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PREPARATION AND EVALUATION OF KETOCONAZOLE NIOSOMAL GEL DRUG DELIVERY SYSTEM BY ULTRASONICATION METHOD

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ABSTRACT

Objective: Niosomal drug delivery system is an effective as well as the advanced path marching towards novel drug delivery system. Ketoconazole is known as an azole antifungal that acts by inhibiting the growth of fungus. The main objective of the study is to achieve control release and prolong the action of ketoconazole by formulating as niosomes. **Method:** In this study the niosomes are formulated by using sonication technique and it is further converted to a gel drug delivery system so as to obtain control release. The results of this study showed that CHO content and the type of surfactant increased drug release rate from niosomes. Formulation consisting the surfactant and CHO ratio 1:0.2 showed higher drug release. **Results**: The λ_{max} of drug was found to be 246 nm and the Beer Lambert's range was found to be 10-50 ug/ml, the globule size of the niosomes by optimum batch was found to be 65.50nm, the viscosity of the niosomes was found to be 450 cps. pH of the optimum formulation was found to be 6. The in-vitro release showed 98.22%drug release in 5 hours. **Conclusion:** From all these studies which were carried out, it was concluded that a gel formulation containing niosomes loaded with Ketoconazole provides control release and long duration of action than containing Ketoconazole in non-niosomal form and it can be developed successfully to improve the antifungal activity.

KEYWORDS: Niosomes, Antifungal Agent, Surfactant, sonication.

INTRODUCTION

The primitive objective of the drug therapy is to acquire a steady state blood or tissue level such that it is therapeutically effective as well as nontoxic for a prolonged period of time. The layout of appropriate dosage regimen was an essential element in achieving the goal.[1,6,7,9] Novel drug delivery systems are direct to transport the drug at a rate desired by the needs of the body duration of the period of treatment and carry the active entity to the site of action.[2,6,8] Targeted drug delivery is indicated for selective as well as effective localization of pharmacologically active moiety at preselected target in therapeutic concentration while inhibiting its entry to non-target normal cellular linings therefore decreasing the toxic effect and increasing therapeutic index.[5,16] Targeted drug delivery is a process where a drug carrier complex delivers the drug (s) completely to the pre-designed targeted cells in a specified manner. To seek the optical drug action, functional molecules could be delivered through a carrier to the site of action and released for doing their task.[8,19] The targeting techniques can be classified as chemical methods, which is achieved by covalent bonding and physical methods.[4,11] Chemical methods include chemical alteration of the parent compound to a derivative, which will be activated only at the target site.[3,9] Many physical methods use the special forms i.e. carriers like liposomes, niosomes, resealed erythrocytes, platelets, magnetic microspheres, nanoparticles, and monoclonal anti-bodies. In current years, the niosomal drug delivery system is generally gaining focus because of its special benefits over conventional drug delivery system.[5,13,16,18] Niosomes consists of the nonionic surfactant vesicles comprising of the lamellar structure which can be uni-lamellar and multi-lamellar which proves to be efficient in giving the desired benefits such as: to promote the effective delivery system to accomplish maximum effective concentration, to produce vesicles that are able of entrapping hydrophilic as well as hydrophobic molecules, alteration of the particle composition or surface could modify the affinity for the target site and the drug release rate, etc.[1,6,10] Niosomes demonstrated to be an encouraging drug carrier and had ability to decrease the side effects of drugs and greater therapeutic efficiency in diversified diseases by inhibiting its action towards the target cells.[8,17] The bilayer structure of niosomes is amphiphilic in characteristic which can be used to transport hydrophilic drugs in its aqueous core and similarly for lipophilic drugs in the bilayer which constitutes of surfactants.[7] Various excipients are added in niosomes which involves nonionic surfactant acting as film forming agent, cholesterol used to stabilize and rigidity agent for the bilayer formation and different charge inducers which gives a charge on the surface of niosomes and provide stability to the prepared formulation consequently by repulsive forces.[1]

