Published in final edited form as:

J Liposome Res. 2011 June ; 21(2): 106–115. doi:10.3109/08982104.2010.483597.

Development and Modeling of Arsenic Trioxide Loaded Thermosensitive Liposomes for Anticancer Drug Delivery

Nicolas D. Winter, Ryan K.J. Murphy, George C. Schatz*, and Thomas V. O'Halloran*

Department of Chemistry, Northwestern University, Evanston, IL 60208

Abstract

A novel delivery system for the anticancer drug arsenic trioxide (ATO) is characterized. The release of ATO from DPPC liposomes with MPPC lysolipid incorporated into the bilayer is measured. There is negligible leakage of ATO from all systems at 25°C. Upon heating the liposomes to 37°C, there is 15% to 25% release over a 24 h time period. The ATO release from the DPPC and DPPC:MPPC(5%) systems levels off after 10 h at 37°C, whereas the DPPC:MPPC(10%) liposomes continue to release ATO over the 24 h timespan. Upon heating the liposomes rapidly to 42°C, through the gel to liquid-crystalline (LC) phase transition, the release rate is substantially increased. The two systems containing lysolipids, DPPC:MPPC(5%) and DPPC:MPPC(10%), exhibit a very rapid release of a significant amount of arsenic in the first hour. In the first hour, the DPPC:MPPC(5%) liposomes release 40% of the arsenic and the DPPC:MPPC(10%) liposomes release 55% of the arsenic. Arsenic release from pure DPPC liposomes is comparable at 37°C and 42°C, indicating presence of lysolipid is necessary for a significant enhancement of the release rate. A coarse-grained molecular dynamics (CGMD) model is used to investigate the enhanced permeability of lysolipid-incorporated liposomes and lipid bilayers. The CG liposomes did not form a gel phase when cooled due to the high curvature, however permeability was still significantly lower at 12°C, below what would be the liquid to gel phase transition temperature. At 50°C and 77°C, above T_m , we find water permeability coefficients on the order of $1.0 \times 10^{-3} \text{ cm s}^{-1}$, in good agreement with experiment. From simulations of flat DPPC:MPPC bilayers we find that a peak in the permeability does coincide with the phase transition from the gel to LC state when the lysolipid MPPC is present. No pores are observed in the simulations, however due to limitations in the model, we cannot rule out the possibility of lysolipid-stabilized pores enhancing the permeability in the experiments.

I. Introduction

While many improvements in cancer treatment have been made, there is still a need for new, more effective therapeutics. In the treatment of solid tumors, it is often difficult to achieve therapeutic levels of anticancer agents at the tumor site without damaging healthy organs and tissues. Ideally, drug systems should target the appropriate cells and release the drug selectively once at the site of treatment. In an attempt to improve the in vivo distribution and activity of anticancer drugs, several systems for drug delivery have been developed.

Liposomes have emerged as a promising delivery system for potent chemotherapeutics.^{1, 2} Liposomes are spherical nanoparticles composed of one or more phospholipid bilayers enclosing an aqueous compartment. Application of mild local heating is a commonly used

To whom correspondence should be addressed schatz@chem.northwestern.edu; t-ohalloran@northwestern.edu.

Supporting Information Available

Methods and data for differential scanning calorimetry and dynamic light scattering experiments. This material is available free of charge via the Internet at <http://pubs.acs.org>.

stimulus for release of drugs encapsulated in either the lipid bilayer or in the encapsulated aqueous compartment of a liposome.³ Mild hyperthermia is not only capable of being directly and narrowly applied, but hyperthermia has been shown to act positively when combined with arsenic treatments, increasing the susceptibility of cells to oxidative stress.^{4–6} Most temperature sensitive (thermosensitive) liposomes take advantage of the enhanced permeability of the bilayer that coincides with a phase transition.⁷ At the phase transition temperature T_m , gel and liquid-crystalline (LC) phases coexist and incompatibilities in molecular packing and hydrophobic matching may afford effective drug release.⁸ Unfortunately, many standard liposome compositions result in liposomes that release drugs slowly at T_m and the use of such systems for temperature sensitive release has failed in preclinical trials.⁹ Thus the development of liposomal systems that release their contents quickly near T_m remains an important goal.

