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Review

Ultrasound, liposomes, and drug delivery: principles for using ultrasound to control the release of drugs from liposomes

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ABSTRACT

Ultrasound is used in many medical applications, such as imaging, blood flow analysis, dentistry, liposuction, tumor and fibroid ablation, and kidney stone disruption. In the past, low frequency ultrasound (LFUS) was the main method to downsize multilamellar (micron range) vesicles into small (nano scale) unilamellar vesicles. Recently, the ability of ultrasound to induce localized and controlled drug release from liposomes, utilizing thermal and/or mechanical effects, has been shown. This review, deals with the interaction of ultrasound with liposomes, focusing mainly on the mechanical mechanism of drug release from liposomes using LFUS. The effects of liposome lipid composition and physicochemical properties, on one hand, and of LFUS parameters, on the other, on liposomal drug release, are addressed.

Acoustic cavitation, in which gas bubbles oscillate and collapse in the medium, thereby introducing intense mechanical strains, increases release substantially. We suggest that the mechanism of release may involve formation and collapse of small gas nuclei in the hydrophobic region of the lipid bilayer during exposure to LFUS, thereby inducing the formation of transient pores through which drugs are released. Introducing PEG-lipopolymers to the liposome bilayer enhances responsivity to LFUS, most likely due to absorption of ultrasonic energy by the highly hydrated PEG headgroups. The presence of amphiphiles, such as phospholipids with unsaturated acyl chains, which destabilize the lipid bilayer, also increases liposome susceptibility to LFUS.

Application of these principles to design highly LFUS-responsive liposomes is discussed.

Contents

Abbreviations: LFUS, low frequency ultrasound; nSSL, nano sterically stabilized liposome; SO, solid-ordered (phase); LD, liquid-disordered (phase); LO, liquid-ordered (phase); mPEG-DSPE, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-(methoxy(polyethylene glycol)-2000) (ammonium salt); HSPC, hydrogenated L-α-phosphatidylcholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; PC, phosphatidylcholine; CAC, critical aggregation concentration; CMC, critical micelle concentration.

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1. Introduction

1.1. Brief history of ultrasound

Historically, acoustics was scientifically studied as early as the 6th century BCE by Pythagoras. In 1638 Galileo showed that pitch is associated with vibration, a study that was later (1877) developed by Lord Raleigh into the "Theory of Sound". Ultrasonics as an independent field of acoustics was developed only during World War I, as a technology capable of detecting enemy vessels (reviewed by Kinsler et al., 1980). The major pioneers in this field were Wood and Gerrard in England, who developed the first hydrophone for locating submarines, and Langevin in France, who suggested that ultrasonic waves could be induced by vibrating piezoelectric devices (reviewed by Goldberg and Kimmelman, 1988). Since then, this field has been studied and developed intensively.

Ultrasonics is a branch of acoustics that deals with vibratory or stress waves at frequencies above those within the hearing range of the average person, i.e., >20 kHz (Suslick, 1988). Stress waves can exist only in media, as they are transmitted from one mass to another by direct contact between the masses. Ultrasonic waves are also termed elastic waves, since it is the elastic property of the medium that is responsible for the sustained vibrations required for ultrasonic wave propagation (Ensminger, 1988).

1.2. Ultrasound physics

The vibrating rate (vibrations per time unit) is defined as frequency (f) and is measured in Hertz (Hz).

Within a single phase of gas, or liquid, or solid, physically being separate elastic media, the rate of propagation of sound waves is a function of the medium's elasticity (K) and density (ρ), and is referred to as the sound wave velocity (c).

$$c = \left(\frac{K}{\rho}\right)^{0.5} \quad (1)$$

The sound wavelength λ describes the distance between two sequential amplitudes. The wavelength is derived from the frequency and the velocity, according to:

$$\lambda = \frac{c}{f} \quad (2)$$

The wave amplitude (A) is measured in units of length or pressure. For the former, the amplitude will describe the maximal distance between molecules in relation to the rest state, while for the latter; the amplitude describes the maximal local pressure.

Values of velocity of sound traveling through various media are presented in Table 1. It can be noticed that sound travels faster through liposomes in the solid-ordered (SO) phase (as in the case of HSPC) in comparison to liposomes in the liquid-disordered (LD) phase (as in the case of EPC) (a detailed explanation of the physical phases of liposomes appears in Section 2.1.1). This can be explained by the fact that sound travels faster through solids in comparison to liquids (Cutnell and Johnson, 1989).

At a given frequency, the particle motion at any point in an ultrasonic wave is sinusoidal, as long as the stresses developed in the waves remain in the linear, elastic range of the medium (Ensminger, 1988). If two sinusoidal waves of slightly differing frequencies are superimposed, their amplitudes are alternately added and subtracted, so that the overall effect is a wave with amplitude equal to the sum of the amplitudes of the individual waves and frequency equal to the difference in the frequencies of the same two waves.

When a disturbance is induced in a mass, the first affected element transfers the energy to the next one in line, and so forth, in a similar manner (propagation), until the energy is dissipated. The elements of the entire mass do not move in unison, due to the fact that mass is elastic and thus deforms under stress. The rate of propagation depends upon the type of wave, the elastic properties of the medium, the density of the medium, and in some cases the frequency (Ensminger, 1988).

1.2.1. Absorbance, dispersion and attenuation

Within an ideal elastic medium there is no energy loss, meaning that kinetic energy is not transformed into heat. However, in actual media there are frictional forces that disrupt the periodic motion of the molecules; thus, part of the kinetic energy is transformed into heat, according to the following equation:

$$I_x = I_o \exp(-2\alpha x) \quad (3)$$

where I_x is the local intensity (at distance x from the source), I_o is the initial intensity of the vibrating surface (the source), α, the absorbance coefficient, is a function of frequency. In general, *as the frequency rises the absorbance rises* (Hill et al., 2004). Similarly, the pressure amplitude (P) decreases due to the absorbance, according to the following equation:

$$P_x = P_o \exp(-\alpha x) \quad (4)$$

where P_x is the local pressure amplitude and P_o is the initial pressure amplitude at the source.

Part of the waves traveling through the medium are dispersed due to matter inconsistency. Dispersion and absorbance cause loss of part of the acoustic energy; the two effects combined are called attenuation (Goss et al., 1978, 1980).

The attenuation (in units of dB) can be estimated using the following equation:

$$\text{attenuation} = \alpha' \times l \times f \quad (5)$$

where α' is the attenuation coefficient, l is the distance of penetration in cm, and f is the frequency in MHz. Values of α' (having units of dB/(MHz × cm)) vary from one tissue to another, for example, at 1 MHz α' is ~40 for air-filled lungs, 20 for bone, 1.0 for kidney, 0.94

Table 1
Velocity of sound traveling through various media.

Media	Density (g/cm^3)	Sound velocity (m/s)	Reference
Air	0.0012	330	Angelsen (2000)
Water (25 °C)	0.9971	1497	Priev et al. (1998)
Biological tissue			
Gas-filled fresh lung	0.4	658	Goss et al. (1980)
Brain, fresh	1.03	1460	Goss et al. (1980)
Fat, fresh	0.94	1479	Goss et al. (1980)
Breast, fresh		1510	Goss et al. (1980)
Serum (25 °C)	1.01	1519	Calculated from Khazanov et al. (2008)
Heart muscle (beef)	1.05	1546	Goss et al. (1980)
Muscle, strained	1.07	1566	Goss et al. (1980)
Liver, fresh	1.06	1570	Goss et al. (1980)
Kidney (beef)	1.04	1572	Goss et al. (1980)
Whole fresh blood	1.06	1580	Goss et al. (1980)
Bone, skull	1.7	2770	Goss et al. (1980)
Aqueous dispersions			
100-nm egg phosphatidylcholine:DHP-PEG^{2000} liposomes (14:1 mole ratio) in water (30 °C), total lipid content 4%	0.995	1497	Priev et al. (1998)
100-nm egg phosphatidylcholine (EPC) liposomes in water (25 °C), total lipid content 4%	1.004	1498	Priev et al. (1998)
4% Globular proteins in 0.01 M phosphate buffer with 0.1 M NaCl, 25 °C	1.014	1508	Calculated from Sarvazyan et al. (1988)
Citrate buffered saline (CBS) (5 mM sodium citrate, 130 mM NaCl, pH 7, 285 mOsmol), 25 °C	1.003	1505	Calculated from Khazanov et al. (2008)
100-nm hydrogenated soybean phosphatidylcholine (HSPC) liposomes in CBS (25 °C), total lipid content 4%	1.007	1507	Calculated from Khazanov et al. (2008)
4% Polypeptides in 0.01 M phosphate buffer with 0.1 M NaCl, 25 °C	1.014	1511	Calculated from Sarvazyan et al. (1988)
4% Fibrillar proteins in 0.01 M phosphate buffer with 0.1 M NaCl, 25 °C	1.014	1515	Calculated from Sarvazyan et al. (1988)
4% Amino acids in 0.01 M phosphate buffer with 0.1 M NaCl, 25 °C	1.014	1524	Calculated from Sarvazyan et al. (1988)

Velocity of sound traveling through other biological media can be found elsewhere (Mast, 2000).

for liver, 0.85 for brain, 0.63 for fat, 0.18 for blood, and 0.0022 for water (Brown et al., 1999; Bushong and Archer, 1991; Goss et al., 1978, 1980; Mast, 2000).

1.2.2. Reflection and refraction

Acoustic waves undergo reflection and refraction when passing an interface between two different media. Thus, two acoustic waves are produced; one continuing into the following medium, and the other being reflected back into the initial medium. The wave that continues into the following medium changes its propagating angle when passing through the interface between the two media.

The fraction of the wave reflected (R) is dependent on the acoustic impedance (Z) of the two media:

$$Z = \rho \times c \tag{6}$$

where ρ is the density of the material, and c is the wave velocity.

R is calculated using the following equation:

$$R = \left[\frac{Z_1 - Z_2}{Z_1 + Z_2}\right]^2 \tag{7}$$

The subscripts of Z_i refer to the first and second medium.

1.2.2.1. Irradiation pressure. The power of a wave, having units of watts (W), describes the sum of acoustic energy per unit of time.

The intensity (I) of a wave describes the acoustic power per unit of area:

$$I = \frac{P^2}{\rho \times c} \tag{8}$$

where P (in units of Pa) indicates the pressure amplitude.

For example, the intensity threshold for hearing in humans, of sound having a frequency of ~4 kHz, is 10^{-12} W/m^2 and defined as decibels (dB) (Gelfand, 2004). Furthermore, the intensity of sound during a normal conversation, held at frequencies of ~1–5 kHz, is in the range of ~10^{-6} W/m^2 (Gelfand, 2004). It should be noted that in cases of human hearing the power of sound (measured in watts), is divided by the surface area of the ear drum (being ~55 mm^2).

Acoustic waves apply force on the surfaces they are reflected from, and apply force on the absorbing medium. When a wave is completely absorbed, the irradiating force (F) is:

$$F = \frac{W}{c} \tag{9}$$

where W is the irradiating power.

When a wave is completely reflected, the irradiating force is:

$$F = 2\frac{W}{c} \tag{10}$$

This force, when applied to particles within a liquid medium, causes motion, called acoustic flow. The velocity (v) of this flow is given by the equation:

$$v = 2\frac{\alpha W}{\mu \rho c} \tag{11}$$

where μ is the viscosity of the fluid and α is the absorbance coefficient.

1.3. Cavitation

Acoustic cavitation is the formation and/or activity of gas- or vapor-filled cavities, i.e., bubbles, in a medium exposed to an oscillating pressure (Flynn, 1964; Neppiras and Noltingk, 1951; Noltingk and Neppiras, 1950). Sources for such bubbles are usually pre-existing stable bubbles that were present in the liquid, or bubbles that were formed when the pressure dropped below the vapor pressure of the liquid. The passage of an ultrasonic wave will cause oscillations of these bubbles, termed cavitation (Suslick et al., 1999).

There are two general types of acoustic cavitation.

Stable cavitation refers to the continuous oscillation of bubbles in response to an oscillating pressure. Thus, the bubble radius varies about an equilibrium value. The vibrating surface of the bubble creates local swirling and fluid convection known as micro-streaming, which induces shear stresses in the fluid near the oscillating bubble (Margulis, 1995).

Inertial cavitation, also known as *transient cavitation*, occurs in a bubble that oscillates with increasingly large amplitudes until the outward expansion exceeds a limiting value, called the bubble resonant radius (R_r), upon which the bubble grows abruptly and then collapses violently (Young, 1989). The increase in the size of the gas bubble over time under an oscillating pressure field is facilitated by a process called 'rectified diffusion', in which more liquid vapors can diffuse *into* the bubble when the pressure drops locally, due to an increase in bubble size and surface area, in comparison to the amount of vapors that diffuses *out* of the bubble during the elevation in pressure, due to contraction of the bubble and decrease in the bubble surface area (Crum, 1984; Young, 1989). Rectified diffusion is also affected by the increase and decrease in the concentration of the gases or vapors in the bubble as the bubble grows and contracts, thereby affecting the concentration gradient across the gas/liquid interface (Crum, 1984; Young, 1989).

During transient cavitation, the collapse of bubbles produces short-lived intense local heating and high pressures. These local cavitation spots have been shown to reach temperatures of 5000 K, pressures of 1000 atm, and have heating and cooling rates of 10^{10} K/s (McNamara et al., 1999; Suslick, 2001). Due to the short life and extremely high heating/cooling rates of cavitation events (several microseconds) they are assumed to be adiabatic (Catania et al., 2006; Suslick et al., 1999). The resonant size of a bubble prior to collapse is determined by the type of gas within the bubble, the medium, and the characteristics of the ultrasonic wave (Suslick and Nyborg, 1990; Young, 1989). For an air bubble in water, the resonant radius of the bubble (R_r in mm) can be estimated using the following equation:

$$R_r \approx \frac{3.28}{f} \quad (12)$$

where f is the ultrasonic frequency in kHz (Young, 1989).

For a given bubble size, there is an ultrasound intensity threshold above which transient cavitation starts to occur. Because the transient cavitation intensity threshold generally decreases as ultrasonic frequency decreases, *transient cavitation occurs more frequently at lower frequencies* (Hoskins et al., 2002). The collapse of the bubble produces a shock wave, micro-streaming, and shear forces near the cavitation event (Margulis, 1995). The collapse of a bubble spawns numerous smaller bubbles, which can grow and eventually collapse.

Apfel and Holland (1991) defined a *Mechanical Index* (MI), which is an indicator of the likelihood of initiation of transient cavitation in media exposed to ultrasound. MI is defined as follows, $MI = (P_{neg}/f^{0.5})$; where P_{neg} is the maximal negative pressure, in MPa, in the sound field, and f is the frequency in MHz. When MI > 0.7 the probability of occurrence of transient cavitation in the medium is high (reviewed in detail in Leighton, 1997). Apfel and Holland (1991) also showed that for ultrasonic frequencies below 1 MHz an acoustic pressure threshold of ~0.2 MPa is sufficient to initiate transient cavitation in aqueous solutions and in blood.

