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Development of ciprofloxacin loaded niosomal gel for the treatment of acne

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ABSTRACT

In the present study niosomes of ciprofloxacin were developed by thin film hydration technique and incorporated into gel formulation suitable for topical application. The niosomes prepared using varying ratio of surfactant (span 20 and tween 20) and cholesterol were evaluated for entrapment efficiency and *in vitro* release. From the results of the release studies it was found that the maximum amount of drug was released from formulations F2 (79.74 %) and F5 (71.68 %) over a period of 12 h at an almost steady rate. The higher amount of drug release along with the higher entrapment efficiency make the formulations F2 and F5 containing surfactant to cholesterol ratio of 1:2 the most promising formulations. The gel loaded with niosomal formulations F2 and F5 were prepared using Carbopol 934 as the gelling agent and propylene glycol as the plasticizer. The drug content in all the formulations ranged from 96.4 to 99.1 % confirming the incorporation of the niosomes into the gel base. The *in-vitro* drug diffusion study of ciprofloxacin niosomal gel was done using dialysis membrane in Franz diffusion cell using phosphate buffered saline (PBS) pH 7.4 enriched with 10% v/v of methanol as the diffusion medium. The formulations NG1 (80.22 %) and NG3 (72.68 %) containing 1% Carbopol 934 were found to release significant amount of drug from the gel over a period of 12 h as compared to the formulations with 2% carbopol.

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Introduction

The primary objective of the novel drug delivery system is to attain a steady state blood or tissue concentration of the drug that is therapeutically effective and non-toxic for an extended period of time[1]. Vesicular systems are prepared by the self-assembly of the lipids/ surfactants to form the bilayers where an aqueous space is present in the core.[2] Drug delivery via vesicular system offer many advantages like increased solubility, high permeability, acts as a carrier for various drugs which exhibits different solubility. It also acts as drug reservoirs, allows drug targeting and control release. It prolongs the residence time of drug in the body, reduces toxicity (if selective uptake is achieved), improves bioavailability and reduces cost of therapy, delays elimination of rapidly metabolizable drug. These systems are widely used in different fields of science like immunology, membrane biology, diagnostic techniques and genetic engineering.[2] Niosomes are lamellar vesicles composed of non-ionic surfactants and cholesterol. Incomparison with liposomes, they offer some advantages, such as lower cost, greaterchemical stability and longer storage time. They also have high compatibility withbiological systems and low toxicity due to their non-ionic nature.[3,4]

Poor water solubility, high metabolic clearance, short half-life and other pharmacokinetic problems hinder the pharmacodynamic efficacy of ciprofloxacin.[5] In order to increase the bioavailability, increase the half-life and prolong the duration of action several formulations like solid dispersions, nanoparticles, solid-lipid nanoparticles, nanoemulsions etc have been formulated. The non-ionic surfactant play important role in solubilizing the drug as well as the permeation enhancers due to their ability to increase membrane fluidity and their capacity to solubilize and extract membrane components.[6] On the basis of these evidences, it was envisioned that non-ionic surfactant based vesicles (niosomes) can help in achieving the goals of increasing bioavailability and half-life of ciprofloxacin.

Material and Methods

Preformulation Studies

The preformulation studies were carried out for confirming the identity of the drug and to ascertain the compatibility amongst the drug and the excipient (polymers) used in formulation.

The organoleptic properties of the procured drug sample were examined using sensory organs and include color, odor, taste and appearance. The solubility of the drug in various solvents was also observed. The melting point of ciprofloxacin hydrochloride was determined by capillary method. Loss on drying was determined by drying the pure drug in an oven at 100°C to 105°C for 3 hours.

Standard curve for ciprofloxacin

Ciprofloxacin 10 mg was weighed and dissolved in methanol in a 100 ml volumetric flask. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 100 µg/ml. Appropriate volume of the above stock solution (1 to 10 ml) from was transferred to volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with methanol to obtain concentrations of 2, 4, 6, 8, and 10 µg/mL. Absorbance of each solution against methanol as blank were measured at 276 nm.

Formulation of ciprofloxacin Niosomes[7]

Ciprofloxacin loaded niosomes were prepared by thin film hydration technique. Accurately weighed quantity of cholesterol and surfactant were dissolved in chloroform methanol mixture ratio (2:1v/v) in a 100 mL volumetric flask. The weighed quantity of drug and dicetyl phosphate was added to the solvent mixture. The solvent mixture was removed from liquid phase using rotary evaporator at 60°C to obtain a thin film on the wall of the flask at a rotation speed of 150 rpm. The complete removal of solvent can be ensured by applying vacuum. The dry lipid film was hydrated with 5 ml phosphate buffer saline of pH 7.4 at a temperature of 60±2°C for a period of 2 hour until the formation of niosomes. All the batches were subjected to sonication process for 2 min using probe sonicator. The ratios of cholesterol and surfactant used in the formulation are presented in Table 1.

