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Formulation Characterization and Optimization of Photosensitive Liposomes for Targeted Drug Delivery Using UV Light Activation

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ABSTRACT

This study explores the formulation, characterization, and optimization of photosensitive liposomes for targeted drug delivery activated by UV light. Liposomes, self-forming spherical vesicles made of phospholipids, are effective carriers for drugs, offering the advantage of localized delivery and reduced systemic distribution. The research focuses on incorporating non-steroidal anti-inflammatory drugs (NSAIDs), specifically Ketoprofen (KP), into liposomes using the reverse phase evaporation (REV) method, achieving high encapsulation efficiency. Optimization studies revealed that increasing cholesterol content in the liposome formulation resulted in larger vesicle sizes but decreased drug entrapment efficiency. Morphological analysis confirmed the presence of both unilamellar and multilamellar vesicles. The physicochemical properties, including vesicle size and drug entrapment efficiency, were significantly influenced by the lipid-to-drug ratio and cholesterol content. A key aspect of this study was the use of UV light to trigger the release of encapsulated KP from the liposomes. UV-induced destabilization of the lipid bilayers facilitated controlled drug release, demonstrating the potential of this method for precise spatial and temporal delivery. The findings suggest that photosensitive liposomes, optimized for drug encapsulation and size, are promising vehicles for targeted drug delivery systems, offering controlled release upon UV light activation. This approach highlights the potential for enhanced therapeutic efficacy and reduced side effects, paving the way for future research and clinical applications.

Keywords: *NSAIDs, Reverse Phase Evaporation, Liposomes, UV light, Photosensitive liposomes*

1. INTRODUCTION

Bangham and colleagues at Cambridge University were the first to describe lipid-oriented vesicles, or liposomes, in 1965[1]. A liposome is a small, spherical, self-forming phospholipid vesicle. They have one or more phospholipid bilayers, with aqueous compartments occupying the space between them. Phospholipids serve as the structural unit representation of liposomes. Cholesterol is the second most significant ingredient and acts as a fluidity buffer. The placement of a medicine within liposomes is ultimately determined by the physicochemical characteristics and makeup of the phospholipids.

There are several ways to categorise liposomes, including the quantity of bilayers they include. Based on this, there are two types of liposomes: multilamellar vesicles and unilamellar vesicles. Large unilamellar vesicles (LUV) and small unilamellar vesicles (SUV) are the two distinct categories into which unilamellar vesicles can be further divided based on their size. A little amount of water volume is enclosed by a single phospholipid bilayer in unilamellar liposomes. Phospholipid bilayers are found in several layers within multilamellar liposomes. These lipid bilayers create phospholipid layers that are concentric and divided by aqueous spaces. All common liposomal formulation techniques involve four distinct steps: first, the removal of organic solvents to create a very thin lipid layer; next, this lipid layer is dispersed into appropriate aqueous media; and finally, the final product is purified and examined for various properties.

Local medication administration offers an appealing substitute for the drug's systemic dispersion. There won't be a need to administer excessively high doses in order to achieve therapeutic concentrations at desired places because of local administration. For many years, many nanocarriers have been created with the purpose of releasing a medicinal compound at the intended location. Of them, liposomes are a good delivery system for more in-depth study to obtain medication release that is triggered. The most difficult part of using liposomes in this case would be releasing the substance that was confined at the site of action. Passive medication release from currently used formulations may result in inadequate and partial drug cargo release. Thus, a number of active release mechanisms have been investigated to cause the release of liposomes, such as heat and pH, metal ion and radiation[2-4].

Light is a safe and efficient way to help release pharmaceuticals encapsulated in liposomes since it can control the radiation's distribution both spatially and temporally. The process by which light-induced isomerisation, cleavage, or polymerisation destabilises the lipid membrane components is what triggers the photochemical activation of liposomal content release. A liposomal bilayer can be modified to contain certain non-stereoidal anti-inflammatory medicines (NSAIDs), such as suprofen, carprofen, diflunisal, KP, and naproxen, in order to create photosensitive liposomes5. Red blood cells were used to test the ability of KP, one NSAID, to lyse cells. In both aerobic and anaerobic settings, this lysis was seen. For this procedure, an aqueous buffer solution with a pH of 6.4 was utilised[5]. KP demonstrated a decarboxylation reaction through the use of intermediate radicals, which under aerobic conditions converted to the compounds 3-benzoylphenyl)ethane, 3-benzoylphenyl)ethyl hydroperoxide, 3-benzoylphenyl)ethanol, and 3-benzoylphenyl)ethanone, and under anaerobic conditions only to the compound 3-benzoylphenyl)ethane. Every photoproduct that was produced showed signs of lytic activity. The findings revealed that the haemolysis caused by KP was caused by a molecular process involving free radicals, superoxide anion, and products of sensitiser photodegradation [6].

