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Factors affecting acoustically triggered release of drugs from polymeric micelles

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Abstract

A custom ultrasonic exposure chamber with real-time fluorescence detection was used to measure acoustically-triggered drug release from Pluronic P-105 micelles under continuous wave (CW) or pulsed ultrasound in the frequency range of 20 to 90 kHz. The measurements were based on the decrease in fluorescence intensity when drug was transferred from the micelle core to the aqueous environment. Two fluorescent drugs were used: doxorubicin (DOX) and its paramagnetic analogue, ruboxyl (Rb). Pluronic P-105 at various concentrations in aqueous solutions was used as a micelle-forming polymer. Drug release was most efficient at 20-kHz ultrasound and dropped with increasing ultrasonic frequency despite much higher power densities. These data suggest an important role of transient cavitation in drug release. The release of DOX was higher than that of Rb due to stronger interaction and deeper insertion of Rb into the core of the micelles. Drug release was higher at lower Pluronic concentrations, which presumably resulted from higher local drug concentrations in the core of Pluronic micelles when the number of micelles was low. At constant frequency, drug release increased with increasing power density. At constant power density and for pulse duration longer than 0.1 s, peak release under pulsed ultrasound was the same as stationary release under CW ultrasound. Released drug was quickly re-encapsulated between the pulses of ultrasound, which suggests that upon leaving the sonicated volume, the non-extravasated and non-internalized drug would circulate in the encapsulated form, thus preventing unwanted drug interactions with normal tissues.

Keywords: Polymeric micelles; Triggered release; Ultrasound; Pluronic; Micellization; Drug delivery; Doxorubicin; Ruboxyl

1. Introduction

The ideal scenario for targeted drug delivery would be to sequester the drug within a container where it could remain until released in a spatially and temporally controlled manner. From the technological perspective, the most attractive drug carriers are polymeric micelles formed by hydrophobic– hydrophilic block copolymers, with the hydrophilic blocks comprised of poly(ethylene oxide) (PEO) chains. These micelles have a core-shell structure with the hydrophobic block forming the core of the micelle and the hydrophilic PEO block (or blocks) forming the shell. Several advantages of these sys tems over other types of drug carriers include: (1) long circulation time in blood because of their resistance to opsonization and clearance by the reticulo-endothelial system (RES); (2) appropriate size (10–30 nm) to escape renal clearance but to allow for the extravasation at the tumor site; (3) simplicity in drug incorporation, compared to covalent bonding of the drug to the polymeric carrier; (4) drug delivery independent of drug character [1]. However, only a few known block copolymers form micelles in aqueous solutions. Their application for drug delivery was studied by Kataoka et al. [1–6] and Kabanov et al. [7–11]. Kabanov et al. reported the

enhanced intracellular uptake and enhanced cytotoxicity of doxorubicin (DOX) delivered with Pluronic copolymers [7–11]. Pluronic is a triblock PEO–PPO–PEO copolymer, where PPO designates poly(propylene oxide); the hydrophobic central PPO blocks form the micelle cores, whereas the flanking PEO blocks form the shell, or corona, which protects the micelles from recognition by the RES. Pluronic structure in aqueous solutions has been extensively investigated by many authors and reviewed by Alexandridis [12]. The phase state of Pluronic micelles can be controlled by choosing members of the Pluronic family with appropriate molecular weight and PPO/PEO block-length ratio, and by adjusting the concentration [12–14]. The hydrodynamic radii of Pluronic micelles at physiological temperatures range between 10 and 20 nm, which make them candidates as potential drug carriers.

Kabanov et al. observed hypersensitization of DOX-resistant cells when the drug was delivered from Pluronic P-85 solutions at concentrations corresponding to copolymer unimers [9]. However, the efficiency of the drug/Pluronic systems dropped above the critical micelle concentration (CMC). Kabanov et al. concluded that the efficiency of Pluronic delivery systems was based on the presence of Pluronic unimers.

