Annals of Biomedical Engineering, Vol. 37, No. 12, December 2009 (© 2009) pp. 2640–2645
DOI: 10.1007/s10439-009-9785-z

A Mathematical Model of Drug Release from Liposomes by Low Frequency Ultrasound

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(*Received 25 May 2009; accepted 19 August 2009; published online 3 September 2009*)

Abstract—Administration of drugs using small (<100 nm) unilamellar liposomes enables effective targeting of tumors and inflamed tissue. Therapeutic efficacy may be enhanced by triggering liposomal drug release in the desired organ in a controlled manner using a noninvasive external signal. Previous studies have demonstrated that low frequency ultrasound (LFUS) can be used to control the release of drugs from liposomes. LFUS irradiation has a twofold effect: (1) it causes the impermeable liposome membrane to become permeable and (2) it induces liposome disintegration. Immediately upon cessation of LFUS irradiation the membrane resumes its impermeable state and liposome disintegration stops. The mathematical model presented here is aimed at providing a better quantitative and qualitative understanding of LFUS-induced liposomal drug release, which is essential for safe and effective implementation of this technique. The time-dependent release patterns are determined by the liposome disintegration patterns and by two key parameters: (a) the average permeability of the membrane to the drug and (b) the ratio between the volume of the entire dispersion and the initial volume of all the liposomes in the dispersion. The present model implies that LFUS irradiation triggers two liposomal drug-release mechanisms: the predominant one is diffusion through the LFUS-compromised liposome membrane, and the less significant one is liposome disintegration.

Keywords—Low frequency ultrasound, Liposome, Controlled drug release, Mathematical model, Doxorubicin, Cisplatin, MPS, Doxil.

ABBREVIATION

LFUS Low frequency ultrasound

INTRODUCTION

Sterically stabilized liposomes <100 nm in diameter have characteristics that make them well suited to serve as a drug delivery system: a prolonged circulation time,[27] a vesicular structure that enables loading of hydrophilic or lipophilic drugs,[2] and an ability to target tumors and inflamed tissue.[1,6] The problem posed by the contradictory requirements for a successful liposomal formulation—stability on the one hand and the capacity to release the drug at a sufficient rate at the target site on the other[2]—has been tackled in various ways. Approaches that have been tried include introducing pH-sensitive[12,21] or light-sensitive[7] constituents into the membrane, and triggering drug release using an external physical signal such as hyperthermia[5,19] or low frequency ultrasound (LFUS).[15,24]

The rationale for using LFUS to control liposomal drug release is based on two findings, first that such signals enhance the permeability of biological membranes for drug and gene delivery,[3,11,14,20,23] and second that the structure of liposome membranes and many of their physiochemical properties are similar to those of biological membranes.[13]

Lin and Thomas showed that LFUS is able to release a loaded dye from sterically stabilized liposomes,[15] while Myhr and Moan reported that a synergistic therapeutic effect occurred when LFUS was applied to tumors implanted in mice and treated with liposomal doxorubicin.[18] Recently it has been shown that LFUS irradiation induces liposomal drug release *in vitro* and that drug release stops upon cessation of irradiation.[24] The extent and profile of drug release have been found to be mostly dependent on the molecular constituents of the lipid bilayer, as well as on irradiation frequency and intensity.[15,24–26] Higuchi[9] performed theoretical investigations of the rate of drug release from solid matrices and dispersed ointment

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bases. Margalit *et al.*[16] studied the thermodynamics of encapsulation and the kinetics of efflux for a series of small molecular weight drugs in multi- and unilamellar liposomes, applying Eyring's absolute rate theory to evaluate the kinetics and mechanism of drug efflux. Huson *et al.*[10] modeled drug release from pellets coated with insoluble polymeric membranes. The latter studies[9,10,16] demonstrate that *diffusion* models provide a good description of the undisturbed mechanism of drug release from carriers into a perfect sink.