Types of niosomes

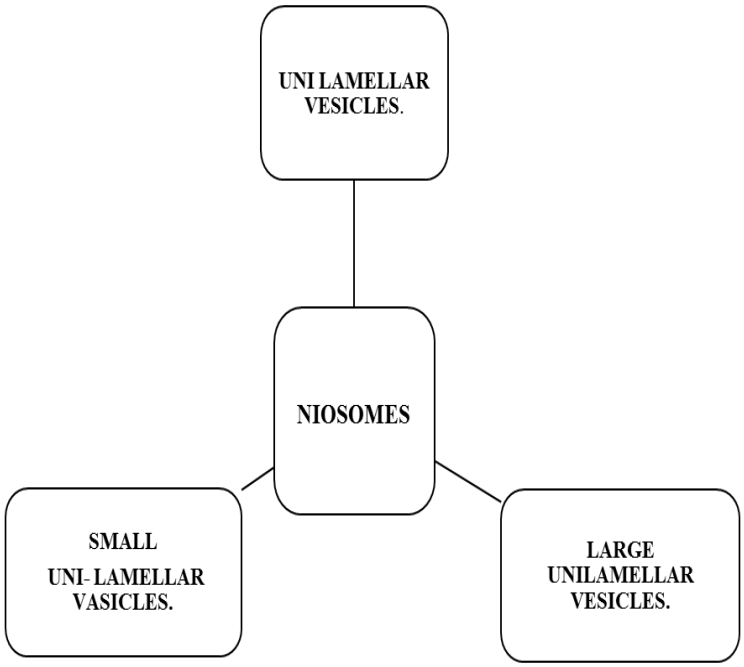

Fig. 1: Types of Niosomes.

1. Multi-lamellar Vesicles (MLV)

Multi-lamellar vesicles comprise several number of bilayer around the aqueous lipid compartment separately (having approximate size vesicles to be 0.5-10 µm diameter). Multi-lamellar vesicles are the most commonly used in noisome preparations.[3,9,13]

2. Large Uni-lamellar Vesicles (LUV)

Larger amount of bio-active materials can be encapsulated in large uni-lamellar vesicles. Niosomes under this classification have a greater aqueous / lipid compartment ratio.[3,9,13] (Size is approximately>0.10µm).

3. Small Uni-lamellar Vesicles (SUV)

The small uni-lamellar vesicles are commonly formulated from multi-lamellar vesicles by sonication process. (Size is approximately 0.025-0.05µm).[3,9,13]

Advantages

- The Niosomal formulations are suspensions of water–based vehicle. This provides high patient compliance instead of oily dosage forms.[1,8]
- Their structure comprises of hydrophilic, amphiphillic and lipophilic moieties together and as a consequence could occupy drug molecules with a broad range of solubility.[6,5]

- This vesicular system acts as a depot which releases the drug moiety in a controlled manner.[1]
- These vesicles have a unique property of being osmotically active and stable, as well as they possess the ability to increase the stability of encapsulated drug.[3,5]
- Handling and storage of surfactants used in these preparations required no special conditions.[1]
- This preparation provides the increased oral bioavailability of poorly absorbed drugs as well as improved skin penetration of drugs.[1,19]
- These Niosomal preparations can be formulated for transporting the drug moiety by oral, parenteral as well as topical routes.[5,8]
- The surfactants are normally biodegradable, biocompatible as well as non-immunogenic.[1]
- These preparations tend to enhance the therapeutic performance of the drug molecules by providing delayed clearance from the systemic circulation thereby protecting the drug from biological environment as well as inhibiting effects on target cells.[1]

Comparison between niosomes and liposomes

a. Liposomes are costly and also comprises the ingredients such as phospholipids which are chemically unstable because of their susceptibility towards the oxidative degradation, they demand special storage paired with handling and also purity of natural phospholipids is distinctive.[1,2]
b. Variability in attributes is present in between liposomes and niosomes, specifically in case of niosomes which are formulated from uncharged single-chain surfactant and also cholesterol whereas in case of liposomes which are synthesized from double-chain phospholipids (neutral or charged).[1,2]
c. Niosomes enact in-vivo as liposomes, which cause prolongation of the circulation of encapsulated drugs and altering its organ distribution as well as metabolic stability. Entrapment of many antineoplastic agents by using carrier vesicles has been demonstrated to reduce the drug induced lethal side effects, whilst maintaining, the anti-tumor efficiency.[1]
d. In the case of liposomes, the characteristics of niosomes rely upon the contents of the bilayer and also on method of their preparation.[1]
e. The entrapment efficacy increases with increase in the concentration and also with respect to lipophilicity of surfactants.[1]