The drop in the efficiency of drug/Pluronic systems above the CMC may result from the recently reported substantial decrease in the intracellular drug uptake from Pluronic micelles [15–17]. This phenomenon may be used advantageously to prevent unwanted drug interactions with healthy cells. However, the challenge is to enhance drug uptake at the tumor site, in which case the drug should either be released from micelles at the tumor site or be taken up together with the micelles by endocytosis. In our study we use ultrasound to increase intracellular drug uptake from (or together with) Pluronic micelles [15–18]. The advantage of using ultrasound is that ultrasound is non-invasive and can penetrate deep into the interior of the body, which optical techniques cannot provide. In previous research, ultrasound was shown to increase the permeability of cell membranes for polyethylene glycol [19] and other compounds [20]; it also enhanced the extravasation of an MRI contrast agent [21]. Ultrasonically enhanced intracellular drug uptake may be caused by the acoustically triggered release of drugs from micelles, upon which the drug is taken up by a regular mechanism specific for a particular drug/cell system. Another mechanism of acoustically activated drug uptake may be related to enhanced endocytosis of drug-sequestering polymeric micelles due to ultrasonically-enhanced permeability of cell membranes. The ultrasound-induced cell membrane permeabilization and poration has been proven [22,23]. In this paper we demonstrate that ultrasonication triggers drug release from micelles and report on the factors that control the extent of this effect. Two anticancer drugs of the anthracyclin family were used in this study: doxorubicin (DOX) and ruboxyl (Rb).

Their encapsulation and distribution in Pluronic micelles was reported earlier [24].

2. Materials and methods

2.1. *Measuring ultrasound-triggered release of DOX and Rb from Pluronic P*-105 *micelles*

The anthraquinone parts of Rb and DOX molecules are inherently fluorescent when excited at a wavelength of 488 nm, making them effective as fluorescent probes. However, Rb and DOX fluorescence is quenched by collisions with water molecules (dynamic quenching). Thus, when Rb and DOX molecules are prevented from collisions with water, for instance by their encapsulation in the hydrophobic core of micelles, their fluorescence increases two- to threefold [24]. This feature was used in this study to measure drug release from micelles under the action of ultrasound.

Real-time measurements of drug release were performed using a specially designed ultrasonic exposure chamber with fluorescence detection, shown schematically in Fig. 1 and described below. The apparatus employed a single-line argon-ion laser (Ion Laser Technology, Model 5500 A) whose beam was divided by a variable beam splitter (a graded metal-film neutral density filter). One portion of the beam was sent directly to a silicon photodetector (Newport Model 818-SL with 835 display) to monitor the laser power. The other portion was directed into the glass cuvette containing the trial solution to be sonicated.

Fluorescence of the drug was excited at an excitation wavelength of 488 nm with an optical power of |0.5 mW in a 2-mm-diameter beam. No photo bleaching

was observed at this light intensity, based on the constant level of drug fluorescence during continuing irradiation for 8 h. The drug release was quantified by measuring the changes in fluorescence emissions before, during, and after the ultrasound exposure. A fiberoptic probe (a sheathed bundle of multimode glass fibers, 3 mm entrance diameter, 0.6 numerical aperture, and 90 cm in length) was used to collect the fluorescence emission. The light passed through a dielectric bandpass filter with a 35 nm bandwidth centered at 535 nm (Omega Optical Model 535DF35) to a sensitive silicon detector (EG&G Model 450-1). The filter effectively cuts off emissions below 500 nm, including any Rayleigh-scattered laser light. The detector signal was digitized using a 12-bit A/D converter (National Instruments) and sent, along with the digitized monitor photodetector signal, to a Macintosh computer for

