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Formulation and Evaluation of Natamycin Niosomal Gel for Transdermal Drug Delivery

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ABSTRACT

The goal of this study was to see how well a topical niosomal gel containing Natamycin delivered the drug. The particle size, shape, entrapment efficiency, and *in vitro* properties of niosomal formulations generated using the thin film hydration process at varied cholesterol and Span 60 ratios were investigated. The mean particle size of the niosomal formulation was discovered to be between 2 and 4 m. Entrapment efficiency was excellent in the niosomal formulation N2 (1: 1) of cholesterol and surfactant (96.72 percent). Niosomal formulation was spherical, according to TEM analysis. At a (1: 1) cholesterol : surfactant ratio, niosomal formulation (N2) had a high percentage of drug release after 24 hours (94.91). Further, a chosen niosomal formulation was employed to create a topical gel, which was evaluated for factors such as pH, viscosity, spreadability, and *in-vitro* potential penetration. In *in-vitro* research, niosomal gel outperformed regular topical gel in terms of skin permeability. Further *in-vitro* testing demonstrated that topical niosomal gel inhibited inflammation better than plain gel and niosomal formulation. According to the findings, topical niosomal gel compositions enable sustained and extended medication administration.

1. INTRODUCTION:

Natamycin, commonly known as pimaricin, is a fungal infection treatment. Natamycin was first developed in 1955 and received FDA approval for medicinal usage in 1978. It's made by fermenting Streptomyces bacteria. Candida, Aspergillus, Cephalosporium, Fusarium, and Penicillium are all fungal infections that Natamycin is used to treat. It's used as a lotion, eye drops, or a lozenge (for oral infections). When given in this manner, natamycin has a very low absorption rate. Because it is poorly absorbed from the gastrointestinal tract when given orally, it is ineffective for treating systemic infections. Yeast infections and oral thrush can also be treated with natamycin lozenges. [1]

The application of a medicine to the skin for a localized impact is known as topical drug delivery. The human body's skin is one of the most common and publicly available structures. An average adult's skin covers around 2 m^2 and gets roughly one-third of the blood that circulates through the body. It aids in the retention of body heat, blood pressure management, and UV protection [2]. Dermal drug delivery has a number of benefits, including longer duration of action, dosing flexibility, reduced side effects, uniform plasma levels, and high patient compliance, but it also has some drawbacks, including the possibility of local irritation, erythema, itching, and low permeability of drugs in the stratum. The top layer of the epidermis, the stratum corneum, is impervious to water and acts as a robust flexible barrier. Corneocytes are keratinized cells that have died and are no longer alive [3]. Many technologies and methods have been researched for drug penetration across this barrier, with the vesicular carrier for drug delivery through the skin being one of the most promising ways.

Dermal delivery using novel drug delivery carriers has a lot of promise. To alleviate the difficulty associated with topical traditional formulation, lipidic and nonlipidic vesicular systems such as liposome, transfersome, ethosome, and niosome are employed. In terms of superior trapping of medicines (payload features), target site-specificity, and handling premature drug release, a drug delivery system employing an unique vesicular carrier, such as a liposome or niosome, has significant benefits over microspheres, nanoparticles, and other carriers (burst effect) [4, 5]. Niosomes were examined as an alternative to liposomes in 1985 because they had various advantages over liposomes, such as being more stable, nontoxic, and cost-effective due to the cheap cost of nonionic surfactants against phospholipids, which are prone to oxidation. Surfactants included into niosomes may also improve medication

effectiveness, potentially by promoting drug absorption by target cells. Niosomes are a nontoxic, biodegradable, and biocompatible alternative to liposomes. They can be used to deliver a wide range of medications since they can entrap hydrophilic, lipophilic, and amphiphilic compounds. NSAIDs, hormones, antibacterial, and antifungal medications are the most often utilised pharmaceuticals for transdermal delivery [6]. The goal of this work was to create a niosomal gel filled with Natamycin for transdermal administration.

