

## Predicting the Spontaneous Vaporisation of Nanodroplets

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### Introduction

Superheated perfluorocarbon droplets have been widely explored as agents for ultrasound imaging and therapy [1] and in other applications such as radiation dosimetry [2]. Submicron, or nano-, droplets offer a number of potential advantages over microbubble agents, e.g. longer circulation half-lives, higher surface area to volume ratio and the ability to perfuse the microvasculature more easily. A key challenge in the use of nanodroplets, however, is the need to avoid spontaneous vaporisation whilst keeping the energy required for acoustic activation within the range of pressures that can be used safely in humans. This is especially important for imaging applications. Perfluoropropane (C<sub>3</sub>F<sub>8</sub>) microbubbles can be condensed to form liquid nanodroplets [3] that offer a good trade-off between thermal stability and acoustic vaporisation threshold. Anecdotal reports, however, suggest that C<sub>3</sub>F<sub>8</sub> droplets can spontaneously vaporise, and may therefore pose a potential safety risk, especially in the presence of concurrent coalescence. The aim of this study was to build on recent theoretical models of droplet vaporisation [4] and investigate the probability of vaporisation as a function of temperature and interfacial tension.

### Methods

The spontaneous vaporisation of a droplet of radius  $r_D$  at temperature  $T$  is modelled as a Poisson process with rate

$$\lambda(r_D, T) = J(r_D, T) \cdot \frac{4}{3} \pi r_D^3$$

where  $J(r_D, T)$  is the volumetric nucleation rate. The half-life of a droplet is then

$$t_{1/2}(r_D, T) = \frac{\ln 2}{\lambda(r_D, T)}$$

while the volume fraction of vaporised droplets as a function of time is

$$\chi(t; T) = \frac{\int_0^t \int_0^\infty \frac{4}{3} \pi r_D^3 PSD(r_D) \lambda(r_D, T) \exp[-\lambda(r_D, T)\tau] dr_D d\tau}{\int_0^\infty \frac{4}{3} \pi r_D^3 PSD(r_D) dr_D}$$

where  $PSD(r_D)$  is the normalised droplet size distribution.

Classical nucleation theory (CNT) predicts a non-zero work of formation at the spinodal, which results in the underestimation of nucleation rates [5]. In order to make the CNT result for the nucleation rate consistent with the vanishing work of formation at the spinodal, we apply a correction factor  $F(r_D, T)$  to the surface tension [6].

The corrected nucleation rate is

$$J(r_D, T) = \frac{N_A}{v_L(T)} \sqrt{\frac{2F(r_D, T)\sigma_{CNT}(T)}{\pi m}} \exp\left[-\frac{F(r_D, T)^3 W_{CNT}^*(r_D, T)}{kT}\right]$$

where

$$W_{CNT}^*(r_D, T) = \frac{16}{3} \pi \frac{\sigma_{CNT}(T)^3}{[p_G(r_D, T) - p_L(r_D)]^2}$$

is the reversible work of formation of a critical nucleus,  $N_A$  is Avogadro's number,  $v_L(T)$  is the molar volume of the superheated liquid,  $\sigma_{CNT}(T)$  is the surface tension at a flat interface,  $m$  is the molecular mass,  $k$  is Boltzmann's constant,  $p_G(r_D, T)$  is the pressure inside the critical nucleus and  $p_L(r_D)$  is the pressure of the superheated liquid. The correction factor, which is equal to one at the binodal, where CNT is expected to hold exactly, and zero at the spinodal, is [6]

$$F(r_D, T) = \left[1 - A x(r_D, T) + \left(A - 1 - \frac{A^3}{108}\right) x(r_D, T)^2 - \frac{A^2}{6} x(r_D, T)^2 \ln x(r_D, T) + \frac{A^3}{108} x(r_D, T)^3\right]^{1/3}$$

where  $x(r_D, T)$  is a nondimensional degree of superheat defined as  $x(r_D, T) = \frac{p_G(r_D, T) - p_L(r_D)}{p_{G,S}(T) - p_S(T)}$

where  $p_{G,S}(T)$  is the pressure inside the critical nucleus at the spinodal, and  $p_S(T)$  the spinodal pressure. The parameter  $A \simeq 0.1481118$  is determined by fitting  $F(x)^3$  to  $W_{DFT}^*(x)/W_{CNT}^*(x)$ , where  $W_{DFT}^*(x)$  is the work of formation determined by density functional theory (DFT) for a Lennard-Jones fluid, using literature data [7]–[9].

