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**Original Research Article**

# Formulation and Evaluation of Anti-acne Niosomal Gel Using Isotretinoin and Lincomycin Combination

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**Abstract:**

Acne vulgaris is a prevalent dermatological concern affecting adolescents and young adults, often causing psychological distress and potential scarring. This multifactorial disorder involves hormonal changes, increased sebum production, bacterial colonization, and inflammation. To address these diverse factors, a combination of drugs with different mechanisms of action in topical formulations has been explored.

This study focuses on the formulation and evaluation of an anti-acne niosomal gel incorporating isotretinoin, a retinoid with anti-inflammatory properties, and lincomycin, an antibiotic targeting Propionibacterium acne. The objective is to develop a stable and efficacious topical formulation that comprehensively addresses the various aspects of acne pathogenesis. Niosomes are chosen as the carrier system for their ability to enhance drug penetration, reduce side effects, and improve drug stability.

Several studies have highlighted the synergistic action of isotretinoin and lincomycin, providing a comprehensive approach to managing acne lesions by addressing both inflammatory and bacterial components. Moreover, their combination has shown a reduced risk of antibiotic resistance compared to monotherapy with antibiotics.

The research involves a systematic evaluation of the formulated niosomal gel, including physicochemical characteristics, in vitro release, and stability studies. By adopting a topical approach, the study aims to minimize systemic side effects and enhance drug delivery to the target site, ensuring localized and effective treatment. The outcomes of this study are anticipated to contribute valuable insights into the potential of an isotretinoin and lincomycin combination in a topical niosomal gel for the advanced and effective treatment of acne vulgaris.

**Keywords:** Acne vulgaris, Isotretinoin, Lincomycin, Topical formulation, Niosomes, Multifactorial acne treatment.

## Introduction:

Acne vulgaris is a prevalent dermatological disorder affecting the pilosebaceous units of the skin, primarily observed in adolescents and young adults[1]. It is characterized by the formation of comedones, papules, pustules, nodules, and cysts, often leading to psychological distress and potential scarring[2]. Various factors contribute to the development of

acne, including hormonal changes, increased sebum production, bacterial colonization by Propionibacterium acnes, and inflammation[3].
To address the multifactorial nature of acne, a combination of drugs with different mechanisms of action has been explored in topical formulations. One such promising combination involves the use of isotretinoin and lincomycin. Isotretinoin, a retinoid, exerts its effects by normalizing keratinization, reducing sebum production, and possessing anti-inflammatory properties. Lincomycin, a lincosamide antibiotic, targets the bacterial component of acne by inhibiting protein synthesis in Propionibacterium acnes[4,5].

Several studies have highlighted the efficacy of isotretinoin and lincomycin combination in the treatment of acne. According to the studies, the synergistic action of these two agents provides a comprehensive approach to managing acne lesions by addressing both inflammatory and bacterial components. Furthermore, their combination has shown a reduced risk of antibiotic resistance compared to monotherapy with antibiotics.

While the oral administration of isotretinoin is a well-established treatment for severe acne, the use of a topical gel containing both isotretinoin and lincomycin presents a promising alternative. Topical formulations minimize systemic side effects and enhance drug delivery to the target site, ensuring localized and effective treatment[6,7].

In this context, the present study focuses on the formulation and evaluation of an anti-acneniosomal gel incorporating isotretinoin and lincomycin. The objective is to develop a stable and efficacious topical formulation that addresses the various aspects of acne pathogenesis. The choice of niosomes as the carrier system is based on their ability to enhance drug penetration, reduce side effects, and improve drug stability[8].

This research aims to contribute valuable insights into the potential of an isotretinoin and lincomycin combination in a topical niosomal gel for the treatment of acne. Through a systematic evaluation of the formulation, including physicochemical characteristics, in vitro release, and stability studies, the study aims to provide a foundation for the development of an advanced and effective anti-acne formulation.

**Aim & Objective:**

To formulate novel niosomal gel containing Isotretinoin and lincomycin for treatment of acne vulgaris. Isotretinoin is a highly lipophilic molecule and is categorized as a Class II drug having high permeability and poor solubility. lincomycin is a BCS III drug which are known to have high solublity but low permeability. With the help of permeation enhancer and solubilizerniosomal gel will be prepared that can help in better penetration and better therapeutic efficacy.

