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Formulation and Characterization of Oxybenzone loaded liposomes

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Abstract

Oxybenzone is a widely used sunscreen molecule incorporated in various sunscreen lotions and creams. The objective of the present investigation was to formulate oxybenzone as liposomes and convert them into the sunscreen creams or lotions to improve stability and enhance sunscreen efficiency and safety of the molecule. Liposomes of oxybenzone were prepared by dissolving varying amounts of lecithin and drug (2: 0.1, 3.5: 0.1 and 6: 0.1). The liposomes were evaluated for particle size, shape, zeta potential, entrapment efficiency and drug release. The liposomes were formulated as sunscreen cream and evaluated for various parameters and in vitro sunscreen efficacy. A maximum of 80.03% oxybenzone release was observed in the formulation OL3 at the end of 12th h while the lowest was observed for formulation OL5 (65.2%)

Keywords: Oxybenzone, sunscreen, liposomes, nitroprusside, cream, release

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Introduction

The intensity of UV-rays reaching the earth has increased due to pollution and heat. On chronic exposure even these beneficial rays exhibit harmful effects like sunburn, erythema, wrinkles, pigmentation, etc. Sunscreen agents are cosmetic products intended to protect the skin from sunlight induced damage.[1,2] Sunscreens are highly popular in the form of lotions, creams, gels, sprays, sticks and oils.[3,4] It is established that sunscreen protection is easy, simple and aesthetic to consumers.[5] Oxybenzone is a widely used sunscreen molecule incorporated in various sunscreen lotions and creams; but due to the toxicity effects associated with molecule and also the low stability of the oily lotions containing oxybenzone, the shelf life of the formulation decreases. This leads to decreased sunscreen efficacy of the molecule. It a well known fact that liposomal formulations, polymeric microspheres, nanostructurd carriers and microsponges have the capacity to improve the stability of incorporated drug molecules.[6] 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 was purchased from Himedia Laboratories, Mumbai; oxybenzone was obtained as gift sample from Gary Pharmaceuticals Pvt. Ltd., Ludhiana. Acetonitrile, acetic acid, glycerol, acetone, isopropyl alcohol, glutaraldehyde, toluene, methanol, trichloroacetic acid and sodium nitroprusside were purchased from Loba Chemie, Mumbai and Qualigens Fine Chemicals, Mumbai. All the chemicals were of AR/LR grade and used as received.

Formulation of Liposomes

Liposomes of oxybenzone were prepared by dissolving varying amounts of lecithin and drug (2: 0.1, 3.5: 0.1 and 6: 0.1) in 50 ml of chloroform using method reported by Shivani et al., 2018. The solution was then transferred to a 500 ml round bottom flask and the chloroform was evaporated under vacuum using rotary evaporator at 63°C to form a thin film. Evaporation was continued for approximately 15 min until dry thin film was formed inside the surface of the flask. To ensure complete evaporation of the organic solvent, the films were vacuum dried for overnight. The film was then hydrated with different amount (90 mL, 120 mL) of phosphate buffer (pH 7.4) solution containing 1g of mannitol and rotated for different hydration time 30 min at 45 ºC. The liposomal suspension was kept overnight to get complete lipid hydration at 4 ºC. The liposomal suspension was sonicated for 10 min using a probe sonicator at 40% of the total power, at room temperature.

Table 1 Formulation formula for oxybenzone liposomes

S.No	Formulation Code	Drug : Lecithin Ratio	Hydration volume (mL)
1	OL	0.1:2	90
2	OL2	0.1:2	120
3	OL3	0.1:3.5	90
4	OL4	0.1:3.5	120
5	OL5	0.1:6	90
6	OL6	0.1:6	120

Evaluation of oxybenzone liposomes

Drug polymer interaction (FTIR) study

FTIR spectroscopy was performed on Fourier transform infrared spectrophotometer. The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wave number range of 4000- 600 cm^{-1}. FTIR study was carried out on oxybenzone, lecithin and physical mixture of oxybenzone and lecithin.

Surface morphology (SEM)

Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture, and to examine the morphology of fractured or sectioned surface. It is probably the most commonly used method for characterizing drug delivery systems, owing in large to simplicity of sample preparation and ease of operation. SEM studies were carried out by using JEOL JSM T-330A scanning microscope (Japan). Dry oxybenzone liposomes were placed on an electron microscope brass stub and coated with in an ion sputter. Picture of oxybenzone liposomes were taken by random scanning of the stub.

