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Rapid release of liposomal contents upon photoinitiated destabilization with UV exposure

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Abstract

The use of liposomes for the delivery of therapeutic agents to tumor sites took a major step forward with the introduction of sterically stabilized liposomes (polyethylene glycol [PEG]-liposomes). Several research groups reported the increased localization of PEG-liposomes at tumor sites. Once PEG-liposomes reach these sites, it can be desirable to increase the rate of release of encapsulated compound(s). The use of radiation for this purpose is attractive, because it can be delivered in a spatially and temporally selective manner. An effective strategy for the photoperturbation of PEG-liposomes relies on the photoinitiated polymerization of reactive lipids in the liposomal bilayer. Previous studies indicated that the inclusion of the photoreactive 1,2-bis[10-(2′,4′-hexadienoyloxy)decanonyl]-*sn*-glycero-3-phosphocholine (bis-SorbPC$_{17,17}$) among the lipids of PEG-liposomes had little effect on their permeability until the PEG-liposomes were exposed to UV light. Photoexposure increased the permeability of the PEG-liposomes 200-fold [Biochim. Biophys. Acta 1511 (2001) 113]. Further study of this phenomena has now revealed that PEG-liposomes can be designed that have extremely low permeabilities to water-soluble fluorescent probes at 37 °C in the dark, yet the permeability can be increased 28,000-fold upon UV irradiation. The large increase in the rate of photoinitiated release of the encapsulated contents may be a consequence of increased phase separation between photoreactive and saturated phospholipids used in the PEG-liposomes.

Keywords: Polyethylene glycol; PEG-liposome; Photoexposure

1. Introduction

Liposomes typically show excellent biocompatibility and capability to encapsulate chemotherapeutic compounds in a manner that recommends them as drug carriers. The advantages of liposomes include the ability to incorporate compounds that are not water-soluble, therapeutic agents do not need to be altered by the addition of a bulky group to mask it from the body's immune system, and chemotherapeutic compounds are kept from interacting at unintended sites. The disadvantages of the liposomes are their recognition by the immune system and their removal from the bloodstream by the reticuloendothelial system (RES) [2]. The disadvantages can be reduced through the use of covalently attached hydrophilic polymers, such as polyethylene glycol (PEG) [3,4].

One of the largest advantages of liposomes is the potential ability for site-specific attack. Liposomes which remain in the blood system for prolonged times tend to accumulate at the cancer sites. Tumors have a greater need for nutrients due to their rapid growth, and one of the ways in which they obtain the nutrients is to increase the permeability of the surrounding blood vessels. This permits long circulating liposomes to escape the vasculature and reach the interstitium, thereby increasing the concentration of the liposomes at the cancer site. The passive leakage of therapeutic agents from liposomes in the interstitium can cause cancer cell death [5]. Liposomes that are retained in the bloodstream are removed by the RES, thus preventing the encapsulated therapeutic agent from attacking other tissue.

Conventional liposomes, without masking or steric stabilization, are rapidly removed by the liver [2] or destroyed by white blood cells after recognition by the immune system [4]. Liposomes that have a masking hydrophilic polymer covalently attached to them have been shown to have a retention half-life, within the human body, about 10 times

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greater than conventional liposomes. While PEG-liposomes are masked from the liver and the immune system, the PEG groups can interfere with the rapid release of the encapsulated chemotherapeutic agents. Therefore, the search for methods to trigger the release of the liposomal contents from PEG-liposomes continues to be important.

The use of light to stimulate the release of encapsulated compounds from liposomes is attractive because it is possible to control the spatial and temporal delivery of the radiation. Liposomes may be made photosensitive by the use of uniquely designed lipids that can alter the liposome properties via photoisomerization, photocleavage, or photopolymerization [6]. A particularly useful characteristic of the latter is the multiplicative nature of the polymerization process [7]. Ultraviolet (UV) light directly initiates the polymerization of phospholipids having the hexa-2,3-dienoyl (sorbyl) functionality at the chain ends. The photopolymerization reaction produces polymers with a low kinetic chain length of about 10 [8]. However, if lipids are substituted with polymerizable groups in both acyl chains, cross-linked polymers networks are formed [9]. Although UV initiated polymerization is not suitable for biological applications due to the high absorbance of UV light by many biomolecules, it does provide a convenient method to test lipid compositions which could, in a clinical setting, be polymerized by more biocompatible techniques such as photosensitization with longer wavelength light [10,11], or by exposure to therapeutic doses of ionizing radiation.

Whatever the means of destabilization, it is important to be able to substantially increase the solute permeability of PEG-liposomes at the desired location. Obviously, the liposome permeability must be low during the several minutes to hours the PEG-liposomes circulate in the bloodstream. Therefore, if less than 1% of the encapsulated agent leaked during a circulation period of 10 h, it would then take more than a month for the rest of the drug to escape the liposome at the tumor site. In cases where it is desirable to release the drug in hours rather than weeks, the permeability must be increased by at least two orders of magnitude. In a previous publication, it was shown that this goal could be achieved [12]. Here we demonstrate that an additional increase of two orders of magnitude can be achieved by a straightforward modification of the photosensitive PEG-liposome composition.

