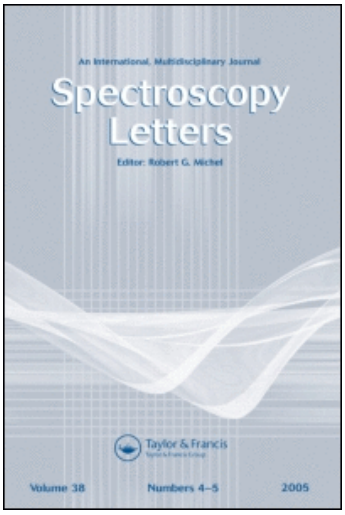


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# Dye Release from Laser Irradiated Liposomes

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# DYE RELEASE FROM LASER IRRADIATED LIPOSOMES

**Key Words:** Dye Release, Laser-Induced, Liposomes.

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## ABSTRACT

The main focus of this paper is to examine the consequence of laser pulses of narrow width impinging on phosphatidylcholine liposomes containing sulforhodamine dye molecules. The release of dye molecules following short-pulsed laser excitation and localized heating was measured and its dependence on laser excitation parameters studied. A characterization of the optimal conditions necessary for release of liposome contents can be applied to the targeted delivery of therapeutic drugs.

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## INTRODUCTION

Liposomes are phospholipid membranes surrounding discrete water-containing compartments. They can simulate vesicles, cells and organelles. Liposomes were discovered accidentally in the early 1960s by Alec Bangham while he was experimenting with reagents and the addition of water to films of phospholipids.[1,2] The potential for their widespread use in the pharmaceutical and biomedical world has evolved over the past three decades. Applications include targeted release of encapsulated drugs in dermatology, radiology and general medicine.[2,3] They are also being used in the delivery of genetic material into bacteria and cells.[4] Among the most significant and promising possible applications of liposomes are the targeted treatment of systemic fungal infections experienced by cancer and AIDS patients.[1,5] Other researchers have expanded the horizon of therapeutic and biotechnological applications for liposome carriers by utilizing them in enzyme replacement therapy,[6] gene transfer,[7] and ophthalmology.[8]

Liposomes use their encapsulating ability as the basis for their function. As a drug vehicle, they transport highly toxic and concentrated drugs by way of adsorption, endocytosis, lipid exchange and fusion processes directly to choice targets in the body.[1,9]

Encapsulation of the toxic drugs prevents assimilation of the drug at untargeted sites, which include vital organs, the central nervous system and blood-forming tissues. Liposomes have been used to entrap compounds, such as steroids, antibiotics and bronchodilators, and those that are anti-malarial, anti-viral, anti-fungal and anti-inflammatory.[9,10]

In order for liposomes to be efficient and useful carriers of clinical significance, the primary obstacles that need to be overcome are those involving stability of prepared liposomes, intercalation in the lipid bilayer membrane, encapsulation within the internal volume, and targeted release of liposome contents.[11] A stable liposome is necessary in order to prevent untimely leakage of contents and at the same time a conveniently destabilized liposome is required for timely release of encapsulated cargo. The stability of liposomes associated with effective storage is improved during preparation by the addition of antioxidants and cholesterol, and by freeze-drying, charging and increasing the number of carbon chains.[12,13] Increased stability of liposomes must be complemented by efficient delivery of their contents to specific sites. Several approaches used for targeted delivery have included endocytosis of target cells,[14] pH-sensitivity involving a lower interstitial pH,[15] heat-

sensitivity dealing with exposure to temperatures greater than the transition temperature of the composite lipid(s),[16] and light-sensitivity involving irradiation.[17]

Substances enclosed in liposomes have been released by heating the liposome environment utilizing radiation. Such heating applications have included the microwave method[8] along with short wavelength laser pulses.[18] A combination of light and heat techniques can be a powerful technique for controlled release of liposome contents. We have utilized the idea of 'direct laser heating' and adapted it for pulsed laser excitation of liposomes containing fluorescent organic dyes. Dyes simulate drugs and their release can be monitored by their fluorescence on exposure to light.