**Materials and Method**

**Materials**

**Table 4.1: List of chemicals**

| S. No | Chemicals | Manufacturer |
|---|---|---|
| 1 | Cholesterol | Merck, India |
| 2 | SPAN 20 | Sigmaldrich, India |
| 3 | SPAN 60 | Sigmaldrich, India |
| 4 | SPAN 80 | Sigmaldrich, India |
| 5 | Sodium Hydroxide | Sigmaldrich, India |
| 6 | Sodium Chloride | Molychem, India |
| 7 | Hydrochloric Acid | Merck, India |
| 8 | Sulphuric Acid | Merck, India |

| | | |
|---|---|---|
| 9 | Potassium Bromide | Merck, India |
| 10 | Phosphate Buffer | Sigmaldrich, India |
| 11 | phosphotungstic acid | Merck, India |
| 12 | HPMC | Sigmaldrich, India |
| 13 | Carbopol | Merck, India |
| 14 | propylene glycol | Merck, India |
| 15 | Glycerin | Sigmaldrich, India |
| 16 | Sodium alginate | Merck, India |
| 17 | Carbapol 934 | Sigmaldrich, India |
| 18 | Xanthan gum | Sigmaldrich, India |
| 19 | Guar gum | Merck, India |
| 20 | Glycerin | Merck, India |
| 21 | Methyl Paraben | Merck, India |
| 22 | Propyl Paraben | Sigmaldrich, India |
| 23 | Propylene glycol | Sigmaldrich, India |
| 24 | Imiquimod | Sigmaldrich, India |
| 25 | formalin | Sigmaldrich, India |

**Pre-formulation studies**

The drug samples were studied for appearance, color, and odour.

**MeltingPoint:**

The melting points of the drugs were determined by an open capillary method using the melting point apparatus[9].

**Partition-Coefficient (Kp):**

The partition coefficient of the drug was determined by shaking equal volumes of oil and the aqueous phase in a separating funnel. A drug solution of 1 mg/ml was prepared in distilled water, and 50 ml of this solution was taken in a separating funnel and shaken with an equal volume of octanol for 10 min and allowed to stand for 24hr with intermittent shaking. Then, the aqueous phase was assayed before and after partitioning using a UV spectrophotometer to get the partition coefficient values[9,10].

**Preliminary solubility studies of drug**

10 mg of (API's) was weighed and solubility was checked in 10 ml water, methanol, ethanol, acetone and chloroform.

**Calibration curve**

**Preparation of stock solution**

Standard stock solution of pure drug containing 1000 µg/ml of Isotretinoin and Lincomycin prepared in phosphate buffer 6.8 pH. The working standard solutions of the drug were obtained by dilution of the stocksolution in the distilled water. Series of solutions with conc. 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/ml of Isotretinoin and Lincomycin were used to prepare calibration curve. Solutions were scanned and proposed methods were applied for the determination of area under curve. Methanol and Water 60:40 % v/v was used as blank solution.

**Preparation of sample stock solution:**

Drug equivalent to100 mg was transferred into a 100 ml volumetric flask (1000µg/ml). From this 10 ml was withdrawn and diluted upto 100 ml with solvent. From this further1 ml was diluted up to 10ml and used as stock solution**.**

**Preparation of calibration curve**

From above working std. stock solution of Isotretinoin and Lincomycin (100 µg/ml), pippete out from stock solution 0.2 to 1 ml and transferred to series of 10 ml volumetric flasks and final volume made up to mark with diluent to form solutions of 2 to 10µg/ml of Isotretinoin and Lincomycin separately. These solutions were then scanned in the range of 1800-400 nm against diluent as blank.

**Characterization of Isotretinoin and Lincomycin by FTIR spectroscopy:**

The infra red spectra of the pure drug were recorded by Shimadzu FT-IR spectrometer. Samples were prepared by KBr disc method (2 mg sample in 100 mg KBr) and examined in the transmission mode. Each spectrum was measured over a frequency range of 4000-400 $cm^{-1}$

**Drug excipient compatibility**

The FTIR studies were carried for the pure drug and drug-polymer physical mixture separately with IR-grade KBr in the ratio of 100:1 and corresponding disks were prepared by applying 5.5 metric ton of pressure in a hydraulic press using FTIR spectrophotometer. The disks were scanned over a wave number range of 4000–400cm.