IN VITRO EXPERIMENTAL BASIS OF THE MODEL

The present model was developed based on the experimental results reported by Schroeder *et al.*, which are summarized below.[24]

Liposomes composed of hydrogenated soybean phosphatidylcholine, m^{2000}PEG-DSPE, and cholesterol were loaded with either (I) the highly potent anti-inflammatory steroid methylprednisolone hemisuccinate sodium salts (MPS), (II) the anticancer chemotherapeutic agent doxorubicin, or (III) the chemotherapeutic agent cisplatin, to form ~100-nm liposomes (in cases I and II) or ~110-nm liposomes (in case III).

Liposomal dispersions (3 mL) were irradiated in a temperature-controlled water bath (37 ± 1 °C) using a 20 kHz ultrasonic processor (VC400 Sonics & Materials, Newtown, USA) at a constant amplitude of 3.3 W/cm^2 for different durations ranging from 0 (control) to 180 s. Immediately after LFUS irradiation any released drug was removed and the drug remaining in the liposomes was quantified, using HPLC for MPS, fluorescence for doxorubicin, and atomic absorption spectroscopy for cisplatin. Nonirradiated liposomes containing each of the three drugs released less than 3% of the loaded drug over the experimental period when kept at 37 °C.[24] Thus, for a period of 0–180 s nonirradiated liposomes may be considered to be practically impermeable.

Schroeder *et al.*[24] reported that during the first ~ 150 s of irradiation, drug release nearly followed the first-order kinetics $dM_L/dt = -k_0 M_L$, or, in its integrated form, $\ln(M_L/M_0) = -k_0 t$, where M_0 (moles) is the initial amount of drug loaded into the liposomes, M_L (moles) is the remaining amount of drug in the liposomes after an irradiation time t (s), and k_0 (1/s) is a first-order release rate constant.[24] A closer inspection reveals that the slope of $\ln(M_L/M_0)$ vs. time decreases monotonically with time throughout LFUS irradiation for all the tested drugs (Figs. 1a–1c).

Schroeder *et al.* also reported a steady decline in the turbidity of the liposomal dispersions with increasing

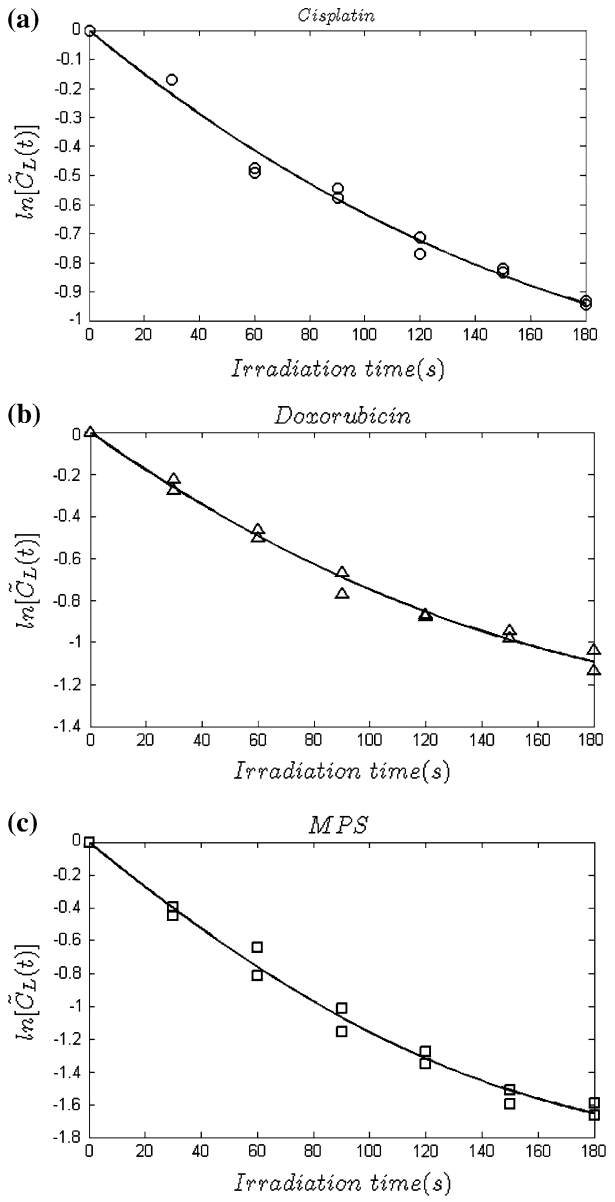

FIG. 1. Natural logarithm of normalized drug concentration in liposomes vs. irradiation time; curves indicate model predictions. (a) Cisplatin: experimental results (circles). (b) Doxorubicin: experimental results (triangles). (c) MPS: experimental results (squares).

