Formulation and Evaluation of Ketoconazole Niosomal Gel

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***Abstract*—Niosomes are an innovative drug delivery system composed of non-ionic surfactant vesicles that encapsulate medications. These nanometric vesicles are produced by hydrating synthetic non-ionic surfactants along with or without cholesterol. Structurally similar to liposomes, niosomes offer additional advantages, making them suitable carriers for amphiphilic and lipophilic drugs. This study aimed to formulate and evaluate a ketoconazole niosomal gel for controlled drug delivery to enhance therapeutic efficacy through sustained release. The objectives included optimizing formulation variables, assessing drug entrapment efficiency, and conducting stability studies under different storage conditions. Ketoconazole, cholesterol, Tween 20, and propylene glycol were used, and the ether injection method was employed for formulation. Characterization studies evaluated physical appearance, pH, viscosity, drug content uniformity, entrapment efficiency, and in vitro drug diffusion. Results indicated significant improvements in drug entrapment efficiency and sustained release, highlighting the controlled release mechanism. Stability studies confirmed that the niosomal gel maintained its properties over time, indicating potential for long-term use. The ketoconazole niosomal gel formulation showed promising results by enhancing antifungal activity through a controlled drug delivery system. The method effectively incorporated the poorly water-soluble drug with high entrapment efficiency. Prolonged drug release from the niosomal gel is expected to reduce administration frequency and adverse effects, enhancing patient compliance. In conclusion, the ketoconazole niosomal gel could offer sustained therapeutic benefits and improved skin penetration compared to conventional formulations.**

***Index Terms*—Niosomes, Controlled Drug Delivery, Ketoconazole, Antifungal Activity.**

I. INTRODUCTION:

Niosomes are a new medicine delivery system where drug is reprised in vesicles composed of bilayers of non-ionic face-active agents, hence the name niosomes. These vesicles are veritably small, bitsy, and nano- metric in scale. Niosomes are non-ionic surfactant vesicles formed by hydrating synthetic nonionic surfactants, with or without cholesterol or other lipids. Structurally analogous to liposomes, niosomes can carry amphiphilic and lipophilic medicines and offer several advantages over liposomes.

1. SURFACTANTS:

Non-ionic surfactants are the preferred choice for vesicle preparation due to their superior stability, compatibility, and lower toxicity compared to anionic, amphoteric, or cationic surfactants. These surfactants are used in various molar ratios to entrap drugs in niosomes of different sizes. They are less toxic, less hemolytic, less irritating to cells, and maintain near physiological pH in solution. Additionally, they act as strong P-glycoprotein inhibitors, which enhance drug absorption and targets specific tissues. The various types of non- ionic surfactants are,

a. Ether linked surfactants
b. Ester linked surfactants
C. Sorbitan Esters
d. Alkyl Amides
e. Fatty Acids and Amino Acid Compounds

2. CHOLESTEROL:

Steroids, such as cholesterol, play a crucial role in altering the fluidity and permeability of the bilayer, making them important components. Cholesterol, a

waxy steroid metabolite, is frequently incorporated into nonionic surfactants to enhance rigidity and maintain orientational order.

This integration occurs without the formation of a bilayer, permitting substantial molar ratios. As an amphiphilic molecule, cholesterol orients its -OH group towards the aqueous phase and its aliphatic chain towards the surfactant's hydrocarbon chain. The rigidity is achieved by the alternating placement of the steroidal skeleton with surfactant molecules, restricting hydrocarbon carbon movement. Cholesterol also prevents leakage by eliminating the gel to liquid phase transition.

3. CHARGE INDUCERS:

Charge inducers enhance vesicle stability by inducing charge on their surfaces, preventing fusion due to repulsive forces and increasing zeta potential values. Negative charge inducers include Dioctyl phosphate, dihexadecyl phosphate, and lipoic acid, while positive charge inducers are sterylamine and cetyl pyridinium chloride.

II. TYPES OF NIOSOMES:

The Niosomes are classified on the basis of

- The number of bilayers (e.g. MLV, SUV)
- Size (e.g. LUV, SUV)
- The method of preparation (e.g. REV, DRV).

The various types of Niosomes are described below:

1. Multilamellar vesicles (MLV):

Multilamellar vesicles, consisting of multiple bilayers surrounding separate aqueous lipid compartments, range in size from 0.5 to 10 µm in diameter. These vesicles are the most commonly used niosomes due to their simple preparation, mechanical stability during long-term storage, and suitability as drug carriers for lipophilic compounds.