Table No 1: Comparison between liposomes and niosomes.[3]

Sr.No.	Liposomes	Niosomes
1	These are much more Expensive	These are very less Expensive
2	Phospholipids are very much prone to oxidative Degradation.	Non-ionic surfactants used here are stable toward oxidation**.**
3	Liposomes require special Process for storage, handling and purification of phospholipids.	No special methods are required for noisome formulations.
4	Phospholipids may have neutral charge.	Non-ionic surfactants do not have charge.

A number of antifungal agents are accessible in the market in form of various topical preparations for e.g. creams, ointments, as well as powders for the use of local dermatological therapy). Ketoconazole, a substituted imidazole is one of the antifungal agents; it has wide spectrum activity against systemic and superficial mycoses. It is easily but incompletely absorbed after oral administration and it is distinct among individuals.[1] General side effects in relation with Ketoconazole therapy involves mild burning at the site of the application, adverse allergic reactions, blisters, irritation, pain along with redness. Topical drug administration is a form of localized drug delivery scheme everywhere in the body through ophthalmic, rectal, vaginal and skin as the topical routes.[5,9,18]

Components of niosomes

Niosomes mainly contains following types of components:

I. Non-ionic surfactants

The non-ionic surfactants arrange themselves in bilayer structure in which the polar which are hydrophilic heads orient facing towards the aqueous bulk (media) while the hydrophobic head arrange in such that the interaction with the aqueous media would be reduced.[7,11,18]

II. Cholesterol

Steroids are essential constituents of the cell membrane and their existence in membrane affects the bilayer fluidity as well as permeability. Cholesterol being a steroid derivative is principally used for the preparation of niosomes. Despite of it not showing any role in the preparation of bilayer, its influence in formation of niosomes as well as manipulation of layer attributes cannot be disregarded. Normally, addition of cholesterol affects characteristics of niosomes such as membrane permeability, rigidity, encapsulation efficiency, ease of rehydration of freeze dried niosomes also including toxicity.[7,10]

III. Charged molecule

Various charged molecules are incorporated to niosomes to increase stability of niosomes with the aid of electrostatic repulsion which restricts coalescence. The negatively charged molecules used in such preparation are diacetylphosphate (DCP) and phosphotidic acid.[7]

DRUG PROFILE

Name: Ketoconazole

IUPAC Nomenclature: cis-1-acetyl-4-[[(2RS, 4RS)-2-(2, 4-dichloro-phenyl)-2-(1H-imidazol-1-ylmethyl)-1, 3-dioxolan-4 yl] methoxyl] phenylpiperazine.

Molecular Weight: 531.4 gm. /mol.

Molecular Formula: $C_{26}H_{28}C_{l2}N_4O_4$

Category: Anti-fungal.

State: White crystalline powder

Color: White to off-white.

Odor: Odorless

Taste: Bitter

Melting Point: 148°C to 152°C

- **MATERIALS AND METHODS**

Table No 2: Materials used for the formulation.

Sr. No.	Materials	Procured from
1	Ketoconazole drug	Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune-44
2	Cholesterol	Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune-44
3	Chloroform	Dr. D.Y. Patil College of Pharmacy, Akurdi, Pune-44
4	Span 80	Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune-44
5	Tween 80	Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune-44
6	Phosphate buffer solution (PBS)7.4	Dr. D. Y.Patil College of Pharmacy, Akurdi, Pune-44
7	Distilled water	Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune-44