2. Experimental

2.1. Materials

The fluorescent probe, 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS), was purchased from Molecular Probes and used without further purification. The collisional quencher, bis-pyridinium-1,4-xylene dibromide (DPX) was prepared from dibromo-1,4-xylene and pyridine. Bis-SorbPC was prepared as described in Ref. [7] and used without further purification. 1,2-Dipalmitoyl-*sn*-phosphatidylcholine (DPPC), 1,2-distearoyl-*sn*-phosphatidylcholine (DSPC), 1,2-diarachidoyl-*sn*-phosphatidylcholine (DAPC), and PEG_{2000}-1,2-distearoyl-*sn*-phosphatidylethanolamine (PEG-DSPE) were obtained from Avanti Polar Lipids. (3-PEG_{2000}-propyl)-*N*-octadecyl-amine $Sorb_{17}$ acid (PEG-IPA) was synthesized by reacting *N*-hydroxysuccinimidyl ester of methoxypoly(ethylene glycol) propionic acid with octadecyl amine. The $Sorb_{17}$ acid is an intermediate in the bis-SorbPC synthesis and is described in Ref. [7].

2.2. Liposome preparation

Lipids were measured volumetrically into separately weighed 10 ml round-bottom flasks from stock solutions of known concentrations. An argon stream was passed over each solution to remove the solvent and the flasks were then placed under high vacuum for a minimum of 4 h to complete the drying. The flasks were weighed to obtain the amount of lipid in each flask. Approximately 2 ml of chloroform were then added to all but one of the flasks and the contents were combined and dried as above. A final weight was taken to ensure the complete transfer of lipids. The lipids were then suspended in a sufficient amount of dye containing pH 7 phosphate buffer to make a 10 mM total lipid concentration. The buffer used for the hydration of the lipids contained ANTS (25 mM), DPX (90 mM) and sodium phosphate (10 mM). The osmolarity of the buffer solution was found to be 277 mosM The lipid suspension was then subjugated to 10 freeze–thaw cycles and then extruded three times through two stacked 600 nm Nuclepore membranes followed by extrusion three times through two stacked 200 nm Nuclepore membranes, and finally extrusion four times through two stacked 100 nm Nuclepore membranes. The resulting ANTS/DPX containing liposomes were eluted through a Sephadex G-75 column with pH 7 buffer solution containing sodium phosphate (10 mM), sodium chloride (139 mM), and having an osmolarity of 277 mosM. The concentration of the resulting liposome suspensions were determined by UV absorbance at 260 nm (sorbyl $\lambda_{max} = 258_{MeOH}$, $\varepsilon = 47{,}100$) of a 30 μl aliquot in 0.97 ml of HPLC grade methanol. The total lipid concentrations of resulting liposomes were between 1 and 3 mM.

2.3. Fluorescence measurements

Fluorescence time based scans were done on 3 ml, 0.15 mM dilutions of the liposome suspensions in pH 7.0 PBS, with 360 nm excitation and 520 nm emission on a Spex Fluorolog 2 fluorometer. The slit width for both excitation and emission monochrometers was 4 mm. Complete leakage was determined after lysis of the liposome by addition of 0.3 ml of 5% (v/v) aqueous Triton X-100 to a 3 ml sample. Photopolymerization was carried to between 20% and 99% by exposures of 1 s up to 8 min to light from a low-pressure Hg pen lamp at 0.02–0.04 W/cm^2. The percent conversion was determined by the change in UV absorbance at 258 nm. A

Corning CS-9-54 filter (>230 nm) was used to prevent photolysis of the polymerization product. Monomer conversion was calculated as

$$\%\,\text{conversion} = (100)(A'^{254}_0)(A^{254}_0 - A^{254}_t)/(A^{254}_0)(A'^{254}_0 - A'^{254}_{20})$$

where A_0 is the initial absorbance of the sample, A'_0 is the initial absorbance of the standard sample, A_t is the absorbance of the sample after time (t) or irradiation, and A'_{20} is the absorbance of the standard sample after 20 min of irradiation.

2.4. Determination of encapsulated ANTS

The total amount of ANTS encapsulated in the liposomes was determined shortly (within 1–2 h) after chromatographic separation of the unencapsulated dye. A 3 ml sample of liposomes having a total lipid concentration of 150 μM was prepared, and the fluorescence at 520 nm with excitation of 360 nm was measured for 45 s. Triton X-100 (0.3 ml at 5% aq. v/v) was added, and the fluorescence measurement was continued for an additional 45 s. The emission intensity of the sample after addition of Triton X-100 was multiplied by 1.1 to adjust for the dilution by the detergent solution and the difference between this and the initial measurement was compared to the emission intensity of standard solutions of ANTS/DPX (5:18 ratio).