LFUS irradiation time.[24] Since dynamic light scattering measurements ruled out the possibility that the effect on turbidity was caused by a decrease in liposome size or the reduction of drug concentration in the liposomes, they suggested that the reduced turbidity could be due to a decrease in the number of liposomes in the dispersion resulting from liposome disintegration.

The present mathematical model is aimed at illuminating this latter phenomenon, as well as elucidating the relative contribution of diffusion and liposome disintegration to the total LFUS-induced drug release.

While various mathematical models have addressed drug release from liposomes, to the *best* of our *knowledge* the model presented below is the first published mathematical model of LFUS-induced drug release that accounts for the effects of disintegrating liposomes and transmembrane diffusion.

CONSTRUCTION OF THE MATHEMATICAL MODEL

The intra- and extra-liposomal domains are both considered to be well mixed, and it is assumed that encapsulated drugs may be released from liposomes by diffusion and/or liposome disintegration.

Adopting a lumped-parameter approach, the release rate may be modeled as:

$$\frac{dM_L}{dt} = \frac{d(V_L C_L)}{dt} = V_L \frac{dC_L}{dt} + C_L \frac{dV_L}{dt}, \qquad (1)$$

where $M_L(t)$, $V_L(t)$ and $C_L(t)$ are the drug content (moles), volume, and average drug concentration of the entire liposome compartment, respectively. The terms $V_L \frac{dC_L}{dt}$ and $C_L \frac{dV_L}{dt}$ represent the instantaneous drug release by diffusion and due to volumetric changes in the liposome compartment, respectively. While ultrasound is known to decrease liposome size, this feature is dependent on lipid composition and on the irradiation characteristics.[22,28] Since the dynamic light scattering measurements described in Schroeder *et al.*[24] have shown that the liposome diameter remained unaffected, independent of irradiation time, all the liposomes in a given dispersion may be assumed to have a similar size.[24] It is therefore assumed that dV_L/dt embodies the volumetric loss due to liposome disintegration. The components of Eq. (1) may be analyzed based on a representative liposome having a constant drug-dependent permeability coefficient and the instantaneous average drug concentration of the entire liposome population. The permeability of the membrane is assumed to result from LFUS-induced pores in the membrane; thus the actual surface area through which diffusion occurs is a fraction of the total liposome surface area. This fraction is implicitly accounted for in the average permeability coefficient h_m.

The following notations are used: R, liposome radius; C_E, drug concentration in the extra-liposome medium; V_E, volume of extra-liposomal medium; $V_T = V_E + V_L$, volume of the entire dispersion.

Fick's first law of diffusion for a single average liposome, $d\left(\frac{4}{3}\pi R^3 C_L\right)/dt = -h_m 4\pi R^2 (C_L - C_E)$, yields, after dividing by $\frac{4}{3}\pi R^3$:

$$\frac{dC_L}{dt} = -\frac{3h_m}{R}(C_L - C_E) \qquad (2)$$

Furthermore, $C_L V_L + C_E V_E = C_0 V_0$, $C_0 = C_L(0)$, $V_0 = V_L(0)$, and therefore after substitution and rearrangements we get:

