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Formulation and characterization of oxybenzone loaded liposomes

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Abstract

In the present study it was attempted to develop liposomal dosage form of oxybenzone and formulate it into cream for topical application with an aim to improvise the sunscreen efficacy of oxybenzone. Ethanol injection method was used formulating the liposomes with varying ratio of lecithin and cholesterol. Surface smoothness of the liposomes was enhanced by an increase in the lecithin concentration, as evident from the TEM photomicrographs. The particle size was found to be reduced with the increasing ratio of lecithin to cholesterol. The drug loading, encapsulation efficiency and drug release from the liposomes followed the similar trend and F1 was found to be having the smallest particle size, highest drug loading and encapsulation efficiency as well as the highest drug released over 8 h. The cream formulations made from the liposomes (F1) and pure drug was found to possess all the physicochemical evaluation parameters within acceptable limits. The sunscreen efficacy of the cream formulations was tested using sodium nitroprusside absorbance method at 395 nm, and it revealed that the liposome loaded formulations had better sunscreen efficiency as compared to the plain drug loaded formulations.

Keywords: Oxybenzone, liposome, cream, sunscreen, ethanol injection

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Introduction

Liposomes are colloidal particles in which phospholipid bilayers encapsulate a portion of the medium into their interior. Liposomal formulations are good for topical application because they can spread excellently to form depots of active ingredients in the horny layer of the skin, which allows for the transport of dermatological and cosmetic agents of different types. Liposomes constitute a dermal drug delivery system that is non-irritating, derma-cosmetically acceptable, and is relatively easy to prepare at a low cost (Pierre and Costa, 2011).

Sunscreen agents are cosmetic products intended to protect the skin from sunlight induced damage. (Nicholas and Pathak 1996; Gordan, 1993) Sunscreens are highly popular in the form of lotions, creams, gels, sprays, sticks and oils. (Mansur JS et al., 1986) Recently micro sponges, microsphere, dendrimer, liposome, nanoparticle incorporated photo-stable and effective sunscreens products have been made available in market (King and Young 1999).

Oxybenzone is a sunscreen agent widely used in the marketed preparations (drugbank.ca, 2021). It effectively absorbs the entire UV B range of light and is also able to absorb some UV A and UV C light. The molecule is known to cause irritation, sensitization, allergy, dermatitis, and systemic & local toxicity (Barry et al., 1995)

The stability of oxybenzone in oily lotions is known to be around 3 weeks, which makes it difficult to be used over longer period of times and the sunscreen efficacy of the molecule is decreased.

It a well known fact that liposomal formulations, polymeric microspheres, nanostructured carriers and microsponges have the capacity to improve the stability of incorporated drug molecules (Farhangi et al., 2017). Hence it was envisioned that formulating oxybenzone as liposomes and converting them into the sunscreen creams or lotions thereof would enable in improved stability and increased sunscreen efficiency and safety of the molecule.

Material and Methods

Lecithin and cholesterol were purchased from Himedia Laboratories, Mumbai; oxybenzone was obtained as gift sample from Yash Pharma Laboratories Pharmaceuticals Pvt Ltd, Maharashtra. Acetonitrile, acetic acid, glycerol, acetone, isopropyl alcohol, glutaraldehyde, toluene, methanol, trichloroacetic acid and sodium nitroprusside were purchased from Oxford Fine Chemicals, Mumbai and Qualigens Fine Chemicals, Mumbai. All the chemicals were of AR/LR grade and used as received.

Preformulation Studies

The procured sample of Oxybenzone was observed for its appearance, color, taste, solubility and melting point in order to characterize the physical parameters.

Calibration curve of Oxubenzone solution

Accurately weighed drug (10 mg) was transferred into clean and dried 100 mL volumetric flask and dissolved in minimum quantity of ethanol. The volume was made upto 100 mL with the solvent system composed of methanol and PBS (pH 7.4) at ratio 50:50. This resulted in 100 µg/mL stock solution. The aliquots were taken into a series of 10 ml volumetric flasks and volume was made up to 10 mL with the solvent system to obtain solutions of varying concentrations. The solutions were filtered through whatman filter paper and absorbance of the filtrate was measured at 295.6 nm using UV-visible spectrophotometer. A graph of concentration v/s absorbance was plotted.

Formulation of Liposomes of oxybenzone

Formulation design

Liposomes were prepared by a modified ethanol injection method. Liposomes of oxybenzone were prepared by dissolving varying amounts of lecithin: cholesterol and fixed amount of drug (table 1) in the defined volume of ethanol (Ambika et al., 2021). The resulting organic phase solution was injected by means of a syringe pump in a defined volume of distilled water under magnetic stirring. Spontaneous liposome formation occurred as soon as ethanolic solution was in contact with the aqueous phase. The liposome suspension was then kept under stirring for 15 minutes at room temperature. Finally, the ethanol and a part of water were removed by rotary evaporation under reduced pressure. Unloaded drug was removed by ultracentrifugation of liposome suspension at 40,000 rpm for 1 hour. The obtained pellets were dispersed in phosphate-buffered saline (PBS) and stored at 4°C.