2. MATERIALS AND METHODS:

2.1 MATERIALS: Natamycin (gift sample) Proquiga Biotech, S.A, CholesterolE. Merck (India) Ltd., Mumbai. Sorbitan Monostearate (Span 60) Loba Chem. Pvt. Ltd., Mumbai. Carbopol 934E. Merck (India) Ltd., Mumbai, Triethanolamine Loba Chem. Pvt. Ltd., Mumbai. MethanolE. Merck (India) Ltd. Ethanol E. Merck (India) Ltd. Chloroform

2.2 METHODS:

2.2.1 Preparation of Niosome Dispersion (Hand Shaking Method): Cholesterol, Span-60, and stearic acid were properly weighed and placed into a clean round bottom flask to make niosomes containing Natamycin in the appropriate proportion. The flask was then immersed in 10ml diethyl ether and vortexed constantly to generate a thin layer along the flask's edges. A suitable amount of natamycin was dissolved in phosphate buffer saline (pH 7.4) and slowly added to the round bottom flask containing a thin layer of surfactant and cholesterol, which was vortexed continuously at room temperature for 30 minutes until the combination was well dispersed. [7, 8]

Table No. 1: Observation of blank noisome dispersion

Batch no.	Span 60 (mg)	Cholesterol (mg)	Observation
NF1	10	10	Dispersion was not obtained
NF2	20	10	Separation was obtained
NF3	30	10	Separation was obtained
NF4	10	20	The dispersion was not obtained
NF5	20	20	0.05%
NF6	30	20	Milky white dispersion was obtained
NF7	10	30	Milky white dispersion was obtained
NF8	20	30	Separation was obtained
NF9	30	30	Separation was obtained
NF10	10	40	Milky white dispersion was obtained
NF11	20	40	0.05%
NF12	30	40	0.05%

2.2.2 Preliminary characterization of Blank niosomal dispersion

i. Organoleptic Properties: The blank dispersions were evaluated for colour, odour, appearance and texture.

ii. Creaming Volume: The blank dispersion was kept undisturbed for 24 hrs in the measuring cylinder and evaluated for separation, creaming and redispersibility.

iii. pH: The pH of blank formulation was checked with a digital pH meter.

iv. Microscopic Evaluation: Formulation of 1 ml was put on slide and evaluated at 400X magnification under a microscope to determine any cluster preparation [9].

Table No. 2: Physical properties of blank niosome

Batch no.	Colour	Odour	pH	Changes after 10 days
NF-6	Milky white	Odourless	4.5-5.6	No changes
NF-7	Milky white	Odourless	4.5-5.6	No changes
NF-10	Milky white	Odourless	4.5-5.6	No changes

v. Effect of Processing Parameters on Formation of Niosomes: After optimizing the concentration of span 60 and cholesterol in the preparation of niosomal dispersions. The processing parameters such as rate of addition & stirring time were optimized.

vi. Effect of Rate of addition: The ether solution was injected rapidly as well as dropwise into the aqueous phase to study its effect on vesicle size.

vii. Effect of Stirring time: The dispersion was stirred for varied time period of 5, 10 and15 minutes [10]. The results are given in table 3.

Table No. 3: Effect of processing parameter on particle size distribution

Rate of addition	
Rapid addition	Non uniform size distribution
Drop wise addition during 15 minutes	Uniform size distribution
Time of stirring	
5 Minutes	Non uniform size distribution
10 Minutes	Non uniform size distribution
15 Minutes	Uniform size distribution

2.2.3 Optimizing & Preparation of Drug Loaded Niosomal Dispersions: Optimization was shown to be a difficult procedure since the many chemicals utilized may interact with one another. So, to simplify this complex procedure, factorial design and statics are used to conduct fewer tests in the optimization phase. It also clarifies how variables affect formulation development [11]. The niosomal dispersion process is a significant one that necessitates the study of some elements as well as their interactions. Statistical techniques like as factorial design can aid in a better understanding of these interactions while needing fewer tests, making formulation development more cost-effective.