When a bubble collapses near a surface, a high-velocity "micro jet" of liquid, reaching several hundred meters per second, is propelled toward the surface, thereby depositing enormous energy densities at the site of impact (Catania et al., 2006; Krasovitski and Kimmel, 2004; Suslick, 1988; Suslick et al., 1999).

1.3.1. Cavitation in solutions enriched with surface-active molecules

When cavitation occurs in aqueous solutions enriched with surface-active molecules (such as phospholipids), surfactants accumulate at the gas/liquid interface of the cavitating bubble, thereby reducing the surface tension of the bubble. The lower surface tension induces an enhanced formation rate of the bubbles; however, on the other hand, these bubbles are less stable and collapse at relatively smaller sizes than bubbles in aqueous solutions without surfactants (Ashokkumar and Grieser, 2007; Crum, 1980, 1984; Jimmy et al., 2008; Sunartio et al., 2007). The smaller size of cavitating bubbles in surfactant-enriched dispersions, in comparison to bubbles cavitating in water alone, is also due to a decayed bubble *fusion* rate due to the presence of surfactants in the water/gas interface (Ashokkumar and Grieser, 2007; Crum, 1980, 1984; Jimmy et al., 2008; Sunartio et al., 2007).

1.4. Medical applications of ultrasound

Ultrasound is widely used in medicine and can be categorized as either low- or high-*intensity*. Low-intensity ultrasound is mainly used to obtain information on the state of matter (as in cases of imaging and flow studies), while high-intensity ultrasound is aimed at manipulating the state of matter (as in cases of kidney stone shattering or tumor and fibroid ablation).

Blood flow and tissue motion are analyzed utilizing the Doppler effect, which exploits the fact that a shift in frequency and amplitude occurs between a wave leaving the transducer and the received wave after being reflected from a moving object. Ultrasonic imaging of tissue is achieved by transmitting an ultrasonic pulse which is partially reflected from the boundary between two tissue structures and then reconstructed into display. In general, for a certain intensity, the higher the frequency the higher the imaging resolution; however, this is at the expense of lower penetration (Hill et al., 2004).

1.4.1. Extracorporeal shock wave lithotripsy

Kidney stones are categorized into four general classifications: calcium stones (75–85% of all cases, composed mostly of calcium oxalate, often combined with phosphate), uric acid stones (5–8%), cystine stones (<1%), and struvite stones (10–15%, composed mostly of ammonium and magnesium phosphate). The shattering of kidney stones by ultrasound, exploits the fact that different substances absorb energy differently, and thereby, the "stones" absorb substantially elevated amounts of energy in comparison to the surrounding tissue, and are shattered, to be excreted in the urine (reviewed in Haupt and Haupt, 2003; Lafon, 2007; Sapozhnikov et al., 2007). See also Section 2.2.1 for examples of the attenuation coefficient of different biological substances.

In physiotherapy, high frequency ultrasound (0.7–3 MHz) is used for local heating of tissue (Ebenbichler et al., 1999; Johns, 2002; Kitchen and Partridge, 1990), as well as accelerating healing of fractures (Hadjiargyrou et al., 1998) and injured muscles (Rantanen et al., 1999; Speed, 2001).

Ultrasound-assisted liposuction is a technique in which high-intensity ultrasound of low- to high frequency (20 kHz to ~1 MHz) is utilized to "liquefy" body fat, thereby facilitating its removal by liposuction (Pine et al., 2003; Rohrich et al., 1998).

High-intensity focused ultrasound (HIFU) combines imaging techniques (such as MRI, CT, or ultrasound) to locate diseased tissue, and then focuses ultrasound beams of high frequency (~1 MHz) and intensity at the point of interest, thereby raising tissue temperature to ~60 °C to cause its ablation (Cohen et al., 2007; Fennessy et al., 2007; Fry et al., 1958; Fry, 1954; Funaki et al., 2007; Jolesz and Hynynen, 2002; Jolesz and McDannold, 2008; Lynn et al., 1942; Ram et al., 2006).

Kost and Langer showed that low frequency ultrasound (LFUS) could be used to increase the permeability of biological barriers, such as the skin, in order to administer drugs or to sample extracellular analyte (Kost et al., 1988, 2000; Lavon and Kost, 2004; Mitragotri and Kost, 2004). Since then, ultrasound has been widely used to increase permeability of different biological barriers, such as cell walls, tumors, and blood clots (Dittmar et al., 2005; Duvshani-Eshet et al., 2006; Frenkel et al., 2006; Gao et al., 2005; Graul, 1950; Karshafian et al., 2005; Khaibullina et al., 2008; Lota and Darling, 1955; Parikov, 1966; Poff et al., 2008; Rapoport et al., 1997; Stone et al., 2007; Sundaram et al., 2003). Schlicher et al. demonstrated that LFUS-facilitated cavitation could disrupt cell membranes in a reversible manner, forming transient pore-like disruptions, <28 nm in diameter, in the plasma membrane (Schlicher et al., 2006). These disruptions have a life-time of up to several minutes, while the cellular repair mechanism and duration are similar to that of cells exposed to wounding induced by mechanical scraping. Unger and others utilized LFUS-facilitated enhanced cellular permeability (referred to also as sonoporation) to improve DNA delivery into cells (Dittmar et al., 2005; Duvshani-Eshet et al., 2006; McCreery et al., 2004).

Examples of common medical uses of ultrasound, and their physical parameters are described in Table 2.

The ability to change the permeability of membranes to control the release of drugs from different drug carriers will be described in Section 2.3.

2. Ultrasound and liposomes: designing ultrasound-responsive liposomes

Advanced drug delivery systems are aimed at targeting the drug to a desired (in most cases, diseased) tissue, where the drug should be released in a therapeutically effective manner. For example, the enhanced permeability and retention (EPR) effect (Maeda et al., 2000) has been utilized to passively target tumors (Gabizon et al., 1994, 2003; Gabizon and Papahadjopoulos, 1988; Papahadjopoulos et al., 1991; Soloman and Gabizon, 2008). Passive targeting has also been utilized to effectively deliver drugs to inflamed tissue (Avnir et al., 2008; Oyen et al., 1996; Schroeder et al., 2008b). Despite effective targeting, the ability to control drug release kinetics at the target site remains a challenge. Several types of triggers have been suggested for releasing drugs from drug delivery systems, including pH (Connor et al., 1984; Ishida et al., 2006; Simões et al., 2004), temperature (Needham et al., 2000), light (Gerasimov et al., 1999; Zhang et al., 2002), and enzymes (Fishel-Ghodsian et al., 1988; Ghadiali and Stevens, 2008; Goldbart et al., 2002; Meers, 2001). Another approach, which enables controlling both the drug release location and profile, using an external trigger, and which has proven to be highly effective, is by use of ultrasound (Frenkel, 2008; Kinoshita et al., 2006; Kost et al., 1989, 1994; O'Neill and Li, 2008; Rapoport, 2007; Schroeder et al., 2007; Steinberg et al., 2007). Ultrasonic waves can be used to induce either thermal or mechanical effects, while drug delivery systems can be designed to respond either to the elevation in temperature or to the mechanical effects of ultrasonic waves, or to both.

In this review, we address mainly non-thermal ultrasonic effects which are associated with drug release from liposomes and nanoliposomes.

2.1. Liposomes, a historical perspective

Bangham (1963) demonstrated that phospholipids dispersed in aqueous media undergo hydration to form vesicular structures of concentric lamellae. Each lamella is a lipid bilayer. Bangham referred to these assemblies as liposomes, however, at first, they were also nicknamed "Banghasomes" (Bangham, 1963, 1995). This discovery confirmed an earlier study that claimed that all plasma and intracellular membranes are based on phospholipid bilayers (Robertson, 1959), and encouraged the use of liposomes as the main model system to study the physicochemical and other properties of biological membranes (Bangham, 1993).

Today, we define liposomes as mostly spherical vesicles in which a single or several continuous lipid bilayer/s separate the external aqueous medium from the intra-liposomal aqueous core, thus creating an intra-liposomal aqueous phase ("milieu interne"). This unique structure enables liposomes to be preferred carriers for a broad spectrum of agents, including drugs (Barenholz, 2001, 2007; Gregoriadis et al., 1993), small interference RNA (siRNA) (Garbuzenko et al., 2008; Whitehead et al., 2009), plasmid DNA (Lasic et al., 1997; Ma et al., 2007; Zuidam and Barenholz, 1999), peptides (Lutsiak et al., 2002; Torchilin et al., 2001), proteins (Weissig et al., 1998), and even subcellular organelles (Eckstein et al., 1997), viruses (Kaneda, 2000; Kim and Park, 2002; Schneider et al., 1983), and bacteria (Carpenter-Green and Huang, 1983; Wagner et al., 1987). The loaded molecules can be hydrophobic, hydrophilic, or amphipathic in nature. Their location in the liposome will be dependent on the physicochemical properties of the loaded agent, being either in the liposome membrane, or in the intra-liposome aqueous phase. The lipid membrane of most liposomes used for drug, protein, or nucleic acid delivery is based mainly on specific phospholipids such as phosphatidylcholines (PC) or sphingomyelins (SPM); such lipids are referred to as liposome-forming lipids (as described below, see Section 2.1.1).

At least 15 liposome-based drugs are now in clinical use and more are under development (Barenholz, 2001; Torchilin, 2005).

2.1.1. Liposome-forming lipids

Lipids that form liposomes are mainly those having a packing parameter (PP) in the range of 0.74–1.0 (Garbuzenko et al., 2005a; Khazanov et al., 2008; Kumar, 1991). The PP is defined as the ratio of the cross-sectional area of the apolar to polar regions of the amphiphile, $PP = V/(l \times A)$, where V and l are the volume and length of the hydrophobic tails, and A is the cross-sectional area of the amphiphile's hydrophilic headgroup (Israelachvili, 1992; Kumar, 1991). When immersed in an aqueous solution, at a concentration higher than the critical aggregation concentration (CAC) and at a temperature above the solid-ordered (SO) to liquid-disordered (LD) phase transition (see below), the lipids aggregate spontaneously to form multilamellar vesicles (MLV) (Kumar, 1991; Lichtenberg and Barenholz, 1988). MLV can then be downsized by various methods to form either large unilamellar vesicles (LUV, >100 nm in diameter) or small unilamellar vesicles (SUV) (Lasic, 1988; Lichtenberg and Barenholz, 1988). Common methods for downsizing liposomes include high-intensity LFUS (Barenholz et al., 1977; Huang, 1969) and extrusion (Clerc and Thompson, 1994; Hope et al., 1985; MacDonald et al., 1991; Mayer et al., 1986; Subbarao et al., 1991). When using lipid *mixtures*, it is possible to predict if liposomes will be formed by calculating the additive PP (i.e., the sum of the PP of each lipid component multiplied by its mole fraction). If the additive PP is in the range of 0.74–1.0, liposomes are likely to be formed (Garbuzenko et al., 2005a; Khazanov et al., 2008; Kumar, 1991).

The lipid bilayer of liposomes can be in one of three phases, based mainly on the packing of the lipid hydrocarbon chains, being either in the solid-ordered (SO, also referred to as crystalline, solid, or gel phase), or liquid-disordered (LD, also called liquid crystalline, fluid, or liquid phase), or liquid-ordered (LO) phase (Barenholz and Cevc, 2000; Lemmich et al., 1997; Mouritsen, 2005; Mouritsen and Jorgensen, 1994).

Most phospholipids have a phase transition of their lipid bilayer, from the SO to the LD phase, and vice versa. The temperature range at which this transition occurs is dependent on the exact molecular

Table 2
Common medical applications of ultrasound.

Common clinical applications of ultrasound				
Use	Device	Description	Frequency	Devices
Tissue ablation	High-intensity focused ultrasound (HIFU)	Tissue is heated locally; ablated tissue is then cleared naturally. In general, protein denaturation occurs after heating tissue to 60 °C for 0.1 s, to 57 °C for 1 s, or to 53 °C for 10 s (Bailey et al., 2003). As a rule of the thumb, decreasing tissue temperature by 1 °C approximately doubles needed heating time.	0.5–1.5 MHz	Sonoblate 500 (Focus Surgery); Exablate 2000 (Insightec, GE); Phillips MR-HIFU system
Kidney stone shattering	Lithotripter	Ultrasonic waves which are absorbed by kidney stones, more than by surrounding tissue, cause their shattering.	0.1–1 MHz	27085 K (Karl Storz), 2167 (Wolf), USL (Circon)
Physiotherapy	Probe	Tissue is heated by absorption of ultrasonic energy.	0.7–3 MHz	Sonic Relief; NovaSonic
Imaging	Probe	An ultrasonic pulse, is partly reflected from the boundary between two tissue structures, and partially transmitted. The reflection depends on the difference in impedance of the two tissues, and is displayed as an image.	3–10 MHz	ImagePoint (HP); Logiq Series (GE)
Dentistry	Micro Probe	The ultrasonic vibrations of the probe, which is pressed to the surface of the tooth, assist in removal of plaque and debris from the tooth surface.	30–150 kHz	Annon Piezo Tooth Cleaner, Piezon (EMS)
Ultrasound-assisted liposuction	Probe	Ultrasound is used to liquefy fat, thus assisting in its removal by vacuum liposuction.	20 kHz to 1 MHz	S-48 (Shanghai Bestzone Machine); Vaser (Sound Surgical Technologies)

Increasing permeability of biological membranes using ultrasound							
Biological membrane	Frequency	Irradiation time	Permeant	Amplitude	Sonicator type	Clinical use	Reference
Skin	<100 kHz	In vitro: <30 min In vivo: <30 s	Glucose, insulin, EMLA cream	2–10 W/cm^2	Probe/Bath	Yes	Mitragotri and Kost (2004)
Cells	24–80 kHz	2 s (in pulses of 0.1 s each) up to 3 h	Calcein, FITC-dextran, FITC-BSA, 16-doxylstearic acid	3.2 W/cm^2 to 80 mW/cm^2	Chamber/Bath	No	Gao et al. (2005); Rapoport et al. (1997); Schlicher et al. (2006)

structure (polar head groups and hydrocarbon chains) of the lipids assembling the bilayer. The main phase transition is characterized by several parameters, such as the temperature range at which it occurs, the temperature at which the maximum change in heat capacity occurs during the phase transition (referred to as T_m), the width at half height of the endothermic curve which represents the cooperativity of the process, while the area under the endothermic curve represents the total enthalpy involved in this phase transition (Barenholz and Cevc, 2000; Biltonen and Lichtenberg, 1993; Mouritsen, 2005). For information on the T_m of different lipids see Marsh (1990) or the LIPIDAT website.

In general, liposomes in the LD phase are more permeable than liposomes in the SO or LO phases. Liposomes undergoing phase transition are even more permeable than liposomes in the LD phase, due to large defects in the bilayer packing which are related to coexistence of SO and LD regions within the bilayer (Jorgensen and Mouritsen, 1995; Leidy et al., 2002; Needham et al., 2000).