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 125 W for 2 min. The suspension was then centrifuged at 3000 rev/min for 2 min. The supernatant was analyzed using UV visible spectrophtometer at 288 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 temperature. 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. As a result of sonication, the liposomes were disrupted to discharge the drug. The discharged drug was determined for the drug entrapment.

In Vitro Drug Release Study

To study the rate and extent of drug release from the liposomes, dissolution of oxybenzone loaded liposomes was studied using USP dissolution test apparatus (USP XXIII). An accurately weighed sample of 10 mL of liposomes was placed in 900 mL of phosphate buffer pH 7.4 and was subjected to dissolution with a paddle speed of 150 rpm at 37 ± 0.5°C. Aliquots (5 mL) were withdrawn at 5 min initially and then at hourly intervals for up to 8 hours and were assayed spectrophotometrically at 288 nm. The percentage of drug released at various time intervals was calculated and plotted against time.[7,8]

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 preparation containing oxybenzone and oxybenzone liposomes

S.No	Ingredients	Quantity of additives in various formulations			
		C1	C2	C3	C4
1	Oxybenzone	3 %w/w	3 %w/w	-	-
2	Oxybenzone liposomes	-	-	3 %w/w	3 %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[9,10]

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[11]

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

Drug Polymer Interaction study (FTIR)

The FTIR spectra of the physical mixture of oxybenzone and lecithin displayed all the characteristic peaks of oxybenzone in the combination spectra, thus indicating that no incompatibility was present between the drug and the polymer (Figure 1).

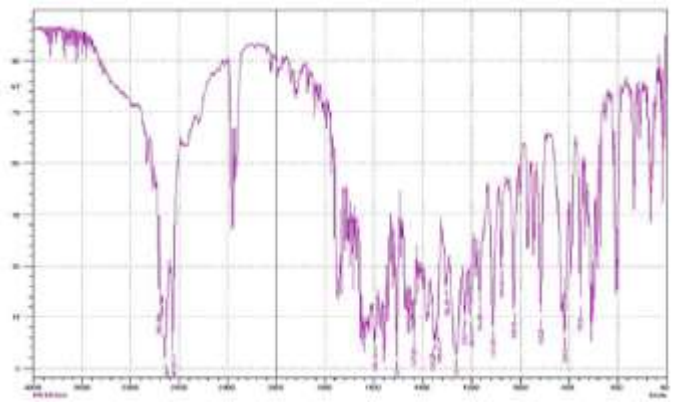

Figure 1 FTIR spectrum of physical mixture of Lecithin and oxybenzone

Surface Morphology (SEM)

The surface morphology of the oxybenzone liposomes was studied by SEM. The SEM photographs of the formulations are shown in Figure 2.

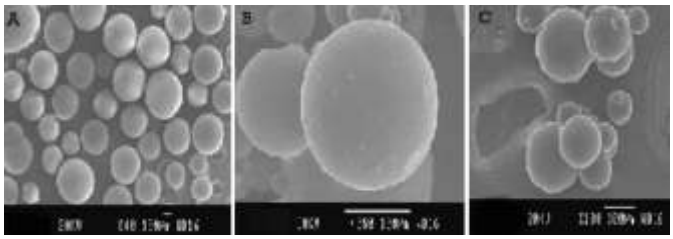

Figure 2 Scanning electron photomicrograph of liposomes at 500x (A), at 1500x (B), and 1000x(C)

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 3. The particle size reduced with increasing the lecithin concentration and decreasing the hydration volume

Table 3 Particle size and zeta potential of the liposomes

S. No	Formulation Code	Average particle size (nm)	PDI	Zeta Potential (MeV)
1	OL1	322	0.675	-22.6
2	OL2	284	0.502	-21.7
3	OL3	228	0.219	-20.8
4	OG4	391	0.501	-23.9
5	OL5	348	0.399	-23.6
6	OL6	297	0.191	-22.5

Drug loading and entrapment efficiency

The percent drug entrapment in various formulations was determined after extracting the drug with trichloroacetic acid and estimating the content of oxybenzone at 288 nm using UV visible spectrophotometry. The result of percent entrapment efficiency and yield are shown in Table 4.