Table No. 4: Factors and levels selected for optimization

S. No.	**Factors**	**Higher-level**	**Middle level**	**Lower level**
1	Span 60	60	50	40
2	Cholesterol	40	30	20

Table No. 5: 3^2 Factorial Batches

S. No.	Batch no.	Span 60 (mg)	Cholesterol (mg)
1	B1(-1,-1)	40	20
2	B2(-1,0)	40	30
3	B3(-1+1)	40	40
4	B4(0,-1)	50	20
5	B5(0,0)	50	30
6	B6(0,+1)	50	40
7	B7(+1,-1)	60	20
8	B8(+1,0)	60	30
9	B9(+1,+1)	60	40

(All batches contain drug 10 mg & stearic acid 5 mg.)

Table No. 6: Formulation chart of Niosome of Blank Niosome

Batch no.	Span 60 (mg)	Cholesterol (mg)	Stearic acid (w/v)
NF1	10	10	0.05%
NF2	20	10	0.05%
NF3	30	10	0.05%
NF4	10	20	0.05%
NF5	20	20	0.05%
NF6	30	20	0.05%
NF7	10	30	0.05%
NF8	20	30	0.05%
NF9	30	30	0.05%
NF10	10	40	0.05%
NF11	20	40	0.05%
NF12	30	40	0.05%

2.2.4 Characterization of Drug Loaded Niosomal Dispersions:

i) Organoleptic Properties: The drug-loaded dispersion was evaluated for colour, odour and appearance.

ii) pH: The pH of niosome dispersion was checked by digital pH meter.

iii) Total Drug Content: Assay of drug-loaded niosomal dispersions were carried out by U.V. method. Two ml of niosomal dispersion was dissolved into 50 ml Phosphate buffer solution having pH6.8. After addition of 1% isopropanol, sample was stirred at 100 rpm to break the niosomes. Drug content was determined using UV spectrophotometer at respective absorption maxima [12].

iv) Transmission Electron Microscopy (TEM): The morphology of drug (Natamycin)and optimized formulation of noisome was examined using a transmission electron microscope (TEM) (Jeol JEM 1230, Tokyo, Japan) at 70 kV after 50-fold dilution of drug and niosome with distilled water. In this technique, one drop of each diluted drug was placed on the surface of a 300-mesh carbon-coated copper grid and left to settle for 3-5 min. The excess fluid was isolated with a filter paper and allowed to dry at room temperature for 10 min before examination under a transmission electron microscope at 70kV [13].

v) Mean Particle Size and Polydispersibility Index: The particle size analyses of the formulation (NF7) were determined using Beckman particle size determination technique. The results of Natamycin loaded noisome are shown in results and discussion part.

vi) Zeta potential (ζ): Zeta potential of the dispersion was determined by Malvern zeta meter. Time duration for zeta potential determination was 60 seconds and charge was find out. Typical graphs for zeta potential of natamycin-loaded noisome was shown in figure 2.

vii) Encapsulation Efficiency: Unentrapped drug from niosomal dispersion was separated by centrifugation method. Niosomes were centrifuged at 20,000 rpm at controlled temperature of 4 ºC for 60min. By using UV spectroscopy unentrapped drug was quantified at respective absorption maxima. The entrapped drug in niosomes verified using following equation. The results of Natamycin loaded noisome are shown in table 8.[14]

$$\text{Entrapment efficiency (\%)} = [(C_t - C_f)/C_t]\ 100,$$

Where, C_t total Drug concentration and C_f free Drug concentration

viii) *In- vitro* Drug Diffusion: The design and development of drug delivery system are greatly aided by in- vitro diffusion studies. To obtain qualitative and quantitative drug release pattern of API through niosomal dispersion and determine correlation among release

mechanism and drug loaded niosomal dispersion and experimental data was determined using dialysis method [15].

In this technique sacks were soaked in saline solution for one day. A 5 ml of drug loaded niosome was transferred into dialysis sack respectively. These drug loaded niosome dialysis bag was placed in a beaker at 370C. One mL samples were analysed by replacing with buffer medium using UV spectrophotometer at intervals 0.5, 1, 2, 3,4, 5, 6, 12 & 24 hours. The results are shown in figure 3 and 4.