- **METHOD OF PREPARATION**

1. Niosomes of ketoconazole

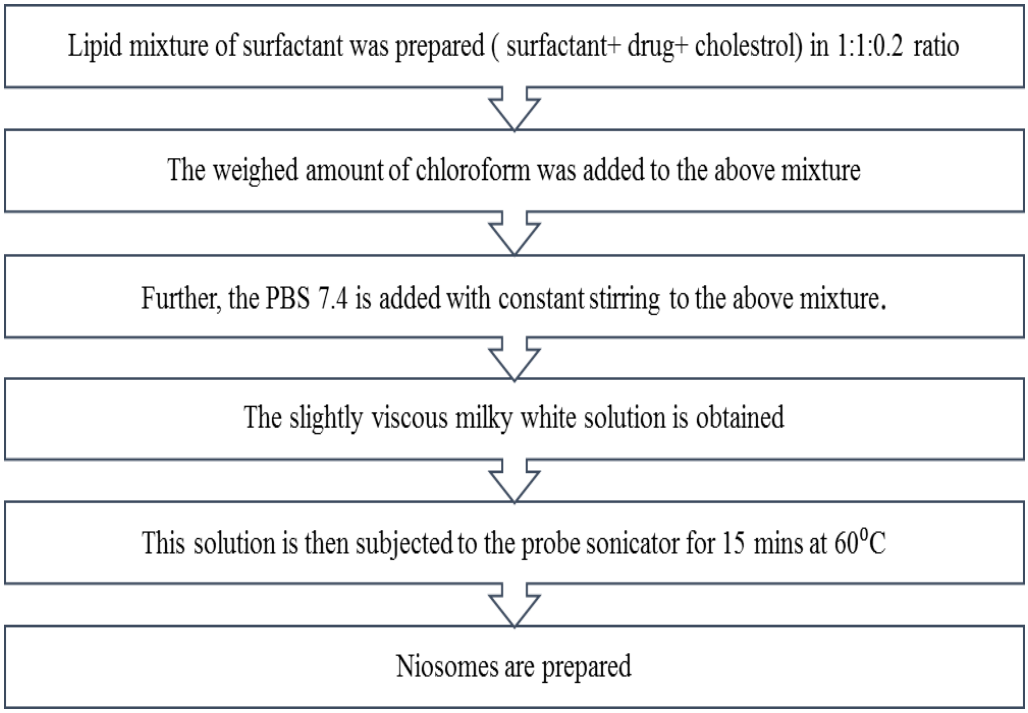

Fig. 2: Procedure for preparation of niosomes.

Niosomes were prepared by a Probe Sonication method with the aid of lipid mixture consisting of surfactant (span 80 and tween 80) and CHO, at different specified ratios as given in table no 3. The cholesterol was weighed according to the ratio and taken into the beaker. To the same beaker add the drug ketoconazole and also weigh the surfactant and transfer to the beaker. The weighed amount of chloroform was added so as to make the drug soluble in the organic phase. Simultaneously the Phosphate Buffer Solution (PBS) was prepared with the distilled water of pH 7.4. Further the aqueous phase was introduced into the organic phase with constant stirring. A milky white slightly viscous solution is produced. This mixture is then subjected to probe sonication for 5 mins at 60°C. The SUV types of niosomes are produced which were further evaluated and converted to the gel.[2,3,19]

Table No 3: Formulation development of niosomes.

Sr. No.	Ingredients	N1	N2
1	Ketoconazole	100	100
2	Span 80	100	-
3	Tween 80	-	100
4	Cholesterol	20	20
5	Chloroform	10	10
6	PBS 7.4	10	10

N1 is the niosomes using span 80; N2 is the niosomes using tween 80

2. Niosomal gel of ketoconazole

The Formulations of niosomes prepared using span 80 and tween 80 containing Ketoconazole equivalent to 2% w/w was incorporated into the gel base composed of carbopol 940 (150 mg), glycerol (250 mg), triethanolamine (quantity sufficient) and distilled water up to 15 g. This gel was further evaluated.[1,2,3]