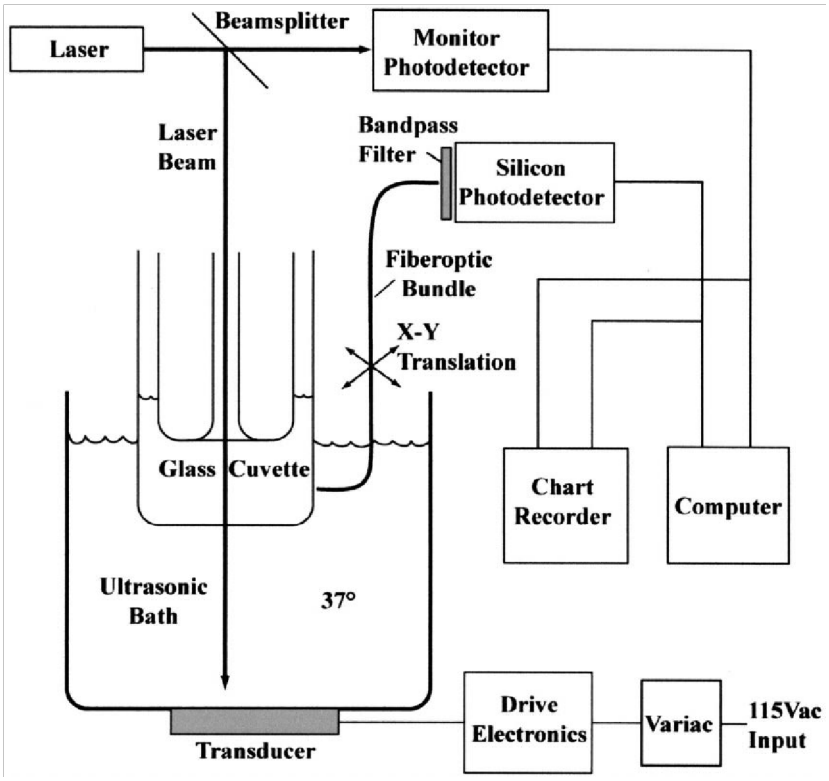

Fig. 1. Experimental arrangement for detection of the fluorescence of drug under ultrasonic exposure. For 20-kHz exposure, the transducer was controlled by different electronics and was inserted into the exposure bath from above.

storage and processing. The analog outputs of both photodetectors were also plotted on a strip chart recorder. The temperature of the ultrasonic exposure chamber was maintained at 378C by circulating thermostated water throughout the sonicating bath. The glass cuvette used to measure drug release had two open tubes for filling or removing drug solution and one sealed tube in the middle to allow the excitation beam to enter the solution through a flat stationary surface. This prevents any distortions that could otherwise arise from waves on the surface of the sonicated liquid. The main chamber of the cuvette was completely filled with the solution, and the excess liquid partially filled the side tubes.

The experimental procedure is described below. Firstly, the fluorescence intensity of the drug in phosphate buffered saline, PBS (F_{PBS}) was measured both with and without ultrasound exposure, with no significant change in the fluorescence intensity observed; then, without any changes in the experimental set-up, the PBS solution was carefully removed and replaced with the drug solution of the same concentration in Pluronic micelles. The base fluorescence intensity of the micellar solution (F_{mic})

was measured, after which CW or pulsed ultrasound was turned on. During the 'ultrasound on' phase, fluorescence dropped as shown in Fig. 2 due to drug release from the hydrophobic core of micelles into the aqueous environment. Digitized data were analyzed to calculate the percentage of drug release from micelles. To reduce the noise, the data were Fourier transformed, and a small magnitude narrow band noise of unknown origin and its next three harmonics were filtered out.

After Fourier filtering, the data were smoothed using a 10-point moving average. An example of the raw and filtered data is presented in Fig. 2 for DOX release from 10% Pluronic micelles.