One of the advantages of photoinduced liberation of the dye from liposomes is that the dye release can occur in a controlled manner. Light-sensitive techniques also allow for controlled timing which is useful in critical stages of clinical or surgical procedures. Heating of liposome carriers following laser excitation with visible pulses combines the advantages of light and heat sensitivities and results in controlled timely release of encapsulated organic dyes. The chief aim of this paper is to compare the impact of 532 nm photons from Nd:YAG lasers of 8 ns and 25 ps pulse widths on 2 $\mu$m

phosphatidylcholine liposomes containing 20 mM and 50 mM sulforhodamine dye and to study the effects of variable energy density of laser pulses. The onset of breakage of the lipid vesicles took place after the critical temperature of 41 $^{0}$C was reached. We postulate the manifestation of a localized thermal mechanism[19] to bring about the phase transition necessary for disruption and breakage of liposomes.



## EXPERIMENTAL

L-α-phosphatidylcholine (DPPC) and dicetyl phosphate (DCP) were obtained from Sigma Chemical Co. The organic dye used was Sulforhodamine 640 from Exciton (equivalent to Kodak Sulforhodamine 101). A modified solvent evaporation-rehydration technique[12] was used to encapsulate the dye within the liposomes. The precursors DPPC and DCP (90:10 mol %) were dissolved in a mixture of chloroform and methanol. Equal volumes of the lipid solution and the dye were prepared with Tris buffer (20 mM, pH 7.7) as solvent. Rehydration of the lipid-dye mixture was done using a water bath maintained at 55°C and the preparation was subsequently cooled to room temperature. The excess unincorporated dye was extracted using Sephadex G-25 columns [Pharmacia PD-10] equilibrated with Tris buffer. Polycarbonate membrane filters [Nucleopore Corp.] were used to obtain vesicles of specific sizes.

Absorption spectra of the dye-liposome complexes were obtained using an UV-VIS spectrophotometer [Perkin-Elmer 330]. A prominent absorption peak occurs around 585 nm together with a clear shoulder at about 545 nm.[19] We postulate the weaker blue-shifted absorption band to be due to the formation of dimers of sulforhodamine molecules, while the strong feature at 585 nm arises due to the dye monomers.[19] As there occurs measurable absorption at 532 nm, the dye-liposome system could be effectively irradiated with frequency-doubled Nd:YAG lasers [Quanta Ray DCR and Quantel/Continuum Corp.].



Tris-buffered and diluted liposomes were transferred to capillary tubes and logitudinally excited with 532 nm pulses of width 8 ns and 25 ps, respectively. Figure 1 shows the experimental arrangement used in irradiating the liposomes. Typically, the 8 ns laser pulse focused to a spot size of about 0.25 cm provided an energy density variation that spanned the range 0.2 - 2.0 $J/cm^2$. The 25 ps laser pulse was focused to a spot size around 0.16 cm and provided an energy density output between 0.038 $J/cm^2$ and 0.39 $J/cm^2$.

The irradiated liposomes were placed transversely in the path of a continuous excitation beam of wavelength 585 nm within an experimental arrangement designed to simulate a fluorescence spectrometer. Fluorescence from

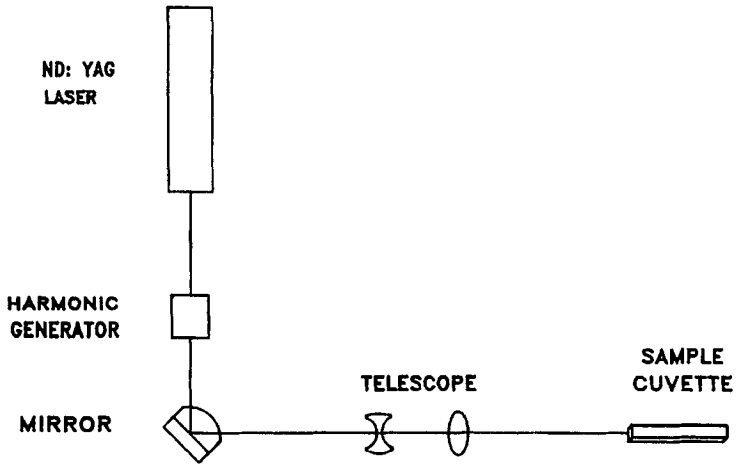


FIG. 1. Experimental Arrangement for the Laser Irradiation of Dye-Encapsulated Liposomes.