2. MATERIALS AND METHODS

Materials

We received Ketoprofen from Anant Pharmaceuticals, located in Maharashtra, India. The suppliers of cholesterol and soybean phosphatidylcholine (SPC) were Fine Chemicals, located in New Delhi, India. The chemicals used, including diethyl ether, methanol, and chloroform, are analytical grade and were acquired from Sigma Aldrich.

Methods

Preparation of blank photosensitive liposomes[7]

In a 50 ml round-bottom flask, the lipid components (cholesterol and SPC) in the molar ratio range of 5:1 to 5:5 were dissolved in the minimal volume of a mixture of methanol and chloroform (9:1 v/v) by gentle spinning. During preparation, KP was combined with the lipid mixture. Nitrogen gas was employed to provide an inert environment while the flask was attached to a rotating evaporator (Nutronics, India). The organic solvent was evaporated using a vacuum of roughly 700 mm Hg until a thin layer of a lipid/cholesterol combination developed on the flask walls.

The obtained thin film of PC and cholesterol was dissolved by vortexing the flask with 3 ml of diethyl ether per 66 µ mol lipid. The aqueous phase was then supplemented with PBS (pH 6.4), vortexed, and bath sonicated at 4ºC (EIE Instruments Pvt. Ltd. India). Sonication was maintained for two to three minutes, or until a stable w/o emulsion formed. The rotary evaporator was connected to the flask containing the two-phase emulsion, and stirring was done while maintaining the nitrogen purge through the flask. The ether was then gradually evaporated by subjecting the contents to a low vacuum (200 mm Hg). The procedure was carried out again until the flask contained a semisolid gel. To break the gel, the flask was taken out and its contents were agitated.

After a brief increase to roughly 300–350 mm Hg, the vacuum pressure was kept there for roughly 15 minutes. Upon eliminating the majority of the ether, the gel transformed into a silky mixture. After taking out the flask, the contents were once more combined. To evaporate the last of the ether, the vacuum was gradually raised to 700 mm Hg and held there for roughly 30 minutes. Following the liposomes' extrusion, the medication that was not entrapped was separated.

The nylon membrane filter with a 5 mm pore size was used to filter the liposomal suspension. A refrigerated centrifuge was utilised with filtrate for 90 minutes at 10,000 rpm and 4ºC. This procedure was used to distinguish between the free and entrapped drugs. After separating the supernatant, the pellet was once again dispersed in pH 6.4 PBS and kept in airtight glass containers at 4 °C until additional testing was conducted.

Physicochemical characterization of liposomal formulations

Morphology and lamellarity

Using images captured with an Olympus BX40 microscope, the morphology and lamellarity of the photosensitive liposomes containing KP that were created using different molar ratios of SPC and cholesterol were determined.

Mean particle size analysis

Using the Nanotrac Particle Size Analyser (Nanotrac NPA250, Microtrac Inc., York, PA, USA), dynamic light scattering based on laser diffraction was used to assess the size distribution profile of photosensitive liposomes. Three separate people carried out the experiment.

Determination of drug entrapment efficiency

Following the centrifugation procedure, the clear supernatant was carefully separated and, after the proper dilution with phosphate buffer saline (PBS) pH 6.4, examined by UV spectrophotometer at 260 nm for KP content. This gives the amount of medication that is not entrapped. After being re-dispersed in PBS pH 6.4, 2% Triton X-100 was added to lyse the liposomal pellet, and it was sonicated for ten minutes. With the use of a UV visible spectrophotometer, the concentration of KP was ascertained at 260 nm following the proper dilution in phosphate buffer pH 6.4. The following formula was used to determine the drug entrapment effectiveness of KP in each formulation:

% entrapment efficiency (% EE) = Q/Q0*100

Where, Q is the amount of KP measured in the liposomes, Q0 is the initial amount of KP added in liposomes.