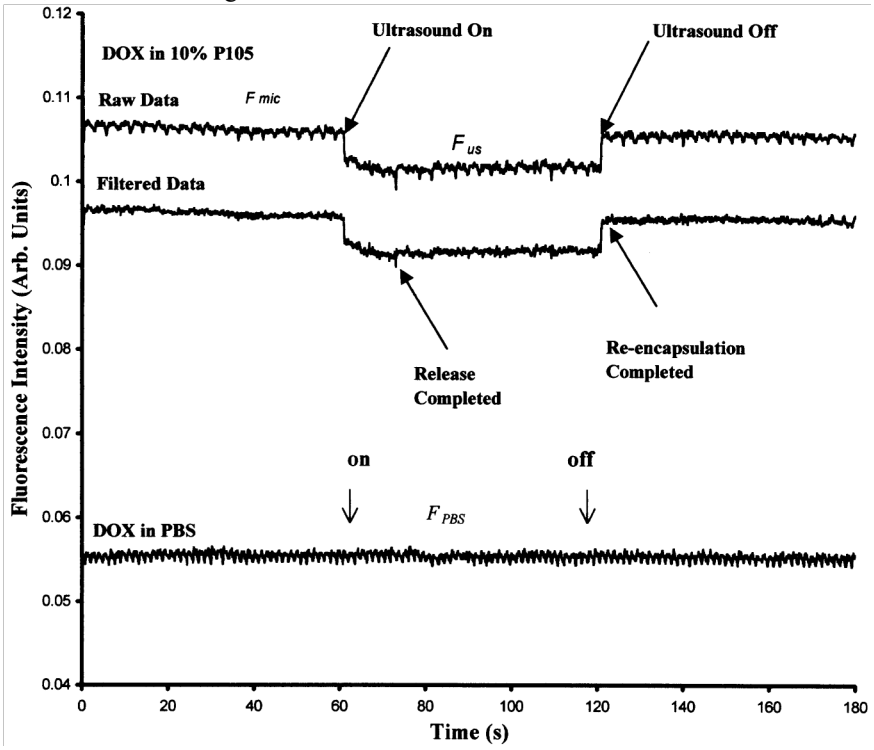

Fig. 2. Example of release profiles of DOX from a 10% Pluronic solution and from PBS. DOX concentration 6.7 mg/ml. Raw and Fourier-filtered data are presented for the 10% Pluronic solution. For the PBS solution, ultrasound was turned on at 60 s and off at 120 s; there was a negligible change of DOX fluorescence under sonication.

The percent of drug release was calculated assuming that F_{mic} - F_{PBS} corresponds to 100% drug release:

Release (%) = $[(F_{mic} - F_{us})/(F_{mic} - F_{PBS})] * 100\%$ (1)

where F_{us} is the fluorescence intensity during expo sure to ultrasound. In our experiments, no changes in the fluorescence spectra of Rb or DOX were observed after ultra sound exposure.

2.2. *Drugs*

Rb was kindly provided by Dr. Shapiro (Institute of Biochemical Physics, Moscow, Russia). DOX was supplied by the University Hospital, University of Utah, Salt Lake City, UT, USA.

2.3. *Micelle-forming polymer*

Pluronic P-105 has an average molecular weight of 6500; it comprises 56 PPO monomer units in the central block and 37 PEO units in each of the side blocks. Pluronic P-105 was kindly supplied by the BASF Corporation.

2.4. *Drug encapsulation in Pluronic micelles*

An aliquot of Rb stock solution in 1:1 C H OH/$_2$ acetone mixture was evaporated in a vacuum evaporator; PBS or Pluronic solution in PBS was added to the solid Rb residue to produce a final Rb concentration of 20 mg/ml. The system was vortexed for 30 s and then sonicated in a sonication bath operating at 90 kHz until Rb was completely dissolved, which usually took about 15 s.

DOX was introduced into PBS or Pluronic solution from a stock solution in PBS to produce a final concentration of 6.7 mg/ml or 5.0 mg/ml (which corresponds to 40 mg/ml and 30 mg/ml of the DOX
dosage form, respectively), followed by a short (15 s) sonication in a sonication bath operating at 90 kHz to facilitate drug encapsulation.

2.5. *Insonation*

We explored drug release as a function of ultrasound frequency in a low-frequency range, from 20 to 90 kHz; both CW and pulsed ultrasound was investigated. The ultrasound power density was varied from 0 to 3 W/cm^2 as measured by a hydrophone as described earlier [20].

The 20-kHz ultrasound was generated by a probe transducer (Sonics and Materials, Newton, CT) inserted into the water bath; sonication at 47 kHz was performed in a Cole–Parmer sonication bath (Cole–Parmer, Mount Vernon, IL); sonication at 67 and 90 kHz was performed in two different Sonicor SC 100 sonication baths (Sonicor Instruments, Copaique, NY). The power density was controlled by adjusting the AC input voltage with a Variac. The 20-kHz ultrasound probe was programmed to generate continuous wave (CW) or pulsed ultrasound of varying power densities and duty cycles; in the pulsed experiments we varied both 'ultrasound on' and 'ultrasound off' durations. For the sonication baths, pulses were generated by turning the instruments on and off manually.

2.6. *Ultrasound-induced radical formation*

Radicalsproduced upon collapse of transient cavitations were trapped with 5,5-dimethyl-1pyrroline-*N*-oxide (DMPO), a radical trap that forms relatively stable adducts with hydroxyl radicals [25].

DMPO was dissolved in PBS at a concentration of 0.1 M. The insonation was performed in darkness; upon the termination of insonation, an aliquot of solution was immediately frozen and kept in liquid nitrogen until the EPR recording.