$$\frac{dC_L}{dt} = -\frac{3h_m}{R(V_T - V_L)}[C_L V_T - C_0 V_0] \qquad (3)$$

In order to calculate the diffusion component $V_L \frac{dC_L}{dt}$, it is necessary to introduce the time-dependent volume of the liposome compartment, $V_L(t)$. This cannot be done by directly employing the optical density of the dispersion reported in Schroeder *et al.*,[24] for, although it is known that the optical density of a particulate dispersion, *OD,* is proportional to the particle concentration $\mathrm{OD} = \alpha V_L$, the proportionality coefficient α is generally unknown.[8] This limitation may be overcome by noting that the normalized optical density function and the normalized liposomal volume are identical due to the cancellation of α: $\mathrm{OD}(t)/\mathrm{OD}(0) = V_L(t)/V_L(0) \equiv \tilde{V}_L(t)$. The value of $\tilde{V}_L(t)$ was derived from an optical density graph and results of biochemical analysis indicating ~23% liposome disintegration within the first 140 s of irradiation.[24] A smooth function $\tilde{V}_L = a \cdot \exp(-\lambda_1 t) + (1-a) \cdot \exp(-\lambda_2 t)$ is chosen to approximate the normalized data, its parameters being determined by least square approximation (confidence intervals in brackets): $a = 0.1607$ [0.110 0.212], $\lambda_1 = 0.443 \times 10^{-1}$ [0.144 0.740] $\times 10^{-1}$, $\lambda_2 = 0.655 \times 10^{-3}$ [0.228 1.08] $\times 10^{-3}$ (Fig. 2). Introducing the non-dimensional forms of the concentration, $\tilde{C}_L(t) = \frac{C_L(t)}{C_L(0)}$, and the liposomal volume, $\tilde{V}_L(t)$, the non-dimensional form of Eq. (1) becomes:

$$\frac{d\tilde{M}_L}{dt} = \frac{d\left(\tilde{V}_L \tilde{C}_L\right)}{dt} = \tilde{V}_L \frac{d\tilde{C}_L}{dt} + \tilde{C}_L \frac{d\tilde{V}_L}{dt} \qquad (4)$$

The non-dimensional form of Eq. (3) becomes:

$$\frac{d\tilde{C}_L}{dt} = -\frac{3h_m}{R\left(\tilde{V}_T - \tilde{V}_L\right)}\left[\tilde{C}_L \tilde{V}_T - 1\right]; \quad \tilde{C}_L(0) = 1, \quad \tilde{V}_L(0) = 1, \qquad (5)$$

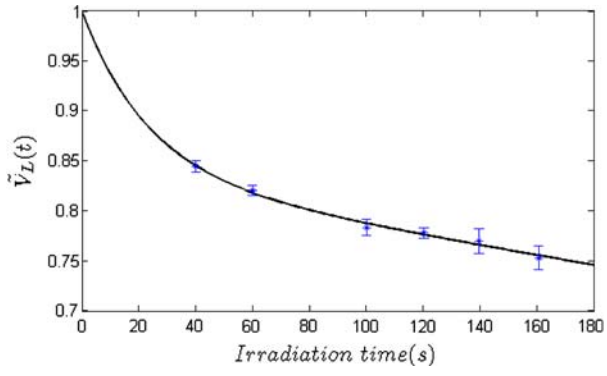

FIG. 2. Normalized volume of liposome compartment vs. irradiation time, obtained from optical density and lipid concentration measurements reported in Schroeder *et al.*[24]

and its solution is:

$$\tilde{C}_{\mathrm{L}} = \frac{1}{\tilde{V}_{\mathrm{T}}}\left[1 + \left(\tilde{V}_{\mathrm{T}} - 1\right)\exp\left(-k_0 \tilde{V}_{\mathrm{T}} \int_0^t \frac{ds}{\tilde{V}_{\mathrm{T}} - \tilde{V}_{\mathrm{L}}(s)}\right)\right];$$
$$k_0 = \frac{3h_{\mathrm{m}}}{R} \tag{6}$$

RESULTS AND DISCUSSION

While the disintegration of a single liposome is a discrete event, its effect on the volume of the entire liposome compartment and its drug concentration is incremental. The volume of the liposome compartment and its average drug concentration were assumed to be continuously differentiable functions of the irradiation time, thus satisfying sufficient requirements for the differentiation in Eq. (1).

