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Release Kinetics of Novel Photosensitive Liposome for Triggered Delivery of Entrapped Drug

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Abstract: Liposomes are nano-sized artificial vesicles of spherical shape composed of natural phospholipids and cholesterol. Doxil®, PEGylated liposomal formulation for the delivery of doxorubicin was the first product based on liposomes. Liposomes are the excellent nano-sized drug delivery system against many diseases like cancer. The basic problems associated with the delivery of drugs like anticancer drugs, are low volume of distribution and damage to normal tissues along with cancerous tissues. To eradicate non therapeutic and toxic effects associated with drugs like anticancer drugs, calcein (CAL) encapsulated reversed phase evaporation vesicles (REVs) carrying photoactive destabilization agent ketoprofen (KPF) in the lipid bilayer were formulated. Effect of UV radiation activation of liposomal membrane incorporated KPF on the destabilization of the liposome bilayer and the release of encapsulated CAL were investigated. Conventional and photosensitive liposomes of phosphatidylcholine (PC):Cholesterol (CHOL) in 5:1 molar ratio were formulated and investigated for size, CAL encapsulation efficiency (EE (%)) and *In-vitro* release. Due to the incorporation of KPF in the photosensitive liposomal membrane approximately 4% increase in the EE (33%) in comparison to conventional liposomes (29%) was observed. Sizes of formulated liposomes were found between 200-400 nm. Exposure to UV radiation resulted in the release of CAL and in 10 hrs 97 % of entrapped CAL was released from photosensitive liposome and only 67% in case of conventional liposome in the same time duration. In this study, the *in vitro* drug release data from conventional liposomes as well as from photosensitive liposomes were fitted to various kinetic models and zero order release was found to be the mechanism of action. It was revealed from present study that this formulation could be considered as an ideal nano-sized system for triggered delivery of drugs like anticancer agents.

Keywords: Release Kinetics, Photosensitive Liposome, Triggered Delivery of Entrapped Drug.

Introduction

The discovery of liposome or lipid vesicle emerged from self forming enclosed lipid bilayer upon hydration. Liposomal drug delivery systems have displayed a vital role in formulation of potent drug to improve therapeutic efficiency[1]. It is highly explored drug delivery system and can be safely and effectively used in various fields like protein /drug delivery, controlled delivery, antiviral therapy, tumour therapy, gene delivery, vaccine delivery, cosmetics and dermatology and others. However, to achieve therapeutic efficacy of the liposomal dosage form the encapsulated associated drug should become available to the target cells[2]. A main reason for this is that accumulation of liposomes in the site of action does not guarantee that the encapsulated drug becomes bioavailable to the target cells[3]. Local administration of drugs has always been attractive because

of the avoidance of systemic distribution of the drug and the need to use excessively high doses to enable effective concentrations at target sites. One of the crucial aspects of liposome use is to achieve the release of components at the target site. In local drug delivery, there are many methods to trigger the controlled release of drugs at the target site; such as the release of contents from liposomes in response to external stimuli of temperature, pH and light[4, 5]. The use of light to stimulate the release of encapsulated compounds from liposomes is attractive, because spatial and temporal delivery of the radiation can be possible to control. Destabilization of the lipid membrane by light-induced isomerization, cleavage, or polymerization results in photochemical activation of content release from liposomal bilayers or its components. Photoisomerizable moieties most frequently used for light-controlled release of liposomal contents are based on azobenzene in the form of Bis-azo PC (1,2-bis(4-n-butylphenylazo-4´- phenylbutyroyl)-L-α-phosphatidylcholine). Photo isomerizable liposome compositions have also been prepared using retinoyl-phospholipids and Liposomal content release was also accomplished by photoisomerization of spiropyran. Photocleavage in controlled released manner primary involves photoinduced cleavage of plasmalogens (naturally occurring lipids) by photodynamic sensitization.The UV-induced cross-linking polymerization of 1,2-bis[10-(2',4'-hexadienoyl oxy)-decanoyl]-*sn*-phosphatidylcholine (bis-sorbPC) in liposomes comprising cholesterol, 1,2-dioleoyl-*sn*-phosphatidylcholine (DOPC), and PEG2000-DOPE, caused an over 100-fold increase in the permeability of an encapsulated fluorescent marker[4]. Red blood cell lysis photosensitized by KPF was investigated. KPF when irradiated takes a decarboxylation process via intermediate radicals, in an aqueous buffer solution at pH 7.4. The overall results suggest for KPF photosensitized hemolysis a molecular mechanism involving free radicals, superoxide anion and sensitizer photodegradation products. The aim of present study was to achieve release of model drug encapsulated in PC based liposomes upon photoactivation of KPF in bilayer by UV light exposure[6].