Evaluation of Cirpofloxacin loaded Niosomes

Encapsulation efficiency[8]

Drug entrapped vesicles were separated from un-entrapped drug by centrifugation method. 0.5 ml of ciprofloxacin loaded niosome preparation was added with 0.5 ml of 10% triton X 100 and mixed well then incubated for 1 hour. The triton X 100 was added to lyse the vesicles in order to release the encapsulated ciprofloxacin. The solution was diluted with phosphate buffer saline pH 7.4 and filtered through Whatman filter paper. The filtrate was measured spectrophotometrically at 276 nm using methanol and triton X 100 mixture as blank.

$$\text{Percent entrapment} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug added}} \times 100$$

***In vitro* release study for niosomal preparation[9]**

The niosomal formulation was taken in a dialysis membrane of 5 cm length and suitably suspended in a beaker containing 100 ml diffusion medium of phosphate buffer saline pH 7.4. The temperature of medium was maintained at 37±0.5°C. The medium was stirred by means of magnetic stirrer at a constant speed. 1 ml of sample was withdrawn at every 1 hour and replaced with 1 ml of fresh buffer, so that the volume of diffusion medium was maintained constant at 100 ml. The withdrawn samples were made upto 10 mL using phosphate buffer saline pH 7.4. The samples were measured spectrophotometrically at 276 nm.

Preparation of ciprofloxacin niosomal gel[10]

Gel formulations were prepared by soaking varying concentration of Carbopol 934 in water for 24 h. The niosomes equivalent to 2% w/w ciprofloxacin were dissolved in ethanol and was added to the gel with continuous stirring. The plasticizer and other ingredients were added and stirred to obtain the gel noisome loaded gel formulation.

Evaluation of Niosomal gel[10,11]

Determination of pH

The pH of each formulation was determined by pH meter. The pH meter was calibrated using standard buffer solutions of pH 4 and pH 7. 1 mL of the formulation was diluted with distilled water and the pH of the solution was recorded by dipping the electrode in the solution.

Drug content

The drug content of the prepared gel was carried out by dissolving accurately weighed quantity of gel equivalent to 10 mg of the drug in 100 ml volumetric flask and volume was made up to 100 ml with methanol. The content was filtered through Whatman filter paper and 5 ml of this solution was taken into a 25 ml volumetric flask and volume was made up to mark with methanol. The content of ciprofloxacin was determined at 276 nm against methanol as blank by using UV-visible spectrophotometer. The drug content was determined from the calibration curve of ciprofloxacin.

Viscosity Determination

Viscosity of the gel was determined using Brook field viscometer DV-1. Temperature of 37±0.5°C was maintained and the spindle no. 52 was lowered into gel formulations which were placed in a beaker. The viscosity of each formulation was determined by applying 10rpm speed.

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 gel (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 gel 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

***In-vitro* drug release study**

Drug release from gel was determined by using Franz diffusion cell. Artificial dialysis membranes were soaked in receptor medium for 12 h prior to use. Phosphate buffer saline (12 ml) pH 7.4 enriched with 10% v/v methanol was added into the receptor chamber maintained at 34 ± 1°C. Gel equivalent to 10 mg of drug was placed into donor compartment and the setup was kept on stirring. Aliquots of 5ml were withdrawn at predetermined time intervals from receptor compartment and replaced with fresh buffer till 12 h. The samples were diluted suitably and analyzed spectrophotometrically at 276 nm and the amount of drug released was determined using calibration curve.

Results and Discussion

The observed organoleptic characteristic of the drug sample is presented in Table 3.

The melting point of the procured drug sample was found to be 254-257°C which was equivalent to the reported standard for ciprofloxacin. The loss on drying of ciprofloxacin pure drug was found to be 0.44%. The drug was soluble in water and phosphate buffer.

Calibration curve of ciprofloxacin

The calibration curve of ciprofloxacin was prepared according to the reported procedure using methanol as the solvent (Figure 1).