Values of $\tilde{V}_{\mathrm{T}}$ and h_{m} were fitted using the least square approximation, and their confidence intervals were estimated by the Bootstrap method.[4] As for each drug (cisplatin, doxorubicin, or MPS) the liposomes were taken from reservoirs with a different liposome concentration, the initial number of liposomes per unit volume was different. Consequently $\tilde{V}_{\mathrm{T}}$ was assessed for each drug separately. The goodness of fit was assessed by the correlation coefficient for each drug. Unlike the permeability, the values of $\tilde{V}_{\mathrm{T}}$ are of no relevance to the properties of the liposomes and/or drug and are therefore not reported here. The calculated permeabilities and their confidence intervals as well as the correlation coefficients are presented in Table 1. The natural logarithm of the experimental results and model predictions of the normalized liposome drug-concentration $\tilde{C}_{\mathrm{L}}(t)$ are shown in Figs. 1a–1c for cisplatin, doxorubicin and MPS, respectively. The good agreement between the model and the experimental data bears witness to the validity of the model and supports the basic assumption that: (a) LFUS-induced drug release may be accurately described as a combination of Fickian diffusion and liposome disintegration and (b) the average permeability can be adequately represented by a constant.

The relative roles of diffusion and liposome disintegration in the overall drug release process can be evaluated from Eqs. (4) and (6), as shown in Figs. 3a–3c for all three drugs. These graphs show that the relative contribution of diffusion $\left|\int_0^t \tilde{V}_{\mathrm{L}}(s)\frac{d\tilde{C}_{\mathrm{L}}(s)}{ds}ds\right|$ (dotted lines) is significantly greater than the relative contribution of

TABLE 1. Estimated parameters and upper and lower bounds of their confidence intervals [L, U].

	Cisplatin	Doxorubicin	MPS
h_{m} ($\mathrm{ms}^{-1} \times 10^{-10}$)	1.4 [0.95, 1.6]	1.6 [1.4, 1.8]	2.4 [2.1, 2.6]
Model correlation coefficient	0.993	0.997	0.997