One method to accomplish this goal has been developed by Needham et al.⁹ who have incorporated a small fraction of lysolipids, which are single chain analogues of standard lipids, into liposomes typically comprised of dipalmitoylphosphatidylcholine (DPPC). Lysolipids, such as monopalmitoylphosphatidylcholine (MPPC) tend to form micellar structures in aqueous solution. When incorporated in small amounts into double tail lipid systems, it is postulated that lysolipids tend to stabilize pores in the lipid bilayer as it undergoes phase transition from gel to LC. Small molecules (such as chemotherapeutic agents) would be able to pass through these pores quite easily.^{10, 11} This technique has already been shown to be effective for the encapsulation and release of the chemotherapeutic Doxorubicin; clinical studies have shown 90% drug release in 20 seconds from liposomes doped with lysolipids.^{12, 13}

A potentially important application of liposomal drug delivery refers to arsenic trioxide (ATO), As_2O_3 . ATO is a highly effective treatment for acute promyelocytic leukemia with complete remission rates of 83–95%.^{14, 15} In addition, ATO has been studied for the treatment of solid tumors in neuroblastoma and liver cancer cells,^{5, 16, 17} however, its toxicity at higher doses has limited its clinical utility. The toxicity can be controlled via liposomal encapsulation, but early attempts to encapsulate ATO have resulted in substantial loss of drug over a short period of time at 4°C.¹⁸ This loss is expected as at physiological pH ATO exists as $As(OH)_3$, which readily diffuses across lipid bilayers. In response to this problem, a method was recently developed by Chen and coworkers¹⁸ to utilize the fact that arsenite forms insoluble compounds with transition metals (Zn^{2+} , Fe^{2+} , Ni^{2+} , and Cu^{2+}). This allows for the loading of much greater density of arsenic drugs into the liposome with longer retention. This technique has been applied to pH responsive liposomes, but has yet to be applied to temperature sensitive systems, such as mixtures of DPPC with a small fraction of MPPC.

In this work, we examine the liposomal encapsulation and release properties of DPPC/MPPC liposomes. The liposomes are comprised of DPPC with 0–15 mol% MPPC, and a primary goal of this work is to estimate liposomal release characteristics over the temperature range 20–45°C (12–77°C in the simulations). The release properties (notably the release time) will be assessed using both experiment and theory. The experiment will focus on the release of $As(OH)_3$ using the transition metal induced loading of ATO mentioned above. The results will be compared with simulation results based on a coarse-grained molecular dynamics (CGMD) approach. Most of the calculated release rates refer to water permeation, which provides a more practical simulation target due to the slow time scale of the $As(OH)_3$ release. However, an analytical model will be developed which provides estimates of release rates that are relevant to $As(OH)_3$.

The CGMD approach provides an effective method for simulating the self-assembly of lipid structures.¹⁹ GGMD simulations significantly reduce the computational size of a system, allowing the simulation of larger systems (such as biological systems) and longer timescales. Marrink and coworkers have modeled the formation, structure, and dynamics of small phospholipid vesicles comprised of DPPC in a previous publication.²⁰ The present calculations build on these simulations by adding the lysolipid MPPC to DPPC liposomes to match the experimental conditions. By calculating the permeability coefficient for water crossing the liposome membrane, we can explore the permeability of DPPC/MPPC liposomes as a function of temperature, allowing us to provide a molecular level interpretation of the experiments.