2.2.5 Niosomal Gel:

Niosomal dispersion showed sustained released but further topical applicability of the developed formulation was enhanced by development of gel formulation. Carbopol934 was selected as polymer for gel preparation.

Preparation of Niosomal Carbopol Gel: Carbopol at different concentrations (1%, 1.5%, 2% w/w) were added to water and kept in a dark for humectation for up to 24 hrs. To obtain a gel pH was adjusted within the range of 6.8 – 7.2 by addition of triethanolamine[16].The optimized batch of the niosomal dispersion was centrifuged at 35000 rpm and the pellet was separated and again dispersed into each concentration of the carbopol gel.

2.2.6 Characterization of Drug Loaded Niosomal Gel:

i) Physical Parameters Characterization: Gel formulations were evaluated for the Colour, pH, Viscosity and Microscopic Appearance.

ii) Spreadability of Gel: For gel dosage form good spreadability value is one of the important properties. Spreadability means gel ability to spread on skin part. Spreading value decide the therapeutic efficiency of gel.

To determine spreadability value spreadability apparatus was used. Spreadability apparatus contains wooden block having two glass plates. Initially gel sample was placed between the two glass plates. Weight near about 300 g was putted on top plate which expelled the air and to form uniform gel layer. Afterwards 100 g weight was put to drag top plate by 10 cm using stringe attached to hook. Time required to move upper plate by 10 cm distance was noted, lesser the time required for dragging the upper plate better is the spreadability value[17].Spreadability value was determined with the help of formula:-

$$S = M \times L / T$$

Where, S is the Spreadability value, L is the Length of the glass slide, M is the Weight tied to the upper plate, and T is the Time taken to separate the glass slides.

iii) Content Uniformity: The content uniformity of the optimized gel was determined by analyzing drug content by analyzing gel samples from five different points from the container. From the container 500 mg of gel sample was withdrawn and dissolved it into distilled water. Sample was stirred at 200 rpm by the addition of methanol to disrupt the niosomes make a require dilution and drug concentration was determined using UV spectrophotometer [16].

iv) Viscosity of gel: Viscosity has a significant role in the performance of topical products. Viscosity of formulation is closely linked to the product characteristics, such as spreadability, ease of application, drug release and stability [18].

v) *In-vitro* Drug Diffusion Study: The diffusion study of the niosomal and conventional gel were carried out in Franz diffusion cell. Gel sample (0.5 g) was taken on cellulose nitrate membrane diffusion studies were carried out at 37±1 0C using 15 ml phosphate buffer (pH 6.8) as the dissolution medium. At time intervals of 0.5, 1, 2, 3, 4, 5, 6, 12 and 24 hours and replaced by fresh medium 2 mL sample were withdrawn and drug content was determined by UV spectrophotometer. The results are shown in figure 5.[19]

3. RESULTS AND DISCUSSION:

3.1 Blank Niosome Dispersion: Non-ionic surfactant concentration from 0.1-0.3% w/v did not give proper dispersion this may be due to insufficient concentration of non-ionic surfactant to form uniform spherical vesicle. Whereas surfactant concentration more than 0.6% %w/v showed cracking this may be due to precipitation of surfactant. So, Batch no. NF6, NF-7B, NF-10 gave milky white dispersion in which the concentration of surfactant was in the range of 0.4-0.6% w/v and this was taken for the further optimization. Therefore, by carried out blank noisome batches we have optimized Chemical parameters and processing parameters. Chemical parameters i.e. concentration of span60 & cholesterol and processing parameters include Rate of addition and time of stirring.

3.2 Drug loaded Noisome Dispersion: To predict the optimum performance of prepared niosomes we have adopted a3^2 factorial design approach. Amount of span-60 and cholesterol

were selected as two independent variables and different performance indicators were studied to determine the effect of the concentration of lipid phase on niosome performance.

3.3 Natamycin-loaded Niosome Dispersion

i) Organoleptic properties: The Natamycin niosomal dispersion was off-white in color, odourless, and fluid in nature. It was stable and did not show sedimentation. Summarize data of all the nine batches of factorial design is shown in table 7.