2.5. Determination of liposome leakage

In order to determine the percent leakage of liposome encapsulated ANTS/DPX, the fluorescence of each sample was measured over 30 s prior to photolysis. Immediately after photolysis, the percent conversion was determined from the sample absorbance with a diode array spectrophotometer, and the fluorescence was measured continuously over several minutes. After the leakage measurement, a 90 s time scan was performed during which a 5% solution of Triton X-100 was added at 45 s. The fluorescence due to 100% leakage was determined from this measurement after correcting for the bleaching of ANTS during photolysis and the dilution factor due to the Triton X-100 solution.

The fluorescent marker, ANTS, has a minor absorption at 258 nm at the sample concentration. For this reason, irradiation of the liposome solution at 230–300 nm causes some bleaching of the ANTS. The percentage of ANTS bleaching was determined by comparing the fluorescence measurement after the addition of Triton X-100 for a photolyzed sample to a similar measurement performed on a sample of non-photolyzed liposomes after dividing each by the initial UV absorbance at 254 nm to factor out any differences in sample preparation,

$$b = I_{\text{photolyzed}}/I_{\text{non-photolyzed}} \times A_{\text{non-photolyzed}}/A_{\text{photolyzed}}$$

A bleaching factor of between 0.8 and 1.0, depending on the length of photolysis, was obtained, which adjusts the baseline (I_0) to what it would be if the amount of ANTS present before photolysis were equal to that present after photolysis.

The percent leakage at any time is given by the following expression:

$$\%\ \text{leakage} = 100 \times (I_t - bI_0)/(1.1I_{100} - bI_0)$$

where I_t is the fluorescence intensity at time (t), I_0 is the fluorescence intensity prior to photolysis, I_{100} is the fluorescence intensity after addition of Triton X-100, and b is the bleaching factor. Because the initial change in concentration inside the liposomes is relatively small, the initial leakage is pseudo-zero order, and the plot is a straight line. The rate of leakage was calculated from the linear region of the plot using a least squares fit.

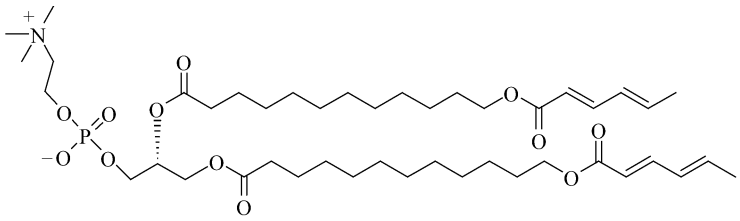
3. Results and discussion

3.1. Liposome composition and permeability

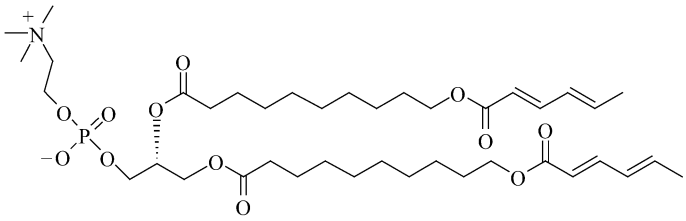
PEG-liposomes were composed of three components, the photosensitive lipid; a saturated lipid D[P,S,A]PC; and a PEG-lipid. Bis-SorbPC$_{17,17}$ or bis-SorbPC$_{19,19}$ was used as the photosensitive lipid. The saturated PC was used as the primary lipid to test the effect of phase separation with the photosensitive bis-SorbPC. PEG-lipid was included as the masking compound, which also prevents unwanted liposome fusion. ANTS and its collisional quencher, DPX were included within the aqueous compartment as a fluorescent probe to investigate liposomal leakage (Scheme 1).

Encapsulation efficiency of the fluorescent probe ANTS was compared to previously published values for liposomes containing cholesterol [1]. The highest values for dye encapsulation in the previously published liposomes was about 33% of the calculated maximum internal volume for 100 nm POPC LUV. Liposome formulations in this publication had a maximum encapsulation value of about 70%. Possible explanations for the difference in encapsulation efficiency include: (1) the absence of cholesterol from the current formulations, and (2) the use of lipids with T_m values above room temperature. All formulations tested in the current study were maintained at room temperature during gel chromatography and dilution. Because room temperature is below the lowest T_m value for the individual lipids, the liposomes can be considered more solid-like in character and less permeable to the encapsulated compounds used in this study.

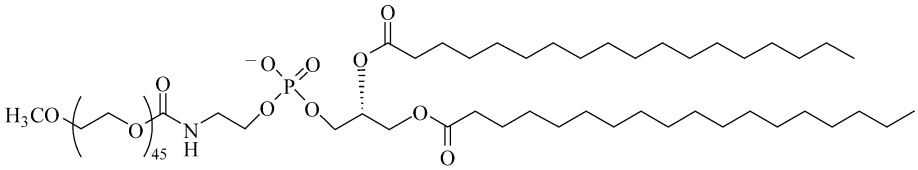
The rate of dark or passive leakage is an indication of how stable the liposomes are prior to irradiation. A sample, 100 μl typically, of the buffer containing the prepared liposomes was placed into a 1 ml cuvette with buffer (900 μl). The UV absorbance was measured and the intensity of the peak at 260 nm was used to calculate the concentration of the solution. An aliquot of the original sample with a