the disrupted liposomes passed orthogonally through a monochromator set at 620 nm and was detected by a photomultiplier tube used in conjunction with a picoammeter. Output from the picoammeter was fed into an A/D converter and then relayed to a microcomputer for signal processing, storage and data analysis.

Heating of the dye-encapsulated liposomes to temperatures greater than the critical gel-liquid crystal phase transition temperature [$T_c$ = 41 $^0$C] brought about their breakage accompanied by a dramatic burst of increased fluorescence from the released dye. The percentage release of sulforhodamine dye was quantitatively determined by comparing the enhanced

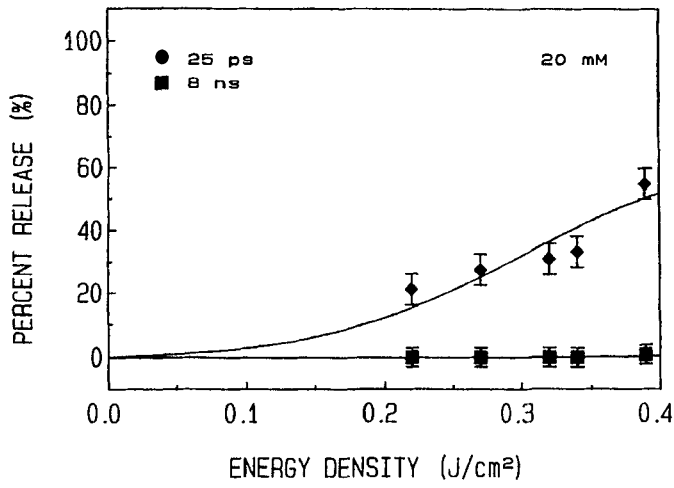


FIG. 2. A Comparison of the Percentage Release of 20 mM Sulforhodamine Dye from 2 μm Liposomes after Exposure to Single 8 ns and 25 ps Laser Pulses.



fluorescence from the liberated dye following pulsed laser excitation with the maximum (saturated) value resulting from additional heating in a hot water bath maintained above 55 $^{0}$C.

## RESULTS AND DISCUSSION

Figure 2 shows a comparison of the percentage release of 20 mM sulforhodamine dye from liposomes of average diameter 2 μm following excitation by *single* 8 ns and 25 ps laser pulses. The comparison was possible in the energy density range 0 to 0.4 J/cm$^2$. As Fig. 2 clearly shows, there was essentially no release of 20 mM

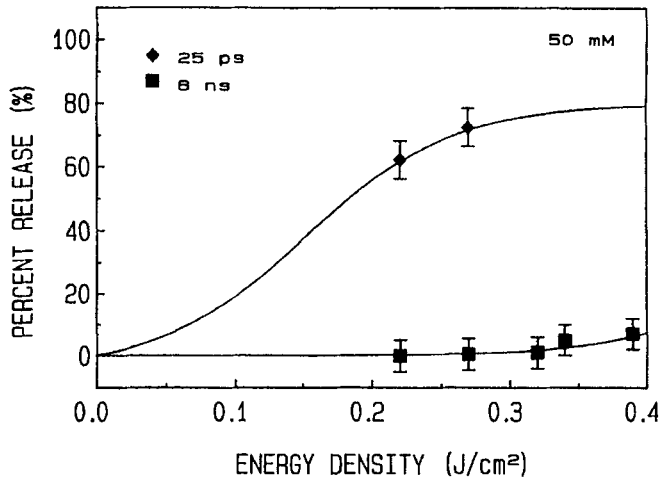


FIG. 3. A Comparison of the Percentage Release of 50 mM Sulforhodamine Dye from 2 $\mu$m Liposomes after Exposure to Single 8 ns and 25 ps Laser Pulses.