The LO phase is a unique intermediate phase between the SO and LD phases, which only occurs when a membrane-active sterol, such as cholesterol, is included in the phospholipid bilayer (Barenholz, 2002; Barenholz and Cevc, 2000). Cholesterol, having packing parameter of ~1.2 (Garbuzenko et al., 2005a), when added to a liposome bilayer at a mole ratio of more than 30% and less than 50%, causes transformation of the lipid bilayer to the LO phase (Barenholz and Cevc, 2000; Mouritsen, 2005). Bilayers in the LO phase, due to their small free volume, are less sensitive to temperature changes, and therefore are less permeable, more stable, more rigid, and have a lower degree of hydration at the polar headgroups of the phospholipids, in comparison to bilayers in the LD phase (Barenholz and Cevc, 2000; Mouritsen, 2005).

The lipid composition of the liposomes, which determines their thermotropic behavior, phase transition temperature range, and T_m (Biltonen and Lichtenberg, 1993; Mouritsen, 2005), determines the liposomes' permeability and therefore the stability of drug loading and rate of drug release (Barenholz and Cevc, 2000; Lasic et al., 1991; Rickwood, 1994).

Adding charged amphiphilic molecules to the liposome membrane increases the liposome electrical charge in a concentration-dependent manner. Charged *bilayers* repel each other, thereby increasing the trapped volume of encapsulated aqueous medium within multilamellar vesicles (MLV) (Barenholz and Cevc, 2000; Zuidam and Barenholz, 1997). In addition, introducing molecules of the same charge into the membrane causes *headgroups* to repel one another in the membrane plane, thereby increasing the permeability of the liposome (Crommelin, 1984; Lichtenberg and Barenholz, 1988; Rickwood, 1994).

Adding the lipopolymer mPEG-DSPE introduces to the lipid bilayer a highly hydrated extended steric barrier that surrounds the liposome (Garbuzenko et al., 2005b; Tirosh et al., 1998; Torchilin and Papisov, 1994).

2.2. Using ultrasound to form liposomes

In the early 1960s it was found that exposing aqueous lecithin dispersions to ultrasound resulted in the formation of dispersions of what was speculated then to be "lecithin micelles" (Attwood and Saundees, 1965; Saunders et al., 1962). It was not observed then that these "micelles" had an inner aqueous core and were actually small unilamellar vesicles. Several years later (Papahadjopoulos and Miller, 1967; Papahadjopoulos and Watkins, 1967), showed that phosphatidylcholine as well as other liposome-forming phospholipid suspensions exposed to 80 kHz LFUS formed small unilamellar vesicles (SUV), Table 3. Huang and coworkers were the first to study these SUV carefully (Huang and Charlton, 1971; Huang, 1969). They treated the SUV as macromolecules and fractionated them using gel permeation chromatography (GPC). These studies provided fundamental understandings regarding liposomes, including phospholipid bilayer structure, liposome dimensions, and the asymmetric lipid distribution between the

Table 3
Using ultrasound for liposome formation and to control liposomal and micellar drug release.

Downsizing large multilamellar liposomes to form small unilamellar liposomes

Frequency	Irradiation time	Liposome diameter after ultrasound	Lipid	Sample volume	Sonicator type	Temperature	Reference
20 kHz	160 min	~25 nm	Egg phosphatidylcholine	N/A	Probe, Branson Sonifier S-125[a]	2 °C	Huang (1969)
80 kHz	20 min	~50 nm	Phosphatidylcholine, phosphatidylserine	25 mL	Bath, Ultrasonic Instr. Model G4oC2H-T4oC1[a]	RT	Papahadjopoulos and Watkins (1967)
10–80 kHz	1 h	25–50 nm	Egg phosphatidylcholine, phosphatidic acid, phosphatidylinositol, phosphatidylethanolamine	0.6 mL	Bath/Probe[a]	16 °C	Johnson et al. (1971)
20–25 kHz	45 min to 1 h	~50 nm	Egg lecithin, dimyristoylphosphatidylcholine, dipalmitoyl-phosphatidylcholine	N/A	Probe, Heat Sys. W350 Sonifier[a]	8 °C, above T_m	Barrow and Lentz (1980)
20 kHz	<30 min	~25 nm	Egg phosphatidylcholine + bovine brain sphingomyelin	2–8 mL	Probe, Heat Sys. W350 Sonifier[a]	0 °C	Barenholz et al. (1977)

Controlled release of drugs by ultrasound from liposomes and micelles

Delivery system	Liposome size	Frequency	Irradiation time	Amplitude	Sonicator type	Temperature	Reference
Liposome	~100 nm	20 kHz	<3 min	3.3 W/cm^2	Probe	37 °C	Schroeder et al. (2007, 2009)
Temperature-sensitive liposomes	~100 nm	1 MHz	12 s (in pulses of 0.1 s each)	1300 W/cm^2	Probe	~42 °C	Dromi et al. (2007)
Micelles	<80 nm	20–100 kHz	<80 s	0–3.5 W/cm^2	Probe/Bath	37 °C	Rapoport (2007); Unger et al. (1998)

N/A = not available.
[a] Amplitude of irradiation is not available.

inner and outer layer of the SUV bilayer. These studies led to better understanding of the importance of liposome curvature on many liposomal properties (reviewed in Lasic, 1996; Lichtenberg and Barenholz, 1988).

Exposing different phospholipids to similar LFUS irradiation conditions formed liposomes of different sizes (Barenholz et al., 1977; Huang, 1969; Johnson et al., 1971; Papahadjopoulos and Watkins, 1967; Szoka and Papahadjopoulos, 1980). Generally, for a given phospholipid formulation, longer irradiation periods and/or higher ultrasonic power resulted in smaller mean size, more homogeneous liposome population, until most of the population reached the minimal size of stable SUV (~20–25 nm) (Olson et al., 1979; Papahadjopoulos and Watkins, 1967; Szoka and Papahadjopoulos, 1980). Barenholz et al. (1977) showed that exposing MLV to ultrasound combined with differential ultracentrifugation resulted in homogeneous dispersions of SUV. Lichtenberg and Barenholz (1988) conducted an in-depth review of the characterization of many physicochemical parameters of ultrasound-formed SUV. Studies by Barenholz et al. (1977); Berden et al. (1975); Hauser and Barratt (1973); Zasadzinski (1986) suggest that SUV are formed by sequential delamination of the outer layers of MLV, thereby resulting in the reduction of MLV size and an increase in SUV population in the dispersion. This correlates well with several other studies (Finer et al., 1972; Lasic, 1988, 1993; Lawaczeck et al., 1976; Mendelsohn et al., 1976) that propose that the mechanism of ultrasound-induced vesicle formation is related to transient cavitation which induces "high energy" inter-vesicle collisions. During the collisions short-lived phospholipid bilayer fragments are freed. These fragments, due to exposure of the hydrophobic regions to the aqueous phase, undergo rapid fusion and closure to rearrange into smaller vesicles (SUV or LUV) (Lasic, 1988). It was also suggested that extreme shear forces, induced by ultrasonic cavitation, narrow the size distribution of liposomal dispersions (Barrow and Lentz, 1980; Finer et al., 1972; Lawaczeck et al., 1976; Maulucci et al., 2005; Moran et al., 2006; Pereira-Lachataignerais et al., 2006). Other studies (Leighton, 1989; Richardson et al., 2007; Tho et al., 2007; Wu, 2007) showed that stable cavitation, which induces acoustic micro-streaming near the surface of the oscillating bubbles and shear forces in the dispersion, can also induce reduction in liposome size.

2.2.1. Formation of smaller molecular assemblies by exposure to ultrasound

Exposing PEGylated liposomes to LFUS resulted in the transformation of a fraction of the liposomes to smaller non-liposomal molecular assemblies of yet unknown nature, coexisting with the liposomes (Lin and Thomas, 2004; Schroeder et al., 2007). This process does not involve any change in the chemical integrity of the liposome lipids (Hauser and Barratt, 1973; Schroeder et al., 2007) and may be explained by the coexistence of liposome-forming lipids (such as PCs) with micelle-forming lipids (such as PEG-DSPE).

Borden et al. (2005), studying the effect of ultrasound-induced destruction of micro-bubbles, showed that exposing less cohesive lipid shells (composed of phospholipids having shorter acyl chains, such as the 14:0 PC DMPC) to ultrasound resulted in formation of micron-scale or smaller particles composed of excess lipid material that "shed" during the ultrasonic pulse. Conversely, exposing more cohesive shells, composed of phospholipids having longer saturated acyl chains such as DSPC (18:0 PC) or DBPC (22:0 PC) to ultrasound, resulted in buildup of lipid strands and globular aggregates several microns in size.

Lin and Thomas (2003a), comparing the effect of LFUS irradiation on PEGylated and non-PEGylated liposomes, showed that when exposing PEGylated liposomes to LFUS, small, <10 nm in diameter, particles are formed coexisting with the liposomes; these particles are not found in dispersions of non-PEGylated liposomes exposed to LFUS.

It seems possible that under intense ultrasonically induced stresses, liposomes undergo transient structural deformations that force a fraction of the lipids out of the liposomes to form other assemblies. This may especially occur in cases where other lipids, having packing parameters out of the liposome range of 0.74–1.0, are present. PEG-DSPE, having a rather low packing parameter of ~0.5 (Garbuzenko et al., 2005a) and a much higher critical aggre-

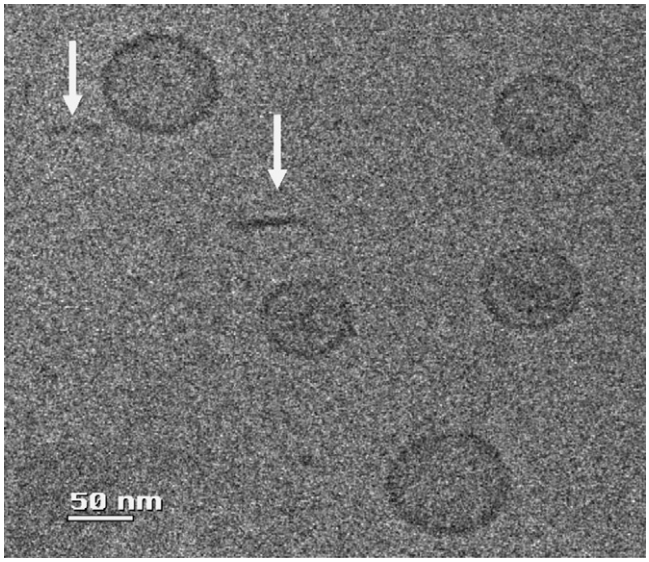

Fig. 1. A cryo-TEM image of a liposomal dispersion after being exposed to LFUS. Possible formation of PEG-DSPE discs (pointed to by white arrows) in the vicinity of ultrasonically irradiated liposomes is seen. Adapted from Schroeder et al. (2007).

gation concentration (CAC) than liposome-forming lipids ($\sim 10^{-5}$ M for PEG-lipids, and $\sim 10^{-10}$ M for zwitterionic phospholipids (Priev et al., 2002)), seems to be a good candidate to be ejected from the lipid bilayer to form micelles (which are smaller assemblies than liposomes) under ultrasound stress. Evidence for the possible formation of PEG-lipid micellar discs (Ickenstein et al., 2003; Johnsson and Edwards, 2003; Leal et al., 2008) in ultrasonically irradiated liposomal dispersions, Fig. 1, have been previously presented (Schroeder et al., 2007).

2.3. Controlled release of drugs from liposomes using ultrasound

Recently, it has been shown that ultrasound can effectively control drug release from liposomes by inducing either thermal or non-thermal effects.

Frenkel and coworkers (Dromi et al., 2007; Frenkel, 2008) studied the ability to release drugs from temperature-sensitive liposomes (also known as thermo-sensitive liposomes) using high-intensity focused ultrasound (HIFU). HIFU is an innovative non-invasive technique which enables targeted treatment of a wide range of clinical conditions by focusing high frequency ultrasonic beams to form a high energy focal point within the body (Cohen et al., 2007; Fennessy et al., 2007; Jolesz and Hynynen, 2002; Jolesz et al., 2004; Jolesz and McDannold, 2008; Ram et al., 2006; Stone et al., 2007; Yang et al., 1991; Zacharakis et al., 2008). The membranes of these liposomes include lipids which have a T_m in the range of 40–45 °C (Needham et al., 2000). During the SO to LD phase transition, the liposomal permeability increases due to coexistence of SO and LD domains in the same membrane, thus disrupting the close and ordered packing of the lipid bilayer and introducing "free volumes" which enable the drug to move across the lipid bilayer from the intra-liposomal aqueous core to the extra-liposomal medium (Jorgensen and Mouritsen, 1995; Leidy et al., 2002; Needham et al., 2000).

The ability to control the release of drugs and other molecules from liposomes using non-thermal effects of LFUS has been studied previously (Lin and Thomas, 2003a, 2004; Pong et al., 2006; Schroeder et al., 2007). It was shown that LFUS-facilitated drug release did not affect the drug's chemical integrity or biological potency (Schroeder et al., 2007), Fig. 2.

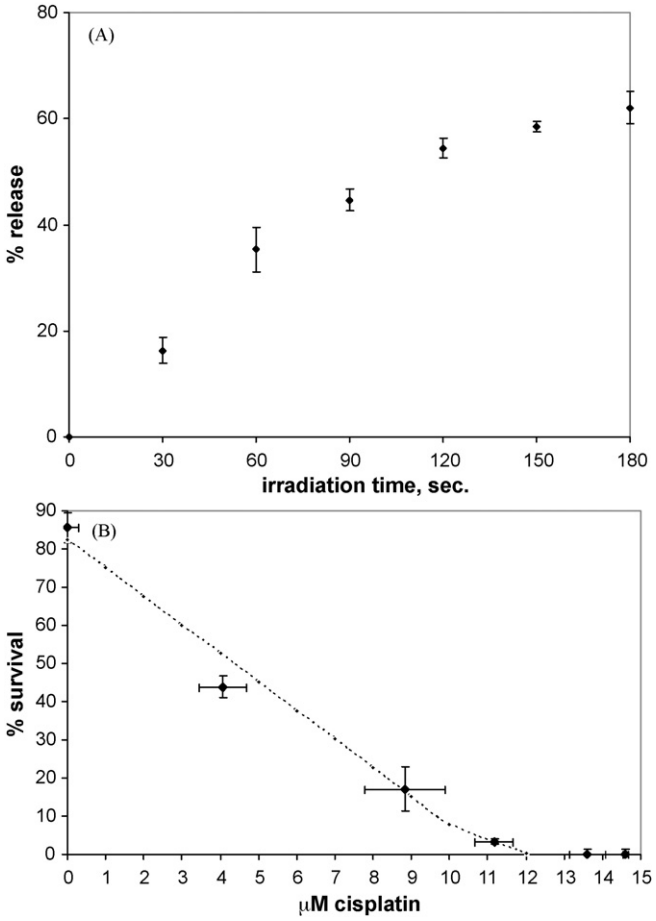

Fig. 2. (A) LFUS (3.3 W/cm^2) induced release of cisplatin from liposomes. (B) Biological potency of released cisplatin on C26 murine colon adenocarcinoma cells in culture (♦); survival was compared to that of equal amounts of free (non-liposomal) cisplatin (dotted line). Data adapted from Schroeder et al. (2007).