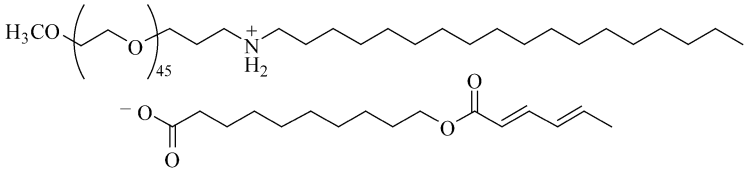
bis-SorbPC$_{19,19}$



bis-SorbPC$_{17,17}$



PEG -DSPE



PEG-IPA

Scheme 1.

known concentration was then placed into the 3 ml fluorometer cuvette to produce a 0.15 mM concentration. An initial reading was performed to obtain a baseline for the amount of dye in the solution but not encapsulated within the liposome's aqueous compartment. Measurements of 90 s would normally be made at 10 min increments without UV irradiation to determine the amount of leakage in the absence of irradiation. This is termed dark leakage.

The T_m values, temperature below which the compounds are in a gel phase and above which are in a liquid crystalline phase, are very important in predicting the thermal stability of liposomes. As the T_m value of the bis-SorbPC is increased, the liposomes exhibit a low dark permeability, at higher temperatures prior to UV irradiation. The T_m value for bis-SorbPC$_{17,17}$ is 29 °C, while the T_m of bis-SorbPC$_{19,19}$ is 42.5 °C. As the temperature was increased above 33 °C, the liposomes containing bis-SorbPC$_{17,17}$ began to show significant dark leakage. The lipid mixtures containing bis-SorbPC$_{19,19}$ were stable to at least 43 °C. The maximum temperature studied here was 43 °C (110 °F) because this is considered the highest natural temperature the human body could attain without causing severe damage. The development of thermotropic liposomes which are sensitive to hyperthermia and destabilized at specific temperatures is an active research topic [13]. The liposome compositions studied here may also deserve consideration as thermotropic liposomes.

The substitution of DSPC for DOPC in liposome formulations incorporating cholesterol indicated a 4-fold decrease in the rate of dark leakage. Removal of the cholesterol from the liposomal formulation caused a further order of magnitude reduction in the rate of dark leakage. Each of these results support the possible explanations for the encapsulation efficiency discussed above.

The ultimate goal of the research is to develop liposomes with very low dark leakage at body temperature, and with much higher rates of photoinduced leakage. To test this, measurements of dark leakage were obtained over temper-

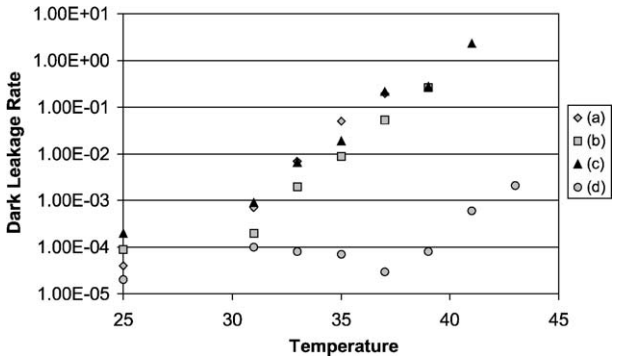


Fig. 1. Log_{10} rates of the dark leakage (% s^{-1}) were measured to determine the stability of the liposomes at specific temperatures. (a) PEG-IPA/DPPC/bis-SorbPC$_{17,17}$ (5:75:20); (b) PEG-IPA/DSPC/bis-SorbPC$_{17,17}$ (5:75:20); (c) PEG-DSPE/DAPC/bis-SorbPC$_{17,17}$ (5:75:20); (d) PEG-DSPE/DAPC/bis-SorbPC$_{19,19}$ (5:75:20). The PEG-IPA represents a synthesized ion pair amphiphile that had no effect on the stability of the liposomes.

atures ranging from room temperature to 43 °C. The dark leakage values are shown in Fig. 1. At 37 °C, the rates for the percent of encapsulated dye that is released per second without irradiation varied from 0.1946 to 0.00003.

3.2. Rates of monomer loss in liposomes

The initial rates of the monomer loss upon UV irradiation were determined to test if there is a difference in photosensitivity between liposome formulations with and without cholesterol. The loss of the monomer was determined by the decrease in UV absorbance of the Sorbyl group as described in the experimental section (Fig. 2). Several time points were plotted and the slope of the initial linear portion was used to calculate the rates. The rates for the loss of monomer in liposomes composed of saturated lipids without cholesterol were 12.2×10^{-7} mol s^{-1} (PEG-DSPE/DAPC/bis-SorbPC$_{17,17}$) and 17.1×10^{-7} mol s^{-1} (PEG-DSPE/DAPC/bis-SorbPC$_{19,19}$). These rates compared favorably with the previously published value of 18.0×10^{-7} mol s^{-1} for liposomes composed of PEG-DOPE/bis-SorbPC$_{17,17}$/cholesterol/DOPC (15:30:40:15) [1]. Liposomes composed of saturated lipids with cholesterol, such as PEG-DSPE/bis-SorbPC$_{17,17}$/cholesterol/DSPC (5:30:35:30), were somewhat less sensitive with a rate of monomer loss of 6.5×10^{-7} mol s^{-1}.

