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Ultrasonic release of doxorubicin from Pluronic P105 micelles stabilized with an interpenetrating network of *N,N* diethylacrylamide

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Abstract

Pluronic P105 micelles sequester hydrophobic drugs and release them upon insonation with low frequency ultrasound; however these micelles dissolve relatively quickly upon dilution. The objective of this research was to determine whether stabilization of these micelles would compromise their ability to sequester and release drug. P105 micelles were stabilized with an interpenetrating network of poly (*N,N*-diethylacrylamide), and ultrasonically-activated release of doxorubicin (Dox) was measured by a fluorescence technique. Results showed that stabilized micelles sequestered the Dox and released it upon insonation at 70 kHz. The amount released was not significantly different from that released from P105 micelles (*P*50.481), and the drug re-encapsulation upon cessation of insonation was complete. This system has potential for controlled drug delivery to insonated tissues *in vivo*.

Keywords: Doxorubicin; Ultrasound; Fluorescence Measurement; Pluronic; Stabilized micelles; Plurogels; Micellar drug delivery

1 . Introduction

Micelles of Pluronic P105 effectively sequester hydrophobic drugs such as doxorubicin (Dox), and yet release the drug upon exposure to low frequency ultrasound [1–5]. However, when diluted below the CMC, the micelles quickly dissolve, which limits the potential *in vivo* use of this ultrasonically activated drug delivery system. The stability upon dilution of P105 micelles has been increased by polymerizing an interpenetrating network of *N,N*-diethylacrylamide (NNDEA) within the core of the P105 micelle [6]. However, it was uncertain whether the stabilized micelles (called Plurogels) would sequester and then release Dox upon exposure to ultrasound (US).

This note reports our investigation of the release of Dox from stabilized P105 micelles at 70 kHz. Stabilizing these micelles is a crucial requirement of this drug delivery system, since these micelles are diluted several-fold when injected into the blood stream.

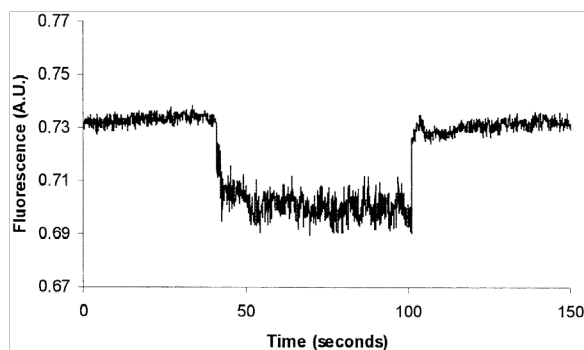


Fig. 1. Change in fluorescence upon insonation at 70 kHz and 2.8W/cm² of 10 mg/ml Dox in Plurogel.

2. Material and methods

Doxorubicin was obtained from the University of Utah Hospital (SLC, UT, USA) in a 1:5 mixture with lactose; it was dissolved in phosphate buffered saline (PBS) and sterilized by filtration through a 0.2-mm filter. Pluronic P-105 was provided by BASF (Mount Olive, NJ, USA); *N,N*-diethylacrylamide (NNDEA) was obtained from Polysciences (Warrington, PA, USA); *N,N*-bis(acryloyl)cystamine (BAC) was obtained from Fluka (Milwaukee, WI, USA); 2,2'-azobis(isobutyronitrile) (AIBN) was obtained from Aldrich (Milwaukee, WI, USA).

To form an interpenetrating network of poly-NNDEA in Pluronic P105 micelles, the following procedure was used [6]. First, a 40-ml aliquot of double distilled water containing 10 wt% P105 was added to a round-bottomed flask. NNDEA monomer was added to give a final concentration of 0.05 wt% monomer.

BAC was added as a cross-linking agent to give a BAC/NNDEA mol ratio of 1:20. AIBN Was added as an initiator, to give an AIBN/NNDEA mol ratio of 1:100. The flask was then connected to a water condenser and purged with nitrogen for at least 1 h without heat. The system was then allowed to polymerize for 24 h at 65 °C with magnetic stirring and a continuous nitrogen purge. Dox stock solution added at room temperature to the resulting Plurogel to make a final concentration of 10 mg/ml. A chamber built to measure the change in the level of fluorescence with and without the application of ultrasound, and to capture the real-time kinetics of drug release was described previously [1].

Ultrasound power was generated by a Sonicor SC-100 ultrasonating bath (Sonicor Instruments, Copaique, NY, USA) operating at 70 kHz. The various power densities insonation intensity was controlled by adjusting a variable a.c. transformer (variac). The insonation intensity as a function of applied voltage was determined using a calibrated hydrophone (Bruel and Kjaer Model 8103, Decatur, GA, USA). The temperature of the bath was maintained at 37 °C using a recirculating thermostatic bath.

3. Results and discussion

The fluorescence intensity of the Dox/Plurogel solution was in the same range as that of 10% P105 solution with the same concentration of Dox, indicating that Dox was sequestered in the core of the Plurogel. When the Dox/Plurogel solution was insonated, the fluorescence decreased, and when insonation stopped, all the fluorescence signal was recovered, as shown in Fig. 1.

Table 1 summarizes release percentages obtained as a result of insonating the Plurogel at various power densities at 70 kHz. The table shows that Dox release increased as the power density increased. Drug release from stabilized micelles was not significantly different from the release observed using Pluronic P105 micelles (P50.481) [1]. For example, 1.66 W/cm² and 70 kHz insonation released 8.2% from P105. All of the power densities used in this experiment were above the threshold of transient cavitation (about 1.0 W/cm² at 70 kHz) [1], and we suspect that transient cavitation plays an important part in the observed release [7].

Table 1

Dox release at various power densities

Power density (W/cm ²)	Release (%)	S.D. (%)
2	6.1	1.1
2.8	9.4	1.3
3.3	9.1	1.5

Our initial hypothesis was that covalent stabilization of the micelles would impede the release of drug by ultrasound. However, this study did not support this hypothesis. With both Pluronic micelles and stabilized Plurogel micelles, the ultrasonic energy produces similar release of Dox. The underlying mechanism is thought to involve cavitation events and the associated high shear forces that shear and disrupt the micelle structure, thus transiently releasing Dox. Once insonation is stopped, the fragments of Plurogel can reassemble and provide a hydrophobic core where the released Dox can be resealed. Thus, Dox is sequestered again even if it is now in a fragmented Plurogel micelle.

The results of these experiments are promising in that Dox from stabilized Plurogels was released at similar power densities to those observed with non-stabilized P105 micelles and reported earlier [1]. These micelles were shown to be stable at concentrations as low as 0.1% [6], suggesting that these stabilized micelles can be used to effectively sequester chemotherapeutic agents and deliver them under the action of ultrasound.

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