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# Kinetics and thermodynamics of acoustic release of doxorubicin from non-stabilized polymeric micelles

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ABSTRACT

This paper studies the thermodynamic characteristics of ultrasound-activated release of Doxorubicin (Dox) from micelles. The release and re-encapsulation of Dox into Pluronic® P105 micelles was measured by recording the fluorescence of a solution of 10 μg/ml Dox and 10 wt% P105 polymer in phosphate-buffered saline, during and after insonation by ultrasound at three temperatures (25 °C, 37 °C and 56 °C). The experimental data were modeled using a previously published model of the kinetics of the system. The model was simplified by the experimental measurement of one of the parameters, the maximum amount of Dox that can be loaded into the poly(propyleneoxide) cores of the micelles, which was found to be 89 mg/ml PPO and 150 mg Dox/ml PPO at 25 °C and 37 °C, respectively. From the kinetic constants and drug distribution parameters, we deduced the thermodynamic activation energy for micelle re-assembly and the residual activation energies for micelle destruction. Our model showed that the residual activation energy for destruction decreased with increasing acoustic intensity. In addition, higher temperatures were found to encourage micelle destruction and hinder micelle re-assembly.



## 1. Introduction

Therapeutic ultrasound (US) has recently been applied for the site-specific delivery of drugs and genetic material. The advantages of US-activated drug and gene delivery include the ability to focus ultrasound on a target tissue [1], the ability to both visualize the tumor and activate the release with the same instrument [2,3], and perhaps most importantly the minimal stress to the patient because there is no surgery or pain associated with the transdermal delivery of acoustic energy. Although US can be used to deliver heat for hyperthermic therapy, most applications that involve drug or gene delivery are done at relatively low intensities and with a minimal deposition of thermal energy. Instead, the therapy relies on non-thermal "mechanical effects", such as radiation pressure and bubble cavitation [1,4]. In brief, radiation pressure is a phenomenon in which acoustic pressure is used to push bubbles, liposomes or emulsions within a tissue, such as pushing microbubbles against the side of a blood vessel [5,6]. Cavitation is the formation and oscillation of bubbles within a fluid medium. Ultrasound can excite these bubbles sufficiently to enhance convection and shear stress near the bubble, or to cause bubble collapse and destruction. The latter has been correlated with the delivery of drugs and genes to cells [1,4,7–16].

The targeted delivery of material using ultrasound has been reported for both *in vitro* and *in vivo* systems. Our group has been investigating the use of ultrasound to release Doxorubicin (Dox) from polymeric micelles to cancerous tissue *in vitro* [8,17–22] and *in vivo* [23–25]. We and others have shown that US is able to release chemotherapy drugs from the cores of the micelles in therapeutically significant amounts [17,18,26,27]. Hosseinkhani and Tabata's research group has similarly shown that ultrasound can be used successfully in gene delivery [28–35]. Our work showed that the amount of drug release increases as the acoustic intensity is increased or as the frequency is decreased [8,18,22].

In a previous publication, we reported the results of a novel kinetic model involving US and bubble cavitation that was capable of representing the kinetics of Dox release from unstabilized Pluronic® P105 micelles under ultrasonic stimulus [36]. The model attempted to capture drug-release kinetics and involved several parameters such as rates of micelle destruction, micelle assembly, drug re-encapsulation, nuclei destruction, and the maximal loading of Dox in micelles.

The transient release and re-encapsulation are processes in which concentration gradients and possibly some fluid convec-

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tion operate to transport the drugs from the carrier to the cell cytosol. These are non-equilibrium phenomena. As such, it is informative and beneficial (in the aspect of drug carrier design) to obtain or derive information related to these processes. For example, it would be useful to know the partition coefficient or the equilibrium distribution of drug between the interior of the micellar carrier and the surrounding aqueous environment. Such data would aid in designing formulations and estimating the expected amount of drug release. Although there is an abundance of mathematical models describing ultrasonic cavitation and destruction of microbubbles [37–42] and liposomes [43–50], there are very few models describing the perturbation of micelles and the associated release of drug.

Herein we use of the above-mentioned drug-release model to represent the acoustic release and re-encapsulation kinetics of Pluronic® P105 micelles at various power densities and temperatures. Then we use the calculated kinetic constants at different temperatures to deduce some thermodynamics parameters relating to micellar destruction, re-assembly and re-encapsulation, including values of the activation and residual activation energies of ultrasonic-induced drug release from micelles.

## 2. Materials and methods

### 2.1. Drug encapsulation in Pluronic® unstabilized/stabilized micelles

Stock solutions of Pluronic® P105 (gift from BASF, Mount Olive, NJ) were prepared by dissolving the polymer in a PBS (phosphate-buffered saline) solution to a final concentration of 10 wt%. Dox (Pharmacia & Upjohn Company Kalamazoo MI) in dosage form (1:5, Dox:lactose) was dissolved into the P105 solutions at room temperature to produce a final Dox concentration of 10 μg/ml in 10 wt% Pluronic®. As a control, the same drug concentration was also prepared in PBS [8,22].