At lower mole fractions of the bis-SorbPC ($\leq 10\%$), monomer loss rates ranged from 1.8×10^{-7} to 3.4×10^{-7} mol s^{-1}. At higher mole fractions, the range is from 8.7×10^{-7} to 2.1×10^{-6} mol s^{-1}. This indicates a small advantage if mole fractions of the polymerizable lipids are above 10%. There is no apparent advantage for the rate of the loss of monomer when comparing liposome formulations with and without cholesterol.

3.3. Liposome photoinduced leakage

An important part of this research was to determine the extent to which UV irradiation of PEG-liposomes would increase their permeability to encapsulated reagents. Photoinduced leakage was determined by encapsulating a fluorescent dye and a collisional quencher (DPX) within the aqueous compartment of the liposome. While the dye and quencher were within the liposome, the dye fluorescence was quenched. The internal volume of the liposome is calculated as 1% of the overall volume of a solution. Once the dye and quencher were able to reach the external medium, the concentration of the quencher was too low to

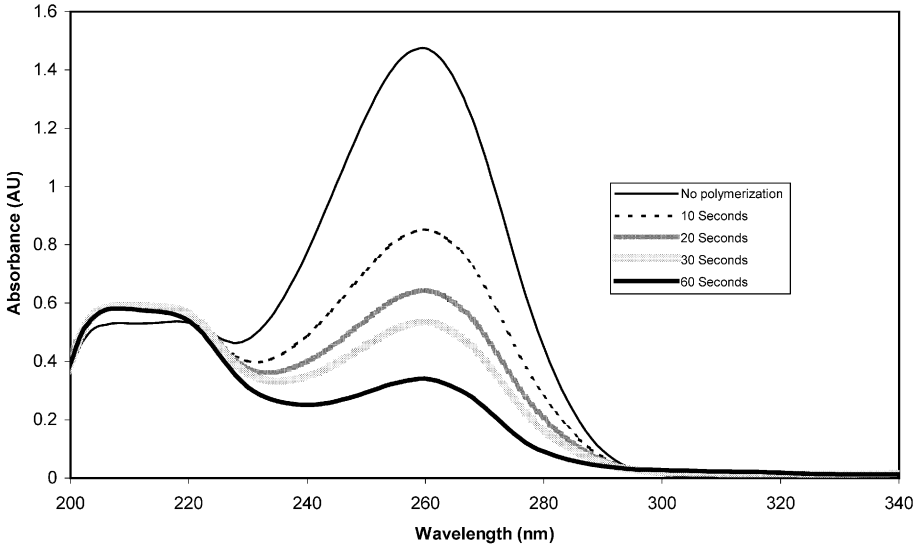


Fig. 2. Sorbyl UV absorption before and after UV irradiation for different time periods. Irradiation of the PEG-DSPE/bis-SorbPC$_{19,19}$/DAPC (5:20:75) liposomes in buffer at room temperature. Irradiation time was from 0 to 60 s with >85% loss of monomer after 60 s. The flat peak between 200 and 220 nm is due to absorbance of the fluorescent dye and quencher (ANTS/DPX) encapsulated within the liposomes for leakage assays.

effectively quench the dye fluorescence, and the increased dye emission could be observed using a spectrofluorometer. The increase in fluorescence intensity after irradiation can be used to quantitatively measure the concentration of the dye released from the liposomes.

Fig. 3 shows a graph of the intensity of the fluorescence over time. The dashed line represents the sample fluorescence prior to irradiation of the sample. The initial reading of 15,000 cps is probably due to the incomplete removal of all the external dye upon passing through the Sephadex column. Triton X-100 treatment of the liposomes at 61 s disrupted them and increased the dye fluorescence by decreasing the quenching efficiency of the DPX. The solid line is the sample fluorescence after the liposomes were irradiated for 10 s with 254 nm light. The large intensity increase from 15,000 to 37,000 cps occurred during the 10 s of irradiation and approximately 5 s it took to transfer the sample cuvette from the light source to the spectrofluorometer.

To determine the rate of leakage, the samples were irradiated for 10 s and the fluorescence measurement was compared to the initial baseline obtained prior to irradiation as described in the experimental section. The fluorescence bleaching factor of the fluorescent probe ANTS at 10 s of UV irradiation is nominal. Rates of leakage were obtained by dividing the percent leakage by the time (in seconds) since the irradiation.