The pressure of the superheated liquid is calculated using the Young-Laplace equation

$$p_L(r_D) = p_{ext} + \frac{2\sigma_D}{r_D}$$

where  $p_{ext}$  is the external pressure and  $\sigma_D$  the interfacial tension of the droplet-water interface. The pressure inside the critical nucleus is calculated using the Kelvin equation

$$p_G(r_D, T) = p_{sat}(T) \exp\left[-\frac{v_L(T)}{RT} (p_{sat}(T) - p_L(r_D))\right]$$

where  $p_{sat}(T)$  is the vapour pressure at a flat interface and  $R$  is the gas constant. The pressure  $p_{G,S}(T)$  inside the critical nucleus at the spinodal is calculated by replacing  $p_L(r_D)$  with  $p_S(T)$  in the expression for  $p_G(r_D, T)$  above. Vapour pressure, molar volume and spinodal pressure are predicted using a volume-translated Peng-Robinson equation of state [10], [11]. The temperature-dependent  $\sigma_{CNT}(T)$  was predicted using the model described in [12].

The droplets used in the serum stability experiments were manufactured by emulsification of 100  $\mu$ l of liquid perfluorobutane in 4 ml of 16:3:1 phosphate buffered saline/propylene glycol/glycerol containing 1,2-distearoyl-sn-glycero-3-phosphocholine and 1,2-distearoyl-sn-glycero-3-phosphoethanol-amine-N-

[methoxy(polyethylene glycol)-2000] in a 9:1 molar ratio at a final concentration of 4 mg/ml, using a probe sonicator (Q125, QSonica, USA) at 50% power for 3 minutes (20 kHz, 33% duty cycle) at  $-5$  °C. The droplets were then purified by three cycles of centrifugation at 11,000g for 6 min and resuspension in the same volume of phosphate buffered saline. A final concentration of  $10^9$  droplets/ml were stained with Laurdan (50  $\mu$ M), incubated in 10% fetal bovine serum and imaged at 5 min intervals in a Zeiss 780 confocal microscope (355 nm excitation laser, BP 450/50 emission filter, C-Apochromat 63x/1.2 objective).

## Results

The exact value of the interfacial tension of droplets obtained from the condensation of phospholipid-shelled microbubbles is not known, and could in fact be substantially lower than the equilibrium interfacial tension of 25 mN/m [13] because of monolayer compression. We investigated a range of interfacial tensions  $\sigma_D$  between 0 and 25 mN/m, and found that half-lives of a few seconds to minutes at 37 °C are predicted for submicron  $C_3F_8$  droplets for values of the interfacial tension lower than about 10 mN/m (figure 1a). These results are in contrast with Mountford's model [4], which predicts long-term stability across the entire range of interfacial tensions (figure 1b).