II. Methods

A. Experimental Methods

Arsenic Loading—Arsenic was loaded into the liposomal systems using the nano-pump method developed by Chen and coworkers.¹⁸ All liposomes are prepared in the absence of cholesterol, as cholesterol has been shown to decrease the sensitivity to temperature by increasing the membrane line tension and stabilizing the lipid bilayer.²¹ Concentrated metal salt solutions were passively loaded into liposomes comprised of varying DPPC:MPPC mixtures (containing 0 to 15 mol% MPPC in DPPC films), which were then sized by repeated extrusion to 100 nm. The extraliposomal metal was removed through gel exclusion chromatography (Sephadex G-50, 300 mM NaCl/20 mM HEPES buffer, pH 6.8) forming an ion gradient across the bilayer. Addition of 150 μ L of 150 mM As₂O₃ solution (pH 12.5) resulted in active loading of arsenic to the liposomes, giving a final As/lipid ratio of 0.5 ± 0.1 after 48 h as shown in Figure 1. Loading was accomplished over an extended period of time (72 h) at room temperature in order to avoid Ni²⁺ leakage that was observed at elevated temperatures. Loaded liposomes were stored for no more than 48 h at 4°C. Figure 1 shows that the DPPC:MPPC(10%) liposomes loaded more As/lipid at a faster rate than the DPPC:MPPC(5%) and DPPC liposomes. This is likely due to the enhancement in permeability due to the inclusion of lysolipid as demonstrated by our simulation results later in this paper. The DPPC:MPPC(5%) liposomes show a slight increase in loading rate as compared to DPPC, but not as great as the DPPC:MPPC(10%) liposomes. In fact, the short time behavior as shown in the bottom panel of Figure 1 indicates a negligible difference in loading rate between the DPPC and DPPC:MPPC(5%) systems. The DPPC:MPPC(10%) liposomes clearly load faster over the entire timespan, however the difference is still fairly minor. This agrees well with the permeability results from the simulations of DPPC:MPPC liposomes, which are discussed in section IIIB.

Stability and Release Assay—The previously loaded sample was removed from 4°C storage and allowed to warm to room temperature, at which point the extraliposomal metal was removed through gel exclusion chromatography (Sephadex G-50, 300 mM NaCl/20 mM HEPES buffer, pH 4.0) reforming an ion gradient across the bilayer and establishing a baseline for release analysis. The solution was readjusted to pH 7.3 and approximately 1.7 mL of the remainder was separated from the bulk. 200 μ L of this sample was set aside to serve as a zero timepoint. The remainder of the sample was heated in a ceramic heating block to the desired temperature. The gel to LC phase transition temperatures were monitored by differential scanning calorimetry (Supporting Information). The phase transition temperatures for the DPPC, DPPC:MPPC(5%), and DPPC:MPPC(10%) systems were found to be 41.9 °C, 41 °C, and 40.5 °C, respectively, in good agreement with the literature.²² 200 μ L aliquots were removed at the timepoints indicated and all extraliposomal metal was again removed through gel exclusion chromatography (Sephadex G-50, 300 mM NaCl/20 mM HEPES buffer, pH 4.0). These samples were collected, diluted with a 2%

nitric acid solution and treated with a 0.5% Triton X-100 solution. The samples were then analyzed with a Vista-MPX Inductively Coupled Plasma Optical Emissions Spectrometer calibrated with solutions of 0, 1, 5 and 10 ppm of the desired elements (As, P, Ni).

B. Coarse-Grained Molecular Dynamics

System and Force Field—For the CGMD simulations we use the MARTINI forcefield developed by Marrink and coworkers.²³ DPPC and MPPC are represented by 12 and 7 coarse-grained (CG) atoms, respectively, as shown in Figure 2. Details of the force field are given elsewhere²³ but we give a brief summary here. The CG atoms interact in a pairwise manner via a Lennard-Jones (LJ) potential. The mapping of real atoms onto CG atoms is done in a 4:1 ratio with approximately 4 nonhydrogen atoms forming one bead. For example, four methylene groups form one lipid tail bead. The CGMD simulations are started from a random configuration of DPPC lipids and water. Two different size DPPC liposomes (12 and 16 nm in diameter) were examined. The 12 nm liposome system consisted of 756 DPPC lipids and 68,000 CG waters (90 CG waters per lipid). The 16 nm liposome system consisted of 1500 DPPC lipids and 242,000 CG waters (161 CG water per lipid). Within 128 ns the lipids aggregate together to form a vesicle encapsulating water. An example of a 16 nm diameter self-assembled liposome composed of DPPC:MPPC(10%) is shown in Figure 3. Note that the MPPC lipids were not present in the liposome self-assembly process but created later by randomly converting some of the DPPC lipids to MPPC lysolipids in the coordinate file. From these liposomes, a range of DPPC/MPPC liposome compositions were created. From the 12 nm liposome 5, 10, 15, 20, 30, 40, or 50% of the DPPC molecules were converted to MPPC. For the 16 nm liposome, 5, 10, 15 or 20% of the DPPC molecules were converted to MPPC. Simulating larger liposomes is not feasible, but to show how the results scale with size, we also simulated DPPC bilayers with varying concentrations of MPPC. The bilayers consisted of 512 lipids and 8000 CG waters (16 CG waters per lipid). As with the 12 nm liposome, 5, 10, 15, 20, 30, 40, or 50% of the DPPC molecules were converted to MPPC.

