \pm 9\%$ of the initial mean diameter over 4 minutes and $89 \pm 3\%$, $70 \pm 12\%$, $50 \pm 14\%$, and $29 \pm 13\%$ over 16 minutes respectively (Fig. 3b).

Simulations indicated that when the syringe was mixed at a rate of 15 rpm, there would be an increase in the number of microbubbles remaining at the center of the syringe. It was estimated that the concentration of the microbubbles with a mean diameter of 1, 2, 3 and 5 μm was greater by $6 \pm 8\%$, $23 \pm 7\%$, $52 \pm 11\%$, and $73 \pm 13\%$ over 4 minutes, and greater by $23 \pm 7\%$, $68 \pm 13\%$, 71

$\pm 11\%$, and $42 \pm 9\%$ over 16 minutes due to mixing, than in the unmixed case (Fig. 3a.). The size distribution of microbubbles in a mixing syringe also decreased less, with the mean diameter greater than the unmixed case by $3 \pm 3\%$, $12 \pm 4\%$, $25 \pm 7\%$, and $36 \pm 11\%$ over 4 minutes, and by $12 \pm 3\%$, $31 \pm 12\%$, $44 \pm 14\%$, and $48 \pm 13\%$ over 16 minutes (Fig. 3b).

Effect of Microbubble Suspension Fluid

For a microbubble population of $1 \mu\text{m}$ in mean diameter, the effect of suspension fluid was analyzed in a 3 mL syringe with no mixing involved. The simulations were run for 3 different densities and viscosities of microbubble suspension fluids including PBS solution ($\rho = 1000 \text{ kg/m}^3$; $\mu = 0.00095 \text{ kg/m.s}$), Tris + 10% Glycerol, 10% Propylene Glycol solution ($\rho = 1033 \text{ kg/m}^3$; $\mu = 0.00188 \text{ kg/m.s}$), and Tris + 20% Glycerol, 10% Propylene Glycol solution ($\rho = 1056 \text{ kg/m}^3$; $\mu = 0.00266 \text{ kg/m.s}$). Simulations indicated that the concentration of the microbubbles in PBS was reduced to $94 \pm 5\%$ of the initial concentration over 4 minutes, whereas there was minimal change (less than 2%) in the microbubbles suspended in the 10% and 20% glycerol solutions. Over 16 minutes, we estimated a reduction in microbubble concentration to $52 \pm 9\%$, $83 \pm 11\%$, and $92 \pm 9\%$ of the initial concentration, for PBS, 10% glycerol, and 20% glycerol, respectively (Fig. 4a).

The size distribution of microbubble populations at the center of an unmixed syringe in 10% Glycerol or 20% Glycerol did not change notably over 4 minutes, whereas mean diameter of microbubbles in PBS decreased to $97 \pm 4\%$. The mean diameters of microbubbles in these 3 solutions decreased to $76 \pm 5\%$, $89 \pm 5\%$ and $95 \pm 5\%$ of their initial mean diameters respectively over 16 minutes (Fig. 4b).

The addition of syringe mixing offset the concentration decrease for bubbles in PBS over 4 minutes, retaining an additional $6 \pm 5\%$ of the distribution. Similarly, at 16 minutes, the PBS, 10% glycerol, and 20% glycerol solutions were $47 \pm 9\%$, $16 \pm 13\%$, and $6 \pm 5\%$ greater than the unmixed case, respectively (Fig. 4a), resulting in maintenance of the original concentration. The mean diameter of microbubbles in PBS, 10%, and 20% glycerol solutions increased by $21 \pm 9\%$, $9 \pm 6\%$, and $3 \pm 5\%$, respectively, above the unmixed case, over 16 minutes.

Changing the mixing rate from 15 rpm to a faster rate of 30 rpm or to a slower rate of 3 rpm did not substantially change the number of microbubbles in the center of the syringe.