Herein, we aim to discuss the non-thermal effects of ultrasound, which have been shown to facilitate drug release, on lipid membranes.

2.4. Ultrasound parameters that affect liposomal drug release

2.4.1. Effect of ultrasound frequency on liposomes of different sizes and lamellarities

Dunn and coworkers (Maynard et al., 1983; Tata and Dunn, 1992) investigated the relaxation kinetics of membranes of single- and multilamellar vesicles composed of DMPC or DPPC by measuring the ultrasonic absorption and velocity in such dispersions. Using 1.42 and 2.11 MHz ultrasound for DMPC (T_m 23.2 °C) and DPPC (T_m 41.4 °C), respectively, they found that enhanced ultrasonic absorbance occurs at the main (SO to LD) lipid phase transition, while below the phase transition ultrasound is hardly absorbed by the membrane. This suggests that liposomal drug release, achieved when working below the phase transition temperature (in the SO phase), may be attributed to mechanical or thermal effects rather than *absorbance* of ultrasound by the lipid bilayer. A previous study, conducted in our lab (Cohen-Levi, 2000), tested the ability to release doxorubicin from Doxil using ultrasound at low or high frequencies (20 kHz, or 1 and 3 MHz). Doxil is a liposomal anti-cancer nanodrug in which doxorubicin is remote (actively) loaded into 100-nm sterically stabilized liposomes (Barenholz, 2007). Exposing Doxil to 20-kHz ultrasound (LFUS) induced effective doxorubicin release both in saline and in human-sourced plasma, reaching 85% and

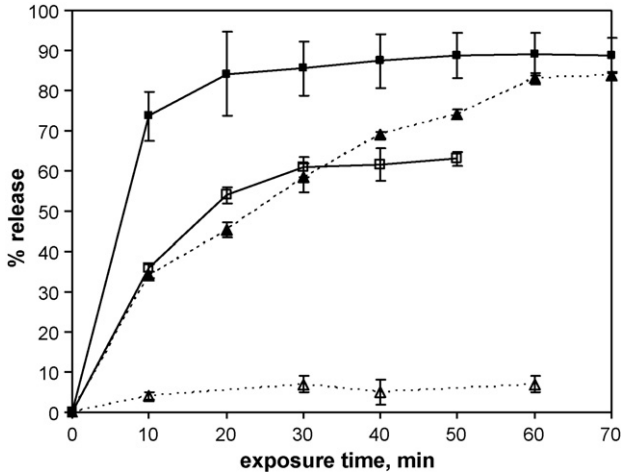

Fig. 3. Controlled release of doxorubicin from liposomes using 20-kHz and 1-MHz ultrasound. Liposomal doxorubicin (Doxil), dispersed at 37 °C in either saline or in human-sourced plasma, was exposed to 20-kHz ultrasound at an amplitude of 1.2 W/cm^2 (■ or □, respectively, for saline or plasma), or to 1-MHz ultrasound at an amplitude of 2.5 W/cm^2 (▲ or △, respectively, for saline or plasma). Data based on Cohen-Levi (2000).

61% release, respectively, after 30 min, Fig. 3. Exposing Doxil to 1-MHz ultrasound released doxorubicin in saline at a slow rate, in comparison to 20-kHz ultrasound, while, in human-sourced plasma, 1-MHz ultrasound released hardly any doxorubicin (~5%), Fig. 3. Even lower release levels were found using 3-MHz ultrasound (data not shown). The difference in release levels using low (20 kHz) or high (1 and 3 MHz) frequency ultrasound is explained by the importance of cavitation in facilitating liposomal drug release (Cohen-Levi, 2000). The lower levels of release in plasma were shown to be related to the presence of the plasma proteins, which seem to absorb a major part of the ultrasonic energy, thereby reducing the extent of cavitation (Cohen-Levi, 2000). See also Table 1 for ultrasound absorbance in protein-enriched solutions.

Another study (Schroeder et al., 2007) showed that the profile of ultrasonically induced liposomal drug release (of several different drugs) follows first-order kinetics. This suggests that release is primarily related to the effect of LFUS on the liposome membrane and on membrane constituents that affect ultrasonic susceptibility.

Addressing the effect of ultrasonic frequency on dye release from liposomes of different sizes and lamellarities, Pong et al. (2006), suggested that when the size of the vesicle is small in comparison to the ultrasound wavelength, the vesicle is exposed to a virtually uniform pressure, and the associated pressure gradient can be considered to be negligible. However, when the dimensions of the vesicle become comparable to the ultrasound wavelength, the pressure is no longer uniform, and produces an associated shear force which acts on the vesicle surface, thereby compromising the integrity of the liposome membrane. Therefore, exposing small unilamellar vesicles (~100 nm in diameter) to high frequency ultrasound, which is ~4 orders of magnitude larger than the liposome (for example, at 1 MHz, $\lambda \approx 1.5$ mm), or to low frequency ultrasound, which is ~6 orders of magnitude larger than the liposome (for example, at 20 kHz, $\lambda \approx 75$ mm), may have a similar mechanism of action on liposomal drug release. The reason that drug release levels are increased when using low frequency ultrasound in comparison to the higher frequencies most likely results of the fact that the intensity needed to induce transient cavitation is lower at low frequencies, see Section 1.3 above.

2.4.2. Cavitation and LFUS-induced drug release

Previous studies, conducted by Rapoport, Pitt and others, testing the ability to release drugs from polymeric *micelles* by ultrasound, show that stable cavitation can induce drug release from micelles, however, transient cavitation increases drug release in a significantly more substantial manner (Husseini et al., 2000; Marin et al., 2002; Pitt et al., 2004; Rapoport, 2007; Rapoport et al., 2002). Kost and coworkers, using ultrasound to control the release of drugs from polymeric matrices, showed that ultrasonic cavitation ruptured drug pockets within the hydrophobic polymer, thereby facilitating drug release (Aschkenasy and Kost, 2005; Traitel et al., 2008).

It has been shown that release of an encapsulated substance from the inner aqueous compartment of 100-nm liposomes using LFUS is increased in the presence of transient cavitation (Lin and Thomas, 2003a, 2004; Schroeder et al., 2007).

2.4.3. Enhancing cavitation to improve drug release from liposomes

The importance of cavitation in facilitating liposomal drug release was exemplified by conjugating lipid micro-bubbles filled with gas (such as perfluorocarbons, PFCs) to drug/gene-loaded liposomes, or by encapsulating a drug together with gas into lipid shells or liposomes. The dispersions were exposed to ultrasound (mostly high frequency), which alone would not induce cavitation, but, cavitation occurred in the presence of these sub-micron to micron sized bubbles (acting as cavitation nuclei). The existence of cavitation near the liposomes was found to enhance drug and gene delivery substantially (Dijkmans et al., 2004; Ferrara et al., 2007; Ferrara, 2008; Gao et al., 2008; Kee et al., 2008; Kheirolomoom et al., 2007; Lawrie et al., 2000; Lentacker et al., 2007; Rapoport et al., 2007; Shaw et al., 2009; Suzuki et al., 2007; Unger et al., 2001, 2004). The main advantages of high frequency ultrasound over low frequency ultrasound are its wide clinical use (for other purposes), and the ability to focus high frequency ultrasound waves to create a high energy focal point, Table 2.

It may be noted that echogenic substances, such as PFCs, can be loaded into liposomes or lipid shells as either liquids or gases. When exposing liposomes loaded with PFCs in the *liquid* state (which is usually highly dense, >1.5 g/mL) to ultrasound, acoustic streaming can be used to direct the liposomes to various organs. Occurrence of cavitation in such dense media is not expected (Dayton et al., 2006).

2.4.4. Transient pore formation by ultrasonic irradiation

Hilgenfeldt, Brochard-Wyarta, Marmottant, and others showed that lipid vesicles placed in an LFUS-induced intense acoustic streaming field undergo transient structural deformations, which induce the forming of transient pores in the membrane, through which the exchange of intra-vesicular and extra-vesicular fluids is enabled (Brochard-Wyarta et al., 2000; Marmottant et al., 2008; Marmottant and Hilgenfeldt, 2003; Sandre et al., 1999). An early study (Papahadjopoulos and Watkins, 1967), demonstrated that SUV, in contrast to MLV, do not rupture under exposure to LFUS; however, their work suggested that under exposure to LFUS the permeability of ions through the lipid bilayer is increased. Lawaczeck et al. (1976) showed that exposing liposomes at a temperature below their T_m to LFUS produced structural defects in the lipid bilayer that enabled rapid permeation of ions from the intra-liposomal aqueous core to the extra-liposomal medium. Mendelsohn et al. (1976), using Raman spectroscopy, showed that exposing liposomes to ultrasound disrupts the close and ordered packing of the hydrophobic chains of liposomal lipids, which may enhance permeability. Lin and Thomas (2004) and Pong et al. (2006) exposed dye-loaded liposomes to ultrasound, and found that dye leakage from liposomes stopped immediately after termination of ultrasonic irradiation.

Schroeder et al. (2007), studying the ability of LFUS to control the release of ions and drugs, having different physical and chemical properties (see Table 4), from liposomes, showed that release

Table 4
Physicochemical properties of different drugs and of acetate released from liposomes by LFUS.

Drug/properties	Doxorubicin	Methyl prednisolone hemisuccinate	Cisplatin	Acetate
Molecular weight	543.52	474.54	300.05	60.05
pK_a	8.68[a]	4.29[a]	5.06,1.82[a]	4.54[a]
Polar surface area, Å^2	206.07[d]	138.2[d]	52.04[d]	37.30[d]
Total area, Å^2	453.8[d]	456.9[d]	154.1[d]	96.4[d]
Non-polar area, Å^2	247.7	318.7	102.06	59.1
ASA_H[c]	436.90[b]	403.70[b]	250.3[b]	118.9[b]
ASA_P[e]	209.40[b]	188.30[b]	50.2[b]	100.9[b]
Intrinsic molar solubility[a]	9.00E−07[a]	1.70E−05[a]	N/A	N/A
Solubility, mM (pH)	0.2(5.5)[a]	30.3(7.6)[a]	3@4 °C 6.3@37 °C 27@65 °C (6.00)[f]	N/A
log P	3.07[a]	2.69[a]	0.04[a]	0.17[a]
log D (pH)	0.31(5.5)[a]	−0.47(7.6)[a]	−1.06(4.00) 0.04(7.00)[a] 0.04(10.0)	−0.28(4.00) −2.60(7.00) −3.85(10.0)
Charge (pH)	1(5.5)[b]	−1(7.6)[b]	0.93(4.00) 0.01(7.00)	−0.22(4.00) −1.00(7.00)

The following programs were used for calculating the properties of the drugs:

[a] Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (using CAS registry database); Calculator Plugins.

[b] Marvin 5.0.4, 2008 ChemAxon.

[c] ASA_H is the solvent accessible surface area of all hydrophobic ($|q_i| < 0.125$) atoms, where $|q_i|$ is the absolute value of the partial charge of the atom.

[d] Molecular Operating Environment (MOE), version 2007.09, Chemical Computing Group Inc. Montreal, Quebec, Canada.

[e] ASA_P: solvent accessible surface area of all polar ($|q_i| > 0.125$) atoms ($|q_i|$ is the absolute value of the partial charge of the atom). Total surface area is the van der Waals surface area. Non-polar surface area was calculated by subtracting the polar surface area from the total surface area.

[f] Data adapted from Zucker et al. (2009), and from Khazanov et al. (2002); Peleg-Shulman et al. (2001).

follows first-order kinetics, thus being dependent on the actual irradiation time and on the concentration gradient of the drug between the intra-liposomal aqueous core and the extra-liposomal medium, suggesting that LFUS induces the formation of transient pore-like defects to the liposome membrane, which reseal after cessation of LFUS irradiation.

2.5. Membrane constituents that affect ultrasound-induced liposomal drug release

2.5.1. Effect of PEG and PEG-lipids on LFUS-induced drug release from liposomes

Liposome membrane composition has a large effect on many liposome properties, including: size, physical phase (SO, LD, or LO) and the temperature range of the phase transition, drug loading efficiency, and stability (Barenholz, 2001, 2003; Barenholz and Cevc, 2000; Barenholz and Crommelin, 1994). The effect of LFUS on release of drugs or other loaded molecules from ∼100-nm SUV composed primarily of EPC ($T_m = -5$ °C), HSPC (hydrogenated soy phosphatidylcholine, $T_m = 52.5$ °C), or DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, $T_m = 41.4$ °C), and containing polyethylene glycol-(PEG) lipopolymers, showed increased release in comparison to non-PEGylated liposomes (Lin and Thomas, 2004; Pong et al., 2006; Schroeder et al., 2008a).

m^{2000}PEG-DSPE (also referred as PEG-DSPE) is a lipopolymer in which a 2000-Da polyethylene glycol is attached to the primary amino group of distearoyl phosphatidylethanolamine. When mixing PEG-DSPE with the other lipids prior to liposome formation, the lipidic moieties of the DSPE integrate into the lipid bilayer, and the highly hydrated (3–4 water molecules per ethylene oxide group) PEG headgroups extend out and surround the liposome in a 4–10-nm corona (Garbuzenko et al., 2005a; Tirosh et al., 1998), thus providing a protective steric barrier which prevents liposomes from being taken up by cells and macrophages, prevents liposome aggregation and fusion, and increases liposome circulation time (Torchilin and Papisov, 1994; Woodle and Lasic, 1992). The degree of extension of the PEG corona from the liposome surface is dependent on the concentration of PEG-DSPE in the liposome and on the PEG moeity length. PEG 2000 below a PEG-DSPE concentration of ∼4 mol%, PEG chains will be in a "mushroom" configuration; from a PEG-DSPE concentration of ∼4 mol% up to ∼7 mol%, PEG will be in a "brush" configuration (Garbuzenko et al., 2005a; Tirosh et al., 1998). The packing parameter of PEG-DSPE is ∼0.5; thus, when present in an aqueous solution *by itself*, it will spontaneously form micelles (Garbuzenko et al., 2005a). The saturation concentration of PEG-DSPE in the liposome lipid bilayer is ∼8 mol%. Above ∼8 mol%, PEGylated liposomes will coexist with PEG-DSPE micelles enriched with liposome lipids (Kalmanzon et al., 1992; Opatowski et al., 2002). The amount of micelles will increase with increasing mol% of PEG-DSPE; the composition of the micelles also changes by increasing the mol% of PEG-DSPE (Garbuzenko et al., 2005b). At higher than 30 mol% of PEG-DSPE, all the liposomes will be converted into PEG-DSPE/PC/cholesterol micelles (Belsito et al., 2001).

Polyethylene glycol (PEG) (non-lipidic) is highly hydrated in aqueous solutions (Tirosh et al., 1998). In contrast to the lipidic PEG-DSPE, which introduces a steric barrier that protects against inter-vesicle interactions including aggregation and fusion (Garbuzenko et al., 2005a,b), introducing non-lipidic PEG to the external medium of liposomes creates an osmotic imbalance, due to the PEG-induced excluded volume effect, which leads to mechanical stress on the membrane that induces liposome fusion (Malinin et al., 2002).