Simulation Details—All simulations were carried out with the GROMACS simulation software.²⁴ Cubic periodic boundary conditions were used. The simulated system is kept at a constant pressure of 1 bar and a constant specified temperature (isobaric-isothermal NPT ensemble) using a Berendsen barostat and thermostat.²⁵ Isotropic and semiisotropic pressure coupling were used for the liposomes and flat bilayers, respectively. Temperatures are chosen in the range from 12–77°C, below and above the gel to LC phase transition temperature of pure CG DPPC bilayers at 22°C.²⁶ Note that in the CG model there is a hysteresis in the phase transition temperature depending on if the bilayer is being heated or cooled. The estimated phase transition temperature of 22°C for the CG DPPC bilayer²⁶ is based on the temperature where a half gel, half LC bilayer is stable. In the same study, it was noted that the apparent transition temperature upon cooling is near 12°C, and upon heating is near 37°C. Since we are only interested in simulating the rapid heating of the lipids we are only concerned with the T_m for heating.

Although the curvature of the small diameter liposomes we have considered is too high to form a stable gel phase (as further discussed below), the diffusion constant of the lipids (and water) is decreased at 12°C which significantly affects the permeability. In addition, an ordered gel phase can be formed for the flat bilayer systems. The flat bilayer systems were simulated at temperatures between 12–52°C for 1 μ s, with the same timestep. The dynamics of the CGMD simulations are significantly faster due to the smoother potential functions as compared with the fully atomistic potentials. This allows faster sampling of configuration space, however one must be careful with the interpretation of time in a CGMD simulation. The timescale presented in the results is an effective timescale. Since the diffusion rate of

water in the CGMD simulation is four times faster than that of real water, the effective timescale is simply the actual timescale of the simulation multiplied by four. Each liposome system was simulated at temperatures of 12, 50, and 77°C for 100 ns with an effective timestep of $t = 80$ fs (20 fs actual timestep).

III. Results and Discussion

A. Experimental Results

After loading the arsenic with the nano-pump method, the arsenic drug is stably entrapped in the temperature-sensitive liposome systems under storage conditions. Release may be triggered through a mild but quick increase in temperature as the permeability of the bilayer to the arsenic drug is sensitive to relatively small ($<4^{\circ}\text{C}$) changes in temperature. Note that release from arsenic entrapped in liposomes by the nano-pump method had previously required a drop in pH to resolubilize the complex,¹⁸ while the DPPC/MPPC system appears to allow for quick release of the drug without a change in external pH. This is promising for potential therapeutic applications.

Arsenic release—Figure 4 shows that release at 25°C was negligible over a 24 hour timespan for all of the liposomal systems: DPPC, DPPC:MPPC(5%), and DPPC:MPPC(10%) which are shown in panels A, B, and C, respectively. Rapid heating of the sample to 37°C results in release of arsenic from all systems, though the release from the DPPC:MPPC(10%) liposomes is clearly much more rapid. The arsenic release curves at 37°C for the DPPC and DPPC:MPPC(5%) liposomes are nearly identical indicating that 5% MPPC is not enough to trigger rapid release at body temperature. However, once the systems are heated rapidly to 42°C , very near the phase transition temperature, release starts rapidly for all of the systems, but quickly levels off between 20 and 30% for DPPC. The systems which include lysolipid release substantially more arsenic within the first hour than does the pure DPPC system. After 1 h, the DPPC:MPPC(5%) and DPPC:MPPC(10%) systems released 40% and 55% of the arsenic, respectively. The DPPC:MPPC(10%) liposomes release the arsenic slightly more rapidly than the DPPC:MPPC(5%) liposomes, ultimately releasing $>60\%$ of the arsenic after 24 h whereas the DPPC:MPPC(5%) system releases only about 50% after 24 h.