The use of saturated PEG-DSPE and DSPC in liposome formulations containing cholesterol has a dramatic effect on the photodestabilization of the liposomes. The photolysis of liposomes composed of PEG-DSPE/bis-SorbPC$_{17,17}$/cholesterol/DSPC (15:30:40:15) to 88% loss of monomer (30 s) at 25 °C produces an increase of 100-fold in the initial rate of leakage (Fig. 4). This is approximately 10 times the increase in the rate of leakage that was observed with the analogous DO-cholesterol liposomes at 37 °C [1]. The greater increase in the relative rates of leakage is partly because of a lower rate of dark leakage and partly because of an earlier onset of photoinduced leakage. The initial rate of leakage at 30 s irradiation in DS-liposomes is about three to ten times higher than that which was observed in DO-cholesterol liposomes, but the rate of dark leakage for DS-cholesterol liposomes is about four to ten times lower than that of DO-liposomes [1]. Variations in the PEG and cholesterol mole fractions of DS-cholesterol liposomes had a significant effect on the rate of release upon photolysis. Liposomes with a composition of PEG-DSPE/bis-SorbPC$_{17,17}$/cholesterol/DSPC (5:30:35:30) show as much as a 100-fold increase in the initial rate of leakage with only 5 s of photolysis.

Liposome formulations prepared without cholesterol showed an even larger enhancement for the rate of release upon photolysis. Table 1 compares the values obtained for the photoinduced leakage of four compositions. The values shown are at 25 °C because of the high permeability of liposomes containing lipids with T_m values below 37 °C. The minimum increase in the rate of release was 280-fold and the highest was 28,000-fold. Liposomes composed of PEG-DSPE/DAPC/bis-SorbPC$_{19,19}$ had a very low dark leakage at 37 °C (3×10^{-5}% s^{-1}) and a photoinduced rate

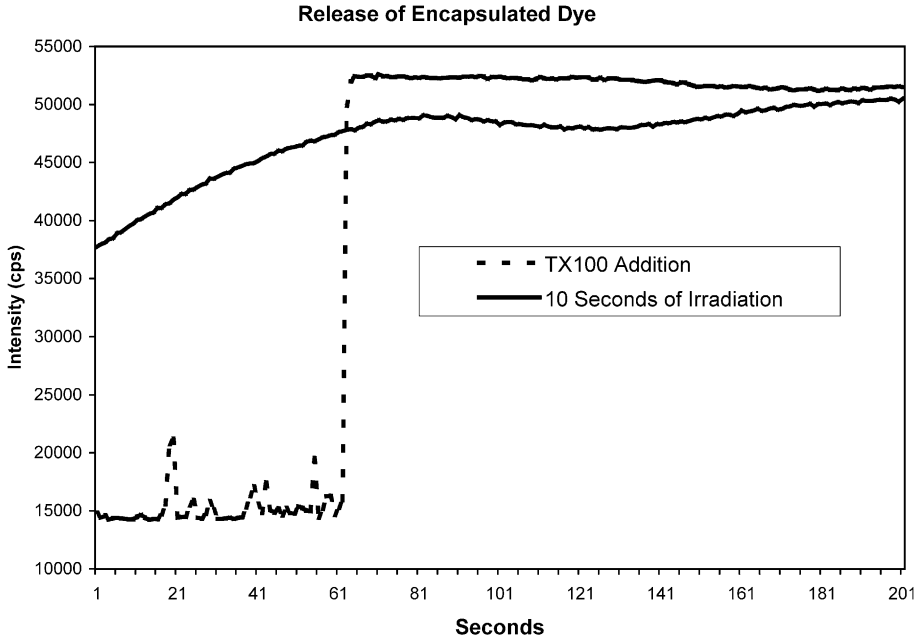


Fig. 3. Fluorescence vs. time shows the fluorescence of our liposomes with or without TX-100 or photolyzed liposomes. The lipid mixture used was PEG-DSPE/bis-SorbPC$_{19,19}$/DAPC (5:20:75). The rapid increase in the intensity is due to the lysis of the liposomes with the use of Triton X-100. The solid line represents the intensity of a sample that has been exposed to 254 nm light for 10 s prior to the start of the fluorescence measurement. The initial increase above the base line is due to the release of the contents during the irradiation and the transfer of the sample cuvette to the spectrofluorometer, which takes less than 15 s.

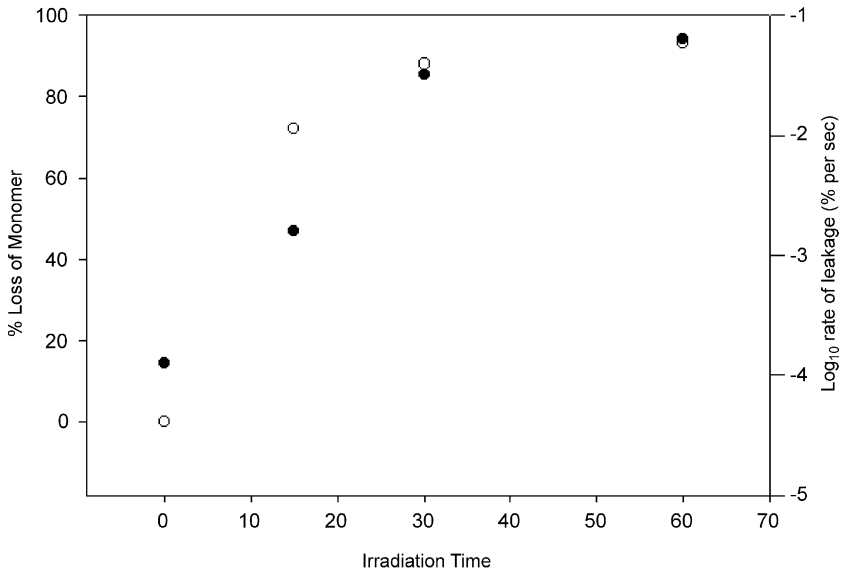


Fig. 4. PEG-DSPE/bis-SorbPC$_{17,17}$/cholesterol/DSPC (15:30:40:15); $\log_{10}$ of the initial rate of leakage vs. irradiation time (filled circles); percent loss of monomer vs. irradiation time (open symbols).