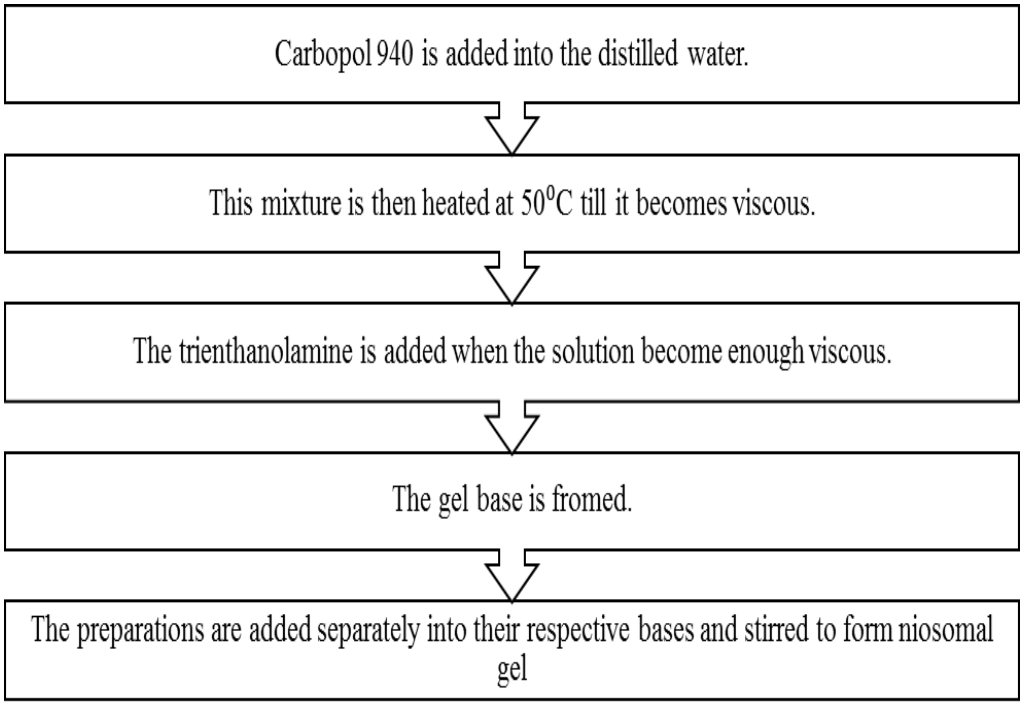

Fig 3: Procedure for preparation of ketoconazole Niosomal Gel.

- **RESULTS AND DISCUSSIONS**

Calibration curve for ketoconazole

The 10mg of the drug ketoconazole was weighed on the digital balance and transferred into the 100ml volumetric flask. The 20ml of methanol was taken into the same volumetric flask so as to dissolve the drug. Further the solution was sonicated in the ultrasound bath sonicator for about 15 mins till it becomes clear solution. This is followed by making the volume by adding the methanol up to the mark on the volumetric flask. The dilutions were then prepared as per the table below and the absorbance was taken in the U. V. Spectrophotometer. The λ_{max} of drug was found to be 246 nm. The Beer Lambert's range was found to be 10-50 ug/ml. The value of the regression coefficient was found to be 0.9996.[2,4,8]

Table No 4: Calibration curve of ketoconazole.

Concentration (µg/ml)	Absorbance
0	0.000
10	0.095
20	0.192
30	0.293
40	0.395
50	0.502

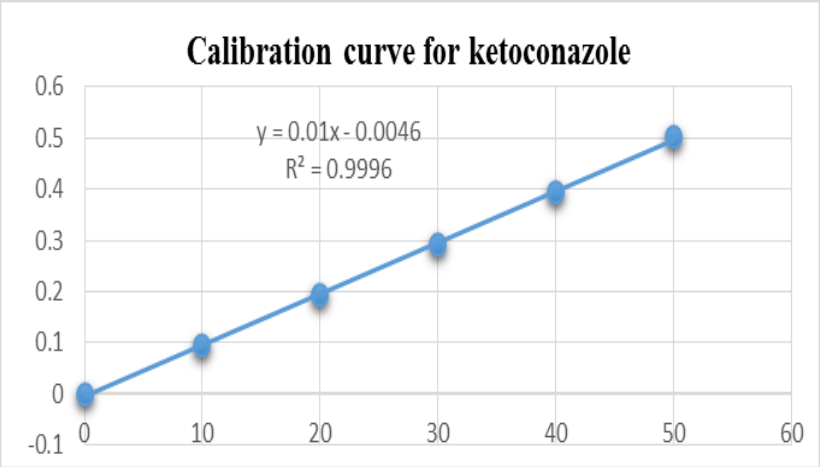

Fig 4: Calibration Curve for Ketoconazole.