Effect of Phase Transition—To get a better sense of the behavior of each system below and above the phase transition temperature we plot the arsenic release of all of the systems at 37°C and 42°C in Figure 5 and Figure 6, respectively. In Figure 5 we see that arsenic release is very slow in the first 2 h for the DPPC and DPPC:MPPC(5%) systems, whereas release is significantly more rapid for the DPPC:MPPC(10%) system; 18% of the arsenic is released in 1 h. The release levels off to 15% after about 10 h for the DPPC and DPPC:MPPC(5%) systems. In the DPPC:MPPC(10%) system arsenic continues to be released for the entire 24 h time period with a final release value of 25%. In Figure 6 the arsenic release curves at 42°C are plotted for all three systems. In heating the liposomes rapidly to 42°C , they go through the gel to LC phase transition and the effects of this can be clearly seen in the release curves. The two systems containing lysolipids, DPPC:MPPC(5%) and DPPC:MPPC(10%), exhibit a very rapid release of a significant amount of arsenic in the first hour. In the first hour, the DPPC:MPPC(5%) liposomes release 40% of the arsenic and the DPPC:MPPC(10%) liposomes release 55% of the arsenic. The release curve for the DPPC:MPPC(5%) system plateaus fairly early at 50% release after approximately 4 h. In contrast, arsenic continues to be released gradually from the DPPC:MPPC(10%) liposomes through the end of the 24 h experiment, with a final release amount of 65%. The arsenic release from the pure DPPC liposomes is not significantly higher at 42°C than it was at

37°C, pointing to the importance of the lysolipid contribution to the significant enhancement of the release rate.

In Figure 7 we report the arsenic release at 45°C, a few degrees past the phase transition temperature, on a minute timescale for both the DPPC and DPPC:MPPC(10%) systems. The DPPC release curve is essentially linear with nearly 25% of the arsenic released after 90 min. The DPPC:MPPC(10%) system does exhibit a more rapid release than pure DPPC in the first 30 min, however after 30 min the release plateaus to a final release value near 30%. So we see that upon heating the systems well past the phase transition temperature, the system loses the rapid release behavior that is seen close to phase transition. Again this points to something unique about the phase transition coupled with the inclusion of lysolipid that enables a significant enhancement in permeability. We were also able to see the influence of the phase transition in the simulations of flat lipid bilayers as we will discuss below.

Release mechanism—Dynamic light scattering over a 24 h period at each of the temperatures shows a monodisperse liposome size throughout the experiment, suggesting that the release achieved is not due to liposome fusion or breakdown of the system (Supporting Information). However, it should be noted that assays completed with entrapped calcein showed a much more dramatic release of the encapsulated species from the liposome (90% release within the first ten minutes of hyperthermia); suggesting that while mild hyperthermia may act as a trigger for arsenic release in temperature sensitive liposomes, escape of arsenic from the liposome at the phase transition may not be the rate-limiting step for release. The arsenic may need to first redissolve by converting from the insoluble nickel(II) arsenite complex back into $\text{As}(\text{OH})_3$ so it may cross the lipid bilayer. This would be especially important if pores are not part of the release mechanism.

B. Simulation Results

Nature of the CG Liposomes—In the simulations we examined the permeability of thermosensitive liposomes, as well as flat lipid bilayers, in an effort to shed light on the mechanism of release. We first characterize the nature of the thermosensitive liposomes by calculating the lateral diffusion constants of the different lipids. Although the liposomes do not form a gel phase in the CG model, we can use the mobility of the lipids as one measure of the rigidity or fluidity of the liposomes at various temperatures.