### 2.2. Measuring ultrasound-triggered release of Dox from Pluronic® P105 micelles

The fluorescence properties of Dox are used to calculate the amount of its release from micelles. A custom ultrasonic exposure chamber with fluorescence detection was used to measure the release of Dox from Pluronic® micelles at three power intensities, at 70 kHz, and three temperatures [22]. An argon-ion laser was directed into an acoustically transparent tube containing the micellar solution to be sonicated. As the drug molecules were excited at 488 nm their fluorescence was collected using a coaxial fiber optic collector. The detector signal was digitized for computer storage and processing. It is well established that fluorescent drugs are able to fluoresce more in hydrophobic than in aqueous environments. Thus, Dox being an aromatic compound, it exhibits a decrease in fluorescence when it leaves the hydrophobic interior of the micelle to interact with surrounding water molecules. By measuring the fluorescence of Dox in PBS (baseline or 100% release) and in 10% Pluronic® solution (100% encapsulation or 0% release), one can calculate the amount of Dox released ultrasonically into water from the decrease in fluorescence. Ultrasound at 70 kHz was generated in a Sonicor 100 bath (Copiague, NY). The intensity was controlled using a variac and monitored using a hydrophone (Bruel and Kjaer model 8103, Decatur, GA). During insonation, fluorescence decreased as expected. Ultrasound was manually turned on for 2 s and then turned off for 2 s. The fluorescence of eight cycles was recorded at each ultrasonic intensity. Although release experiments were conducted at 15 different power densities, in this paper we analyzed the data collected at the highest three acoustic intensities where the most significant release was observed. For more details refer to our previous article [8].

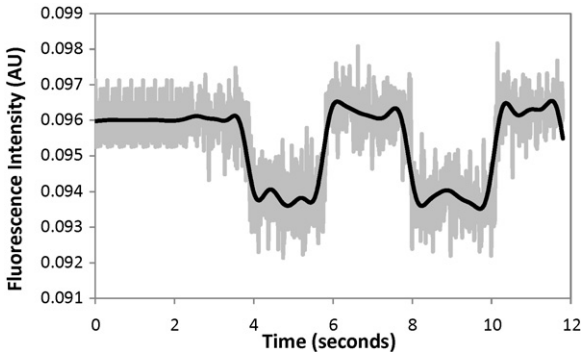


**Fig. 1.** Raw and filtered fluorescence data at 25 °C and 0.765 W/cm$^2$ at 70 kHz. Light gray points show raw data, and the solid black line shows the same data filtered using wavelets.

### 2.3. Data analysis

The raw fluorescence data exhibit large levels of high frequency noise. Hence, an efficient filtering (denoising) method is needed to remove this noise while preserving the important transitions that represents the rapid change in fluorescence. Conventional low pass filtering techniques do not offer a viable solution to this problem since the signal transitions will be severely blurred. As such, wavelet transform is well suited to the denoising of signals with sharp transients. A multi-level wavelet transformation is done to obtain the wavelet coefficients that represent the various frequency sub-bands of the signal. These coefficients are then thresholded to remove the high frequency noise and, at the same time, preserve the important transitions in the signal. Fig. 1 shows the result of filtering the high frequency noise of the raw florescence data via wavelet denoising.

After wavelet denoising, the eight cycles were averaged for each temperature and acoustic intensity. The timing for each cycle was set at zero at the point of the initial rapid decrease in fluorescence. The eight cycles were overlayed and averaged from zero to 1.5 s to provide the release portion of the data. Fig. 2a illustrates the preprocessing of fluorescence data at 56 °C and 0.675 W/cm$^2$.

Since the pulsing of ultrasound was done manually, the cycle time was not precisely 2 s. For this reason, the re-encapsulation portion, occurring after the ultrasound is turned off, does not precisely coincide for all eight cycles. Instead, the eight cycles were overlayed a second time, to align the re-encapsulation portion, and averaged from 1.1 to 2.2 s. This is illustrated for 56 °C and 0.675 W/cm$^2$ in Fig. 2b. The final averaged signal for the entire 2-s cycle was obtained by averaging the release and re-encapsulation portions, including the overlapping section between 1.1 and 1.5 s.

### 2.4. Measuring the amount of drug at saturation

The maximum amount of Dox which can be encapsulated in a 10% P105 solution, $E^{sat}_{tot,o}$, was estimated by first measuring the concentration of Dox for the entire solution at saturation, $[Dox]^{sat}$, which includes both the encapsulated drug and the drug dissolved in the PBS solution. This was accomplished by adding Dox in 2 mg increments to a 10% P105 solution until a precipitate was observed. After every addition of the drug, the resulting solution was vortexed and observed for any drug settling at the bottom. In 2 ml of 10% P105, 11.22 mg of Dox dissolved before any precipitate was observed at 25 °C, while at 37 °C, it took 18.83 mg of Dox to saturate 2 ml of a 10% P105 micelle solution.