(8.5×10^{-1}% s^{-1}), which was also an increase of 28,000. The photoinduced rate corresponds to 1.9×10^{-8} mol s^{-1}.

3.4. Calorimetry of lipid formulations

It was apparent in the data shown in Fig. 1 that as the T_m values for the individual lipids increased, the liposomes became more stable at higher temperatures. The T_m values for the individual lipids are:

bis-SorbPC$_{17,17}$	29 °C	bis-SorbPC$_{19,19}$	42.5 °C
DPPC	41 °C	DSPC	55 °C
DAPC	66 °C	PEG-DSPE	60 °C

To determine the T_m values of the liposomes, multilamellar vesicles were formed through the freeze–thaw method described earlier. The concentration of the lipid mixture was 5–9 mM. The samples were run from a temperature 10 °C below the lowest T_m value to at least 10 °C above the highest T_m value. Each scan was repeated four times per sample and the observed values (Fig. 5) for the endotherms of the liposomes were compared to the literature values. Some lipid mixtures exhibited two peaks or transitions in the multilamellar vesicles. In most of the lipid mixtures, only one peak appeared which closely represented the T_m value of the nonpolymerizable lipid. The values for the main transition temperature were always several degrees lower then published values for the individual lipids, which indicates a mixing of the polymerizable and nonpolymerizable lipids tends to shift the T_m to a lower value. In the mixture PEG-DSPE/bis-SorbPC$_{17,17}$/DAPC (5:20:75), where the T_m values differed by more then 35 °C, the two distinct endotherms were observed indicating phase separation of the polymerizable and nonpolymerizable lipids.

Table 1
Comparison of the rates of release (% s^{-1}) before and after irradiation at room temperature

Irradiation time (s)	PEG-IPA/ DPPC bis-Sorb$_{17,17}$ (5:75:20)	PEG-IPA/ DSPC bis-Sorb$_{17,17}$ (5:75:20)	PEG-DSPE/ DSPC bis-Sorb$_{17,17}$ (5:75:20)	PEG-DSPE/ DAPC bis-Sorb$_{19,19}$ (5:75:20)
0	0.00004	0.00009	0.0002	0.00002
10	1.1111	0.0258	0.1101	0.1021
× increase	28,000	290	550	5100

Irradiation times were for 10 s using the light from a low-pressure Hg pen lamp. Rate of release for the liposomal formulation PEG-DSPE/DAPC/bis-SorbPC$_{19,19}$ (5:75:20) in mol s^{-1} is 1.9×10^{-8}.

3.5. Mechanism of release

The use of lipids with major differences in T_m's can favor the formation of domains at temperatures below the lowest individual T_m. Lipids are in a solid phase at a temperature below the T_m and phase separation has been observed (Fig. 6), but the solid phase separation may not be accompanied by liquid phase separation at higher temperatures. Lamparski and O'Brien found the liposome composition DOPC/bis-SorbPC$_{17,17}$ (75:25) could not be stimulated to release an encapsulated compound upon UV irradiation at room temperature [7]. The T_m of DOPC is −20 °C, therefore at room temperature, the lipid would be in a liquid crystalline phase. Lipids in a liquid crystalline phase have rapid lateral diffusion rates within the bilayer and may be able to compensate for fissures that form, thus hindering the stimulated release of encapsulated com-