Effect of UV light on liposomes

The impact of UV radiation was investigated in an Indian Kompakt UV cabinet equipped with a UV B light. A 10.0 cm exposure distance was maintained. In this experiment, both standard and KP-containing REVs were employed. A control sample that had not been exposed was used. For a minimum of five minutes, both conventional and KP-containing REVs were exposed. A phase contrast microscope was used to obtain pictures of the subject both before and after exposure. Figure 4 displayed the results[7, 8].

3. RESULT AND DISCUSSION

Preparation and evaluation of liposomal formulations for optimization

In the current investigation, KP was used to manufacture both conventional and photosensitive liposomal formulations using the REV procedure as a working method. The benefit of using this liposomal preparation technique is that it can achieve an encapsulation efficiency of up to 50%.[9].

Morphology and lamellarity

The biological features of liposomal formulations are attributed to many physico-chemical aspects of liposomes, including vesicle size, shape, stability, and the number of membrane bilayers (lamellae) per vesicle. The quantity of bilayers contained in the liposomes directly correlates with the size of the vesicles and the volume entrapped.[10]

Figure 1 displays the photomicrographs of the produced liposome (PL-1) utilising the REV method that were taken for this investigation. Upon closely examining the photomicrograph, it was observed that most of the liposomes that were generated were spherical and multi-lamellar, comprising several concentric phospholipid bilayers. Additionally, some unilamellar structure was seen, which may have resulted from the sonication technique used to prepare the liposomes.

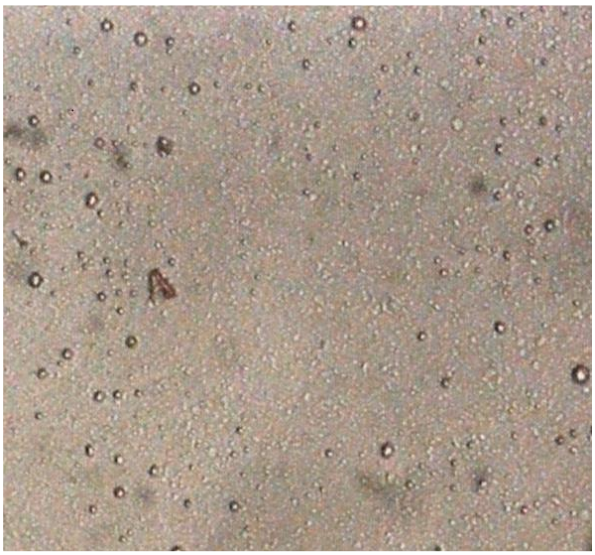

Figure 1: Representative photomicrographs of liposomes formulated by REV method

It is possible to formulate unilamellar, oligolamellar, and multilamellar vesicles, among other product kinds. They all have distinct qualities. While oligolamellar are challenging to create, unilamellar possesses unique surface properties. The controlled polymer-induced approach is one of the processes used to convert unilayer vesicles into bilayer vesicles.[11] Because multilamellar vesicles have greater permeation barriers, drugs are retained in them for a significantly longer period of time.

Effect of cholesterol content on the physicochemical properties of liposomes

Using the REV technique, many batches of liposomal formulations were created, and their physiochemical characteristics—such as particle size and percentage entrapment efficiency (% EE)—were subsequently optimised. We changed the amount of cholesterol and saw an impact on the properties indicated above. Tables 1 and 2 showed the results. It was observed that the amount of cholesterol utilised had an effect on the vesicle size of the generated liposomal formulations. In addition, when the cholesterol content of liposomal formulations increased, EE dropped. The SPC:CHOL ratio was adjusted from 5:1 to 5:5. Figures 2 and 3 provide an example of a prepared liposome's vesicle size analysis.