**Formulation and development of niosomes**

**Preparation of niosomes Film hydration method:**

Mixture of surfactant (Span 20, 60, 80) and cholesterol (equivalent to 50 mg) were dissolved in 10 ml of chloroform. The solvent was slowly evaporated using a rotary flash evaporator ( at 80 rpm, 60°C) under low pressure to produce thin lipid film. In another conical flask, weighed amount of drug (according to dose)was transferred and dissolved in required quantity of Phosphate buffer saline (pH 7.4). The mixture was sonicated for 5 min. by the hand and again resonicated for 5 min. The prepared niosomes were allowed to equilibrate at room temperature. Niosomal dispersion was then kept in refrigerator at 4°C. Total 9 batches of niosomes were prepared according to the variant composition of surfactant Span 20,Span 60 and Span 80 with cholesterol shown in Table 3.1. The batches were labelled as NF1 toNF9. All the prepared niosomes were subjected to evaluation for the selection of best batch among the others[9,11].

**Evaluation of niosomes[12,13]**

**Optical microscopy study:**

The particle size of the niosomal suspension was determined by optical microscopy. A drop of niosomal suspension was placed on a glass slide. A cover slip was placed over the noisome suspension and the average vesicle size was measured by an optical microscope (Motic digital microcope) and by using a pre-calibrated ocular eyepiece micrometer. The prepared vesicles were studied under 40X magnification to observe the formation of vesicles.

**Drug content**

Niosomal suspension equivalent to 10mg taken in a volumetric flask of 100 ml and volume was made up by phosphate buffer pH 7.4 after that 1ml of this mixture was diluted to 10ml by phosphate buffer pH 7.4 and the percentage dug content was observed at 291 nm using UV spectrophotometer.

**Estimation of entrapment efficiency**

The Entrapment efficiency of niosomes was estimated by ultra-centrifugation method where the niosomal dispersions were centrifuged at 14000 rotations per minute for 90 minutes. The clear supernatant from the resulting solution was diluted appropriately using phosphate buffer pH 7.4 and analyzed for the drug concentration spectrophotometrically. The percentage encapsulation efficiency(EE%) was calculated using following equation[14].

**Drug Entrapment Efficiency (%) was calculated as follows:**

$EE\% = [(T-C)/T] \times 100$

Where, T = total amount of drug (calculated both in supernatant and sediment) C =amount of drug found only in the supernatant.

**Preparation of isotretinoin and lincomycin niosomal gel**

The composition of gel formulae is designed in Table Isotretinoin(0.05% w/w) and Lincomycin (2% w/w) was dissolved in a hot mixture containing propylene glycol (25% w/w)and glycerin (10% w/w) as moistening agent. The gel formulations were prepared by dispersing weighed amount of polymers Carbopol 940, HPMC, sodium alginate, guargum and xanthan gum in water with constant stirring using magnetic stirrer at a moderate speed. Then, mixture containing drug was added. The pH of gel were adjusted using TEA. Finally, preservatives methyl and propyl paraben were added slowly with continuous stirring. The prepared gels were packed in wide mouth glass containers covered with screw-capped plastic lid. The containers covered with an aluminum foil and were kept in dark and cool place.

| Ingredients | Formulations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 |
| Drug niosomal dispersion | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| HPMC | 2 | 4 | - | - | - | - | - | - | - | - |
| Sodium alginate | - | - | 2 | 4 | - | - | - | - | - | - |
| Carbapol 934 | - | - | - | - | 2 | 4 | - | - | - | - |
| Xanthan gum | - | - | - | - | - | - | 2 | 4 | - | - |
| Guar gum | - | - | - | - | - | - | - | - | 2 | 4 |
| Glycerin | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Methyl Paraben | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Propyl Paraben | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Propylene glycol | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Water up to | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Drug delivery, dyes and paints, cosmetics, adhesives, coatings, agriculture, and textiles. HPMC is also soluble in polar organic solvents, making it possible to use both aqueous and nonaqueous solvents. It has unique solubility properties with solubility in both hot and cold organic solvents. HPMC possesses increased organo-solubility and thermo-plasticity compared to other methyl cellulose counterparts. It forms gel upon heating with gelation temperature of 75–90$^{o}$C.