- **Evaluation Parameters for Ketoconazole Niosomes**

1. Physical appearance

The niosomes formulated by the above methods were examined for physical appearance, homogeneity, consistency as well as alteration in viscosity. The results demonstrated that the organogels were found to be creamy white in color, homogenous with absence of grittiness; having required consistency without alteration in viscosity for specified period of time. This verifies the stability of niosomes.[1,3,8]

2. Morphological characterization

The vesicle formation was confirmed with the aid of optical microscopy with 45x resolution. The niosomal suspension was taken over a glass slide which is further fixed with the use of drying at room temperature, the dry thin film of niosomal suspension observed under the microscope in the formation of vesicles. The photographic image of the niosomes was taken from the microscope with the aid of a digital camera.[1,2,3]

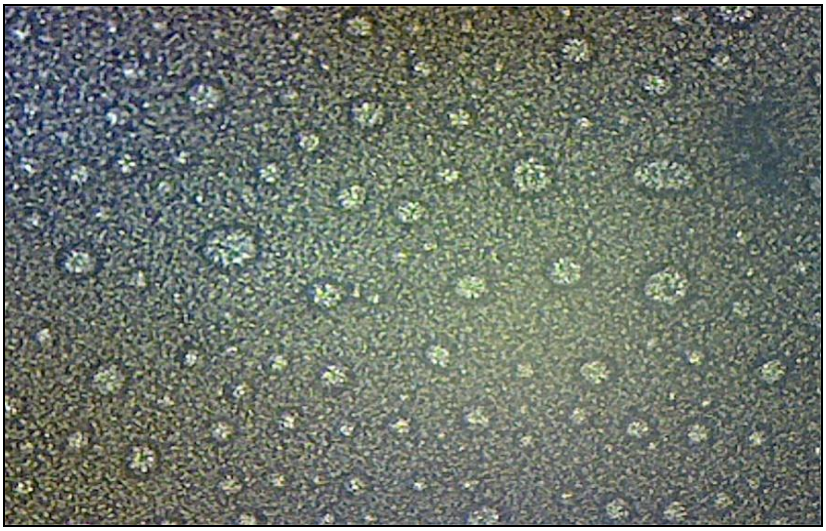

Fig 5: Optical microscopy of niosomes.

3. Particle size analysis: For N1 formulation

Table No 5: Globule size analysis of N1 formulation.

Globule number	Globule size (in nm)
1	51.86
2	81.42
3	56.20
4	30.88
5	21.60
6	66.32
7	45.90
8	100.01
9	76.25
10	57.78
11	99.01
12	87.62
13	100.22
14	54.16
15	34.22
16	45.87
17	69.03
18	98.003
19	33.25
20	100.44

Arithmetic mean = $\frac{\sum X}{n} = \frac{1310.54}{20}$ = 65.50 nm

For N2 formulation

Table No 6: Globule size analysis of N2 formulation.

Globule number	Globule size (in nm)
1	41.86
2	21.42
3	66.20
4	40.88
5	21.60
6	88.32
7	53.90
8	100.67
9	64.25
10	57.78
11	99.56
12	77.62
13	100.123
14	57.16
15	46.22
16	32.87
17	96.03
18	89.003
19	23.25
20	100.99

$$\text{Arithmetic mean} = \frac{\sum X}{n} = \frac{1415.86}{20} = 70.793 \text{ nm}$$

4. Viscosity

The niosomes formulated by using the span 80 surfactant was further evaluated for change in the viscosity by subjecting it to Brookfield Viscometer. In this study, spindle No: CP 52 with a required speed of 0.01 rpm was used to examine the viscosity of the preparation.[2,7]

Table No 7: Viscosity of N1 and N2 Formulation.