2. Large unilamellar vesicles (LUV):

These niosomes have a high aqueous/lipid compartment ratio, allowing them to entrap larger volumes of bio-active materials with minimal use of membrane lipids.

3. Small unilamellar vesicles (SUV):

Small unilamellar vesicles are typically prepared from multilamellar vesicles using methods such as sonication and French press extrusion. Electrostatic stabilization is achieved by including dicetyl phosphate in 5(6)-carboxyfluorescein (CF)-loaded Span 60-based niosomes.

METHOD OF PREPARATION

ETHER INJECTION METHOD:

Niosomes are created by gradually adding a surfactant solution in diethyl ether to warm water at 60°C. This involves injecting the surfactant-ether mixture through a 14-gauge needle into an aqueous solution, resulting in the formation of single-layered vesicles as the ether evaporates. The diameters of these vesicles can range from 50 to 1000 nm, depending on the specific conditions used.

FORMULATION ASPECTS & FACTORS AFFECTING NIOSOME FORMATION

1. SURFACTANTS:

The formation of bilayer vesicles instead of micelles relies on the hydrophilic-lipophilic balance (HLB) of the surfactant, the chemical structure of the components, and the critical packing parameter (CPP).

a) Chemical structure of the surfactant:

The entrapment efficiency of nonionic surfactants depends on the chain length and size of their hydrophilic head group. Surfactants with stearyl (C18) chains exhibit higher efficiency than those with lauryl (C12) chains. The Tween series of surfactants, with a long alkyl chain and a large hydrophilic moiety combined with cholesterol in a 1:1 ratio, have the highest entrapment efficiency for water-soluble drugs.

b) HLB value of surfactant and phase transition temperature of surfactant:

The HLB value of surfactants significantly influences drug entrapment in vesicles:

- Surfactants with HLB values between 14 -17 are unsuitable for producing niosomes.
- An HLB value of 8.6 results in the highest entrapment efficiency for niosomes.
- Entrapment efficiency decreases as the HLB value drops from 8.6 to 1.7. For an HLB of 6, cholesterol must be added to the surfactant to form bilayered vesicles. For lower HLB values, cholesterol enhances vesicle stability.
- The phase transition temperature (Te) of the surfactant also affects entrapment efficiency. For eg: Span 60, with its more Te, exhibits the largest entrapment efficiency.

2. CHOLESTEROL:

Incorporating cholesterol in niosomes increases both their hydrodynamic diameter and entrapment efficiency. Cholesterol has a dual effect: it increases the chain order of liquid-state bilayers, while decreasing the chain order of gel-state bilayers. At high concentrations, it transforms the gel state into a liquid-ordered phase.

The amount of cholesterol required depends on the HLB value of the surfactants. As the HLB value exceeds 10, more cholesterol is needed to compensate for the larger head groups. Higher entrapment of Minoxidil is observed in Brij76 niosomes with higher cholesterol content, whereas no significant increase is seen in Brij52 (HLB 5.3) niosomes. Beyond a certain cholesterol level, entrapment efficiency decreases, possibly due to a reduction in volume diameter (CPP < 0.05).

3. DRUG:

Entrapment of drugs in niosomes likely increases vesicle size due to interactions between the solute and surfactant head groups. This interaction boosts the charge and mutual repulsion of the surfactant bilayers, causing an increase in vesicle size. For polyoxyethylene glycol (PEG) coated vesicles, some drugs are entrapped in the long PEG chains, which reduces the tendency for size increase. Additionally, the hydrophilic-lipophilic balance of the drug impacts the degree of entrapment.

4. TEMPERATURE OF HYDRATION:

The hydration temperature is pivotal in determining the shape and size of niosomes. For optimal conditions, it should exceed the gel-to-liquid phase transition temperature of the system. Variations in temperature within a niosomal system impact the assembly of surfactants into vesicles and can also trigger changes in vesicle shape.

5. PH OF HYDRATION MEDIUM:

The entrapment efficiency of niosomes is significantly influenced by the pH of the hydration medium. High entrapment of flurbiprofen is reported at acidic pH, with a maximum encapsulation efficiency of 94.6% at pH 5.5. The encapsulated fraction of flurbiprofen increases about 1.5 times as pH decreases from 8 to 5.5, and decreases significantly at pH > 6.8. The lowest entrapment occurs at pH 7.4 and 8, with no significant difference between these values.