The lateral diffusion constant of DPPC and MPPC as a function of MPPC concentration at different temperatures is shown in Figure 8. The lateral diffusion constant of the lipids can be obtained from the slope of a plot of the mean square displacement. The diffusion constants are on the order of $10^{-7} \text{ cm}^2 \text{ s}^{-1}$, in good agreement with experimental measurements^{27, 28} and previous CG lipid simulations.¹⁹ Clearly the diffusion constant of both DPPC and MPPC increases with increasing temperature. Interestingly, at the colder temperature of 12°C, the diffusion constants of DPPC and MPPC increase with increasing MPPC concentration, particularly when a composition of 50% MPPC/50% DPPC is reached. This could be due to the increased free volume and mismatches in packing as more MPPC lysolipids are added to the system. Although the diffusion constants of DPPC or MPPC at 12°C are about a factor of 2 smaller than the diffusion constant at 50°C, they are still on the order of $10^{-7} \text{ cm}^2 \text{ s}^{-1}$ which indicates that the bilayer of the liposome is still in the LC phase. DPPC lipids in the gel phase have a diffusion constant on the order of $10^{-9} \text{ cm}^2 \text{ s}^{-1}$.²⁹ The estimated fluid to gel phase transition temperature for the CG model is near 22°C,²⁶ however the significant curvature of the liposome (especially one as small as our simulated liposome) tends to stabilize the fluid phase. We must keep in mind this lack of an

observed phase transition when interpreting the liposome permeability results from the CGMD simulations.

Permeability of Liposomes—To measure the permeability of the DPPC/MPPC liposomes, we use CG water as our probe molecule. Since CG water represents 4 molecular waters, it is comparable in size and polarity to $\text{As}(\text{OH})_3$, the species thought to be released from the experimental liposomes in this work. However, because the sampling time of the simulations is sped up by approximately a factor of 4, this compensates for the coarse-graining of the water such that values close to experimental water permeabilities are obtained, as discussed below. Over the course of the CGMD trajectory, we monitor the flux of water across the liposome bilayer. We then calculate the permeability coefficient from the following equation derived from Fick's law:³⁰

$$P = \frac{J}{\Delta C A} \quad (1)$$

where P is the permeability coefficient in cm s^{-1} , J is the unidirectional flux in mol s^{-1} , ΔC is the effective water concentration (55.5 mol dm^{-3}), and A is the surface area. The value of J is taken to be the average of the water flux into and out of the liposome (over long enough time periods these numbers should be equal). Figure 9 and Figure 10 show the permeability coefficient of water for the 12 nm diameter and 16 nm diameter liposomes, respectively. We were able to calculate the permeability coefficient for a wider range of MPPC concentrations in the smaller (12 nm) liposome due to the smaller system size. At 12°C , the permeation rate is close to zero as few waters possess enough energy to cross the bilayer. At 50°C , the permeability coefficient ranges from 0 to $1.2 \times 10^{-3} \text{ cm s}^{-1}$ in the 12 nm liposome and 0.5×10^{-3} to $1.0 \times 10^{-3} \text{ cm s}^{-1}$ in the 16 nm liposome. At 77°C , the permeability coefficient ranges from 3.5×10^{-3} to $5.5 \times 10^{-3} \text{ cm s}^{-1}$ in the 12 nm liposome and from 3.4×10^{-3} to $4.0 \times 10^{-3} \text{ cm s}^{-1}$ in the 16 nm liposome. These values are in good agreement with experimentally measured permeability coefficients of pure DPPC liposomes.^{31, 32} Note that the range of the permeability coefficient is larger in the 12 nm liposome system due to the larger range of MPPC concentrations examined. In both liposomes, at both 50°C and 77°C , we see a slight but steady increase in the permeability of water as the concentration of MPPC is increased. Although the MPPC lysolipid is thought to stabilize pores in the bilayer of the DPPC liposome, we do not observe pores in our simulations. The enhanced permeability upon addition of MPPC is most likely due to an increased free volume fraction in the bilayer caused by mismatches and a "missing tail" on the MPPC. A quantitative analysis of the free volume properties of DPPC/MPPC bilayers will be discussed in a forthcoming paper.³³ From the permeability coefficient we can estimate the time for release of the liposome cargo. It is straightforward to show that:³⁰