EXPERIMENTAL RESULTS

Effect of Suspension Fluid and Mixing

Particle sizing measurements at the initial time points indicated that the microbubble population tested had an initial mean diameter of approximately $1 \mu\text{m}$. Microbubbles solutions pumped out of the syringe mixer were observed to decrease in both concentration and mean diameter over time, although the higher viscosity solutions substantially reduced loss in microbubble concentration and size. After 4 minutes of injection from a 3 mL syringe without mixing, the concentration of microbubbles decreased to $82 \pm 8\%$ and $88 \pm 6\%$ of the initial concentration for PBS and 10% glycerol, respectively (Fig. 5a). There was no notable change in the 20% glycerol solution. Over 16 minutes, the concentration decreased to $65 \pm 8\%$, $78 \pm 8\%$, and $88 \pm 6\%$ of the initial concentration for PBS, 10% glycerol, and 20% glycerol, respectively. Microbubble mean diameters decreased to $92 \pm 4\%$, $94 \pm 5\%$, and $91 \pm 5\%$ of the initial over 4 minutes and to $84 \pm 3\%$, $89 \pm 10\%$, and $86 \pm 7\%$ of the initial over 16 minutes, respectively (Fig. 5b).

With the addition of mixing, the concentration of microbubbles was maintained at the approximately the initial concentration over 4 minutes in the 10% and 20% glycerol buffers, and at $92 \pm 12\%$ of the initial concentration in PBS. After 16 minutes, concentrations were $78 \pm 3\%$ and $85 \pm 9\%$ of their initial values for PBS and 10% glycerol, respectively, whereas the

final bubble concentration in the 20% glycerol buffer remained at nearly 100% of the initial value.

The 10% glycerol solution was observed to most closely preserve the microbubble population diameter, with changes in mean diameter of $94\pm5\%$ and $89\pm10\%$, over 4 and 16 minutes respectively without mixing. With mixing, it was observed that the mean diameter of the microbubbles increased to slightly greater than measured initially ($103\pm4\%$) at 4 minutes, and mean diameter was $98\pm13\%$ of the original over 16 minutes (Fig. 5b). The application of mixing had a negligible effect on mean microbubble diameter in the 20% glycerol solution.

Effect of Syringe Diameter and Dilution

The 3 mL syringe with a PBS buffer was chosen as the standard for these experiments, however, switching the size of the syringe was found to have a small effect on the bubble distribution during a continuous injection. Both with and without mixing, contrast agents pumped from the larger 10 mL syringe appeared to have a higher concentration than with the smaller 3 mL syringe. Over 4 minutes, the concentration of microbubbles pumped from the 3 mL syringe was $82\pm7\%$ without mixing and $92\pm12\%$ with mixing, compared to $88\pm6\%$ without mixing, and almost no change in concentration from the original with mixing (Fig. 6a). There were only very small changes in mean diameter as a function of syringe size (Fig. 6b).

DISCUSSION

By the use of simulations, it was demonstrated that changes in microbubble population at the center of a syringe were mainly dependent on the viscosity and density of the microbubble suspension fluid as well as the size of the microbubbles. Without mixing, we estimated that a population of microbubbles with a mean diameter of $1\text{ }\mu\text{m}$ would be reduced to 96% of the original concentration, whereas a population of microbubbles with a mean diameter of $5\text{ }\mu\text{m}$ would be reduced to 25% of its initial concentration. Although the population of bubbles experimentally studied for this manuscript had a mean diameter near $1\text{ }\mu\text{m}$, simulations indicate that our findings would be much more significant for contrast agents having larger diameters. For each diameter range, simulations indicated that the addition of syringe mixing should reduce changes in microbubble concentration and size distribution over time.

The experimental results were in agreement with simulations, however, experimental data also indicated microbubble loss that was not predicted in the model. We hypothesize that this additional microbubble loss was due to the natural demise of microbubbles due to gas diffusion into solution and other microbubble destruction mechanisms which were not accounted for. The change in microbubble population due to non-ultrasound induced microbubble decay was outside of the scope of this manuscript, but is being explored by other researchers (Borden and Longo 2002, Lozano and Longo 2009).

The concentration of microbubbles diluted in PBS changed by 20% over the first 4 minutes of injection without mixing. We hypothesize that a change in population of this magnitude would influence accuracy in a quantitative contrast imaging exam. Indeed, the Lohmaeir study (2004) illustrates a significant decrease in scattered ultrasound intensity from a perfusion phantom over several minutes using a horizontal stationary syringe.