Table No. 7: Evaluation of Natamycin Niosomal batches

Batch no.	Appearance	Odour
NT-1	Milky white	Odourless
NT-2	Milky white	Odourless
NT-3	Milky white	Odourless
NT-4	Milky white	Odourless
NT-5	Milky white	Odourless
NT-6	Milky white	Odourless

ii) pH and Drug Content: The pH of the niosome was measured by using a digital pH meter. pH was found to be in the range of 5.1-6.2. The drug content of the niosomal was found to be 89.78– 96.41%.

iii) Entrapment Efficiency: The Entrapment efficiency was found in range of 79.78 to 98.41. Formulation NT-4 shows highest % entrapment efficiency values.

Table No. 8: pH, drug content and Entrapment Efficiency of Natamycin Niosomal batches

Batch no.	pH	Drug Content	Entrapment Efficiency
NT-1	5.7	91.98%	90.18 %
NT-2	5.5	89.78%	79.78 %
NT-3	6.1	94.7%	92.73 %
NT-4	5.9	96.41%	93.41 %
NT-5	6.2	93.76%	90.16 %
NT-6	5.3	95.87%	92.17 %

iv) Morphology of noisome by transmission electron microscopy (TEM): According to analysis of the noisome batch NT4 was selected for morphological examination. Fig. 1 confirmed the formation of a vesicle with a round shape having nano-size particles.

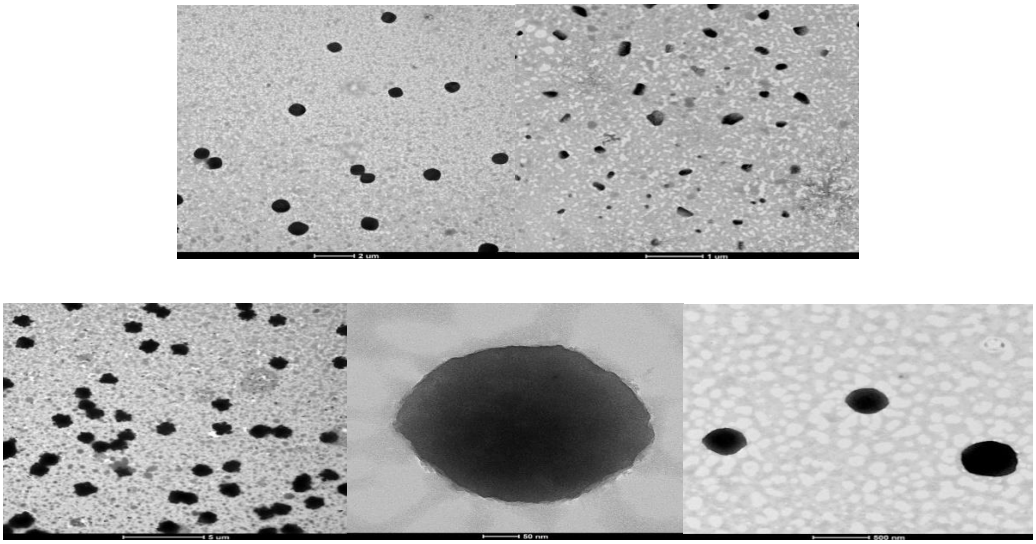


Figure No. 1: TEM image of optimized formulation of Niosome

v) Vesicle size: The mean vesicle size of drug loaded niosomes of the different batches according to the factorial design ranged between 2.52 - 3.42 µm. The polydispersvity index (PdI)was in the range of 0.370 – 0.420 for drug loaded niosomes which indicates a narrow vesicle size distribution. The mean vesicle size, PdI and zeta potential of all six batches of factorial design.

vi) Zeta (ζ) Potential Determination: The values of ζ potential of the drug-loaded niosomal formulation were in the range of-20.29 to -30.55mV. Values of ζ potential showed that the drug-loaded niosome had sufficient charge and mobility to inhibit aggregation of vesicles.