Table 1: Effect of cholesterol content on physicochemical properties of liposomal formulations

Formulation Code	PC	Chol	Ketoprofen (mg)	Mean Vesicle Size (nm)	% EE
PL-1	5	1	25	126.6 ± 4.2	25.8 ± 3.0
PL-2	5	2	25	147.0 ± 3.8	40.7 ± 2.1
PL-3	5	3	25	162.9 ± 3.5	54.6 ± 5.4
PL-4	5	4	25	186.4 ± 4.1	46.4 ± 5.9
PL-5	5	5	25	192.6± 3.2	29.8 ± 6.0

All data are expressed the means ± standard deviation (n = 3)

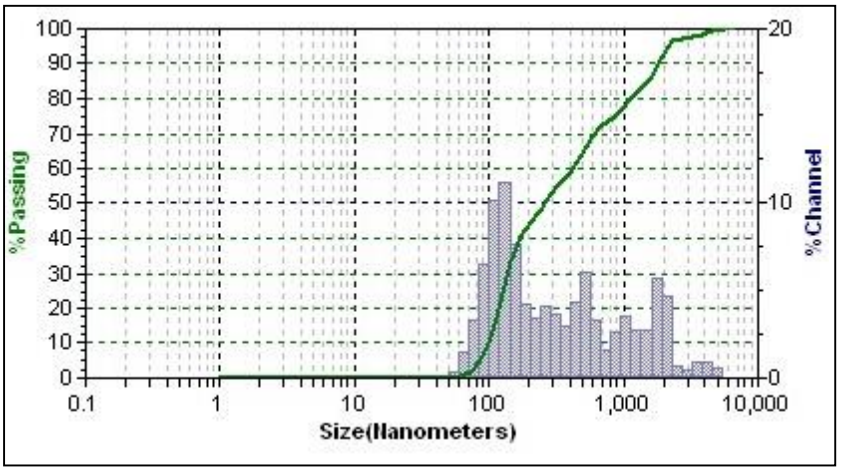

Figure 2: Representative vesicle size analysis of 5:1 liposomal formulation with 25 mg KP

Table 2: Effect of cholesterol content on physicochemical properties of liposomal formulations

Formulation Code	PC	Chol	Ketoprofen (mg)	Mean Vesicle Size (nm)	% EE
PL-1	5	1	50	523.00 ± 8.46	61.07 ± 3.44

PL-2	5	2	50	641.00 ± 10.99	55.11 ± 5.19
PL-3	5	3	50	679.00 ± 12.04	41.28 ± 3.07
PL-4	5	4	50	829.00 ± 8.38	33.51 ± 4.77
PL-5	5	5	50	870.00 ± 9.70	21.82 ± 5.91

All data are expressed the means ± standard deviation (*n = 3)*

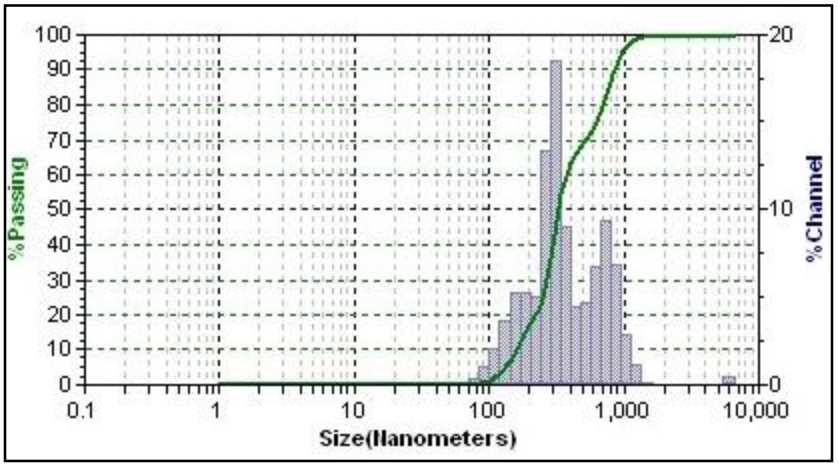

Figure 3: Representative vesicle size analysis of 5:1 liposomal formulation with 50 mg KP

The second most crucial component that must be adjusted and managed throughout preparation in order to achieve the intended drug release at the target site and a well-predicted and defined in vivo or ex vivo performance is vesicle size and dispersion.[12] To obtain a homogenous formulation with an optimised size range, the preparation process should be chosen in this way. In this regard, the REV approach was judged appropriate. In the current investigation, raising the molar ratio of cholesterol from 5:1 to 5:5 (SPC:CHOL) resulted in an increase in vesicle size. This result unequivocally demonstrated that when the cholesterol concentration increased, more stiff bilayer vesicles formed.[13–14]

% entrapment efficiency (% EE) reduced with increasing cholesterol concentration, from a molar ratio of 5:1 to 5:5 (SPC:CHOL).[15–16] The act of cholesterol building up in the phospholipid bilayer assembly's cavities and the resulting increase in packing density and stiffness are related to its function.[17] Because cholesterol fills up spaces, lipophilic medicines' bilayer loading may be reduced. Lipophilic drugs and cholesterol compete for the packing area in the phospholipid bilayer during the vesicle production process. Furthermore, bilayer drug loading is likewise diminished by reduced membrane fluidity.[18]

Effect of quantity of KP content on physicochemical properties of liposomal formulations

The percentage of weight medication content is another variable that can be optimised to determine its impact on various physical attributes. Optimising this factor can also lead to an improvement in particle size.