Table 4 Drug entrapment efficiency of liposomes

S.No.	Formulation code	Drug Loading (%)	Entrapment efficiency (%)
1	OL1	75.96	63.17
2	OL2	75.74	57.18
3	OL3	88.46	56.20
4	OL4	93.2	49.47
5	OL5	72.62	49.40
6	OL6	91.80	72.56

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 OL1 to OL6 are represented in Table 5 and Figure 3. A maximum of 80.03% oxybenzone release was observed in the formulation OL3 at the end of 12th h while the lowest was observed for formulation OL5 (65.2%)

Table 5 *In vitro* release data of oxybenzone from liposomes

Time (h)	% cumulative drug release					
	OL1 ± SD	OL2 ± SD	OL3 ± SD	OL4 ± SD	OL5 ± SD	OL6 ± SD
0	0	0	0	0	0	0
1	9.32 ± 0.33	7.61 ± 0.20	9.32 ± 0.33	6.81 ± 0.40	5.7 ± 0.57	5.7 ± 0.57
2	12.23 ± 0.34	11.23 ± 0.30	12.23 ± 0.34	10.4 ± 0.30	9.46 ± 0.62	9.46 ± 0.62
3	22.27 ± 0.33	23.14 ± 0.82	25.27 ± 0.33	19.14 ± 0.63	25.41 ± 0.52	25.41 ± 0.52
4	31.24 ± 0.75	30.68 ± 0.41	33.24 ± 0.75	25.08 ± 0.40	29.67 ± 0.61	29.67 ± 0.61
5	41.71 ± 0.55	36.41 ± 0.32	42.71 ± 0.55	32.50 ± 0.66	34.67 ± 0.83	34.67 ± 0.83
6	47.23 ± 0.63	43.44 ± 0.61	51.23 ± 0.63	39.07 ± 0.45	38.27 ± 0.76	38.27 ± 0.76
7	52.05 ± 0.64	49.63 ± 0.61	62.05 ± 0.64	43.87 ± 0.54	41.67 ± 0.69	41.67 ± 0.69
8	55.34 ± 0.60	54.47 ± 0.63	67.34 ± 0.60	48.32 ± 0.43	45.19 ±0.70	45.19 ±0.70
9	58.03 ± 0.70	59.36 ± 0.40	70.03 ± 0.70	52.03 ± 0.69	51.48 ± 0.55	51.48 ± 0.55
10	61.23 ± 0.94	64.44 ± 0.85	74.23 ± 0.94	57.48 ± 0.49	59.08 ± 0.59	59.08 ± 0.59
11	62.47 ± 0.69	70.81 ± 0.76	77.47 ± 0.69	61.12 ± 0.54	64.53 ± 0.64	64.53 ± 0.64
12	64.12 ± 0.79	75.36 ± 0.89	80.03 ± 0.79	67.37 ± 0.72	65.20 ± 0.74	71.49 ± 0.74

SD-standard deviation (n=3)

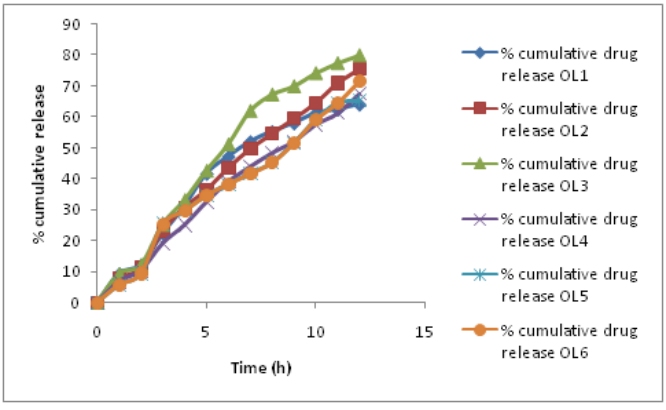

Figure 3 % cumulative release of oxybenzone from liposomes

Sunscreen Formulations

The data of the evaluated formulations C1-C4 are represented in Table 6; all the parameters were found to be in acceptable limits.

Table 6 Physicochemical data of sunscreen formulations

Formulation	pH	Viscosity	Spreadability (g.cm/sec)	Extrudability (%)
C1	6.5	6000	18.12	95
C2	6.3	6800	14.08	84
C3	6.2	6200	17.56	92
C4	6	7100	14.6	87

The consistency of the formulations was found to be proper in all the batches and the viscosity increased 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 7.

Table 7 Sunscreen efficacy measured as absorbance of sodium nitroprusside solution at 395 nm.

Formulation	Absorbance of sodium nitroprusside solution
C1	2.684
C2	2.026
C3	1.662

C4	1.002
Covered with cellophane membrane only	2.85

Sun exposure method using sodium nitroprusside solution was used for testing the *in vitro* sunscreen efficacy of the formulations. 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 C4 had the best sunscreen efficacy, which may be imparted to the oxybenzone-loaded liposomes.

Conclusion

The present study was aimed to improvise the sunscreen efficacy of oxybenzone and also to improve its safety profile. It was attempted to develop liposomal dosage form of oxybenzone and formulate it into cream for topical application. 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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