By reducing the molar substitution of hydroxyl propyl group, the glass transition temperature of HPMC can be reduced to 40$^{o}$C. HPMC forms flexible and transparent films from aqueous solution. HPMC films are generally odorless and taste less and can be effectively used in reducing absorption of oil from fried products such as

French fries because of their resistance to oil migration. It is extensively used in the food industry as a stabilizer, as an emulsifier, as a protective colloid, and as athickener. HPMC is used as a raw material for coatings with moderate strength, moderate moisture and oxygen barrier properties, elasticity, transparency, and resistance to oil and fat. It is also used as a tablet binder and as a tablet matrix for extended release. The potential application of HPMC in biomedical field has attracted great attention of both scientists and academicians because of it sexcellent biocompatibility and low toxicity.

Carbopol polymers are polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol. They are primary produced from polymer having particles of about 0.2 to 6.0 micron in diameter. Carbomer-934 is to be a polymer of acrylic acid cross-linked with alkyl-sucrose: CH=CHCH2-O-sucrose. Carbomer-934P is the pharmaceutical grade of Carbomer-934(Lubrizol,2006).

Molecular weights for Carbomers - 934: Approx. 500,000 to 4,000,000. g(CIR,1979)

Color: White, light, acidic, hygroscopic powder.

Particle size: Flocculated powder having a median diameter of 2 to 7 microns. Solubility/swelling properties: They are insoluble due to their crosslinked nature and high molecular weight. They get swellin water and some polar solvents, producing viscous dispersions.

**Topical Applications:** Carbopol 934 is very well suited to aqueous formulations of the topical dosage forms such as hydrogel. Many commercial topical products available today have been formulated with these polymers. They provide the following plentiful advantage to topical formulations:

Carbopol polymers have a long antiquity of safe and effective use in topical gels, creams and ointments. They are also supported by board toxicology studies.

Carbopol polymers have been shown to have extremely low irritancy properties and are non-sensitizing with repeat usage. —Carbopol polymers provide a magnificent vehicle for drug delivery. Due to their high molecular weight, they are not able to penetrate the skin or affect the therapeutic efficacy of the drug. Xanthan gum soluble in both cold and hot water and is generally not affected by changes in pH value. Xanthan gum will dissolve in most acids or bases. Xanthangumas is with all hydrocolloids bind water.

The viscosity of xanthan gum is stable at low pH values and at high temperatures for a long period of time and is not affected by the addition of large amounts of salt.

By itself, xanthan drastically increases the viscosity (thickness) of any liquid it isadded to in very low concentrations. In high concentrations, it will form a mucusy paste that looks like a gel but is not technically a gel.

Xanthan gum has a synergistic effect in combination with locust bean gum and konjac (gel formation) as well as with guar gum (higher viscosity). The unique rheological and synergistic properties of its aqueous solutions, xanthan gum is used in many applications as a suspending agent and emulsion stabilizer, a foam enhancer or an improver of dough volume. The viscoelasticity between xanthan andlocust bean gums is due to the cross-linking between smooth region of locust beangumand disordered segment of xanthan.

**Evaluation of niosomal gel[15-17]**

**Visual examinations**

All prepared gel formulations were inspected for their color, syneresis, and presence of lumps by visual inspection after the gels have been kept in the containers.

**Homogeneity**

All prepared gels were tested for homogeneity after the gels have been set in the container. They

were tested for their appearance and presence of any aggregates and results for the same were noted.

**Grittiness:** The formulations were evaluated microscopically for the presence of particles if any. No appreciable particulate matter was seen under light microscope.

**Spread ability test:** A sample of 0.5g of each formulation was pressed between two slides(divided into squares of 5mm sides) and left for about 5min where no more spreading was expected. Diameters of spreaded circles formed due to press were measured in cm and were taken as comparative values for spread ability.