Materials and Methods

Materials

Soya phosphatidylcholine (SPC) and dialysis bags (cellulose tubing 25 m long, 10 mm inflated diameter) were purchased from HiMedia, India. CHOL was purchased from Sisco Research Laboratories Mumbai, India. KPF (RS)2-(3-benzoylphenyl)-propionic acid was purchased from Chemco, India. CAL (3,3´-bis[N,N-bis(carboxymethyl)aminomethyl]-fluorescein) was purchased from Central Drug House New Delhi, India. All other chemicals and solvents used were purchased from local suppliers and were of analytical grade unless mentioned.

Formulation of reverse phase evaporation vesicles (REVs)

Liposomes were prepared by REV method. Both conventional and photosensitive liposomes were prepared (PC:CHOL 5:1 to 5:5 molar ratio) for study. Ketoprofen (drug-to-lipid ratio 0.25 wt/wt) was incorporated to membrane by dissolving in a mixture of chloroform and methanol (2:1v/v). CAL (1 ml, 100 mM) was encapsulated in the liposomes. The shape and morphology of these liposomes were observed with the help of transmission electron microscope (Morgagni, 268, FEI, Electron microscope, Netherlands) after negative staining[7-10].

Determination of Fluorescent Marker (CAL) or fluorescence measurements

The amount of CAL was determined spectrofluorimetrically at an exitation wavelength (λex) of 494 nm and an emission wavelength (λem) of 517 nm with Jasco Spectrofluoremeter (Japan, Model FP-6500). A calibration curve of CAL (FI Vs Concentration) was also prepared.

Light treatment

Light-triggered release of entrapped contents from liposomes was evaluated after exposure to UV. Effect of exposure to UV was studied in a Kompakt UV cabinet, India fitted with a UV lamp bulb. Optimized formulations were characterized for *in vitro* CAL release study using dialysis tube method after suitable light treatments. Release from unexposed samples was also performed as a control. Morphology of liposomes was examined before and after exposure by a phase contrast microscope on 40 X magnification (Nikon Eclipsce E200, U.S.A.).

In situ CAL release

This liposome mixture (1 ml) was dialyzed in centrifuge tubes containing 50 ml phosphate-buffered saline (PBS; pH 7.4). The amount of CAL released from the liposomes was determined by measuring the FI of the release medium at predetermined time points. Release studies of all REV performed after 10 min exposures to UV light. Release from unexposed REV samples was used as control sample. The release studies were started immediately after the exposures. CAL release was expressed as % release and plotted as a function of time[10, 11].

Kinetic analysis of CAL release

Evaluation of release kinetics of drug from liposome was performed to find the effects of different factors on optimization factors and control of drug release. In this study, the *in vitro* drug release data were fitted to various kinetic models such as zero order, first order, Higuchi, Korsmeyer-peppas and Hixon-crowell. The data were analyzed using DD solver software and correlation coefficient values (R) were presented in table. Based on R values, best fit model was determined[12-15].

Results and Discussion

Formulation of photosensitive liposomes

Several formulations of liposomes were prepared to study the effect of lipid-cholesterol ratio. Five different batches of both conventional and photosensitive liposomes containing various drug/SPC molar ratios from 5:1 to 5:5 were prepared. Separation of liposomes was achieved by centrifugation at 16,500 rpm for 90 min at –5 °C. The liposomal concentrate was washed twice with PBS pH 7.4. TEM micrograph was taken and clearly showed the formation of liposomes (Figures 1). Most of the liposomes formed appeared spherical and symmetrical in shape and were mainly unilamellar in nature. Sizes of formulated liposomes were found between 200-400 nm.