$$C_I(t) = C_I(0)e^{(-3Pt/R)} \quad (2)$$

where $C_I(t)$ is the concentration of the material inside a liposome of radius R at time t . From Eq. 2 we can calculate the halftime for release as $t_{1/2} = 0.693R/3P$. Assuming a permeability coefficient of approximately $10^{-3} \text{ cm s}^{-1}$ for water, the halftime to empty a 100 nm diameter liposome is 1 ms. Phospholipids in the LC state are remarkably permeable to water, while the permeability of other uncharged polar solutes is generally at least two orders of magnitude smaller.³⁰ The release of $\text{As}(\text{OH})_3$ is therefore likely to be slower, particularly due to its larger size. A molecule of more comparable size is glycerol, for which the experimental permeability across phospholipids is measured to be $5 \times 10^{-6} \text{ cm s}^{-1}$.³⁴ This value of P gives a halftime of release of 231 ms for a 100 nm diameter liposome. Unfortunately the time resolution of the $\text{As}(\text{OH})_3$ release curves does not allow for an accurate comparison, but our calculations suggest that a lower bound to the $\text{As}(\text{OH})_3$ release

time, not taking into account the possible rate limiting step of dissolution of $\text{As}(\text{OH})_3$, is on the order of one second.

Permeability of Flat Lipid Bilayers—To study the effect of the gel to LC phase transition on the permeability we also simulated flat lipid bilayers of DPPC and various concentrations of MPPC. The phase transition does indeed show up in simulations of the flat bilayer systems. The phase transition can be verified by monitoring the area/lipid, the diffusion coefficient of DPPC, or the radial distribution function as the temperature is changed. For quantitative details of these properties for DPPC/MPPC bilayers, see ref.,³³ but here we report the general result that there is a peak in permeability for DPPC/MPPC bilayers near the phase transition temperature similar to what is seen in the experimental work of this paper as well as previous experiments.²² Figure 11 shows the permeability coefficient of water through the bilayer as a function of temperature for 20 and 50 mol% MPPC as well as for a pure DPPC bilayer. The results for 5, 10, 15, 30, and 40% have similar profiles but are not shown for clarity. The magnitude of the permeability coefficients for water passing through the bilayers is similar to results for the liposomes. The peak in permeability appears near 39°C which is close to the calculated phase transition temperature upon heating. The experimental T_m is near 42°C in good agreement with our results. Even though pure DPPC does go through a phase transition, we do not observe a definite "peak" in the permeability near the phase transition temperature in contrast to the bilayers that contain the lysolipid, which do exhibit a well-defined peak near T_m . However, we do not observe lysolipid-stabilized pores in the bilayer simulations and instead attribute the peak in permeability due to mismatches enhanced by the presence of MPPC at the grain boundaries of the partially melted gel phase as well as a higher free volume due to the "missing tail" on the MPPC. Also, the limited size of our simulated liposomes and bilayers may suppress the formation of pores that would otherwise form in larger systems. In addition, our model does not accurately reflect the conical shape of the MPPC molecule, so we cannot rule out the possibility of lysolipid-stabilized pores enhancing the permeability near the phase transition in the experiments.

IV. Conclusions

In this work, we have characterized a novel delivery system for the anticancer drug ATO. We have measured the release of ATO from DPPC liposomes with MPPC lysolipid incorporated into the bilayer. There was negligible leakage of ATO from all systems at 25°C. Upon heating the liposomes to 37°C, there was 15% to 25% release over a 24 h time period. The ATO release from the DPPC and DPPC:MPPC(5%) systems leveled off after 10 h at 37°C, whereas the DPPC:MPPC(10%) liposomes continued to release ATO over the 24 h timespan. Upon heating the liposomes rapidly to 42°C, they go through the gel to LC phase transition and the release rate is substantially increased. The two systems containing lysolipids, DPPC:MPPC(5%) and DPPC:MPPC(10%), exhibited a very rapid release of a significant amount of arsenic in the first hour. In the first hour, the DPPC:MPPC(5%) liposomes release 40% of the arsenic and the DPPC:MPPC(10%) liposomes release 55% of the arsenic. The arsenic release from the pure DPPC liposomes was not significantly more rapid at 42°C than it was at 37°C, indicating presence of lysolipid is necessary for a significant enhancement of the release rate.