Simulations and experimental results demonstrated that both the use of a dilution buffer incorporating a viscosity agent and the use of a mechanical syringe mixer were able to better maintain the concentration and size distribution of the contrast agents over time. The mechanical mixer by itself improved the consistency of the distribution of contrast agents pumped from the syringe – however, we were surprised to find that a similar result could be achieved by simply using the Tris-glycerol buffer without mixing. One reason for the improved

consistency could be the reduced microbubble floatation velocity in the higher viscosity medium. However, it is likely that the addition of the propylene glycol and glycerin also had a stabilizing effect directly on the microbubbles. Barrack (2009) and Talu (Talu et. al 2006) have also observed that microbubbles tend to be more stable in solutions including propylene glycol and glycerin. The combination of both a mixer and viscosity agents resulted in very good consistency of contrast agent concentration, and mixed solutions containing 20% glycerol buffer maintained the microbubble concentration at nearly the same as the original as far out as 16 minutes.

If PBS alone was to be used with a mechanical mixer, the application of a larger syringe appeared slightly better at keeping the contrast agents mixed. Although data was not shown, we did also repeat experiments with both 1:10 and 1:100 dilutions of microbubbles. The dilution did not have a notable effect on rate of change of contrast agent population.

Doubling the rotation speed to 30 rpm or reducing it to 3 rpm in our model predicted no notable impact on the microbubble concentration within the syringe. This result is in agreement with the experimental results reported by Lohmaier et al. (2004), and is likely due to the fact that the microbubble rise velocities are very slow relative to the mixing rate - the order of 0.56 $\mu\text{m/s}$ for a microbubble with a diameter of 1 μm .

One observation that should be noted was that after being added and diluted in the syringe, some of the microbubbles floated almost immediately to the top of the syringe. Based on our observations, these microbubbles were much greater than 10 μm in diameter, and were too buoyant to remain mixed in the syringe, regardless of the rotational or buffer parameters we evaluated. If the injection syringe was oriented vertically, rather than horizontally as in our studies, the initial aliquot pumped out of the syringe pump would likely include a population of these very large microbubbles. Hence, a horizontal injection orientation is recommended for consistent administration of contrast agents in the sub-10 μm diameter range.

The main limitations of this study included several simplifications in the model, such as the assumption that the fluid moves with the syringe without slipping, that the bubble velocity is constant, and that the bubble diameter distribution was Gaussian. Most microbubble distributions are not modeled accurately by Gaussian distribution, however, description of the mean and standard deviation was convenient for data presented in this manuscript, and we believe that this a reasonable approximation for a proof of concept. Experimental error arose from pipetting errors in transferring the microbubbles from the syringe output to the particle sizer, error in the particle sizer itself, and natural bubble disintegration over time. Under certain conditions, mean concentrations slightly greater than the initial concentration were observed. This was attributed to experimental measurement errors.

CONCLUSION

These results should help researchers and clinicians better understand the changes in a lipid-encapsulated ultrasound contrast agent population over time, and take the necessary actions to maintain consistent contrast agent concentration and distribution. In order to maintain maximum consistency with contrast agent distribution during a prolonged injection period, it is recommended that users consider both the application of a dilution buffer with greater viscosity than PBS and the use of a syringe mixing device.

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Figure 1.
Photograph of Genie Plus Pump with custom syringe mixer apparatus.

Figure 2.

Diagram of syringe cross-section illustrating forces acting on a microbubble which were considered in the model. Also illustrated are the axis of syringe rotation, and the center volume within which microbubble distribution was evaluated over time. Figure not to scale.

Figure 3.

Simulated change in concentration and mean diameter of microbubble populations with initial average diameters of 1, 2, 3 and 5 μm with and without mixing, as expressed as percent of the initial concentration and initial mean diameter.

Figure 4. Simulated change in concentration and mean diameter of microbubble populations with initial average diameter of 1 μm diluted in buffers containing PBS only, 10% glycerol, and 20% glycerol. Data are expressed as percent of the initial concentration and initial mean diameter.

Figure 5.

Experimentally measured change in concentration and mean diameter of microbubble populations with initial average diameter of $\sim 1 \mu\text{m}$ diluted in buffers containing PBS only, 10% glycerol, and 20% glycerol. Data are expressed as percent of the initial concentration and initial mean diameter.

Figure 6.

Experimentally measured change in concentration and mean diameter of microbubble populations with initial average diameter of $\sim 1 \mu\text{m}$ diluted in a PBS buffer in 3 mL and 10 mL syringes, with and without mixing. Data are expressed as percent of the initial concentration and initial mean diameter.