Table 1 Formulation formula for oxybenzone liposomes

Formulation Code	Drug (mg/mL)	Lecithin (mg/mL)	Cholesterol (mg/mL)	Ethanol (mL)	Water (mL)
F1	2	7.5	2.5	25	50
F2	2	7	3	25	50
F3	2	6.5	3.5	25	50
F4	2	6	4	25	50
F5	2	5.5	4.5	25	50
F6	2	5	5	25	50

Evaluation of oxybenzone liposomes

Drug polymer interaction (FTIR) study

FTIR spectroscopy was performed on Fourier transform infrared spectrophotometer. The samples were placed in the sample cell of the instrument and scanned from 400-4000 cm^{-1}. FTIR study was carried out on oxybenzone, lecithin and physical mixture of oxybenzone, lecithin and cholesterol.

Surface morphology (TEM)

A drop of liposomal suspension was put on a wax bed and a drop of 2% ammonium molybdate solution was added to it. The preparation was allowed to take up the stain for 5 minutes. The stained preparation was mounted on the surface of a formvar grid. Excess liquid was removed by adsorption onto a filter paper and the grid was then

inserted into the assembly of the transmission electron microscope and photographs were obtained.

Particle size analysis and zeta potential

Particle size and zeta potential measurements of the liposomes were carried at 25 ºC by photon correlation spectroscopy on Malvern Zetasizer. All samples were kept in refrigerator at 4 °C prior to characterization.

Determination of Drug Loading

A suspension of an accurately weighed amount of oxybenzone liposomes in methanol was sonicated at for 2 min. The suspension was then centrifuged at 3000 rev/min for 5 min. The supernatant was analyzed using UV visible spectrophtometer at 295.6 nm.

Entrapment efficiency

The liposomal formulation was centrifuged at 4000 rpm for 15 min at 4°C temperature using cooling centrifuge to separate the free drug. The supernatant was again centrifuged at 12000 rpm for 30 min at 4°C. As a result, a transparent solution of supernatant and liposome pellet was attained. The pellet consisting of liposomes was redispersed in distilled water prior to other studies.

A weighed quantity of the pellet was mixed with 10 ml of mixture of methanol: water ratio (7:3 v/v) followed by 5 min of sonication. The liposomes were disrupted to discharge the drug which was determined for the drug entrapment using UV spectrophtometry.

In Vitro Drug Release Study

To study the rate and extent of drug release from the liposomes, dissolution of oxybenzone loaded liposomes was studied. An accurately weighed sample of 10 mL of liposomes was placed in 900 mL of methanolic phosphate buffer pH 7.4 [PBS 7.4/methanol (3:2)] and was subjected to dissolution with a paddle speed of 150 rpm at 37 ± 0.5°C. Aliquots (5 mL) were withdrawn at at hourly intervals for up to 8 hours and were assayed spectrophotometrically at 295.6 nm by appropriate dilution. The percentage of drug released at various time intervals was calculated and plotted against time. (Shaikh KS et al., 2010; Rekha and Manjula, 2011)

Sunscreen Formulations

Different formulations containing varying amounts of free oxybenzone and other additive are shown in Table 2. Free oxybenzone, and liposomes bearing oxybenzone were incorporated in cream base.

An oil in water type of cream formulated. Firstly the desired concentration of oil phase i.e., stearic acid and lanolin were taken and heated in mineral oil at temperature not exceeding 70°C. The prescribed ratio of oxybenzone was dissolved in the oil phase. Separately, the water and triethanolamine were mixed together to prepare the aqueous phase. Both the phases were mixed together while triturating to obtain a consistent cream.

Table 2 Sunscreen cream of oxybenzone and oxybenzone liposomes

S.No	Ingredients	Quantity			
		LC1	**LC2**	**LC3**	**LC4**
1	Oxybenzone	5 %w/w	5 %w/w	-	-
2	Oxybenzone liposomes	-	-	5 %w/w	5 %w/w
3	Mineral Oil	10 g	10 g	10 g	10 g
4	Stearic acid	15 g	10 g	15 g	10 g
5	Lanolin	5 g	7 g	5 g	7 g
6	Triethanolamine	2 mL	2 mL	2 mL	2 mL
7	Water	48 mL	48 mL	48 mL	48 mL

Evaluation of sunscreen cream (Purushottamrao et al., 2010)

The prepared cream formulations were evaluated for official and non official specifications.

pH of the formulation

Accurately weighed quantity of 5 g of each cream formulation was mixed separately with 45 mL of distilled water and the pH of the solution was determined with the help of digital pH meter.