3. Results and discussion

3.1. *Drug release under the continuous wave and pulsed ultrasound*

Using the ultrasonic exposure chamber with real time fluorescence detection, we measured drug release from micelles under CW or pulsed ultrasound in the frequency range of 20 to 90 kHz. Examples of the release profiles of drugs from

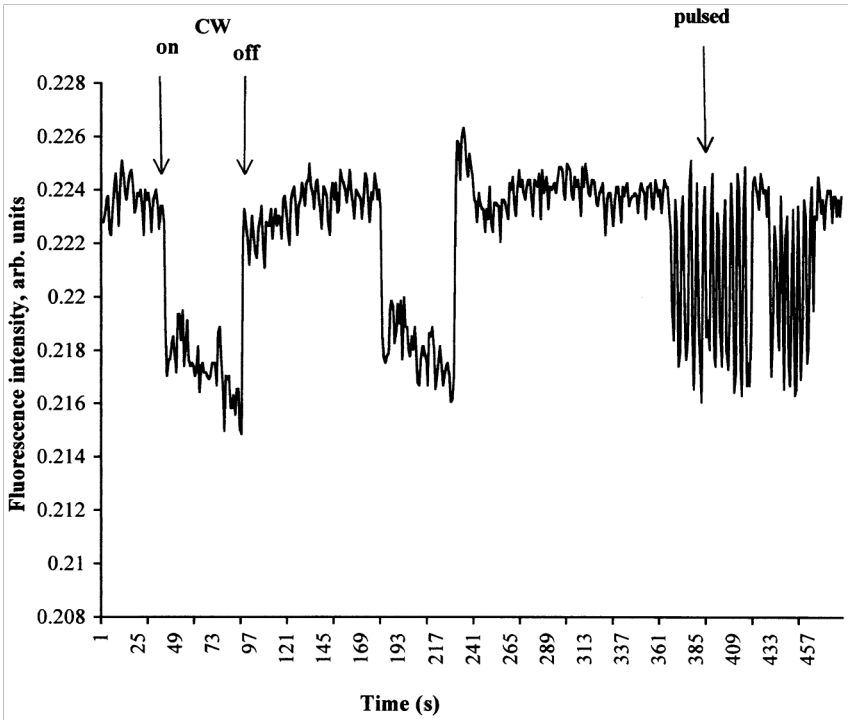

Fig. 3. Rb release profile from 10% Pluronic P-105 micelles at CW and pulsed sonication; Rb concentration 20 mg/ml; ultrasound frequency 47 kHz; power density 3.5 W/cm .[2]

10% Pluronic micelles are shown in Fig. 3 for Rb at 47-kHz sonication and in Fig. 4 for DOX at 20-kHz sonication, respectively; both CW and pulsed ultrasound with various duty cycles were explored.

The drop in fluorescence intensity during the 'ultrasound on' phase indicates drug release from the hydrophobic environment of Pluronic micelle cores into the aqueous environment, which may result either from ultrasound-induced drug diffusion out of micelles or from micelle degradation under sonication.

Figs. 3 and 4 reveal fast re-encapsulation of the released drug during the 'ultrasound off' phase of pulsed ultrasound. This is a satisfying finding because it suggests that upon leaving the sonicated volume, the non-extravasated and non-internalized drug would circulate in the encapsulated form, thus preventing unwanted interactions with normal tissues. This is supported by the negligible adsorption or binding of Rb to blood proteins (albumin, fibrinogen) reported elsewhere [26].

When pulse duration was longer than 0.5 s, only negligible differences were observed between the magnitude of the drug release under pulsed ultrasound and that under CW ultrasound (Figs. 3 and 4).

3.2. *Effect of ultrasound frequency*

We explored drug release as a function of ultrasound frequency in a low-frequency range, using the following transducers: 20 kHz, 47 kHz, 67 kHz, and 90 kHz. Drug release from micelles was most efficient at 20 kHz, despite low power densities; to get comparable release at higher frequencies, much higher power densities have to be used (Table 1). This is in agreement with the observations of the effect of ultrasonic frequency on the transdermal penetration of various drugs studied by Mitragotri et al. [27].