This increased encapsulation efficiency at lower pH is likely due to the ionizable carboxylic acid group of flurbiprofen. At lower pH, a higher proportion of unionized flurbiprofen forms, which partitions more readily into the lipid bilayer than the ionized species. At these lower pH levels, niosome formulations should be examined using optical microscopy for the presence of drug precipitates both before and after centrifugation and washing. This ensures determination of the drug concentration in the hydration medium for optimal encapsulation in niosomes.

III. SEPARATION OF UNENTRAPPED DRUG:

The removal of unentrapped solute from vesicles can be achieved through various techniques, including:

1. DIALYSIS:

The aqueous niosomal dispersion is dialyzed in dialysis tubing against phosphate buffer, normal saline, or glucose solution.

2. GEL FILTRATION:

The unentrapped drug is removed by gel filtration of the niosomal dispersion through a Sephadex-G-50 column and elution with phosphate-buffered saline or normal saline.

3. CENTRIFUGATION:

The niosomal suspension undergoes centrifugation, leading to the separation of the supernatant. The pellet is then washed and re-suspended, resulting in a niosomal suspension devoid of unentrapped drug.

METHODOLOGY

METHODS USED:

ETHER INJECTION METHOD:

- Here the non-ionic surfactant, cholesterol and drug were weighed separately and dissolved by using Diethyl ether: Methanol (1:1) mixture.
- The above organic layer was taken in a 10 ml syringe having 14 guaze needle fitted in it.
- In separate beaker adequate amount of distilled water was taken and keep stirring
- Maintain the temperature between 55°C and 65°C.
- Vaporization of ether and methanol leads to the formation of single layered niosome.
- Finally, the Niosomes were stored at 4°C in a refrigerator.

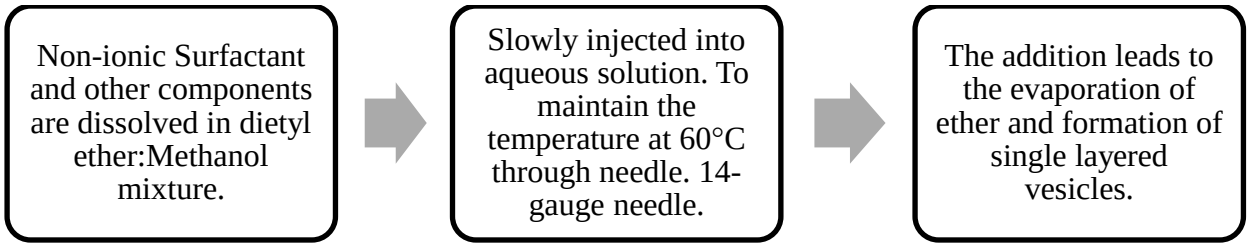

Figure No: 1

IV. PREFORMULATION STUDIES

1. Organoleptic Properties:

- Colour-colourless
- Odour- Characteristic odour
- State-Crystalline form
- Melting point-150°C

2. Bulk characterization studies:

- Bulk density: We found the bulk density of drug was 0.428
- Tapped density: We found the tapped density of drug was 0.507
- Angle of repose: We found the angle of repose of drug was 0.66, it means that it possessed excellent flow.

Flow Character	Angle of repose
Very good	<20
Good	20-30
Poor	30-34
Very poor	>40

Table No: 1 Angle of repose

Hausner's ratio: We found the Hausner's ratio of drug was 1.18, it means that it has a good or free flow property.

Hausner's ratio	Hausner's ratio
1.00-1.11	Excellent/very free flow
1.12-1.18	Good/free flow
1.19-1.25	Fair
1.26-1.34	Passable

Table No: 2 Hausner's ratio

Carr's Index**:** We found that the Carr's index of drug was 18.45; it means that it possessed excellent flow properties.

3. Particle Size & Shape

- Sieving size- 45 mesh

4. Solubility Profile:

- Solubility: Soluble-Ethanol, Insoluble-water
- Partition coefficient: The partition coefficient of Ketoconazole in n-octanol and water was obtained 4.35
- Maximum wavelength of drug: We found maximum wavelength of drug by using UV-Visible spectrophotometer was 246 nm.

V. EVALUATION OF KETOCONAZOLE NIOSOMAL GEL

Physical Appearance: Clarity, color, unity and the presence of foreign patches in the Niosomal gel was determined.