Development of ciprofloxacin niosomes

In this study, ciprofloxacin loaded niosomes were prepared by thin film hydration technique using cholesterol and non-ionic surfactants such as span 20 and tween 20. Chloroform-

methanol mixture (2:1v/v) was used as solvent. After evaporation of solvent from the formulation, thin film was formed. The thin film was hydrated and removed by phosphate buffer saline pH 7.4. Size of the vesicles in formulation was reduced by sonicating the formulation in Probe sonicator. Formulations with different ratios of surfactant and cholesterol were prepared. Several physicochemical characteristics of niosomes such as morphology, vesicle size determination, drug release profile was investigated. Dicetyl phosphate (DCP) was included in the formulation as charge inducing agent. The inclusion of charge inducing agent (DCP) prevented the aggregation and fusion of vesicles. Integrity and uniformity also maintained by dicetyl phosphate.

Percentage drug entrapment efficiency

The unentrapped drug from niosomes was removed by centrifugation technique. The results are presented in Table 4.The entrapment efficiency of the niosomes is governed by the ability of formulation to retain drug molecule in aqueous core or in bilayer membrane of vesicles. After removal of unentrapped drug, the entrapment of all formulation was studied. Entrapment efficiency was varied with varying the surfactant and cholesterol ratio. Various factors like lipid concentration, drug to lipid ratio, and cholesterol content are liable to affect the entrapment efficiency.

The entrapment efficiency using both the surfactants was determined and it was found that a 1:2 ratio of surfactant and cholesterol exhibited the maximum entrapment of the drug in the core.

***In vitro* release study**

The release of ciprofloxacin from niosomes was determined using the membrane diffusion technique. Release study was carried for 12 hours and results were plotted (Figure 2).

From the results of the release studies it was found that the maximum amount of drug was released from formulations F2 (79.74 %) and F5 (71.68 %) over a period of 12 h at an almost steady rate. The higher amount of drug release along with the higher entrapment efficiency make the formulations F2 and F5 containing surfactant to cholesterol ratio of 1:2 the most promising formulations. These two formulations were further processed to formulate gel loaded with niosomes containing ciprofloxacin.

The gel loaded with niosomal formulations F2 and F5 were prepared using Carbopol 934 as the gelling agent and propylene glycol as the plasticizer. The niosomes were suspended in ethanol prior to mixing with the Carbopol gel employing cold gelling procedure. The gel formulations were evaluated for various parameter to ascertain the most suitable formulation.

Evaluation of niosomal gel

The gel formulations were prepared using two concentrations of the gelling agent and were evaluated for physical appearance, pH, viscosity, drug content and in vitro diffusion of the drug.The gel formulations were found to be off-white in color, homogenous and sticky in feel. The pH of the all the formulations was 6.4 to 6.9 making them suitable for topical application. The formulations were found to possess sufficient viscosity to make then suitable for application to the surface and extrusion from the collapsible tube in which they were packed.

The drug content in all the formulations ranged from 96.4 to 99.1 % confirming the incorporation of the niosomes into the gel base. The results of the evaluation parameters are presented in Table 5.

***In vitro* drug diffusion from gel formulations**

The *in-vitro* drug diffusion study of ciprofloxacin niosomal gel was done using dialysis membrane in Franz diffusion cell using phosphate buffered saline (PBS) pH 7.4 enriched with 10% v/v of methanol as the diffusion medium (Figure 3).

The results of the *in vitro* diffusion study revealed that increasing the concentration of the gelling agent (Carbopol 934) decreased the release of drug from the gel. The formulations NG1 (80.22 %) and NG3 (72.68 %) containing 1% Carbopol 934 were found to release significant amount of drug from the gel over a period of 12 h as compared to the formulations with 2% carbopol. Thus it was concluded that 1% Carbopol 934 was an appropriate concentration for formulating the niosomal gel.

Conclusion

The objective of the present investigation was to develop non-ionic surfactant based delivery system for topical application of ciprofloxacin for treatment of fungal diseases. The idea was to increase the bioavailability and skin permeation of ciprofloxacin and decrease the side effects like skin rashes that are associated with the contact of drug to skin. Niosomes are known to present a solution to these side effects and the study proved that niosomes of ciprofloxacin could be easily prepared and formulated as gel for topical application that can provide good skin contact and improve drug bioavailability.