3.3. *Effect of power density*

For all frequencies studied, the release of drug increased with increasing power density (Table 2). This was true for both stationary release under CW

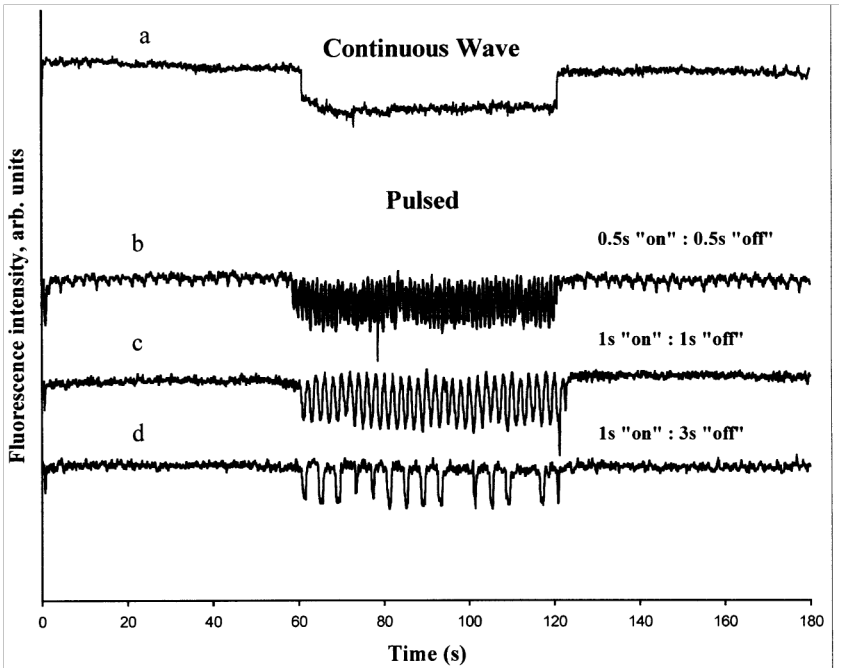

Fig. 4. DOX release profiles from 10% Pluronic P-105 micelles at CW (a) and pulsed (b–d) sonication; DOX concentration 6.7 mg/ml; ultrasound frequency 20 kHz, power density 0.058 W/cm ; pulse sequence: (b) 0.5 s on: 0.5 s off; (c) 1 s on: 1 s off; (d) 1 s on: 3 s off.[2]

Table 1
Effect of ultrasound frequency on DOX release from 10% Pluronic P-105 micelles; DOX concentration 6.7 mg/ml

Frequency	20 kHz			67 kHz			
Power density, W/cm^2	0.033	0.047	0.058	0.15	1.35	1.66	2.8
DOX release %	4.660.5	6.561.1	9.260.56	0	5.462.3	8.162.1	10.760.8

ultrasound and peak release under pulsed ultrasound (data not shown).

3.4. *Effect of drug lipophilicity*

At the same frequencies and power densities, the release of DOX from Pluronic micelles was noticeably higher than that of Rb (Table 2). This may be due to the deeper insertion of Rb into the interior of Pluronic micelles reported earlier [24]. This indicates that drug lipophilicity is an important factor determining the extent of acoustically activated drug release from micelles.

3.5. *Effect of Pluronic and drug concentration*

We did not observe much difference between Rb

Power density, W/cm² CW	Drug release (%)	
	DOX	Rb
1.35	5.462.3	0.860.25
1.66	8.162.1	3.260.9
2.8	10.760.8	5.561.5

Table 2
Effect of power density on Rb and DOX release from 10% Pluronic P-105 micelles; ultrasound frequency 67 kHz

or DOX) release from 10% and 1% Pluronic micelles; however, significantly higher release of Rb and DOX was observed from 0.1% solutions at all frequencies and power densities studied. For Rb, release was between 11 and 13% from 0.1% solution vs. 5.5% from 10% solution (at 67 kHz and 2.8 W/cm power density). Data for DOX at 20 kHz are presented in Fig. 5 (note that measurements of drug release from Pluronic solutions of low concentrations are somewhat less accurate than those for 10% or 1% solutions because of decreased differences between drug fluorescence in Pluronic and PBS). Higher drug release from Pluronic solutions of lower concentrations may be due to higher local drug concentration in the core of Pluronic micelles when the number of micelles is low, i.e. at Pluronic concentrations only slightly above the corresponding CMC (which is 0.03% for P-105 at 378C, based on data presented in Ref. [28]). This is corroborated by the finding that for the same Pluronic concentration of 10%, drug release indeed increased with increasing initial concentration of drug in the solution. At a concentrationof 6.7 mg/ml, DOX releasewas 10+- 1% (mean S.D.), while at a concentration of 5 mg/ml, the release was only 5.561% (at 67 kHz and 2.8 W/cm power density). The lower drug release at the lower drug concentration could be attributed to a higher ratio of PPO to DOX in the hydrophobic core of Pluronic micelles, which favors hydrophobic interaction. We postulate that increased hydrophobic interaction reduces the percentage of drug that can be released from the micelle core upon the application of ultrasound. This is confirmed by the above-mentioned lower release of Rb in comparison to DOX. At higher local drug concentrations in micelle cores, drug/PPO hydrophobic interactions are replaced by weaker drug/drug interactions, which facilitates drug release.