PH: PH of the Niosomal gel was determined using digital pH cadence.

Viscosity: Brookfield viscometer was used to determine the density of the Niosomal gel.

Drug content uniformity: The drug content of the niosomal gel was determined by dissolving 10 mg of the drug in a 100 ml volumetric flask, followed by the addition of 50% n-propanol for niosome lysis. The volume was then adjusted to 100 ml with methanol. The solution was filtered, and absorbance was measured using a UV spectrophotometer at 225 nm.

Entrapment Efficiency: "The free drug concentration in the supernatant was determined at 225 nm using a UV-Visible Spectrophotometer. This was achieved by centrifuging 0.5 g of gel, equivalent to 10 mg of ketoconazole, diluted to 10 ml with distilled water at 15,000 rpm for 60 minutes at 4°C using a high-speed cooling centrifuge to separate the niosomes from the unentrapped drug.

The % drug entrapped was calculated from the

$$\text{formula} = \frac{\text{Amount entrapped}}{\text{total amount}} \times 100$$

In-vitro drug diffusion studies: In- vitro prolixity studies of the Niosomal gel were carried out using dialysis membrane. The medicine release from the Niosomal gel was determined from the collected samples. The analysis of the collected samples was done under UV spectrophotometer at 225 nm.

FOURIER-TRANSFORM INFRARED SPECTROSCOPY:

The FTIR study of medicine and excipients admixture was studied and set up to be no any other peaks. The results of FTIR peaks revealed that medicine and excipients admixture wasn't show any commerce and suitable for the expression and development.