Sr. No.	Formulation	Observation
1	N1	450cps
2	N2	530cps

Evaluation of ketoconazole niosomal gel

i. Physical appearance

The prepared gels were examined for physical appearance, homogeneity, consistency as well as alteration in viscosity. The results showed that the gels prepared were creamy white in color, homogenous with absence of grittiness; possessing semisolid consistency without alteration in viscosity for specified period of time. This verifies the stability of gels.[1,3,8,12]

ii. pH determination

As, topical systems are directly used on the skin; therefore the pH should be always compatible with the pH of skin. An acidic or basic pH results in skin irritation or disturbance of the skin structure.[1,2,3]

Table No 8: The pH of the noisome formulations.

Sr. No.	Formulation	Observation
1	NG1	6.75
2	NG2	5.28

NG1 is the niosomal gel formulated using **N1** niosomes, **NG2** is the niosomal gel formulated using **N2** niosomes.

iii. Extrudability study

Extrudability test is based upon the examination of weight desired to extrude 0.5 cm ribbon of gel in 10 sec. from a lacquered collapsible aluminum tube. Extrudability is dependent upon the viscosity of that gel. Lesser the viscidity of the gel, lower the force required to remove it from tube, thus shows better extrudability.[1,2,3]

Extrudability = Weight applied to extrude gel from tube (gm.) / Area (cm2)

Table No 9: Extrudability of both the formulations.

Sr. No.	Formulation	Extrudability
1	NG1	+++
2	NG2	++

NG1 is the niosomal gel prepared by using **N1** niosomes, **NG2** is the niosomal gel formulated using **N2** niosomes.

iv. In-vitro diffusion study

In-vitro diffusion was performed by modified Franz diffusion cell. A glass cylinder having both ends open, 10 cm in height, having 3.7 cm outer diameter as well as 3.1 cm in inner diameter was used as diffusion cell. A cellophane membrane was choose and placed over the beaker comprising of the water. The water was heated until the membrane separates outs. Further it was fixed to one end of the cylinder by using an adhesive. Approximately 1gm of gel was placed in the cell (donor compartment) and this cell was immersed in a beaker comprising the 500 ml of phosphate buffer (pH 6.8) acting as receptor compartment. Sample consisting of 5 ml of the receptor compartment was removed at 1 hour interval of time for a

period 8 hours with same amount to be replaced to maintain sink condition. These samples were analyzed at 226 nm against blank with the aid of UV Spectrophotometer. Amount of ketoconazole released at different time intervals was measured with the help of calibration curve with phosphate buffer (pH 6.8) as well as plotted against time vs. in-vitro release profiles of ketoconazole from various gel formulations are represented in table no 10 and 11.[1,2,3]

For NG1 formulation

Table No 10: In-vitro drug release profile of NG1.

Sr. No.	Time (in mins.)	% Cumulative drug release
1	0	00
2	10	20.08
3	20	28.44
4	30	35.65
5	40	41.23
6	50	48.66
7	60	58.66
8	120	63.79
9	180	77.88
10	240	86.65
11	300	98.22

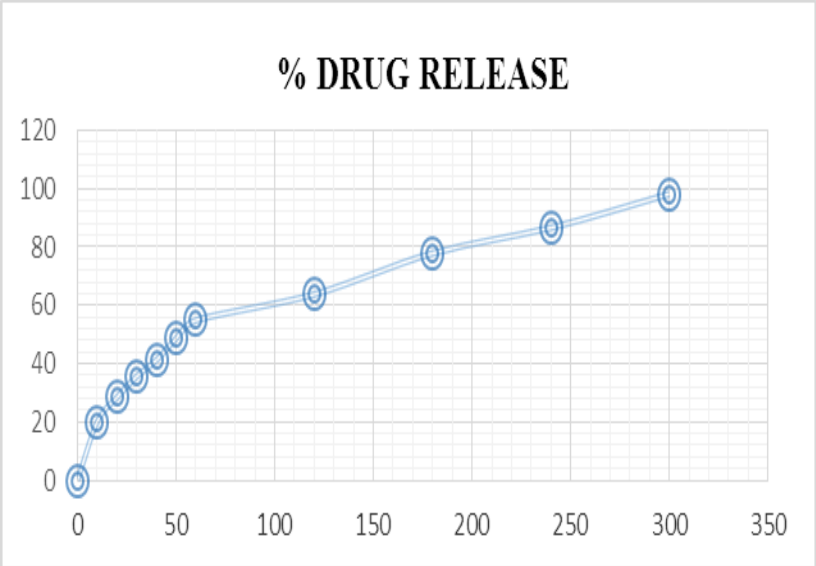

Fig 6: Drug release graph for NG1 Formulation.