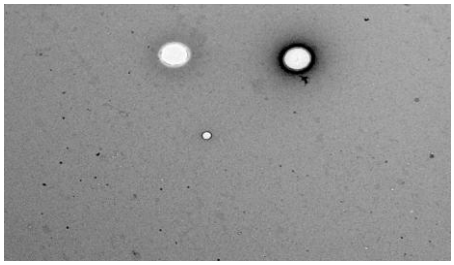

Fig.1 Transmission electron microscopic photograph of prepared liposomes

Determination (Of encapsulated CAL or) of CAL loading efficiency of Liposomes

Results revealed that at 5:1 PC/Cholestrol molar ratio showed maximum entrapment efficiency (33.47±2.51% & 29.66±3.05) for both conventional and photosensitive liposomes Fig.2 & 3. Optimized formulation was characterized for *in vitro* CAL release study using dialysis tube method after suitable treatments with using PBS (pH 7.4) as dialysis media. Results shown that photosensitive liposome released approximately 100 % of entrapped CAL in 10 hrs and only 67% in case of conventional liposome. Effect of UV radiation exposure duration on *in situ* CAL release from both the standard and the KPF containing liposomes were examined.

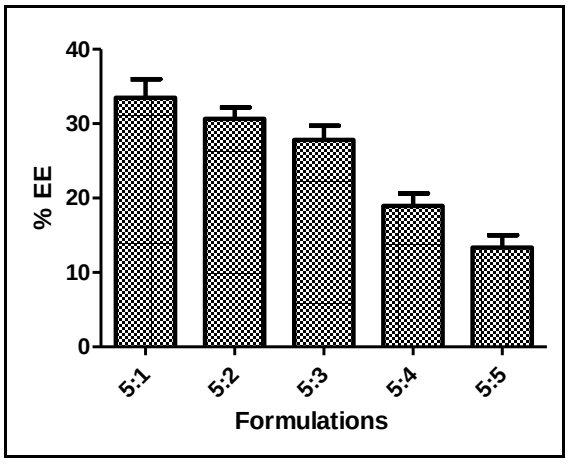

Fig. 2 % EE of formulations of photosensitive liposomes

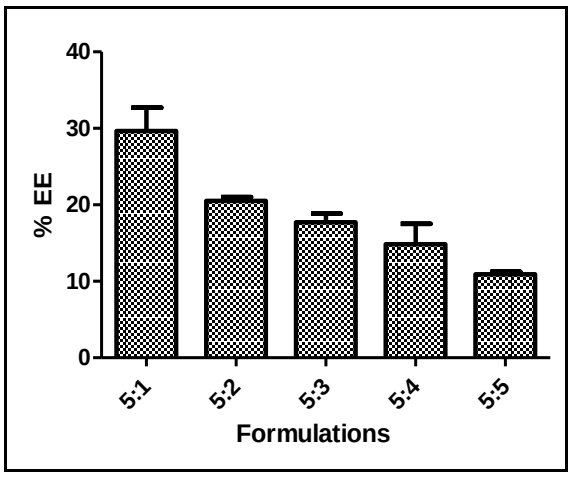

Fig. 3 % EE of formulations of conventional liposomes

Light treatment

It was observed that the CAL release from the REV increased significantly ($p < 0.05$) after UV light exposure. Shape was observed before and after exposure for photosentive liposomes using phase contrast microscope. It was observed visibly that size increased after exposure Fig.4.