The interaction of ultrasound with PEGylated lipopolymers (either inserted in the liposome lipid bilayer (Barenholz, 2007; Gabizon et al., 1994; Woodle and Lasic, 1992), or as PEG-lipid micelles added to preformed liposomes (Uster et al., 1996)), in comparison to the interaction of ultrasound with free, non-lipidic, PEG, was studied on HSPC:cholesterol liposomes (60:40, mol:mol) (Schroeder et al., 2008a). Adding non-lipidic PEG (2000 Da) to liposome dispersions affected LFUS-induced drug release in a bell-shaped manner. Release increased up to a PEG:phospholipid mole ratio of ∼1.2:1, while at higher PEG concentrations, drug release decreased. These data suggest that at low concentrations, the dominant effect of non-lipidic PEG added to a liposome dispersion is an increase in membrane instability (Malinin et al., 2002; Needham et al., 1997), which increases liposome responsivity

to LFUS. However, at higher PEG concentrations, the dominant effect becomes absorption of ultrasonic energy by the highly hydrated PEG molecules (Tirosh et al., 1998), thereby reducing the energy available to affect the liposome membrane, and reducing release.

The effect of adding PEG-DSPE micelles to liposomal dispersions has a different profile. Up to a PEG-DSPE:phospholipid mole ratio of ~1:10, drug release increased (Lin and Thomas, 2004; Schroeder et al., 2008a), above this mole ratio, release decreased. PEG-DSPE, below a mole ratio of PEG-DSPE:phospholipid of ~1:10, readily disassembles from its micellar form and incorporates into the liposome membrane, leading to the formation of PEGylated liposomes in which the PEG-DSPE is present in the external leaflet of the liposomes (Schneider et al., 1996; Uster et al., 1996). Adding PEG-DSPE (as micelles) to preformed liposomes, at a mole ratio above ~1:10 PEG-DSPE:phospholipid, induces formation of PEG-DSPE micelles enriched with liposome phosphatidylcholine (PC) and cholesterol (PEG-DSPE:PC:cholesterol); these micelles coexist with PEGylated liposomes (Garbuzenko et al., 2005a). It was proposed (Schroeder et al., 2008a) that the gain in LFUS-induced drug release at low PEG-DSPE:phospholipid mole ratios is due to enhanced absorption of ultrasonic energy by PEG moieties extending from the liposome surface, thereby focusing ultrasonic energy near the liposome membrane. Increasing PEG-DSPE above a 1:10 mole ratio reduces LFUS-induced drug release in a concentration-dependent manner, most likely due to the dominant competitive effect of ultrasound absorption by the highly hydrated PEG moieties extending also from the PEG-DSPE:PC:cholesterol *micelles*. These results agree with an earlier study (Lin and Thomas, 2003b) that showed that adding small amounts (<10 mol%) of Pluronic P105 (a micelle-forming ethylene oxide/propylene oxide block copolymer) micelles to non-PEGylated liposomal dispersions resulted in an increase in LFUS-induced liposomal dye release, probably due to the insertion of the pluronic P105 molecules into the liposome membrane.

Testing the effect of PEG chain length on LFUS-induced liposomal dye release showed that at PEG-DPPE:PC mole ratios lower than 1:10, ultrasound-induced dye release from liposomes containing short PEG-lipids (PEG350-DPPE) was similar to that of the longer PEG2000-DPPE (Lin and Thomas, 2003a, 2004). Thus suggesting, that the lateral surface pressure of the headgroup (which is higher for PEG2000 in comparison to PEG350) is not a critical factor for ultrasound sensitization of liposomes (Lin and Thomas, 2003a, 2004). However, at higher PEG-DPPE:PC mole ratios, in which a coexistence of PEGylated liposomes and of PEG-DPPE micelles (and of monomeric PEG-DPPE lipids) occurs (Garbuzenko et al., 2005a; Priev et al., 2002), dye release rate from the liposomes was higher for the shorter PEG350-DPPE in comparison to the longer PEG2000-DPPE (Lin and Thomas, 2003a). This suggests that the longer PEG2000 moiety absorbs a higher level of ultrasonic energy in comparison to the shorter PEG350. This may be due to the fact that in an equimolar dispersion of PEG2000 and PEG350 there are many more ethylene oxide *groups* in the dispersion containing the longer PEG chains. The reason that when incorporated in the liposomes no difference in LFUS-induced release is noticed for the two polymer chain lengths (Lin and Thomas, 2003a) may be because the contribution of the PEG part which is close to the lipid bilayer is most important, however this requires further investigation.

2.5.2. Effect of surfactants on LFUS-induced liposomal drug release

Huang and MacDonald (2004) showed that incorporating 4 mol% of the micelle-forming molecule diheptanoylphosphatidylcholine (DHPC, di-C7-PC) into liposomes increased the release of encapsulated calcein upon ultrasonic irradiation. Similarly, it was shown that introducing phospholipids with unsaturated *cis* double bonds to the bilayer also increased liposomes' susceptibility to ultrasound (Pong et al., 2006). It seems that incorporating lipids that introduce structural irregularities leading to an increase in free volume in the membrane and disrupting the close packing of acyl chains within the membrane, increases liposome susceptibility to LFUS.

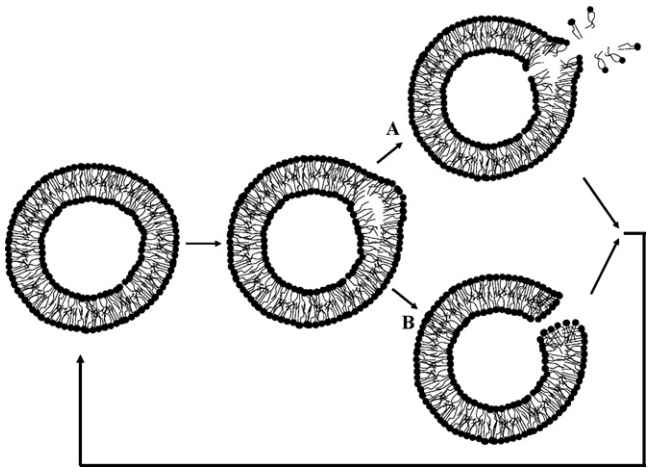

Fig. 4. A schematic representation of the formation of transient pores in the liposome membrane by ultrasound. The transient pores may occur due to formation of small gas nuclei in the hydrophobic region of the lipid bilayer under the effect of an ultrasonic field. The pores may be either hydrophobic (A) or hydrophilic (B) in nature, and tend to reseal after short periods of time. The formation of transient pores may free membrane fragments from the liposomes, which will then form into smaller lipid aggregates.

Enhanced liposomal dye release occurred also when exposing liposomes to ultrasound in the presence of surfactants which share the structural feature of a polymeric/oligomeric ethylene glycol headgroup (Triton X 20 and 80, and Tween 100 and 450) (Lin and Thomas, 2003a, 2004). Surfactants are known to penetrate and weaken the membrane (Needham et al., 1997) and most likely thereby to facilitate ultrasound-induced liposome permeabilization. In the cases of Triton and Tween (similar to the cases mentioned above regarding PEG-DPPE with different PEG chain lengths), up to a certain concentration of the surfactants, no difference in release was noticed for surfactants having short or long headgroups. However, at higher concentrations, release in the presence of the shorter headgroup was higher than release in the presence of the longer headgroup (Lin and Thomas, 2003a), most likely due to absorption of ultrasonic energy by the longer headgroups.

The major parameters that affect ultrasound-induced liposomal drug release are presented in Table 5.

2.6. A proposed mechanism of ultrasound-induced drug release

Optimizing LFUS-induced liposomal drug release requires better understanding of the mechanism by which transient pores are formed in the liposome membrane. It is suggested that when a liposome is exposed to an oscillating ultrasonic field, gas bubble nuclei may be formed in the hydrophobic region of the lipid bilayer. These nuclei grow until they permeate the membrane, forming a transient pore through which the drug is released; thereafter, the membrane relaxes and resumes its initial impermeable state. Such transient pores may be either hydrophilic or hydrophobic in nature (schematically presented in Fig. 4). It seems possible that formation of gas nuclei in the hydrophobic region of the lipid bilayer may be favorable energetically. Furthermore, these transient disruptions may be the reason for the disassembly of ~23% of the liposomes. As in cases of multiple poration of a single liposome membrane,

Table 5
Major parameters that affect ultrasound-induced liposomal drug release.

Ultrasound parameters affecting liposomal drug release	
Frequency	Liposomal drug release induced by LFUS is associated mainly with mechanical effects, such as transient cavitation. Liposomal drug release induced by high frequency (>1 MHz) ultrasound is associated mainly with thermal effects.
Amplitude	Ultrasonic amplitude controls the extent of mechanical (such as cavitation) or thermal effects. Using LFUS, the threshold for cavitation has been shown to be ~1.2 W/cm^2. Using HIFU to induce local hyperthermia, the amplitude plays an important role in heating.
Cavitation	Acoustic cavitation, i.e., the formation, growth, and intense imploding of gas bubbles in solutions exposed to ultrasound, increases liposomal drug release substantially. Gas nuclei may form in the extra-liposomal medium, in the liposome membrane, or in the intra-liposomal aqueous core. The acoustic power needed to induce cavitation is lower at lower frequencies; therefore, cavitation is more prevalent when using lower frequency ultrasound (Mason and Lorimer, 1988).
Hyperthermia	Thermo-sensitive liposomes are designed to undergo a phase transition (from solid-ordered (SO) to the liquid-disordered (LD) phase) at a temperature slightly higher than physiological temperature. Local heating induces phase transition and results in drug release from the liposomes. Local heating can be induced using high-intensity focused ultrasound (HIFU). HIFU is associated with high frequency ultrasound.
Membrane constituents that affect ultrasound-induced liposomal drug release	
Lipid composition	Surface-active molecules, such as detergents, or phospholipids having unsaturated acyl chains, that disrupt the close packing of the lipid bilayer, increase liposomes' responsivity to ultrasound. It seems that these molecules weaken van der Waals interactions between the acyl chains, thereby increasing free volume and making the lipid bilayer more susceptible to mechanical strains induced by ultrasound.
Physical state of the bilayer	Increased absorbance of ultrasonic energy by the lipid bilayer occurs during the SO-to-LD phase transition.
PEG moieties	Introducing to the liposome membrane polyethylene glycol (PEG) conjugated to a lipid increases liposomal drug release. It seems that PEG moieties absorb ultrasound, thereby focusing ultrasonic energy at the liposome surface.
Thermo-sensitive lipids	Mixing in the liposome membrane lipids such as MPPC or DMPC (Needham et al., 2000), which lower the SO-to-LD phase transition to a temperature slightly above the physiological temperature, enables releasing drugs by local hyperthermia. Drug release occurs due to the coexistence of LD and SO domains in the lipid bilayer, which disrupt the close packing of the lipid bilayer and facilitate drug release.

This table summarizes data described throughout this paper. Only new references that were not cited in the paper were cited in the table.

fragments may be released to the media to then form smaller lipidic assemblies. In a similar scenario, regarding the formulation of echogenic liposomes, the formation of a gas compartment within the hydrophobic region of the lipid bilayer of liposomes has been suggested by Huang and MacDonald (2004). Formation of gas nuclei in the hydrophobic zone of the lipid bilayer may also affect the additive packing parameter of the liposome lipids (Garbuzenko et al., 2005a) in such a way that will force PEG-lipids out of the lipid bilayer to form PEG-lipid micelles or micellar discs.

3. Conclusions

Herein we present a comprehensive analysis of the mechanism of ultrasound-induced drug release from liposomes. Liposome constituents, especially PEG-lipopolymers, which seem to absorb ultrasonic energy, and molecules that compromise the close packing of lipids in the bilayer have a large effect on increasing ultrasonic responsivity. The mechanism of release seems to be formation of transient pores in the lipid bilayer, through which drugs are released from the inner aqueous core of the liposomes to the extra-liposomal medium. The principles described here may be applicable for ultrasonically induced transient pores in other lipid-based vesicles and assemblies, and may be used to design novel ultrasound-responsive drug delivery systems.

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References

Angelsen, B.A., 2000. Ultrasound Imaging. Emantec, Trondheim, Norway.
Apfel, R.E., Holland, C.K., 1991. Gauging the likelihood of cavitation from short-pulse, low-duty cycle diagnostic ultrasound. Ultrasound Med. Biol. 17, 179–185.
Aschkenasy, C., Kost, J., 2005. On-demand release by ultrasound from osmotically swollen hydrophobic matrices. J. Control. Release 110, 58–66.
Ashokkumar, M., Grieser, F., 2007. The effect of surface active solutes on bubbles in an acoustic field. Phys. Chem. Chem. Phys. 9, 5621–5728.
Attwood, D., Saundees, L., 1965. A light-scattering study of ultrasonically irradiated lecithin sols. Biochim. Biophys. Acta 98, 344–350.
Avnir, Y., Ulmansky, R., Wasserman, V., Even-Chen, S., Broyer, M., Barenholz, Y., Naparstek, Y., 2008. Amphipathic weak acid glucocorticoid prodrugs remote-loaded into sterically stabilized nanoliposomes evaluated in arthritic rats and in a Beagle dog: a novel approach to treating autoimmune arthritis. Arthritis Rheum. 58, 119–129.
Bailey, M.R., Khokhlova, V.A., Sapozhnikov, O.A., Kargl, S.G., Crum, L.A., 2003. Physical mechanisms of the therapeutic effect of ultrasound. J. Acoust. Phys. 49, 369–388.
Bangham, A.D., 1963. Physical structure and behavior of lipids and lipid enzymes. Adv. Lipid Res. 1, 65–104.
Bangham, A.D., 1993. Cell Membranes: Biochemistry, Cell Biology, & Pathology. HP Publishing Co., New York.
Bangham, A.D., 1995. Surrogate cells or Trojan horses. The discovery of liposomes. Bioessays 17, 1081–1088.
Barenholz, Y., 2001. Liposome application: problems and prospects. Curr. Opin. Colloid Interface Sci. 6, 66.
Barenholz, Y., 2002. Cholesterol and other membrane active sterols: from membrane evolution to "rafts". Prog. Lipid Res. 41, 1–5.
Barenholz, Y., 2003. Relevancy of drug loading to liposomal formulation therapeutic efficacy. J. Liposome Res. 13, 1–8.
Barenholz, Y., 2007. Amphipathic weak base loading into preformed liposomes having a transmembrane ammonium ion gradient: From the bench to approved Doxil. In: Gregoriadis, G. (Ed.), Liposome Technology, Volume II: Entrapment of Drugs and Other Materials into Liposomes. CRC Press, Boca Raton, FL.
Barenholz, Y., Cevc, G., 2000. Structure and Properties of Membranes in Physical Chemistry of Biological Surfaces. Marcel Dekker, New York.
Barenholz, Y., Crommelin, D.J.A., 1994. Liposomes as Pharmaceutical Dosage Forms. Marcel Dekker, New York.
Barenholz, Y., Gibbes, D., Litman, B.J., Goll, J., Thompson, T.E., Carlson, R.D., 1977. A simple method for the preparation of homogeneous phospholipid vesicles. Biochemistry 16, 2806–2810.
Barrow, D.A., Lentz, B.R., 1980. Large vesicle contamination in small, unilamellar vesicles. Biochim. Biophys. Acta 597, 92–99.
Belsito, S., Bartucci, R., Sportelli, L., 2001. Lipid chain length effect on the phase behaviour of PCs/PEG:2000-PEs mixtures. A spin label electron spin resonance and spectrophotometric study. Biophys. Chem. 93, 11–22.