dye from the liposomes on irradiation with individual 8 ns pulses. However, picosecond excitation shows an initial release of 21 % for a pulse of energy density 0.22 J/cm$^2$, and then it increases gradually to a peak release of about 55 % for a pulse of energy density 0.39 J/cm$^2$. A similar trend is observed for release of 50 mM dye from the liposomes following nanosecond and picosecond exposure. Figure 3 illustrates that there is essentially a flat release response for an 8 ns pulse over the energy density domain 0 to 0.3 J/cm$^2$; whereas a 25 ps pulse of energy density 0.22 J/cm$^2$ caused a dye release of 62 % and that of 0.27 J/cm$^2$ gave 73 % release. Figures 2 and 3 reveal two important facts concerning dye

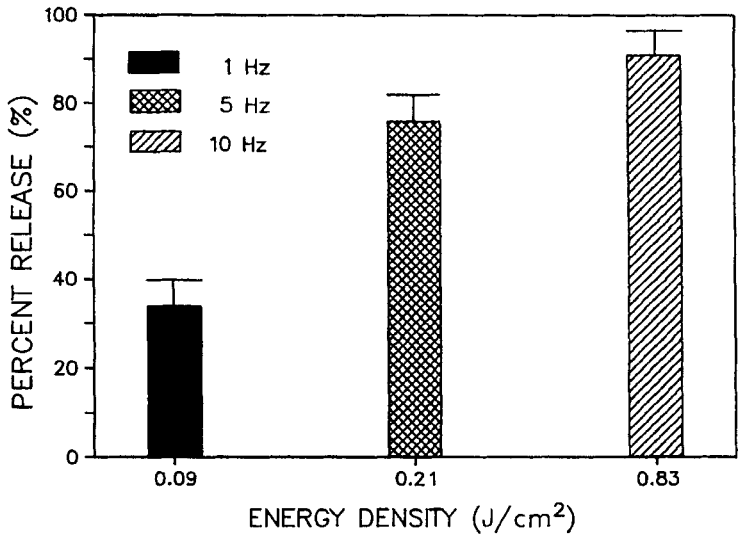


FIG. 4. Percent Release of 50 mM Sulforhodamine Dye from 2.2 μm Liposomes Following Excitation with 10 Laser Shots at 1, 5, and 10 Hz.



release from liposomes of a specific size: (i) there is enhanced release for greater concentration of the encapsulated dye following pulsed laser excitation, and (ii) there is more release with a narrower laser pulse.

Another series of experiments was performed using a timed-shutter and the picosecond laser running at 1, 5 and 10 Hz. Unfiltered liposomes of average size 2.2 μm containing 50 mM dye were used for irradiation. The average energy of the 25 ps pulses was maintained around 6.5 mJ, while the laser repetition rate was varied. Burn patterns on laser paper yielded measurements of 0.01,

0.03 and 0.09 $cm^2$ for the pulse cross-sections for 1, 5 and 10 Hz, respectively. The corresponding energy densities for the picosecond pulses at 1, 5 and 10 Hz, were 0.09, 0.21 and 0.83 $J/cm^2$, respectively. Figure 4 summarizes the data for percent release of the dye obtained with 10 laser shots at each frequency setting. The error bars reflect the uncertainty in the release measurements when several liposome samples were subjected to irradiation under identical conditions. The data clearly show that it is the energy density rather the average pulse energy which determines the quantity of sulforhodamine liberated from the disrupted liposomes.



Our results indicate that the concentration of the encapsulated dye, the laser pulse width and the associated energy density are the critical factors governing the efficiency of dye release from irradiated liposomes. A localized thermal mechanism appears responsible for the breakage of liposomes following pulsed laser excitation and the subsequent release of the encapsulated dye.

## ACKNOWLEDGMENTS

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