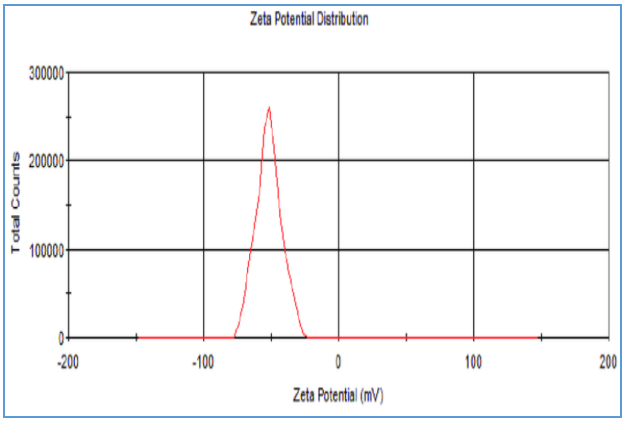
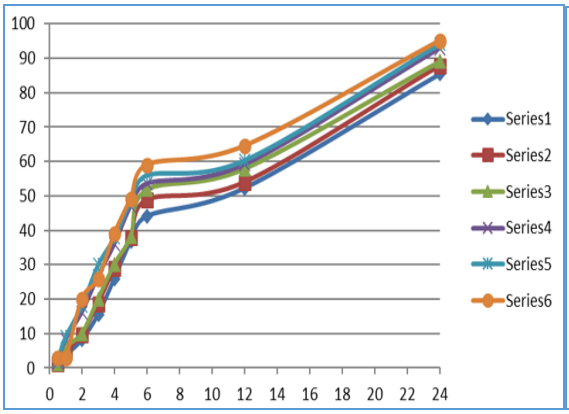


Figure No. 2: Zeta Potential of optimized formulation of niosome

vii) *In-vitro* Drug Diffusion Profile: The *in-vitro* release of all developed niosome formulations was carried out by diffusion method. The studies revealed that the rate of drug release depends on the percentage of drug entrapment efficiency. Drug diffusion from niosomal dispersion and conventional dispersion were found to be 95% and 88% respectively. Niosomal dispersion showed sustained release than conventional dispersion. It was confirmed by *ex-vivo* study using porcine skin.



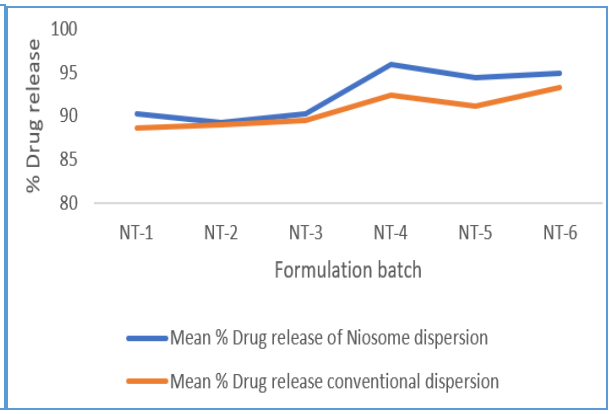


Figure No. 3: Cumulative % drug release Figure 4: Natamycin niosome *in-vitro* diffused profile

3.4 Formulation of Natamycin Loaded niosomal Gel: The gels were prepared by dispersion method using HPMCK4M and Carbopol 934 indifferent ratios as shown in table 9.

Table No. 9: Formulation of niosomal gels using Carbopol 934, HPMCK4M

Formula code	Niosomal suspension (ml)	Carbopol 934 (%)	HPMCK4M (%)	Propylene glycol (ml)	Triethanolamine (%v/v)	Water (up to 30 ml)
NTG-1	10	0.5	-	5	0.5	Q.S.
NTG-2	10	1.0	-	5	0.5	Q.S.
NTG-3	10	1.5	-	5	0.5	Q.S.
NTG-4	10	-	0.5	5	0.5	Q.S.
NTG-5	10	-	1.0	5	0.5	Q.S.
NTG-6	10	-	1.5	5	0.5	Q.S.