Berden, J.A., Barker, R.W., Radda, G.K., 1975. NMR studies on phospholipid bilayers. Some factors affecting lipid distribution. Biochim. Biophys. Acta 375, 186–208.
Biltonen, R.L., Lichtenberg, D., 1993. The use of differential scanning calorimetry as a tool to characterize liposome preparations. Chem. Phys. Lipids 64, 129–142.
Borden, M.A., Kruse, D.E., Caskey, C.F., Zhao, S., Dayton, P.A., Ferrara, K.W., 2005. Influence of lipid shell physicochemical properties on ultrasound-induced microbubble destruction. IEEE Trans. Ultrason. Ferroelectr. Freq. Control 52, 1992–2002.
Brochard-Wyarta, F., de Gennes, P.G., Sandrea, O., 2000. Transient pores in stretched vesicles: role of leak-out. Physica A 278, 32–51.
Brown, B.H., Smallwood, R.H., Barber, D.C., 1999. Medical Physics and Biomedical Engineering. CRC Press, Boca Raton, FL.
Bushong, S.C., Archer, B.R., 1991. Diagnostic Ultrasound. Mosby Inc., New York.
Carpenter-Green, S., Huang, L., 1983. Incorporation of acylated wheat germ agglutinin into liposomes. Anal. Biochem. 135, 151–155.
Catania, A.E., Ferrari, A., Manno, M., Spessa, E., 2006. A comprehensive thermodynamic approach to acoustic cavitation simulation in high-pressure injection systems by a conservative homogeneous two-phase barotropic flow model. J. Eng. Gas Turbines Power 128, 434.
Clerc, S.G., Thompson, T.E., 1994. A possible mechanism for vesicle formation by extrusion. Biophys. J. 67, 475–476.
Cohen-Levi, D., 2000. Ultrasound for targeted delivery of cytotoxic drugs from liposomes. MSc Thesis (Kost, J., Barenholz, Y., supervisors). Faculty of Engineering Sciences, Ben Gurion University, Beer Sheva, Israel, pp. 115.
Cohen, Z.R., Zaubermann, J., Harnof, S., Mardor, Y., Nass, D., Zadicario, E., Hananel, A., Castel, D., Faibel, M., Ram, Z., 2007. Magnetic resonance imaging-guided focused ultrasound for thermal ablation in the brain: a feasibility study in a swine model. Neurosurgery 60, 593–600.
Connor, J., Yatvin, M.B., Huang, L., 1984. pH-sensitive liposomes: acid-induced liposome fusion. PNAS 81, 1715–1718.
Crommelin, D.J., 1984. Influence of lipid composition and ionic strength on the physical stability of liposomes. J. Pharm. Sci. 73, 1559–1563.
Crum, L.A., 1980. Measurements of the growth of air bubbles by rectified diffusion. J. Acoust. Soc. Am. 68, 203–211.
Crum, L.A., 1984. Acoustic cavitation series: part five rectified diffusion. Ultrasonics 22, 215–223.
Cutnell, J.D., Johnson, K.W., 1989. Physics. Wiley, New York.
Dayton, P.A., Zhao, S., Bloch, S.H., Schumann, P., Penrose, K., Matsunaga, T.O., Zutshi, R., Doinikov, A., Ferrara, K.W., 2006. Application of ultrasound to selectively localize nanodroplets for targeted imaging and therapy. Mol. Imaging 5, 160–174.
Dijkmans, P.A., Juffermans, L.J.M., Muster, R.J.P., van Wamel, A., ten Cate, W., Gilst, v., Visser, C.A., de Jongc, N., Kamp, O., 2004. Microbubbles and ultrasound: from diagnosis to therapy. Eur. J. Echocardiogr., 5.
Dittmar, K.M., Xie, J., Hunter, F., Trimble, C., Bur, M., Frenkel, V., Li, K.C.P., 2005. Pulsed high-intensity focused ultrasound enhances systemic administration of naked DNA in squamous cell carcinoma model: initial experience. Radiology 235, 541–546.
Dromi, S., Frenkel, V., Luk, A., Traughber, B., Angstadt, M., Bur, M., Poff, J., Xie, J., Libutti, S.K., Li, K.C.P., Wood, B.J., 2007. Pulsed-high intensity focused ultrasound and low temperature sensitive liposomes for enhanced targeted drug delivery and antitumor effect. Clin. Cancer Res. 13, 2722–2727.
Duvshani-Eshet, M., Baruch, L., Kesselman, E., Shimoni, E., Machluf, M., 2006. Therapeutic ultrasound-mediated DNA to cell and nucleus: bioeffects revealed by confocal and atomic force microscopy. Gene Ther. 13, 163–172.
Ebenbichler, G.R., Erdogmus, C.B., Resch, K.L., Funovics, M.A., Kainberger, F., Barisani, G., Aringer, M., Nicolakis, P., Wiesinger, G.F., Baghestanian, M., Preisinger, E., Fialka-Moser, V., 1999. Ultrasound therapy for calcific tendinitis of the shoulder. N. Engl. J. Med. 340, 1533–1538.
Eckstein, M., Barenholz, Y., Bar, L.K., Segal, E., 1997. Liposomes containing Candida albicans ribosomes as a prophylactic vaccine against disseminated candidiasis in mice. Vaccine 15, 220–224.
Ensminger, D., 1988. Ultrasonics: Fundamentals, Technology, Applications. Marcel Dekker, New York.
Fennessy, F.M., Tempany, C.M., McDannold, N.J., So, M.J., Hesley, G., Gostout, B., Kim, H.S., Holland, G.A., Sarti, D.A., Hynynen, K., Jolesz, F.A., Stewart, E.A., 2007. Uterine leiomyomas: MR imaging-guided focused ultrasound surgery—results of different treatment protocols. Radiology 243, 885–893.
Ferrara, K., Pollard, R., Borden, M., 2007. Ultrasound microbubble contrast agents: fundamentals and application to gene and drug delivery. Annu. Rev. Biomed. Eng. 9, 415–447.
Ferrara, K.W., 2008. Driving delivery vehicles with ultrasound. Adv. Drug Deliv. Rev. 60, 1097–1102.
Finer, E.G., Flook, A.G., Hauser, H., 1972. Mechanism of sonication of aqueous egg yolk lecithin dispersions and nature of the resultant particles. Biochim. Biophys. Acta—Lipids Lipid Metab. 260, 49–58.
Fishel-Ghodsian, F., Brown, L., Mathiowitz, E., Brandenburg, D., Langer, R.S., 1988. Enzymatically controlled drug delivery. PNAS 85, 2403–2406.
Flynn, H.G., 1964. Physics of Acoustic Cavitation in Liquids in Physical Acoustics. Academic Press, New York.
Frenkel, V., 2008. Ultrasound mediated delivery of drugs and genes to solid tumors. Adv. Drug Deliv. Rev. 60, 1193–1208.
Frenkel, V., Etherington, A., Greene, M., Quijano, J., Xie, J., Hunter, F., Dromi, S., Li King, C.P., 2006. Delivery of liposomal doxorubicin (Doxil) in a breast cancer tumor model: investigation of potential enhancement by pulsed-high intensity focused ultrasound exposure. Acad. Radiol. 13, 469–479.
Fry, F.J., Ades, H.W., Fry, W.J., 1958. Production of reversible changes in the central nervous system by ultrasound. Science 127, 83–84.
Fry, W.J., 1954. Intense ultrasound; a new tool for neurological research. J. Ment. Sci. 100, 85–96.
Funaki, K., Fukunishi, H., Funaki, T., Sawada, K., Kaji, Y., Maruo, T., 2007. Magnetic resonance-guided focused ultrasound surgery for uterine fibroids: relationship between the therapeutic effects and signal intensity of pre-existing T2-weighted MR images. Am. J. Obstet. Gynecol. 196, e1–6.
Gabizon, A., Catane, R., Uziely, B., Kaufman, B., Safra, T., Cohen, R., Martin, F., Huang, A., Barenholz, Y., 1994. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. Cancer Res. 54, 987–992.
Gabizon, A., Horowitz, A.T., Goren, D., Tzemach, D., Shmeeda, H., Zalipsky, S., 2003. In vivo fate of folate-targeted polyethylene-glycol liposomes in tumor-bearing mice. Clin. Cancer Res. 9, 6551–6559.
Gabizon, A., Papahadjopoulos, D., 1988. Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors. PNAS 85, 6949–6953.
Gao, Z., Kennedy, A.M., Christensen, D.A., Rapoport, N.Y., 2008. Drug-loaded nano/microbubbles for combining ultrasonography and targeted chemotherapy. Ultrasonics 48, 260–270.
Gao, Z.G., Fain, H.D., Rapoport, N., 2005. Controlled and targeted tumor chemotherapy by micellar-encapsulated drug and ultrasound. J. Control. Release 102, 203–222.
Garbuzenko, O., Barenholz, Y., Priev, A., 2005a. Effect of grafted PEG on liposome size and on compressibility and packing of lipid bilayer. Chem. Phys. Lipids 135, 117–129.
Garbuzenko, O., Zalipsky, S., Qazen, M., Barenholz, Y., 2005b. Electrostatics of PEGylated micelles and liposomes containing charged and neutral lipopolymers. Langmuir 21 (21), 2560–2568.
Garbuzenko, O.B., Saad, M., Betigeri, S., Zhang, M., Vetcher, A.A., Soldatenkov, V.A., Reimer, D.C., Pozharov, V.P., Minko, T., 2008. Intratracheal versus intravenous liposomal delivery of siRNA. Antisense oligonucleotides and anticancer drug. Pharm. Res..
Gelfand, S.A., 2004. Hearing, an Introduction to Psychological and Physiological Acoustics. Marcel Dekker, USA.
Gerasimov, O.V., Boomer, J.A., Qualls, M.M., Thompson, D.H., 1999. Cytosolic drug delivery using pH- and light-sensitive liposomes. Adv. Drug Deliv. Rev. 38, 317–338.
Ghadiali, J.E., Stevens, M.M., 2008. Enzyme-responsive nanoparticle systems. Adv. Mater., online.
Goldbart, R., Traitel, T., Lapidot, S.A., Kost, J., 2002. Enzymatically controlled responsive drug delivery systems. Polym. Adv. Technol. 13, 1006–1018.
Goldberg, B.B., Kimmelman, B.A., 1988. Medical Diagnostic Ultrasound: A retrospective on its 40th anniversary. Kodak, Rochester, New York.
Goss, S.A., Johnston, R.L., Dunn, F., 1978. Comprehensive compilation of empirical ultrasonic properties of mammalian tissues. J. Acoust. Soc. Am. 64, 423–457.
Goss, S.A., Johnston, R.L., Dunn, F., 1980. Compilation of empirical ultrasonic properties of mammalian tissues. II. J. Acoust. Soc. Am. 68, 93–108.
Graul, E.H., 1950. Effect of ultrasonic waves on cell membrane. Arch. Dermatol. Syph. 191, 409–411.
Gregoriadis, G., Florence, A.T., Patel, H.M., 1993. Liposomes in Drug Delivery. CRC Press, Boca Raton, FL.
Hadjiargyrou, M., McLeod, K., Ryaby, J.P., Rubin, C., 1998. Enhancement of fracture healing by low intensity ultrasound. Clin. Orthop. 355, 216–229.
Haupt, G., Haupt, A., 2003. In vitro comparison of 4 ultrasound lithotripsy devices. J. Urol. 170, 1731–1733.
Hauser, H., Barratt, M.D., 1973. Effect of chain length on the stability of lecithin bilayers. Biochem. Biophys. Res. Commun. 53, 399–405.
Hill, C.R., Bamber, J.C., ter Haar, G.R., 2004. Physical Principles of Medical Ultrasonics. John Wiley and Sons, New York.
Hope, M.J., Bally, M.B., Webb, G., Cullis, P.R., 1985. Production of large unilamellar vesicles by a rapid extrusion procedure. Characterization of size distribution, trapped volume and ability to maintain a membrane potential. Biochim. Biophys. Acta—Biomembr. 812, 55–65.
Hoskins, P., Thrush, A., Martin, K., Whittingam, T., 2002. Diagnostic Ultrasound: Physics and Equipment. Cambridge University Press.
Huang, C., Charlton, J.P., 1971. Studies on phosphatidylcholine vesicles. Determination of partial specific volumes by sedimentation velocity method. J. Biol. Chem. 246, 2555–2560.
Huang, C.H., 1969. Phosphatidylcholine vesicles. Formation and physical characteristics. Biochemistry 8, 344–352.
Huang, S.L., MacDonald, R.C., 2004. Acoustically active liposomes for drug encapsulation and ultrasound-triggered release. Biochim. Biophys. Acta 1665, 134–141.
Husseini, G.A., Myrup, G.D., Pitt, W.G., Christensen, D.A., Rapoport, N.Y., 2000. Factors affecting acoustically triggered release of drugs from polymeric micelles. J. Control. Release 69, 43–52.
Ickenstein, L.M., Arfvidsson, M.C., Needham, D., Mayer, L.D., Edwards, K., 2003. Disc formation in cholesterol-free liposomes during phase transition. Biochim. Biophys. Acta 1614, 135–138.
Ishida, T., Okada, Y., Kobayashi, T., Kiwada, H., 2006. Development of pH-sensitive liposomes that efficiently retain encapsulated doxorubicin (DXR) in blood. Int. J. Pharm. 309, 94.
Israelachvili, J.N., 1992. Intermolecular and Surface Forces. Academic Press, London.
Jimmy, B., Kentish, S., Grieser, F., Ashokkumar, M., 2008. Ultrasonic nebulization in aqueous solutions and the role of interfacial adsorption dynamics in surfactant enrichment. Langmuir 24, 10133–10137.