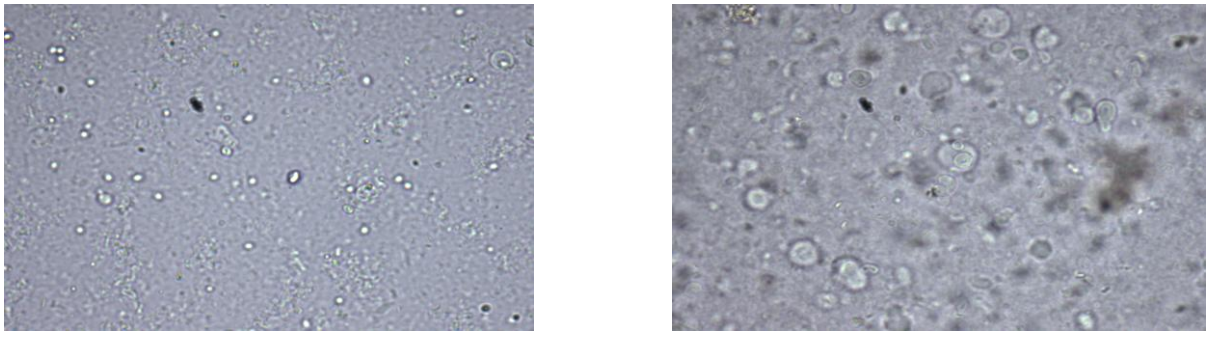

Fig. 4 Phase contrast microscopic photographs of liposome before and after light treatment

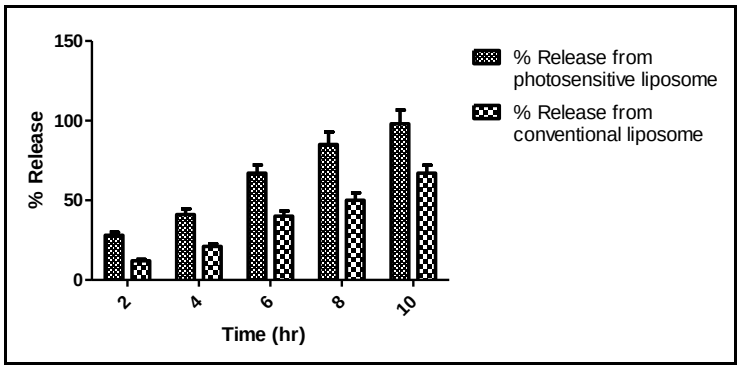

Fig. 5 (%) Release Vs time interval for conventional and photosensitive liposomes

Kinetic analysis of CAL release

Evaluation of release kinetics of drug from liposome was performed to find the effects of different factors on optimization factors and control of drug release. In this study, the *in vitro* drug release data from conventional liposomes were fitted to various kinetic models such as zero order (Fig. 6 A), first order (Fig. 6 B), Higuchi (Fig. 6 C), Korsmeyer-peppas (Fig 6 D) and Hixon-crowell (Fig 6 E) and from fig.7 A to 7 E for photosensitive liposomes. The data were analyzed using DD solver software and correlation coefficient values (R) were presented in the table. Based on R values, best fit model was determined Table.1. The release data were plotted according to different models, and these curves were used to draw some conclusions regarding the mode of drug release from the liposomes. The kinetic analysis proved that the zero order best fit the release data. This was confirmed by high values of regression coefficients obtained in all cases, which illustrates that the release from liposomal formulation has the most regular and showed the least interaction between the drug and lipids.