Viscosity measurement

The viscosity of each formulation was measured at 10 rpm by using Brookfield DV-1 viscometer employing a S94 spindle.

Spreadability

Spreadability of the formulations was determined using indigenously developed apparatus. The apparatus consisted of a wooden block provided with a pulley at a one end. A rectangular ground glass was fixed on the block. An excess of cream (3-5 g) was placed on this plate sandwiched using another glass plate having the dimensions as that of fixed ground plate. A 1 kg weight was placed on the top of the plates for 5 minutes to expel air and to provide a uniform film of the cream between the plates. Excess of the ointment was scrapped off from the edges. Weight of 80 g was hung on the hook of the top plate with the help of string attached to the hook and the time (in seconds) required by top plate to cover a distance of 10 cm was noted. Spreadability of the formulation was determined by the following formula:

$$S = M * L/T$$

where,

S – spreadability

L – distance travelled by the glass slide

T – time in seconds

M - weight in the pan

Tube extrudability

The formulations were filled in clean, lacquered aluminum collapsible tubes with nozzle of 5mm opening and pressure was applied on the tubes with the help of finger. Tube extrudability was determined by measuring amount of cream that extruded through the tip when the pressure was applied on tube.

Sunscreen Efficacy Testing (Kaidbey and Klingman, 1978)

A solution 0.05% w/w of sodium nitroprusside was prepared in distilled water and 40 ml of this solution was placed in the petriplates. These petriplates were

covered with a cellophane membrane. One petriplate containing sodium nitroprusside solution was left uncovered to expose it directly to sunlight. Then 2 g of the preparation were spread uniformly over the membrane as a layer. The petriplates were exposed to sunlight for 2 h during mid-day. After exposure to sunlight, the samples were analyzed using UV method for absorbance of sodium nitroprusside at 395 nm.

Results and Discussion

Preformulation Studies

The observed physical characteristics and the determined melting point of oxybenzone are detailed in the Table 3.

Table 3 Physical characteristics and melting point of oxybenzone

Property	Observation
Color	Light Yellow
State	Crystalline powder
Odor	Odorless
Melting Point	62–65˚C

The oxybenzone powder was found to be soluble in methanol, ethanol, chloroform, acetone; slightly soluble in phosphate buffer and insoluble water.

The calibration curve of oxybenzone was obtained by measuring the absorbance of appropriately diluted stock solution at 295.6 nm in the solvent system (methanol:PBS pH7.4; 1:1) and plotting the graph of absorbance v/s concentration (figure 1).

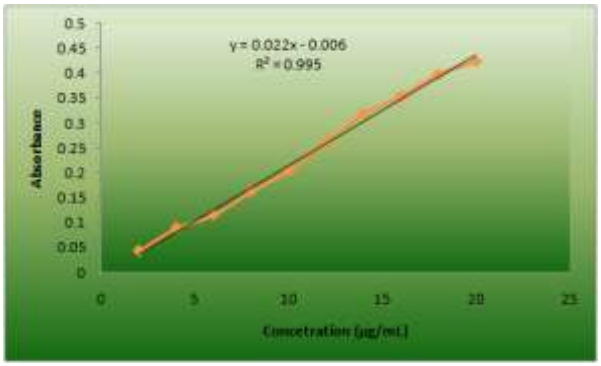

Figure 1 Calibration curve of oxybenzone

Drug Polymer Interaction study (FTIR)

The FTIR spectra of the physical mixture of oxybenzone, lecithin and cholesterol displayed all the characteristic peaks of oxybenzone in the combination spectra, thus indicating that no incompatibility was present between the drug and the polymer.

Surface Morphology (TEM)

The surface morphology of the oxybenzone liposomes was studied by TEM. The TEM photographs of the formulations are shown in Figure 5.5.

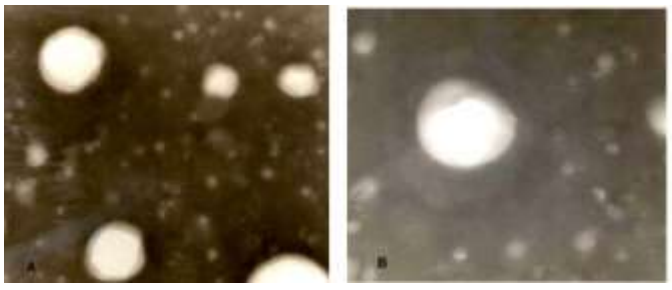

Figure 2 Transmission electron photomicrograph of liposomes at 500x (A), at 1500x (B)

The surface of the liposomes was smooth and the liposomes formed were spherical in shape as shown in the photomicrographs.