Johns, L.D., 2002. Nonthermal effects of therapeutic ultrasound: the frequency resonance hypothesis. J. Athletic Training 37, 293–299.
Johnson, S.M., Bangham, A.D., Hill, M.W., Korn, E.D., 1971. Single bilayer liposomes. Biochim. Biophys. Acta 233, 820–826.
Johnsson, M., Edwards, K., 2003. Liposomes, disks, and spherical micelles: aggregate structure in mixtures of gel phase phosphatidylcholines and poly(ethylene glycol)-phospholipids. Biophys. J. 85, 3839–3847.
Jolesz, F.A., Hynynen, K., 2002. Magnetic resonance image-guided focused ultrasound surgery. Cancer J. 8 (Suppl. 1), S100–S112.
Jolesz, F.A., Hynynen, K., McDannold, N., Freundlich, D., Kopelman, D., 2004. Noninvasive thermal ablation of hepatocellular carcinoma by using magnetic resonance imaging-guided focused ultrasound. Gastroenterology 127, S242–S247.
Jolesz, F.A., McDannold, N., 2008. Current status and future potential of MRI-guided focused ultrasound surgery. J. Magn. Reson. Imaging 27, 391–399.
Jorgensen, K., Mouritsen, O.G., 1995. Phase separation dynamics and lateral organization of two-component lipid membranes. Biophys. J. 69, 942–954.
Kalmanzon, E., Zlotkin, E., Cohen, R., Barenholz, Y., 1992. Liposomes as a model for the study of the mechanism of fish toxicity of sodium dodecyl sulfate in sea water. Biochim. Biophys. Acta 1103, 148–156.
Kaneda, Y., 2000. Virosomes: evolution of the liposome as a targeted drug delivery system. Adv. Drug Deliv. Rev. 43, 197–205.
Karshafian, R., Bevan, P.D., Burns, P.N., Karshafian, R., Samac, S., Banerjee, M., Bevan, P.D., 2005. Ultrasound-induced uptake of different size markers in mammalian cells. IEEE Trans. Ultrason., 13–16.
Kee, P.H., Abruzzo, T.A., Smith, D.A., Kopechek, J.A., Wang, B., Huang, S.L., MacDonald, R.C., Holland, C.K., McPherson, D.D., 2008. Synthesis, acoustic stability, and pharmacologic activities of papaverine-loaded echogenic liposomes for ultrasound controlled drug delivery. J. Liposome Res. 18, 263–277.
Khaibullina, A., Jang, B.S., Sun, H., Le, N., Yu, S., Frenkel, V., Carrasquillo, J.A., Pastan, I., Li, K.C., Paik, C.H., 2008. Pulsed high-intensity focused ultrasound enhances uptake of radiolabeled monoclonal antibody to human epidermoid tumor in nude mice. J. Nucl. Med. 49, 295–302.
Khazanov, E., Barenholz, Y., Gibson, D., Najajreh, Y., 2002. Novel apoptosis-inducing trans-platinum piperidine derivatives: synthesis and biological characterization. J. Med. Chem. 45, 5196–5204.
Khazanov, E., Priev, A., Shillemans, J.P., Barenholz, Y., 2008. Physicochemical and biological characterization of ceramide-containing liposomes: paving the way to ceramide therapeutic application. Langmuir 24, 6965–6980.
Kheirolomoom, A., Dayton, P.A., Lum, A.F., Little, E., Paoli, E.E., Zheng, H., Ferrara, K.W., 2007. Acoustically-active microbubbles conjugated to liposomes: characterization of a proposed drug delivery vehicle. J. Control. Release 118, 275–284.
Kim, H.S., Park, Y.S., 2002. Gene transfection by quantitatively reconstituted Sendai envelope proteins into liposomes. Cancer Gene Ther. 9, 173–177.
Kinoshita, M., McDannold, N., Jolesz, F.A., Hynynen, K., 2006. Noninvasive localized delivery of Herceptin to the mouse brain by MRI-guided focused ultrasound-induced blood-brain barrier disruption. PNAS 103, 11719–11723.
Kinsler, L.E., Frey, A.R., Coppens, A.B., Sanders, J.S., 1980. Fundemaentals of Acoustics. John Wiley and Sons, New York.
Kitchen, S.S., Partridge, C.J., 1990. A review of therapeutic ultrasound. Physiotherapy 76, 593–600.
Kost, J., Leong, K., Langer, R., 1988. Ultrasonically controlled polymeric drug delivery. Makromolek. Chem. 19, 275.
Kost, J., Leong, K., Langer, R., 1989. Ultrasound-enhanced polymer degradation and release of incorporated substances. Proc. Natl. Acad. Sci. U S A 86, 7663–7666.
Kost, J., Liu, L.S., Gabelnick, H., Langer, R., 1994. Ultrasound as a potential trigger to terminate the activity of contraceptive delivery implants. J. Control. Release 30, 77–81.
Kost, J., Mitragotri, S., Gabbay, R., Pishko, M., Langer, R., 2000. Transdermal monitoring of glucose and other analytes using ultrasound. Nat. Med. 6, 347–350.
Krasovitski, B., Kimmel, E., 2004. Shear stress induced by a gas bubble pulsating in an ultrasonic field near a wall. IEEE Trans. Ultrason. 51, 973–979.
Kumar, V.V., 1991. Complementary molecular shapes and additivity of the packing parameter of lipids. PNAS 88, 444–448.
Lafon, C., 2007. Physics for Medical Imaging Applications. Springer, Netherlands.
Lasic, D.D., 1988. The mechanism of vesicle formation. Biochem. J. 256, 1–11.
Lasic, D.D., 1993. Liposomes: From Physics to Applications. Elsevier, Amsterdam.
Lasic, D.D., 1996. Handbook of Nonmedical Applications of Liposomes. CRC Press, New York.
Lasic, D.D., Martin, F.J., Gabizon, A., Huang, S.K., Papahadjopoulos, D., 1991. Sterically stabilized liposomes: a hypothesis on the molecular origin of the extended circulation times. Biochim. Biophys. Acta 1070, 187–192.
Lasic, D.D., Strey, H., Stuart, M.C.A., Podgornik, R., Frederik, P.M., 1997. The Structure of DNA-Liposome Complexes. J. Am. Chem. Soc. 119, 832–833.
Lavon, I., Kost, J., 2004. Ultrasound and transdermal drug delivery. Drug Discov. Today 9, 670–676.
Lawaczeck, R., Kainosho, M., Chan, S.I., 1976. The formation and annealing of structural defects in lipid bilayer vesicles. Biochim. Biophys. Acta 443, 313–330.
Lawrie, A., Brisken, A.F., Francis, S.E., Cumberland, D.C., Crossman, D.C., Newman, C.M., 2000. Microbubble-enhanced ultrasound for vascular gene delivery. Gene Ther. 7, 2023–2027.
Leal, C., Rögnvaldsson, S., Fossheim, S., Nilssen, E.A., Topgaard, D., 2008. Dynamic and structural aspects of PEGylated liposomes monitored by NMR. J. Colloid Interface Sci. 325, 485–493.
Leidy, C., Kaasgaard, T., Crowe, J.H., Mouritsen, O.G., Jorgensen, K., 2002. Ripples and the formation of anisotropic lipid domains: imaging two-component supported double bilayers by atomic force microscopy. Biophys. J. 83, 2625–2633.
Leighton, T.G., 1989. Transient exitation of insonated bubbles. Ultrasonics 27, 50–53.
Leighton, T.G., 1997. The Acoustic Bubble. Academic Press, New York.
Lemmich, J., Mortensen, K., Ipsen, J.H., Honger, T., Bauer, R., Mouritsen, O.G., 1997. The effect of cholesterol in small amounts on lipid bilayer softness in the region of the main phase transition. Eur. Biophys. J. 25, 293–304.
Lentacker, I., De Smedt, S.C., Demeester, J., Van Marck, V., Bracke, M., Sanders, N.N., 2007. Lipoplex-loaded microbubbles for gene delivery: a trojan horse controlled by ultrasound. Adv. Funct. Mater. 17, 1910–1916.
Lichtenberg, D., Barenholz, Y., 1988. Liposomes: preparation, characterization, and preservation. Methods Biochem. Anal. 33, 337–462.
Lin, H.Y., Thomas, J.L., 2003a. PEG-Lipids and oligo(ethylene glycol) surfactants enhance the ultrasonic permeabilizability of liposomes. Langmuir 19, 1098–1105.
Lin, H.Y., Thomas, J.L., 2003b. Pluronic P105 Sensitizes Cholesterol-Free Liposomes to Ultrasound. IEEE, pp. 311–312.
Lin, H.Y., Thomas, J.L., 2004. Factors affecting responsivity of unilamellar liposomes to 20 kHz ultrasound. Langmuir 20, 6100–6106.
Lota, M.J., Darling, R.C., 1955. Changes in permeability of the red blood cell membrane in a homogeneous ultrasonic field. Arch. Phys. Med. Rehabil. 36, 282–287.
Lutsiak, M.E., Kwon, G.S., Samuel, J., 2002. Analysis of peptide and lipopeptide content in liposomes. J. Pharm. Pharm. Sci. 5, 279–284.
Lynn, J.G., Zwemer, R.L., Chick, A.J., 1942. The biological application of focused ultrasonic waves. Science 96, 119–120.
Ma, B., Zhang, S., Jiang, H., Zhao, B., Lv, H., 2007. Lipoplex morphologies and their influences on transfection efficiency in gene delivery. J. Control. Release 123, 184–194.
MacDonald, R.C., MacDonald, R.I., Menco, B.P., Takeshita, K., Subbarao, N.K., Hu, L.R., 1991. Small-volume extrusion apparatus for preparation of large, unilamellar vesicles. Biochim. Biophys. Acta 1061, 297–303.
Maeda, H., Wu, J., Sawa, T., Matsumura, Y., Hori, K., 2000. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J. Control. Release 65, 271–284.
Malinin, V.S., Frederik, P., Lentz, B.R., 2002. Osmotic and curvature stress affect PEG-induced fusion of lipid vesicles but not mixing of their lipids. Biophys. J. 82, 2090–2100.
Margulis, M.A., 1995. Sonochemistry and Cavitation. Gordon and Breach Science Publishers, Amsterdam.
Marin, A., Sun, H., Husseini, G.A., Pitt, W.G., Christensen, D.A., Rapoport, N.Y., 2002. Drug delivery in pluronic micelles: effect of high-frequency ultrasound on drug release from micelles and intracellular uptake. J. Control. Release 84, 39–47.
Marmottant, P., Biben, T., Hilgenfeldt, S., 2008. Deformation and rupture of lipid vesicles in the strong shear flow generated by ultrasound-driven microbubbles. Proc. R. Soc. A 484, 1781–1800.
Marmottant, P., Hilgenfeldt, S., 2003. Controlled vesicle deformation and lysis by single oscillating bubbles. Nature 423, 153–156.
Marsh, D., 1990. CRC Handbook of Lipid Bilayers. CRC Press, Boca Raton, FL.
Mason, T.J., Lorimer, J.P., 1988. Sonochemistry: Theory, Applications, and Uses of Ultrasound in Chemistry. Ellis Horwood, Chichester, New York.
Mast, D., 2000. Empirical relationships between acoustic parameters in human soft tissues. Acoust. Res. Lett. 1, 37–42, Online.
Maulucci, G., De Spirito, M., Arcovito, G., Boffi, F., Castellano, A.C., Briganti, G., 2005. Particle size distribution in DMPC vesicle solutions undergoing different sonication times. Biophys. J. 88, 3545–3550.
Mayer, L.D., Hope, M.J., Cullis, P.R., 1986. Vesicles of variable sizes produced by a rapid extrusion procedure. Biochim. Biophys. Acta 858, 161–168.
Maynard, V.M., Magin, R.L., Strom-Jensen, P.R., Dunn, F., 1983. Ultrasonic absorption by ultrasound. IEEE Ultrason. Symp., 806–809.
McCreery, T.K., Sweitzer, R.H., Unger, E.C., 2004. DNA delivery to cells in culture using ultrasound. Methods Mol. Biol. Gene Transfer Tech., 287–291.
McNamara, W.B., Didenko, Y., Suslick, K.S., 1999. Sonoluminescence temperatures during multibubble cavitation. Nature 401, 772–775.
Meers, P., 2001. Enzyme-activated targeting of liposomes. Adv. Drug Deliv. Rev. 53, 265–272.
Mendelsohn, R., Sunder, S., Bernstein, H.J., 1976. The effect of sonication on the hydrocarbon chain conformation in model membrane systems: a Raman spectroscopic study. Biochim. Biophys. Acta 419, 563–569.
Mitragotri, S., Kost, J., 2004. Low-frequency sonophoresis. A review. Adv. Drug Deliv. Rev. 56, 589–601.
Moran, C., Ross, J., Cunningham, C., Butler, M., Anderson, T., Newby, D., Fox, K., McDicken, W., 2006. Manufacture and acoustical characterisation of a high-frequency contrast agent for targeting applications. Ultrasound Med. Biol. 32, 421–428.
Mouritsen, O.G., 2005. Life As a Matter of Fat. The Emerging Science of Lipidomics. Springer-Verlag, Berlin.
Mouritsen, O.G., Jorgensen, K., 1994. Dynamical order and disorder in lipid bilayers. Chem. Phys. Lipids 73, 3–25.
Needham, D., Anyarambhatla, G., Kong, G., Dewhirst, M.W., 2000. A new temperature-sensitive liposome for use with mild hyperthermia: characterization and testing in a human tumor xenograft model. Cancer Res. 60, 1197–1201.
Needham, D., Stoicheva, N., Zhelev, D.V., 1997. Exchange of monooleoylphosphatidylcholine as monomer and micelle with membranes containing poly(ethylene glycol)-lipid. Biophys. J. 73, 2615–2629.
Neppiras, E.A., Noltingk, B.E., 1951. Cavitation produced by ultrasonics: theoretical conditions for the onset of cavitation. Proc. Phys. Soc. B 64, 1032–1038.
Noltingk, B.E., Neppiras, E.A., 1950. Cavitation produced by ultrasonics. Proc. Phys. Soc. B 63, 674–685.