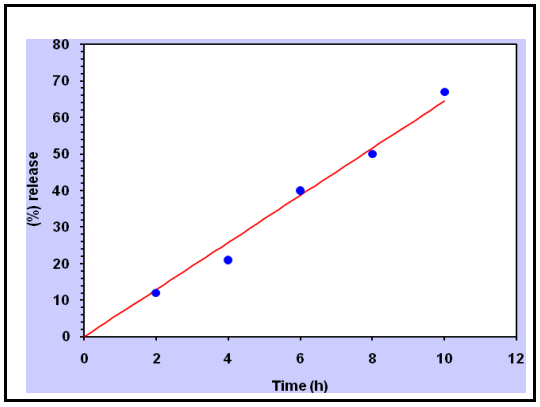

Fig. 6 A: Zero order plot of CAL release from conventional liposomes

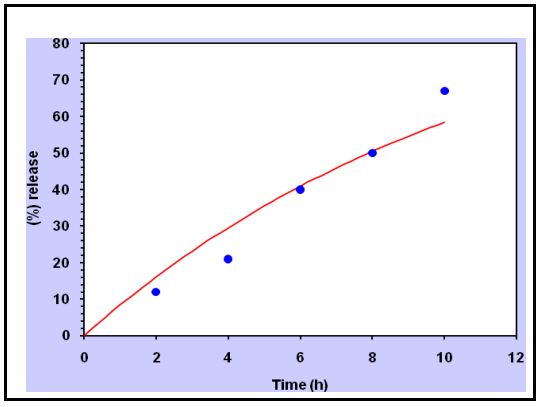

Fig. 6 B: First order plot of CAL release from conventional liposomes

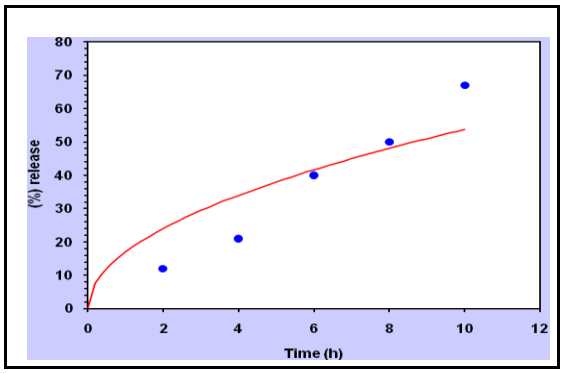

Fig. 6 C: Higuchi's plot of CAL release from conventional liposomes

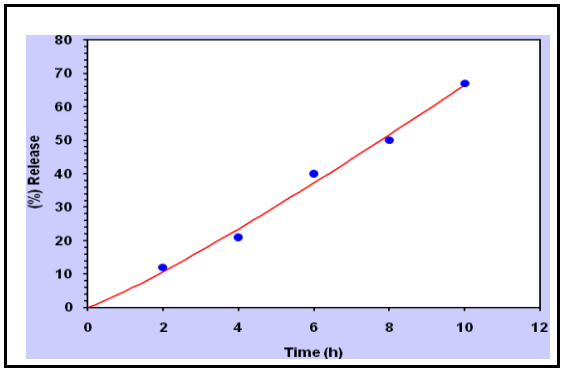

Fig. 6 D: Korsmeyer's plot of CAL release from conventional liposomes

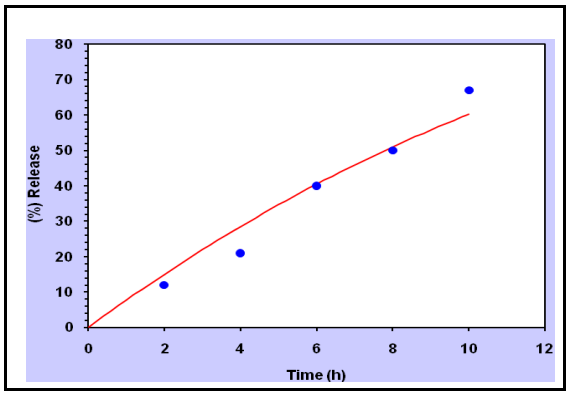

Fig. 6 E: Hixon Crowell plot of CAL release from conventional liposomes

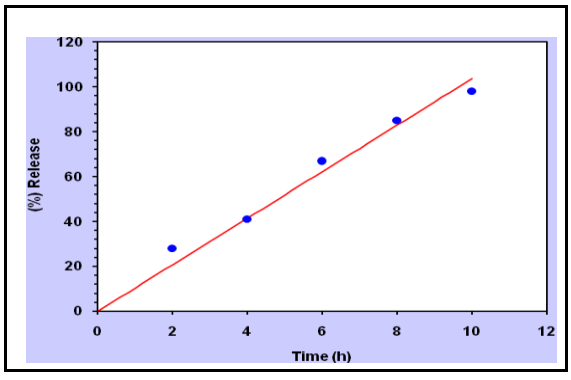

Fig. 7 A: Zero order plot of CAL release from photosensitive liposomes

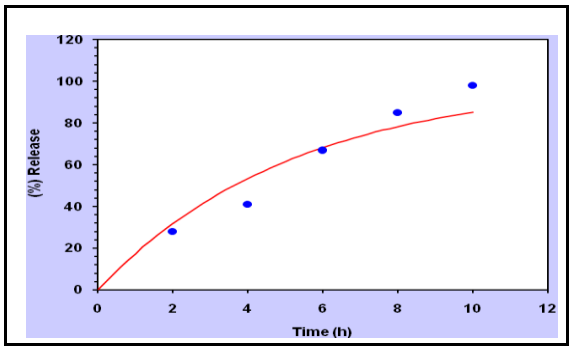

Fig. 7 B: First order plot of CAL release from photosensitive liposomes

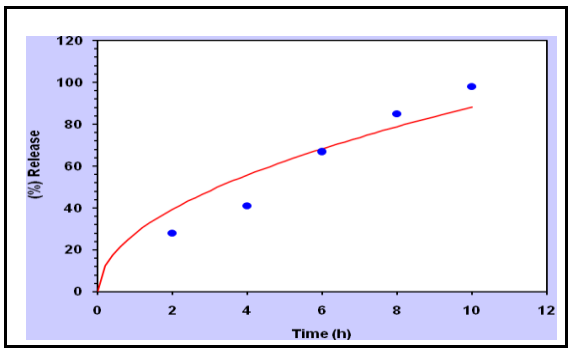

Fig. 7 C: Higuchi's plot of CAL release from photosensitive liposomes

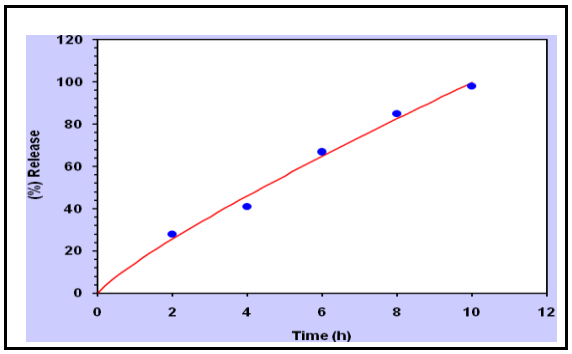

Fig. 7 D: Korsmeyer's plot of CAL release from photosensitive liposomes