3.6. *Radical formation under sonication*

In parallel with measuring drug release, we measured the threshold for transient cavitation by trapping radicals that were produced upon the collapse of cavitation bubbles. Cavitation threshold increased with increasing ultrasound frequency; at 20 kHz, radicals were observed even at a power density as low as 0.01 W/cm , which is consistent with the relatively high efficiency of 20-kHz ultrasound for drug release from micelles. At 67 kHz, no radicals and no drug release were observed below a power density of 1.0 W/cm . These data are summarized in Table 3.

The data suggest that transient cavitation plays an important role in triggering drug release from micelles. We hypothesize that shock waves produced by transient cavitation events disrupt micelles and release drug into aqueous environment. During the ‘ultrasound off’ phase, the micelles are restored and drug is re-encapsulated.

Based on the results presented above, acoustically activated micellar drug delivery may develop into an effective therapeutic technology for targeted delivery of drugs to solid tumors.

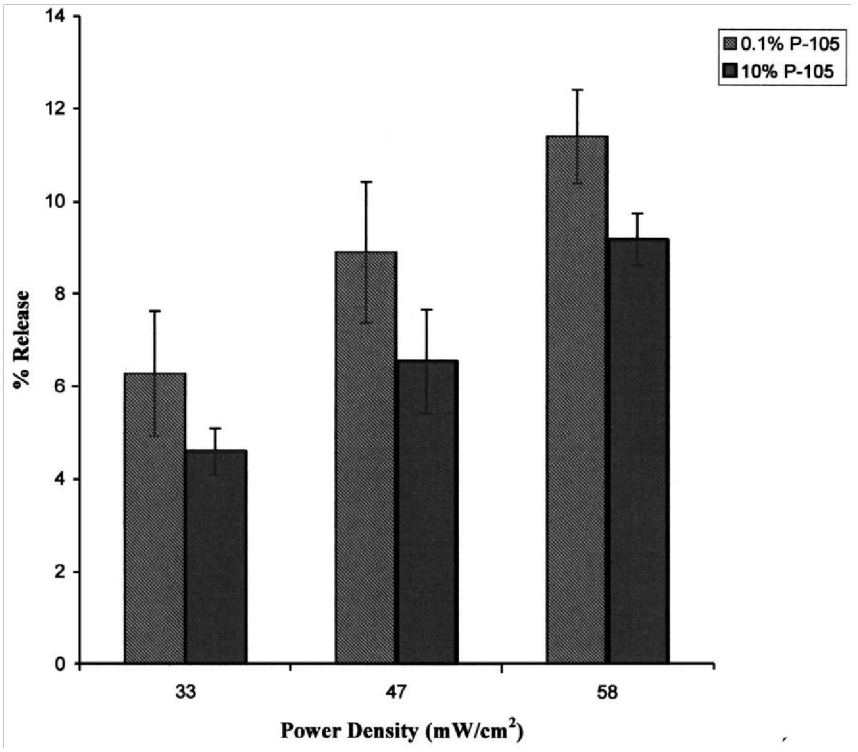

Fig. 5. DOX release from 0.1% and 10% Pluronic micelles at 20 kHz; DOX concentration 6.7 mg/ml.

Table 3

Correlation between the formation of transient cavitation and Rb release from Pluronic micelles

Ultrasound frequency, kHz	Power density, W/cm²	Radical formation	Drug release from Pluronic micelles
20	0.021	1	Traces
	0.033	1	1
	0.047	1	1
	0.058	1	1
47	3.54	1	1
67	0.15	2	2
	1.0	Traces	2
	1.35	1	1
	1.66	1	1
	2.80	1	1
90	0.22	2	2
	0.83	2	2
	1.66	Traces	Traces

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References