For NG2 formulation

Table No 11: In-vitro drug release profile of NG2.

Sr. No.	Time (in mins)	% Cumulative drug release
1	0	00
2	10	28.66
3	20	38.77
4	30	45.66
5	40	52.88
6	50	65.24
7	60	70.1
8	120	75.66
9	180	78.9
10	240	82.56
11	300	86.99

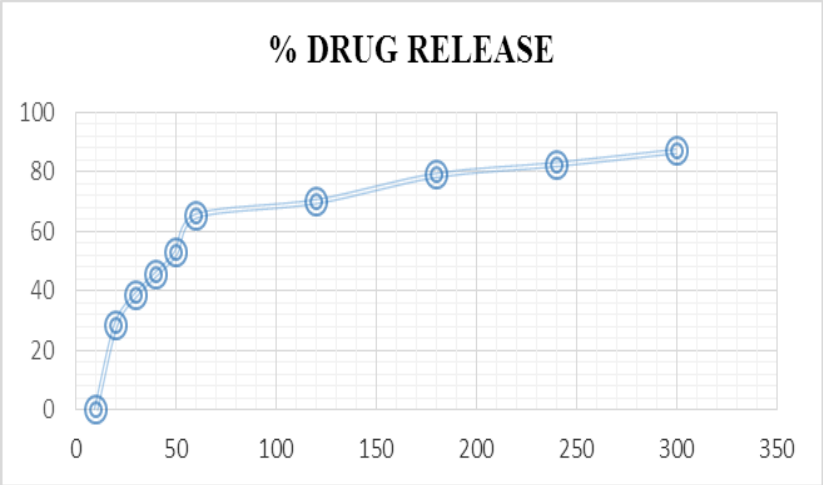

Fig 7: Drug Release graph for NG2 Formulation.

- **SUMMARY AND CONCLUSION**

Niosomes drug delivery system is an effective way towards novel drug delivery. Niosomes are comprised principally of non-ionic surfactants and cholesterol. Niosomes may be formulated by different methods such as ether injection method, hand shaking method, sonication method, reverse phase evaporation method, remote loading method, extrusion method as well as micro fluidization method.[1,4,8] The characteristics of niosomes are influenced by additives, methods of preparation, drug properties, amount, structure as well as type of surfactant used, cholesterol content and resistance to osmotic stress. In general, as a drug delivery device, which when compared to liposomes, niosomes have property to be osmotically active and are quite chemically stable and improve the stability of the drug which is encapsulated and delivered.[5,9,15] These do not require special conditions for handling, protection or storage as well as industrial manufacturing. Although, they provide flexibility in

structural characteristics, they can be designed as required. Niosomes provides various benefits over other drug delivery devices as well as have found suitable in pharmaceutical field.[7]

Besides, niosomes acts as drug carriers they have indicated many advantages like being cheap and chemically stable, but they are associated with notable problems with respect to physical stability like fusion, aggregation, sedimentation as well as leakage on storage.[5] Hence the niosomes are entrapped into the gel so as to improve their physical stability.[1,8]

The results of this study demonstrate that CHO content and the type of surfactant modified the entrapment efficiency as well as drug release rate from niosomes. Formulation having surfactant and CHO ratio 1:0.2 provided higher drug release. The N1 formulation was found to be with optimum result and NG1 formulation showed good results. Hence the niosomes with span 80 was found to be optimum. From all these studies, it can be concluded that a gel formulation comprising niosomes loaded with Ketoconazole demonstrated prolonged action than formulations comprising Ketoconazole in non-niosomal form as well as it can be developed successfully to improve the antifungal activity.[2,3]

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