3.5 Characterization of Natamycin Loaded Niosomal Gel

i) Appearance: The appearance was checked visually. After gelling the clarity of the formulation colour was determined by visual examination under light, alternatively against white and black background. The colour of niosome is observed to be about pale yellow to colourless with translucent appearance.

ii) Determination of pH: The pH of formulations was in the range of 5.23 to 6.45 which is considered acceptable to avoid the risk of skin irritation upon application to skin. The optimized formulation (NTG2) pH was found to be 6.45. There was no significant change in pH values as a function of time for all formulations.

iii) Drug Content determination: The resultant solution was suitably diluted with methanol and absorbance was measured at 427nm.The NTG2 formulation shows maximum % Drug content i.e., 94.23% and thus selected asa final formulation. The results are shown in Table 10.

iv) Spread ability study: Niosomal gels agent exhibited spreadability values ranging from 12.64-15.89 g.cm/s. The spreading coefficient of various niosomal gel formulations are given below in table 10.

v) Determination of Viscosity: The average viscosity of formulations lies in the range from 1937.49 to 3650.63 cps. The Viscosities of all gel formulations are shown in Table 10 and was found to be decreased on increasing the shear rate i.e., pseudo plastic behavior was noted.

Table No. 10: pH and Drug content of Natamycin Niosomal gel

Batch no.	pH	Drug Content	Spreadability g.cm/s	Viscosity (cps)
NTG-1	5.32	88.18%	12.64	2358.87
NTG-2	6.45	94.23%	15.89	3650.63
NTG-3	5.99	92.73%	15.15	1937.49
NTG-4	6.09	90.21%	14.09	2498.09
NTG-5	6.25	92.61%	14.25	3234.09
NTG-6	5.87	93.17%	13.87	2909.98

vi) *In-vitro* drug release study: The in vitro drug release studies were carried out across cell membranes. The results of in-vitro release after incorporation of niosomes in gel. The cumulative percentage drug release for 8 hrs was highest for formulation NTG2 using carbopol 934.An *in-vitro* drug release was conducted for the optimized niosomal formulation NT4, the optimized niosomal gel formulation NTG2 and the marketed formulation. The Niosomal formulation showed higher drug release than the gel and marketed formulation. The results are shown in figure 5.

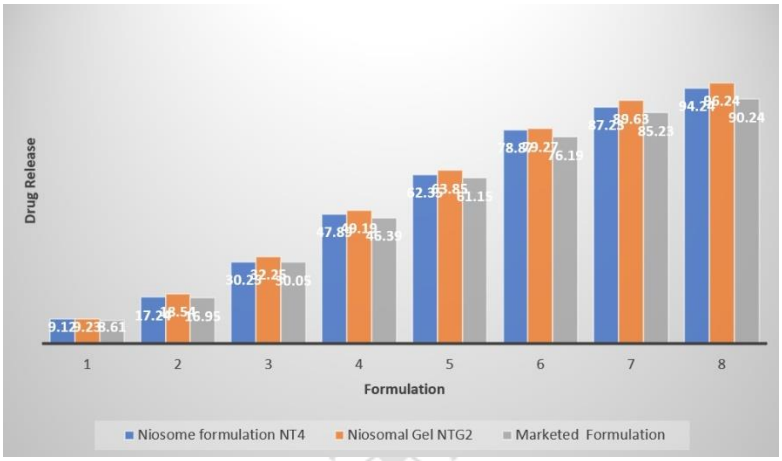


Figure No. 5: Comparison between, noisome, niosomal gel, and Marketed formulation

CONCLUSION:

Gel-embedded nanometric systems are included in niosomal gels. Nanometric structures have a greater surface area, which makes them ideal for the application of pharmacological ingredients that allow uniform drug release. Such structures have been examined as alternatives to traditional formulations that concentrate on chemical skin permeability enhancers. Furthermore, because nanostructure systems are small, they are easy to apply to the skin in dermatological products. According to a study on skin irritation, created niosomal gel has the main irritation index of zero. As a result, the formulation was determined to be skin-safe and non-irritating. As a result, a safe, effective, and non-irritant surfactant-based topical formulation for the treatment of fungal illness has been created. The results achieved with traditional formulations were identical to those obtained with niosomal preparations. As a result, the produced formulation demonstrated strong antifungal efficacy.

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