Following the formulation of the first batch and optimisation based on the percentage of EE and vesicle size, additional optimisation was carried out using an increasing quantity of KP. The physical characteristics of the batches were assessed in terms of vesicle size and % EE after they were prepared using the REV method.

It was evident that as the percentage w/w amount of KP added increased, the liposomal formulations' vesicle size increased (Table 4.2). The percentage of EE was found to increase proportionately in all liposomal formulations when the amount of KP was increased from 25 mg to 50 mg. The current investigation demonstrated a notable increase in particle size as KP concentration increased. Furthermore, the percentage EE of the liposomal formulations in the current investigation was discovered to be consistently reliant on the percentage of KP added to the liposomal composition. To a certain extent, the percentage EE of the liposomal formulations increased as the percentage KP content increased. The passive association of KP with the liposome membrane, which would increase with increasing accessible drug, may be the cause of the increase in the % EE with increase in KP concentration.[19–20] Drug-to-lipid ratios have been shown to have a comparable effect on the percentage of EE in liposome-encapsulated doxorubicin preparations, which have demonstrated efficacy against a variety of human neoplasms.[21] It was evident that the generated liposomal formulations' vesicle sizes grew as the percentage of weight-

weight KP added increased.[22] Nonetheless, there was a noticeable increase in size, with an average size of 500–800 nm being attained. For our investigation, this size is either useless or very useful. Therefore, this study's findings indicate that you should either utilise less KP or implement any appropriate method.

Effect of UV light exposure on drug release from Liposomes

The impact of UV exposure was investigated in an India-equipped Kompakt UV cabinet with a UV light. A 10-cm exposure distance was maintained. Five minutes was the exposure time. Qualitative changes in photosensitive liposome vesicle size were noted in response to UV light (Figure 4).

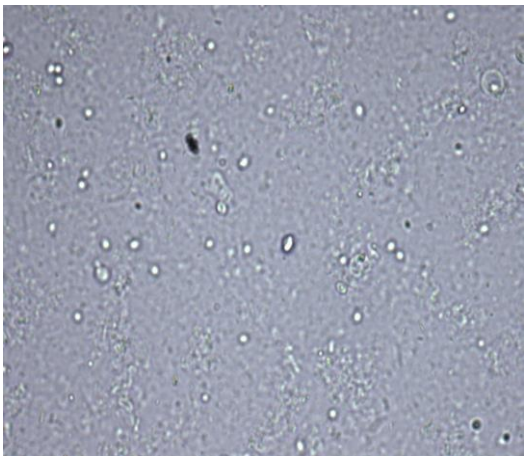

Figure 4. A Before

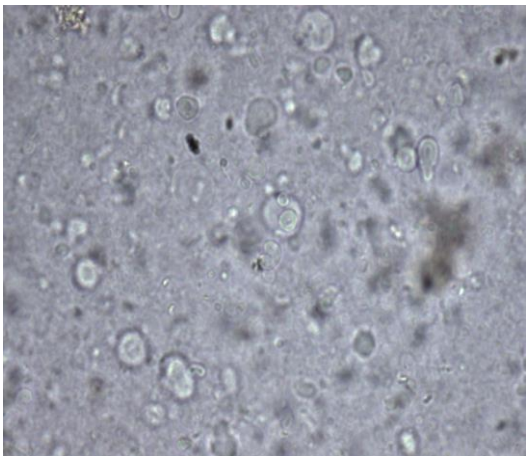

Figure 4. B After

Figure 4. Phase contrast microscopic photographs of liposomes before and after light treatment

4. CONCLUSION

A rational design of drug delivery systems that can satisfy various therapeutic needs by fitting the intended spatial and temporal drug release schedules requires an understanding of the various mechanisms underlying the complex drug release that occurs throughout the swellable system.

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