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Table 1 Composition of Ciprofloxacin Niosomes

Formulation Code	Ciprofloxacin (mg)	Surfactant	Surfactant: Cholesterol (µM)
F1	100	Span 20	100:100
F2	100	Span 20	100:200
F3	100	Span 20	100:300
F4	100	Tween 20	100:100
F5	100	Tween 20	100:200
F6	100	Tween 20	100:300

Table 2 Composition of gel formulation

Ingredients	NG1	NG2	NG3	NG4
Ciprofloxacin noisome with span 20 (%w/w)	2	2	-	-
Ciprofoxacin noisome with Tween 20 (%w/w)	-	-	2	2
Carbopol 934 (%w/w)	1	2	1	2
Propylene glycol (% w/w)	10	10	10	10
Ethanol (mL)	5	5	5	5
Triethanolamine (% w/w)	0.7	0.7	0.7	0.7
Water (g)	15	15	15	15

Table 3 Organoleptic properties of ciprofloxacin

S.No	Test	Observation
1	Color	White to off-white
2	Odor	Odorless
3	Appearance	Solid, crystalline
4	Taste	Tastless

Table 4 Entrapment efficiency

Formulation Code	Surfactant: Cholesterol Ratio	Surfactant Used	Percentage Entrapment Efficiency (%)
F1	100:100	Span 20	63
F2	100:200	Span 20	74
F3	100:300	Span 20	62
F4	100:100	Tween 20	68
F5	100:200	Tween 20	71
F6	100:300	Tween 20	61

Table 5 Evaluation of the niosomal gel formulations

Formulation code	Color	Appearance	pH	Viscosity (cps)	Drug content (%)	Spreadability (g.cm/sec)
NG1	Off White	Sticky	6.43	8320	96.4	18.12
NG2	Off White	Sticky	6.67	8670	99.12	14.08
NG3	Off White	Sticky	6.9	8417	97.15	17.56
NG4	Off White	Sticky	6.84	8648	98.97	14.6

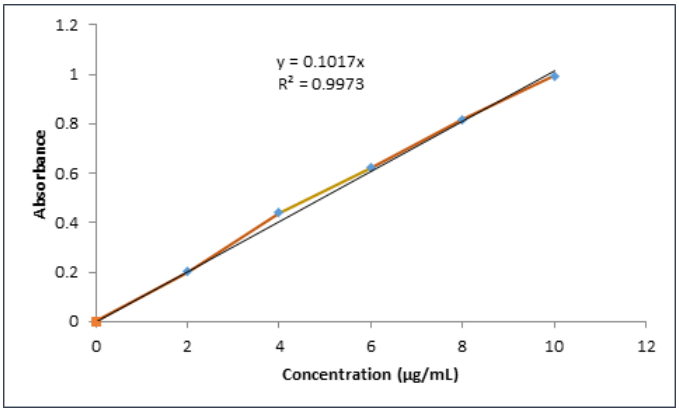

Figure 1 Calibration curve of ciprofloxacin

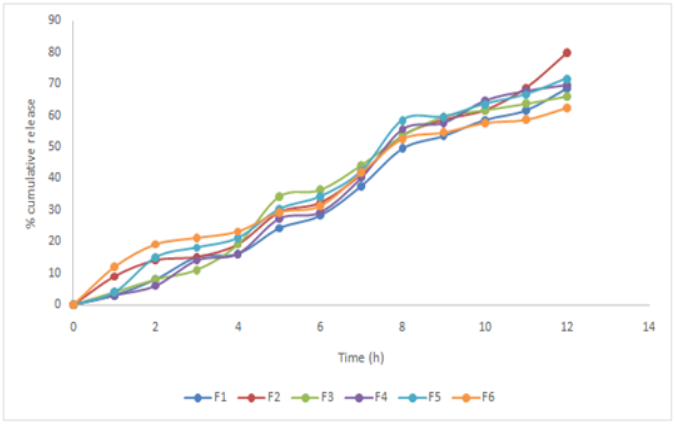

Figure 2 Comparative i*n vitro* release from niosome formulations

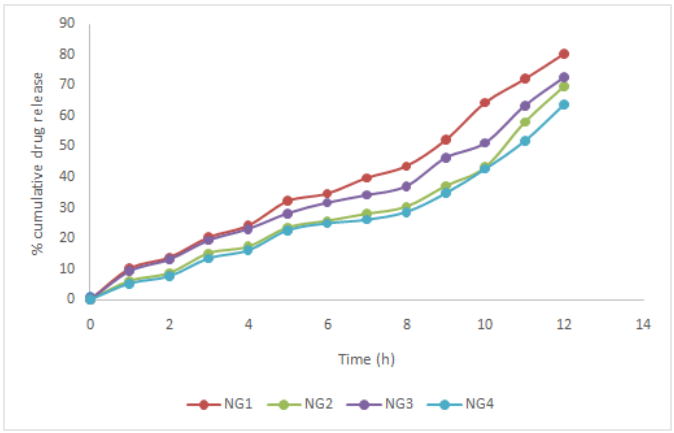

Figure 3 *In vitro* release profile of ciprofloxacin from niosomal gel formulations