Particle size and zeta potential

The particle size of all liposomal formulations represented in table 4. The particle size was found to be reduced with the increasing ratio of lecithin to cholesterol. Cholesterol is one of the common additives included in the liposome formulation in order to improve their bilayer characteristics (i.e., the fluidity of the bilayer membrane or its stability). The increased concentration of cholesterol directly impacted the size of the particles by decreasing the lecithin concentration during the

Table 4 Particle size, zeta potential, drug loading and entrapment efficiency of the liposomes

Formulation Code	Average particle size (nm)	Drug Loading (%)	Entrapment efficiency (%)	Zeta Potential (mV)
F1	242	46.1	87.6	-20.8
F2	297	41.3	78.4	-21.7
F3	348	33.8	64.2	-22.7
F4	385	29.4	55.8	-23.9
F5	463	25.2	47.8	-20.4
F6	784	20.8	39.5	-25.4

The zeta potential of the liposomes was negatively due to cholesterol and sufficiently high to impart electrostatic repulsion against neighboring particles, preventing them from aggregating in the solution and thereby imparting stability to the particles.

Drug loading and entrapment efficiency

Drug loading and drug encapsulation in the liposomes was also found to be the highest in F1 indicating that the hydrophobic drug was uniformly distributed in the matrix of the phospholipid and precipitated as globules during the diffusion from organic to aqueous phase (Table 4).

In vitro release of oxybenzone from liposomes

The *in vitro* release study of oxybenzone from the liposomes exhibited prolonged and controlled release of oxybenzone. The results of the *in vitro* release studies of the formulations F1 to F6 are represented in Figure 3. A maximum of 80.03% oxybenzone release was observed in the formulation F1 at the end of 8 h while the lowest was observed for formulation F6 (65.2%).

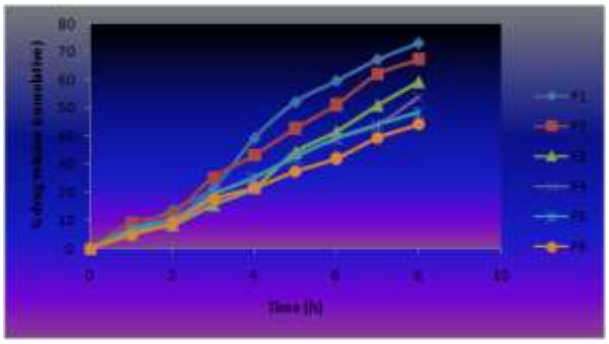

Figure 3 Percent cumulative release of oxybenzone from liposomes

The liposomal formulation F1 was selected as the best formulation as it exhibited the lowest particle size, highest drug loading and encapsulation

efficiency as well as the maximum drug release over the period of 8 h. Formulation F1 was hence used for formulation of the sunscreen cream and comparing the results with plain oxybenzone.

Sunscreen Formulations

The data of the evaluated formulations LC1-LC4 are represented in Table 5; all the parameters were found to be in acceptable limits.

Table 5 Physicochemical data of sunscreen formulations

Formulation	pH	Viscosity	Spreadability (g.cm/sec)	Extrudability (%)
LC1	6.4	4975	17.46	93
LC2	6.6	5720	13.17	81
LC3	6.4	5100	17.14	90
LC4	6.3	5690	41.22	82

The consistency of the formulations was found to be proper in all the batches and the viscosity increased while the spreadability and tube extrudability decreased in formulations with higher amount of Lanolin.

Sunscreen efficacy

The sunscreen efficacy of the cream formulations were evaluated using sodium nitroprusside method and the results is reported in Table 6.

Sun exposure method using sodium nitroprusside solution was used for testing the *in vitro* sunscreen efficacy of the formulations.

Table 6 Sunscreen efficacy measured as absorbance of sodium nitroprusside solution at 395 nm.

Formulation	Absorbance of sodium nitroprusside solution
LC1	1.56
LC2	1.41
LC3	1.22
LC4	0.98
Covered with cellophane membrane only	1.77

Sodium nitroprusside is known to be photosensitive in aqueous solution and on exposure to direct sunlight it degrades to yield prussian blue and nitric oxide (NO). The spectrophotometric measurement has been employed to determine the stability of sodium nitroprusside; with most emphasis on increase in the absorbance at 390–395 nm with degradation. It was observed that formulation LC4 had the best sunscreen efficacy, which may be imparted to the oxybenzone-loaded liposomes.

Conclusion

The present work was able to demonstrate that liposomes loaded with the lipophilic sunscreen drug oxybenzone could be effectively prepared. It was concluded from the results that the encapsulation of

oxybenzone in liposomes was able to increase the efficiency of the sunscreen agent.

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