We also used a coarse-grained molecular dynamics model to investigate the enhanced permeability of lysolipid-incorporated liposomes and lipid bilayers. Due to the ability of water to cross the bilayer on a timescale relevant to our simulation timescale, we used it as our permeability probe. The CG liposomes did not form a gel phase when cooled due to the high curvature, but permeability was significantly lower at 12°C, below what would be the liquid to gel phase transition temperature. At 50°C and 77°C, above T_m , we find water

permeability coefficients on the order of $1.0 \times 10^{-3} \text{ cm s}^{-1}$, in good agreement with experiment. Models of release rate from liposomes for molecules other than water were developed which suggest a lower bound of 1 s for ATO, which is faster than we can access in our experiments. However the trends in release rate with temperature and lipid composition do match what is seen in the simulations. In addition, our estimates of release timescales are similar to what Needham and others found for Doxorubicin.^{12, 13}

From the simulations of flat DPPC:MPPC bilayers we found that the a peak in the permeability does coincide with the phase transition from the gel to LC state when the lysolipid MPPC is present. No pores were observed in our simulations, however due to limitations in our model, we cannot rule out the possibility of lysolipid-stabilized pores enhancing the permeability in the experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Cancer Institute division of the U.S. National Institutes of Health as part of the Center of Cancer Nanotechnology Excellence at Northwestern University (U54CA119341).

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Figure 1.
Kinetics of arsenic loading into liposomes of various MPPC concentrations as indicated in the legend. The bottom panel shows the short time behavior.

Figure 2.
Coarse-grained representations of DPPC, MPPC, and water mapped onto the atomistic structures.

Figure 3.

A self-assembled 16 nm diameter liposome (90%/10% DPPC/MPPC) from a 128 ns coarse-grained molecular dynamics trajectory at 50°C. The DPPC choline group is in green, the phosphate group in orange, the glycerol group in white, and carbon tail beads in cyan. Waters are shown in blue and the MPPC lysolipids are shown in red. Note that the MPPC lipids were added after self-assembly of the liposome. See text for details. The image was created in Visual Molecular Dynamics.³⁵

Figure 4.

Panel A shows the arsenic release at 25°C, 37°C, and 42°C for the pure DPPC liposomes. Panels B and C show analogous results for the DPPC:MPPC(5%) and DPPC:MPPC(10%) liposomes, respectively.

Figure 5.
The arsenic release at 37°C (below the phase transition temperature) for the DPPC, DPPC:MPPC(5%), and DPPC:MPPC(10%) liposomal systems.

Figure 6.

The arsenic release at 42°C (the phase transition temperature) for the DPPC, DPPC:MPPC(5%), and DPPC:MPPC(10%) liposomal systems.

Figure 7.
The arsenic release at 45°C (above the phase transition temperature) for the DPPC and DPPC:MPPC(10%) liposomal systems. Note that the timescale is in minutes.

Figure 8.

The top panel shows the diffusion constant of DPPC in the 12 nm liposome as a function of MPPC concentration at 12°C (dashed line), 50°C (dotted line), and 77°C (solid line). The bottom panel is the analogous plot for the diffusion constant of MPPC. Error bars on shown only on the 12°C curves for clarity.

Figure 9.

The water permeability coefficient P as a function of MPPC concentration for the smaller (12 nm diameter) liposome. Error bars on shown only on the 77°C curve for clarity.

Figure 10.

The water permeability coefficient P as a function of MPPC concentration for the larger (16 nm diameter) liposome. Note the different range in the x-axis as compared to Figure 9. Error bars are shown only on the 77°C curve for clarity.

Figure 11.

The water permeability coefficient P as a function of temperature for DPPC bilayers with varying concentrations of MPPC (0, 20, and 50% MPPC). The results for 5, 10, 15, 30, and 40% have similar profiles but are not shown for clarity.