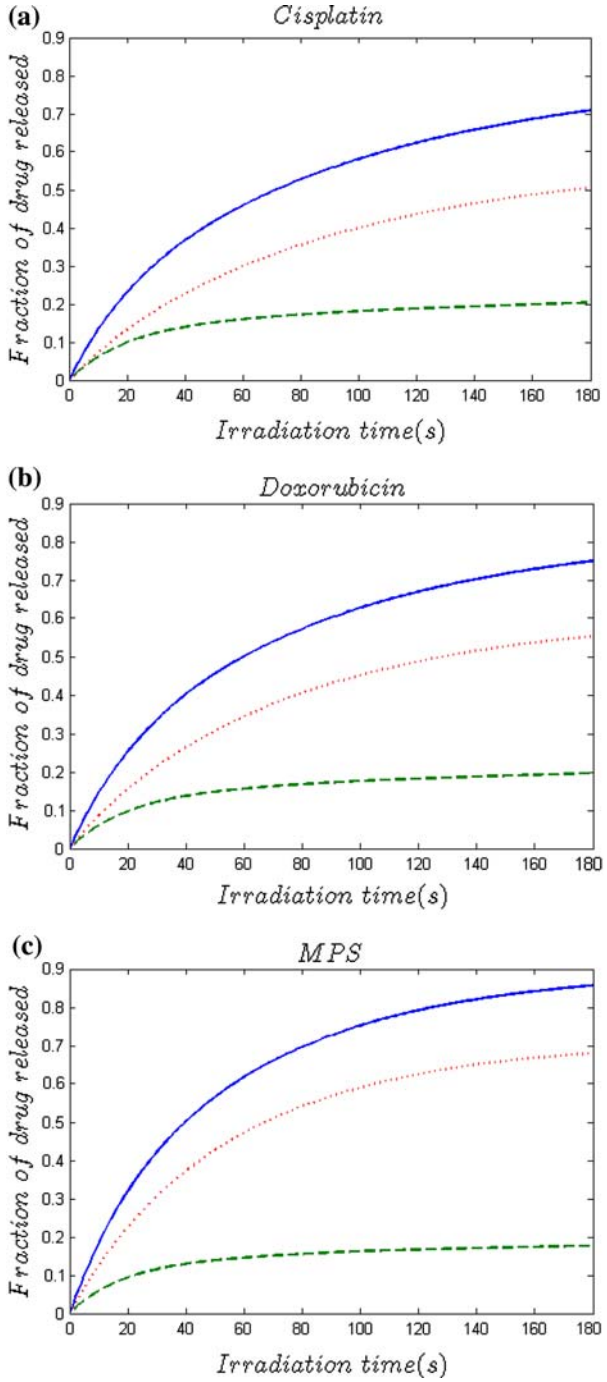

FIG. 3. Model prediction of total normalized amount of drug released during the irradiation period 0–*t* (continuous line); normalized amount released by diffusion (dotted line); and normalized amount released by liposome disintegration (dashed line). (a) Cisplatin, (b) Doxorubicin and (c) MPS.

liposome disintegration $\left|\int_0^t \tilde{C}_L(s)\frac{d\tilde{V}_L(s)}{ds}ds\right|$ (dashed lines). The total drug released $\tilde{C}_L(0)\tilde{V}_L(0) - \tilde{C}_L(t)\tilde{V}_L(t)$ is shown by the continuous lines. Exposing liposomes to LFUS induces drug release by diffusion; therefore, their drug content decreases with irradiation time. As a result, the average amount of drug released from disintegrating liposomes is larger at earlier stages than at later stages of the irradiation period.

As LFUS induces intense mixing of the dispersion due to cavitation micro-streaming, and since the diffusion rate through the membrane is significantly lower than that of the medium, both the intra- and the extra-liposomal medium drug concentrations may be considered to be homogeneous at all times.

It is hypothesized that the permeability of each liposome is triggered by LFUS irradiation and that this permeability stops immediately upon cessation of irradiation. Liposome disintegration, on the other hand, may be affected by several parameters, including inter-liposomal collisions, liposome collisions with vial walls, cavitation in the immediate vicinity of liposomes, and the actual distance of liposomes from the probe. The non-dimensional liposome volume was obtained from the optical density data provided in Schroeder *et al.*[24] and fitted to a sum of two weighted exponentials with two different weights and two different decaying rate constants, as described above.[24]

The good agreement between the lumped-parameter model predictions and the experimental results suggests that the main resistance to drug transport is within the liposome membrane. It further corroborates the assumption that the average permeability coefficient h_m may be approximated as a constant throughout irradiation. This implies that LFUS irradiation does not produce cumulative effects in the liposome membrane, in contradistinction to biological membranes, which continue to be permeable for long periods of time after cessation of the irradiation.[14,17]

The dominance of diffusion in the release process explains the monotonic decrease in the slope of the $\ln(M_L/M_0)$ curve with irradiation time (Figs. 1a–1c). Early in the irradiation period the extra-liposome drug concentration C_E is small. As a result the release process initially appears as diffusion to a perfect sink, with a nearly constant release rate. As time progresses the drug buildup in the extra-liposomal medium acts to decrease the diffusion rate from the intra-liposome compartment to the extra-liposome medium (Eq. 2).

CONCLUSIONS

In this study a mathematical model is presented of ultrasonically induced drug release from liposomes. The model offers an accurate description of the experimental results and hence provides a tool for predicting the amount of drug that will be released from liposomes subjected to LFUS irradiation, as well as for evaluating the relative roles of liposome disintegration and diffusion in the release process. The model suggests that this process is dominated by transmembrane diffusion.

ACKNOWLEDGMENTS

The authors thank Prof. Eitan Kimmel for valuable discussions and Ms. Ilana Nisky for her assistance in the statistical analysis. AS is grateful to the Israel Ministry of Science and Technology for the Eshkol Scholarship, as well as to the Barenholz Fund and to The Research Council of Norway for their support.

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