VI. RESULTS AND DISCUSSIONS

1. FOURIER- TRANSFORM INFRARED SPECTROSCOPY

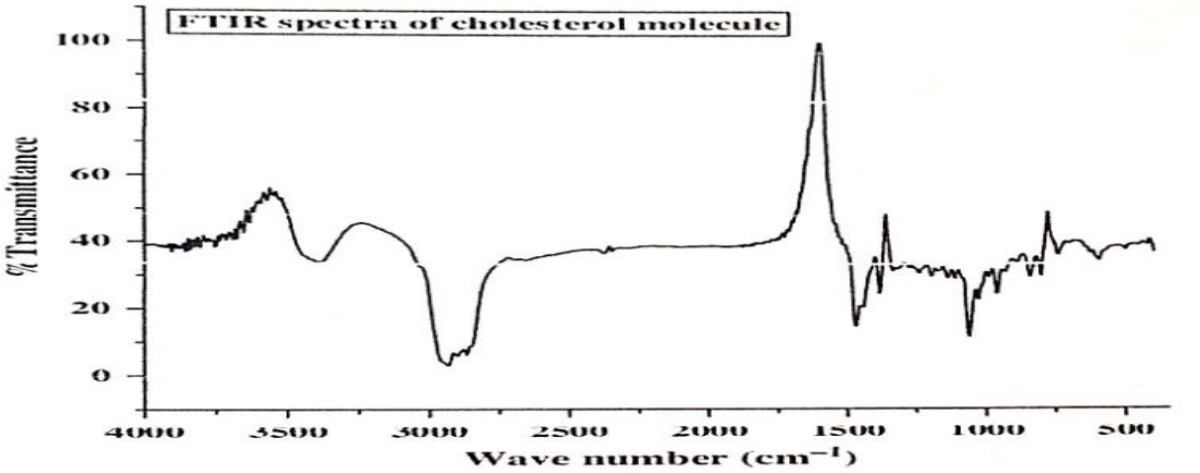

Figure No: 2 Fourier-Transform Infrared Spectroscopy

FTIR SPECTRA FOR KETOCONAZOLE MOLECULE

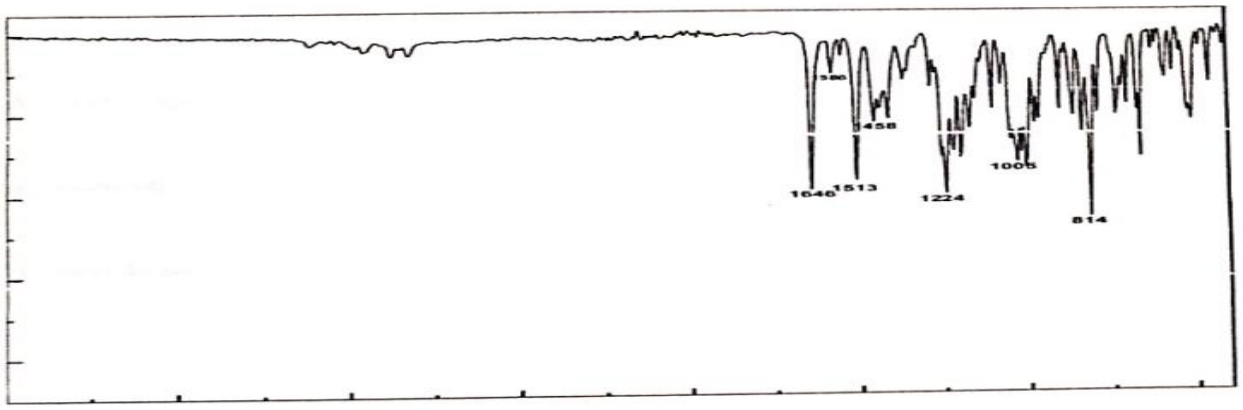

Figure No: 3 FTIR Spectra for Ketoconazole Molecule

2. ORGANOLEPTIC CHARACTERS:

PARAMETERS	POLYMER
Colour	Colourless
Odour	Characteristic
State	Crystalline
Melting point	150°c

Table No: 3 Organoleptic Characters

3. BULK CHARACTERS:

PARAMETERS	RATIO
Bulk Density	0.428
Tapped Density	0.507
Angle of Repose	0.66
Carr's Index	18.45

Table No: 4 Bulk Characters

4. SOLBILITY PROFILE:

a) Soluble: Ethanol
b) Insoluble: Water
c) UV SPECTRUM:

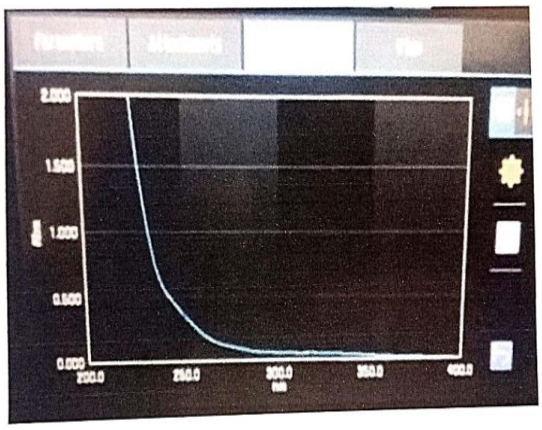

Figure No: 3 UV Spectrum

d) PARTITION CO-EFFICIENT: 4.35

5. IN VITRO RELEASE PLOT:

S.NO.	Time(in mins)	Concentration
1.	0	0
2.	10	28.7
3.	20	38.8
4.	30	45.57
5.	40	52.97

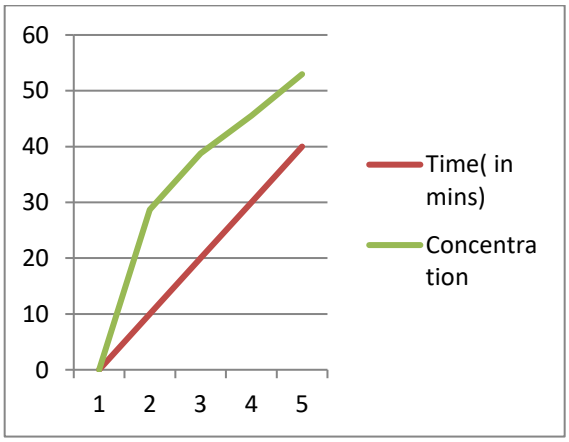

Figure No: 4 In-Vitro Release Plot

VII. CONCLUSION:

A gel formulation containing niosomes loaded with ketoconazole demonstrates prolonged action compared to formulations with non-niosomal ketoconazole. This suggests it can be developed to enhance anti-fungal activity. Ketoconazole niosome gel, a transdermal drug delivery system, offers several advantages. First, cholesterol powder is safe as it consists of biocompatible and biodegradable synthetic polymers. Ether injection methods are effective for incorporating poorly water-soluble drugs like ketoconazole into niosomes with high entrapment efficiency. The prolonged release of the drug from niosomes indicates a reduced frequency of administration and fewer adverse effects, improving patient compliance. Using the drug in gel form enhances its penetration.

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