O'Neill, B.E., Li, K.C., 2008. Augmentation of targeted delivery with pulsed high intensity focused ultrasound. Int. J. Hyperthermia 24, 506–520.
Olson, F., Hunt, C.A., Szoka, F.C., Vail, W.J., Papahadjopoulos, D., 1979. Preparation of liposomes of defined size distribution by extrusion through polycarbonate membranes. Biochim. Biophys. Acta Biomembr. 557, 9–23.
Opatowski, E., Kozlov, M.M., Pinchuk, I., Lichtenberg, D., 2002. Heat evolution of micelle formation, dependence of enthalpy, and heat capacity on the surfactant chain length and head group. J. Colloid Interface Sci. 246, 380–386.
Oyen, W.J., Boerman, O.C., Storm, G., van Bloois, L., Koenders, E.B., Claessens, R.A., Perenboom, R.M., Crommelin, D.J., van der Meer, J.W., Corstens, F.H., 1996. Detecting infection and inflammation with technetium-99m-labeled Stealth liposomes. J. Nucl. Med. 37, 1392–1397.
Papahadjopoulos, D., Allen, T.M., Gabizon, A., Mayhew, E., Matthay, K., Huang, S.K., Lee, K.D., Woodle, M.C., Lasic, D.D., Redemann, C., 1991. Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. PNAS 88, 11460–11464.
Papahadjopoulos, D., Miller, N., 1967. Phospholipid model membranes. I. Structural characteristics of hydrated liquid crystals. Biochim. Biophys. Acta 135, 624–638.
Papahadjopoulos, D., Watkins, J.C., 1967. Phospholipid model membranes. II. Permeability properties of hydrated liquid crystals. Biochim. Biophys. Acta 135, 639–652.
Parikov, V.A., 1966. Injection of tetracyline into the tissues of cow udders by ultrasonics. Veterinariia 43, 88–91.
Peleg-Shulman, T., Gibson, D., Cohen, R., Abra, R., Barenholz, Y., 2001. Characterization of sterically stabilized cisplatin liposomes by nuclear magnetic resonance. Biochim. Biophys. Acta. 1510, 278–291.
Pereira-Lachataignerais, J., Pons, R., Panizza, P., Courbin, L., Rouch, J., Lopez, O., 2006. Study and formation of vesicle systems with low polydispersity index by ultrasound method. Chem. Phys. Lipids 140, 88.
Pine, J.L., Smith, L.J., Haws, M.J., Gingrass, M.K., 2003. Ultrasound-assisted lipoplasty. Plast. Surg. Nurs. 23, 101–108, quiz 109.
Pitt, W.G., Husseini, G.A., Staples, B., 2004. Ultrasonic drug delivery—a general review. Expert Opin. Drug Deliv. 1, 37–56.
Poff, J.A., Allen, C.T., Traughber, B., Colunga, A., Xie, J., Chen, Z., Wood, B.J., Van Waes, C., Li, K.C., Frenkel, V., 2008. Pulsed high-intensity focused ultrasound enhances apoptosis and growth inhibition of squamous cell carcinoma xenografts with proteasome inhibitor bortezomib. Radiology 248, 485–491.
Pong, M., Umchid, S., Guarino, A.J., Lewin, P.A., Litniewski, J., Nowicki, A., Wrenn, S.P., 2006. In vitro ultrasound-mediated leakage from phospholipid vesicles. Ultrasonics 45, 133–145.
Priev, A., Samuni, A., Tirosh, O., Barenholz, Y., 1998. The role of hydration in stabilization of liposomes: resistance to oxidative damage of PEG-grafted liposomes. In: Gregoriadis, G., McCormack, B. (Eds.), Targeting of Drugs 6: Strategies for Stealth Therapeutic Systems. Plenum Press, New York, pp. 147–167.
Priev, A., Zalipsky, S., Cohen, R., Barenholz, Y., 2002. Determination of critical micelle concentration of lipopolymers and other amphiphiles: comparison of sound velocity and fluorescent measurements. Langmuir 18, 612–617.
Ram, Z., Cohen, Z.R., Har Nof, S., Tal, S., Faibel, M., Nass, D., Maier, S.E., Hadani, M., Mardor, Y., 2006. Magnetic resonance imaging-guided high-intensity focused ultrasound for brain tumor therapy. Neurosurgery 59, 949–956.
Rantanen, J., Thorsson, O., Wollmer, P., Hurme, T., Kalimo, H., 1999. Effects of therapeutic ultrasound on the regeneration of skeletal myofibers after experimental muscle injury. Am. J. Sports Med. 27, 54–59.
Rapoport, N., 2007. Physical stimuli-responsive polymeric micelles for anti-cancer drug delivery. Prog. Polym. Sci., 962–990.
Rapoport, N., Gao, Z., Kennedy, A., 2007. Multifunctional nanoparticles for combining ultrasonic tumor imaging and targeted chemotherapy. J. Natl. Cancer Inst. 99, 1095–1106.
Rapoport, N., Marin, A., Christensen, D.A., 2002. Ultrasound-activated drug delivery. Drug Deliv. Syst. Sci. 2, 37–46.
Rapoport, N., Smirnov, A.I., Timoshin, A., Pratt, A.M., Pitt, W.G., 1997. Factors affecting the permeability of *Pseudomonas aeruginosa* cell walls toward lipophilic compounds: effects of ultrasound and cell age. Arch. Biochem. Biophys. 344, 114–124.
Richardson, E.S., Pitt, W.G., Woodbury, D.J., 2007. The role of cavitation in liposome formation. Biophys. J. 93, 4100–4107.
Rickwood, B.D.H.D., 1994. Liposomes—A Practical Approach. Oxford University Press, London.
Robertson, J.D., 1959. The ultrastructure of cell membranes and their derivatives. Biochem. Soc. Symp. 16, 3–43.
Rohrich, R.J., Beran, S.J., Kenkel, J.M., Adams, W.P., DiSpaltro, F., 1998. Extending the role of liposuction in body contouring with ultrasound-assisted liposuction. Plast. Reconstr. Surg. 101, 1090–1102.
Sandre, O., Moreaux, L., Brochard-Wyarta, F., 1999. Dynamics of transient pores in stretched vesicles. PNAS 96, 10591–10596.
Sapozhnikov, O.A., Pishchalnikov, Y.A., Maxwell, A.D., Bailey, M.R., 2007. Calibration of PVDF hydrophones using a broad-focus electromagnetic lithotripter. IEEE Ultrason. Symp..
Sarvazyan, A.P., Shilnikow, G.V., Priev, A.I., 1988. An ultrasound study of immunochemical systems. IEEE Ultrason. Symp. Proc., 919–922.
Saunders, L., Perrin, J., Gammack, D., 1962. Ultrasonic irradiation of some phospholipid sols. J. Pharm. Pharmacol. 14, 567–572.
Schlicher, R.K., Radhakrishna, H., Tolentino, T.P., Apkarian, R.P., Zarnitsyn, V., Prausnitz, M.R., 2006. Mechanism of intracellular delivery by acoustic cavitation. Ultrasound Med. Biol. 32, 915–924.
Schneider, J., Falk, H., Hunsmann, G., 1983. Virosomes constructed from lipid and purified Friend leukaemia virus glycoprotein. J. Gen. Virol. 64 (Pt 3), 559–565.
Schneider, T., Sachse, A., Leike, J., Röβling, M., Schmidtgen, M., Drechsler, M., Brandl, M., 1996. Surface modification of continuously extruded contrast-carrying liposomes: effect on their physical properties. Int. J. Pharm. 132, 9–21.
Schroeder, A., Avnir, Y., Weisman, S., Najajreh, Y., Gabizon, A., Talmon, Y., Kost, J., Barenholz, Y., 2007. Controlling liposomal drug release with low frequency ultrasound: mechanism and feasibility. Langmuir 23, 4019–4025.
Schroeder, A., Honen, R., Brandl, M., Gabizon, A., Barenholz, Y., Kost, J., 2008a. Ultrasound: An Effective Tool for Controlling the Release of Drugs from PEGylated Liposomes. CRS.
Schroeder, A., Honen, R., Turjeman, K., Gabizon, A., Kost, J., Barenholz, Y., 2009. Ultrasound triggered release of cisplatin from liposomes in murine tumors. J. Control. Release 137, 63–68.
Schroeder, A., Sigal, A., Turjeman, K., Barenholz, Y., 2008b. Using PEGylated nanoliposomes to target tissue invaded by a foreign body. J. Drug Target. 16, 591–595.
Shaw, G.J., Meunier, J.M., Huang, S.L., Lindsell, C.J., McPherson, D.D., Holland, C.K., 2009. Ultrasound-enhanced thrombolysis with tPA-loaded echogenic liposomes. Thromb. Res..
Simões, S., Moreira, J.N., Fonseca, C., Düzgüneş, N., de Lima, M.C., 2004. On the formulation of pH-sensitive liposomes with long circulation times. Adv. Drug Deliv. Rev. 56, 947–965.
Soloman, R., Gabizon, A.A., 2008. Clinical pharmacology of liposomal anthracyclines: focus on pegylated liposomal Doxorubicin. Clin. Lymphoma Myeloma 8, 21–32.
Speed, C.A., 2001. Therapeutic ultrasound in soft tissue lesions. Rheumatology (Oxford) 40, 1331–1336.
Steinberg, Y., Schroeder, A., Talmon, Y., Schmidt, J., Khalfin, R.L., Cohen, Y., Devoisselle, J.-M., Begu, S., Avnir, D., 2007. Triggered release of aqueous content from liposome-derived sol–gel nanocapsules. Langmuir 23, 12024–12031.
Stone, M.J., Frenkel, V., Dromi, S., Thomas, P., Lewis, R.P., Li, K.C., Horne, M., Wood, B.J., 2007. Pulsed-high intensity focused ultrasound enhanced tPA mediated thrombolysis in a novel in vivo clot model, a pilot study. Thromb. Res. 121, 193–202.
Subbarao, N.K., MacDonald, R.I., Takeshita, K., MacDonald, R.C., 1991. Characteristics of spectrin-induced leakage of extruded, phosphatidylserine vesicles. Biochim. Biophys. Acta 1063, 147–154.
Sunartio, D., Ashokkumar, M., Grieser, F., 2007. Study of the coalescence of acoustic bubbles as a function of frequency, power, and water-soluble additives. JACS 129, 6031–6036.
Sundaram, J., Mellein, B.R., Mitragotri, S., 2003. An experimental and theoretical analysis of ultrasound-induced permeabilization of cell membranes. Biophys. J. 84, 3087–3101.
Suslick, K.S., 1988. Ultrasound: Its Chemical, Physical, and Biological Effects. VCH Publishers, New York.
Suslick, K.S., 2001. Sonoluminescence and Sonochemistry in Encyclopedia of Physical Science and Technology. Academic Press, San Diego.
Suslick, K.S., Didenko, Y., Fang, M.M., Hyeon, T., Kolbeck, K.J., McNamara III, W.B., Mdleleni, M.M., Wong, M., 1999. Acoustic cavitation and its chemical consequences. Philos. Trans. R. Soc. Lond. A 357, 335–353.
Suslick, K.S., Nyborg, W.L., 1990. Ultrasound: its chemical, physical, and biological effects. J. Acoust. Soc. Am. 87, 919–920.
Suzuki, R., Takizawa, T., Negishi, Y., Utoguchi, N., Maruyama, K., 2007. Effective gene delivery with liposomal bubbles and ultrasound as novel non-viral system. J. Drug Target. 15, 531–537.
Szoka Jr., F., Papahadjopoulos, D., 1980. Comparative properties and methods of preparation of lipid vesicles (liposomes). Annu. Rev. Biophys. Bioeng. 9, 467–508.
Tata, D.B., Dunn, F., 1992. Interaction of ultrasound and model membrane systems: analyses and predictions. J. Phys. Chem. 96, 3548–3555.
Tho, P., Manasseh, R., Ooi, A., 2007. Cavitation microstreaming patterns in single and multiple bubble systems. J. Fluid Mech. 576, 191–233.
Tirosh, O., Barenholz, Y., Katzhendler, J., Priev, A., 1998. Hydration of polyethylene glycol-grafted liposomes. Biophys. J. 74, 1371–1379.
Torchilin, V.P., 2005. Recent advances with liposomes as pharmaceutical carriers. Nat. Rev. Drug Discov. 4, 145–160.
Torchilin, V.P., Papisov, M.I., 1994. Why do polyethylene glycol-coated liposomes circulate so long? J. Liposome Res. 4, 725–739.
Torchilin, V.P., Rammohan, R., Weissig, V., Levchenko, T.S., 2001. TAT peptide on the surface of liposomes affords their efficient intracellular delivery even at low temperature and in the presence of metabolic inhibitors. PNAS 98, 8786–8791.
Traitel, T., Goldbart, R., Kost, J., 2008. Smart polymers for responsive drug-delivery systems. J. Biomater. Sci. Polym. Ed. 19, 755–767.
Unger, E.C., Hersh, E., Vannan, M., Matsunaga, T.O., McCreery, T., 2001. Local drug and gene delivery through microbubbles. Prog. Cardiovasc. Dis. 44, 45–54.
Unger, E.C., McCreery, T.P., Sweitzer, R.H., Caldwell, V.E., Wu, Y., 1998. Acoustically active lipospheres containing paclitaxel: a new therapeutic ultrasound contrast agent. Invest. Radiol. 33, 886–892.
Unger, E.C., Porter, T., Culp, W., Labell, R., Matsunaga, T., Zutshi, R., 2004. Therapeutic applications of lipid-coated microbubbles. Adv. Drug Deliv. Rev. 56, 1291–1314.
Uster, P.S., Allen, T.M., Daniel, B.E., Mendez, C.J., Newman, M.S., Zhu, G.Z., 1996. Insertion of poly(ethylene glycol) derivatized phospholipid into pre-formed liposomes results in prolonged in vivo circulation time. FEBS Lett. 386, 243–246 (4).

Wagner, N., Gutweiler, M., Pabst, R., Dose, K., 1987. Coreconstitution of bacterial ATP synthase with monomeric bacteriorhodopsin into liposomes. A comparison between the efficiency of monomeric bacteriorhodopsin and purple membrane patches in coreconstitution experiments. Eur. J. Biochem. 165, 177–183.
Weissig, V., Whiteman, K.R., Torchilin, V.P., 1998. Accumulation of protein-loaded long-circulating micelles and liposomes in subcutaneous Lewis lung carcinoma in mice. Pharm. Res. 15, 1552–1556.
Whitehead, K.A., Langer, R., Anderson, D.G., 2009. Knocking down barriers: advances in siRNA delivery. Nat. Rev. Drug Discov. 8, 129–138.
Woodle, M.C., Lasic, D.D., 1992. Sterically stabilized liposomes. Biochim. Biophys. Acta 1113, 171–199.
Wu, J., 2007. Shear stress in cells generated by ultrasound. Prog. Biophys. Mol. Biol. 93, 363–373.
Yang, R., Reilly, C.R., Rescorla, F.J., Faught, P.R., Sanghvi, N.T., Fry, F.J., Franklin Jr., T.D., Lumeng, L., Grosfeld, J.L., 1991. High-intensity focused ultrasound in the treatment of experimental liver cancer. Arch. Surg. 126, 1002–1009, discussion 1009–10.
Young, F.R., 1989. Cavitation. McGraw-Hill, Maidenhead, UK.
Zacharakis, E., Ahmed, H.U., Ishaq, A., Scott, R., Illing, R., Freeman, A., Allen, C., Emberton, M., 2008. The feasibility and safety of high-intensity focused ultrasound as salvage therapy for recurrent prostate cancer following external beam radiotherapy. BJU Int. 102, 786–792.
Zasadzinski, J.A., 1986. Transmission electron microscopy observations of sonication-induced changes in liposome structure. Biophys. J. 49, 1119–1130.
Zhang, Z.Y., Shum, P., Yates, M., Messersmith, P.B., Thompson, D.H., 2002. Formation of fibrinogen-based hydrogels using phototriggerable diplasmalogen liposomes. Bioconjugate Chem. 13, 640–646.
Zucker, D., Marcus, D., Barenholz, Y., Goldblum, A., 2009. Liposome drugs' loading efficiency: a working model based on loading conditions and drug's physicochemical properties. J Control Release 139, 73–80.
Zuidam, N.J., Barenholz, Y., 1997. Electrostatic parameters of cationic liposomes commonly used for gene delivery as determined by 4-heptadecyl-7-hydroxycoumarin. Biochem. Biophys. Acta 1329, 211–222.
Zuidam, N.J., Barenholz, Y., 1999. Characterization of DNA-lipid complexes commonly used for gene delivery. Int. J. Pharm. 183, 43–46.