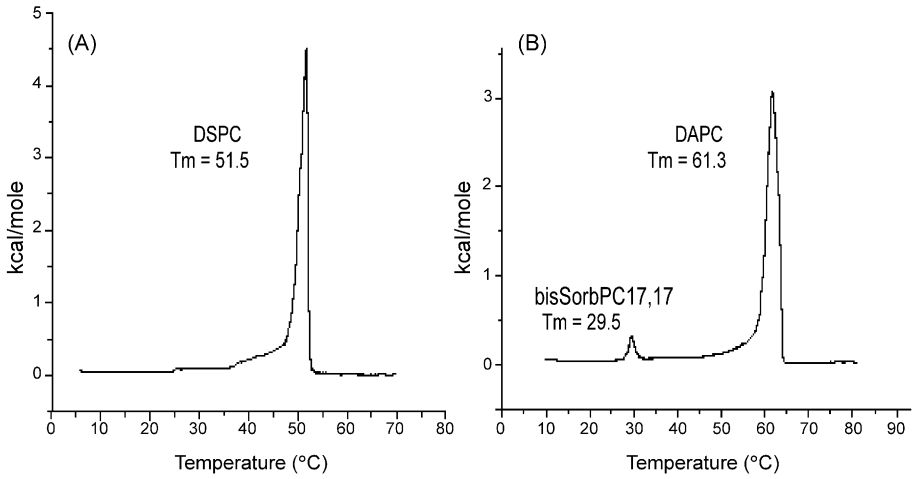


Fig. 5. Differential scanning calorimetry (DSC) data for the lipid mixtures: (A) PEG-DSPE/bis-SorbPC$_{17,17}$/DSPC (5:20:75), (B) PEG-DSPE/bis-SorbPC$_{17,17}$/DAPC (5:20:75). The T_m values for DSPC and DAPC are slightly lower than published values. This may be explained by the incorporation of bis-SorbPC$_{17,17}$ and/or PEG-DSPE as impurities within the domains.

pounds. Irradiation of solid state, phase-separated liposomes show a greater then 4-fold increase in rate of monomer loss compared to liposomes in a liquid crystalline state. The close association of photoreactive groups in solid state, phase-separated systems could account for the increased rate for monomer loss upon irradiation.

The destabilization and subsequent release of the encapsulated components may take place by the contraction of the polymerizable domain (Fig. 6) or distortion of the polymerized domain. The polymerizable lipids are drawn together by the covalent bonds that are formed in the hydrophobic tails upon irradiation. There may be a lag time between the contraction of the polymerized domain and the movement of the nonpolymerized domain to fill the vacated area. During this time, small fissures may exist between the domains until the nonpolymerized lipids are able to fill these spaces, or the fissures may not be filled if they appear in the interior of the polymerized domain.

Another possibility is that the shape of the polymerized domain cannot be incorporated into the lipid bilayer and cause a distortion that leads to micelle or disc formation. Micelle or disc formations would lead to unfavorable interactions between the hydrophilic head groups of the poly(lipid) domain and the hydrophobic tails of the lipid bilayer. Diffusion of the poly(lipid) domain would be favored which could lead to vacated areas and subsequent release of the encapsulated compounds.

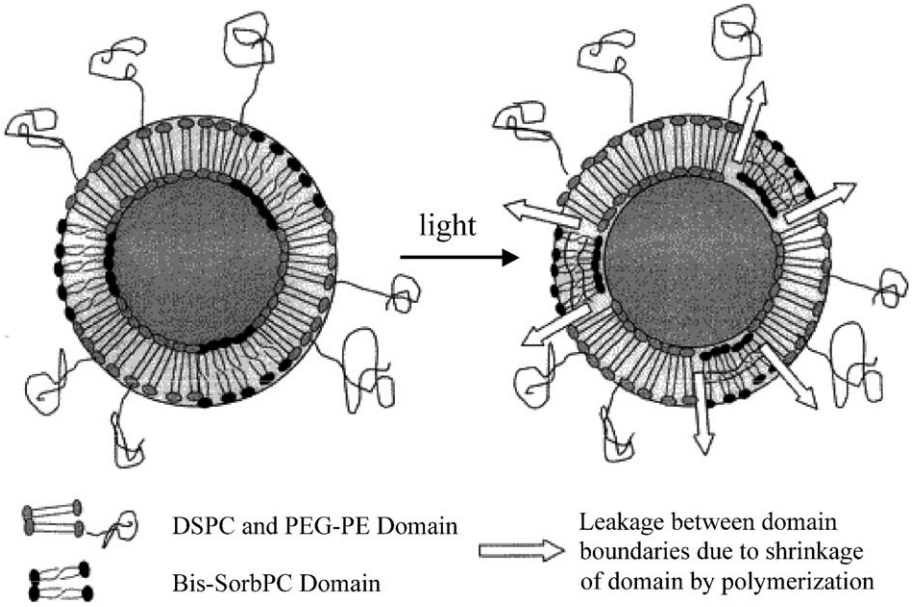


Fig. 6. Schematic cross-section of PEG-liposomes that represents the domains of bis-SorbPC and stearoyl lipids. The photopolymerization-induced reduction in the surface area of the polymerizable domains (black areas) during UV irradiation is shown on the right.

4. Conclusions

Liposomes containing a saturated lipid (DAPC), photosensitive lipid (bis-SorbPC$_{19,19}$), and a PEG lipid (PEG-DSPE) have been shown to be thermally stable at temperatures above body temperature (37 °C) and have been shown to be responsive to UV irradiation. Phase separation of the lipids based on T_m values may have provided the enhancement to the photoreactivity and increased release of the liposomal contents (greater then 28,000-fold compared to unirradiated liposomes). The large increase in the rate of release was observed with only 10 s of UV irradiation at 254 nm with a Hg pen lamp. Total release of the liposomal contents was observed within 2 min. This compares very favorably to the commercially available liposomal drug delivery formulations that rely on passive release over days and weeks. Unfortunately, UV irradiation is not a biocompatible form of polymerization and in order to perform such experiments in vivo it will be necessary to utilize PEG-liposomes that are sensitive to other forms of polymerization.

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