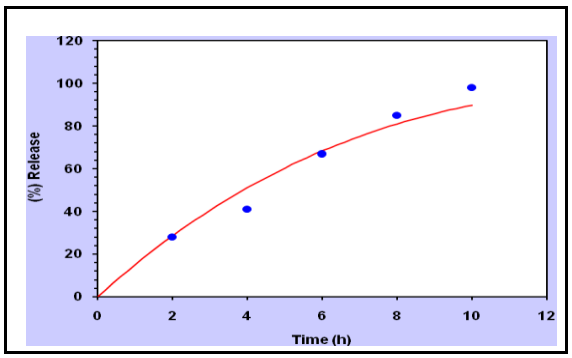

Fig. 7 E: Hixon Crowell plot of CAL release from photosensitive liposomes

Table.1: Drug release kinetic data of different lipoomal formulation derived from various mathematical models

Formulations	Corelation coefficients (R) values				
	Zero order	**First order**	**Higuchi**	**Korrsmeyer-peppas**	**Hixon-crowell**
Conventional liposomes (5:1 molar ratio)	0.9824	0.9153	0.7463	0.9874	0.9423
Photosensitive liposomes (5:1 molar ratio)	0.9672	0.8896	0.8587	0.9828	0.9445

Conclusion

According the results of the present study, incorporation of CAL in photosensitive liposome has many advantages over the conventional liposomes, including triggered release and delivery to the site of action. These liposomes can be used to get local drug effect at site of the action of the drug. This will minimize the side effects associated with drugs and enhancement of therapeutic efficiency.

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