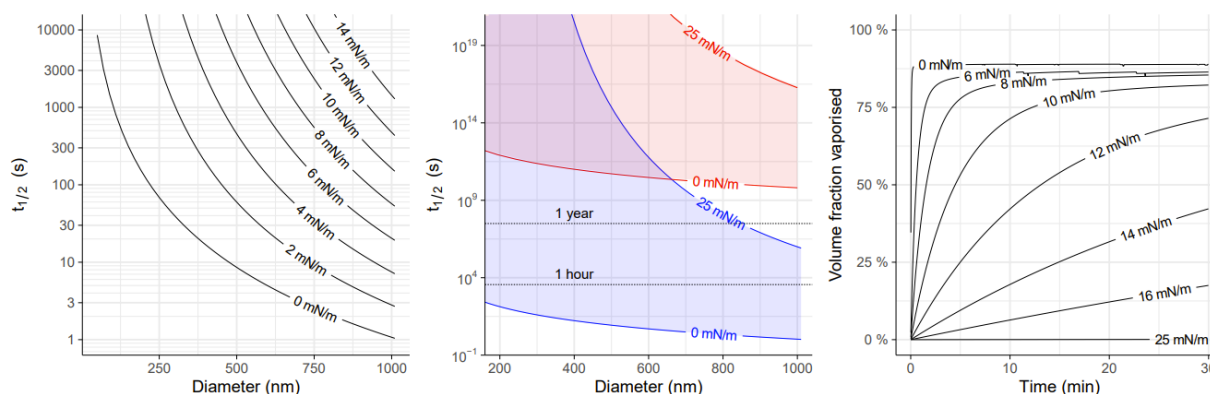


Figure 1. a) Predicted half-life of  $C_3F_8$  droplets at 37 °C as a function of diameter and droplet-water interfacial tension; b) Range of half-lives predicted by our model (blue) and [4] (red) at 37 °C for droplet-water interfacial tensions between 0 and 25 mN/m; c) Time evolution of the volume fraction of vaporised droplets at 37 °C for a starting population with lognormal size distribution (median diameter 400 nm, coefficient of variation 50%).

We also modelled the time evolution of the volume fraction of vaporised droplets at 37 °C starting from a polydisperse size distribution of median diameter 400 nm modelled as lognormal with coefficient of variation 50%. A substantial fraction of the total droplet volume, reaching and even exceeding 50%, can be vaporised within typical *in vitro* and *in vivo* experimental time scales in a physically plausible range of interfacial tensions (figure 1c). It is important to note that our predictions do not take into account the possibility of heterogeneous nucleation, which would further increase the vaporisation rate.

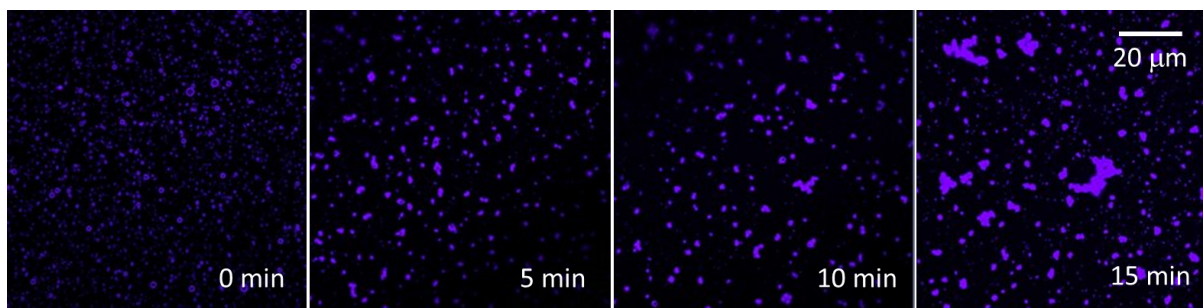


Figure 2. Time course confocal microscopy images of Laurdan-stained perfluorobutane droplets in fetal bovine serum at 37 °C.

During our experimental investigations of droplets stability, we also observed accelerated vaporisation of perfluorobutane droplets in fetal bovine serum compared to phosphate buffered saline (PBS) at 37 °C. Confocal microscopy of fluorescently labelled droplets revealed the formation of large aggregates within 15 minutes of incubation, which, if followed by coalescence, would be a possible explanation for the observed vaporisation behaviour. (figure 2).

## Conclusions

Acoustically activatable nanodroplets show great potential but there are challenges for their clinical translation, especially in diagnostic applications. Anecdotal observations of spontaneous vaporisation of C<sub>3</sub>F<sub>8</sub> droplets have remained unexplained so far. In this study, however, we find that the rate of spontaneous vaporisation at 37 °C can be substantially higher than previously predicted. A model has been derived which predicts the half-life of a population of C<sub>3</sub>F<sub>8</sub> droplets and the corresponding increase of the vaporised volume fraction over time, showing the potential for vaporisation to proceed to a significant degree within an experimentally relevant time scale. We have also shown accelerated aggregation of perfluorobutane droplets in fetal bovine serum, which could explain their increased vaporisation rate when compared to PBS. Further work is needed to develop the model to include heterogenous nucleation and predict acoustic activation thresholds. The inherent instability of low boiling point perfluorocarbon droplets should be taken into account in preclinical work and, most importantly, in clinical translation.

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