**pH determination**

The pH of the formulated gels was determined using digital pH meter(Systonic). Readings noted.

**Viscosity studies**

The measurement of viscosity of the prepared gels was done using Brookfield viscometer. The gel was evaluated using spindle no. 64.

**Extrudability**

The prepared gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 gwas placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The amount of gel extruded was calculated (>90%extrudability:Excellent,>80%extrudability:Good,and>70% extrudability: Fair).

***In-vitro* Release of Niosome Gel**

*In vitro* release study was performed using Modified-Franz diffusion cell. Niosomal gel formulation (0.045% w/w) was used in the study. Phosphate buffered saline (0.01M,pH7.4) was used as a release medium(acceptor compartment). Gel sample (1 g) was placed on a cellulose nitrate membrane (0.1 mmpore diameter), which acted as a diffusion barrier (donor compartment). The assembly was water jacketed to maintain 32±0.5 $^{0}$C. Aliquots of samples were withdrawn at different time intervals during a time period of 48 h and were analyzed using the validated HPLC procedure.

**Stability studies**

The purpose of stability testing is to provide evidence on how the quality of an active substance or pharmaceutical product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light. In any, rationale design and evaluation of dosage forms for drugs, the stability of the active component is the major criteria in determining their acceptance or rejection. During the stability studies the product is exposed to normal conditions of temperature and humidity. However, the studies take a longer time and hence it would be convenient to carry out the accelerated stability studies where the product is stored under extreme conditions of temperature. To assess the drug and formulation stability, stability studies were done according to ICH guidelines. As per ICH requirements, stability testing of the optimized gel formulation was carried out. Gel was packed in clean, lacquered, collapsible aluminum tubes and different replicates were held in a humidity chamber at 25 ± 2 ° C and 60 ± 5% RH. Gel was evaluated at an interval of 0 - 6months for alteration of appearance, pH, and drug content and in vitro release profile.

**Results and Discussion**
**Preformulation studies**

**Melting point**

Isotretinoin 172-177°C
Lincomycin 156-158°C
**Partition coefficient**
Isotretinoin: 2.24
Lincomycin: 5.94
**Solubility studies of drug**

**Table 4.1: Solubility analysis data of drugs**

| Solvent | Isotretinoin | Lincomycin |
|---|---|---|
| Water | Insoluble | 90mg/ml |
| DMSO | 60mg/ml | 89mg/ml |
| DMF | 4mg/ml | 20mg/ml |
| Ethanol | 8mg/ml | 0.8mg/ml |
| Chloroform | 50mg/ml | 25mg/ml |

**Calibration curve of Isotretinoin**

The calibration graph for Isotretinoin was generated using Isotretinoin 5-30 µg / mL solution in PBS 6.8 pH and measured the absorbance at 344nm. Figure 4.1 showed the calibration graph for Isotretinoin. Table 4.2 showed the absorbance achieved for the given concentrations. A regression equation Y = 0.0046X + 0.142 and $R^2$ value 0.9994 is shown in the calibration curve (Table 4.2). The result found that the concentration of drugs around 5-30 µg / ml followed the law of Beer Lambert, as the coefficients of regression was 0.9994.

**Calibration curve of Lincomycin**

The calibration graph for Lincomycin was generated using Lincomycin 5-30 µg / mL solution in PBS 6.8 pH. They measured the absorbance at 196nm. Figure 4.2 showed the calibration graph for Isotretinoin. Table 4.2 showed the absorbance achieved for the given concentrations. A regression equation Y = 0.0046X + 0.1316 and $R^2$ value 0.9993 is shown in the calibration curve (Table 4.2). The result found that the concentration of drugs around 5-30 µg / ml followed the law of Beer Lambert, as the coefficients of regression was 0.9993.

**Table 4.2: Absorption of Isotretinoin in Phosphate Buffer 6.8pH**

| Conc. µg/ml | Absorbance | |
|---|---|---|
| | Isotrentinoin | Lincomycin |
| 20 | 0.231 | 0.219 |
| 40 | 0.345 | 0.324 |
| 60 | 0.435 | 0.417 |
| 80 | 0.511 | 0.499 |
| 100 | 0.606 | 0.589 |

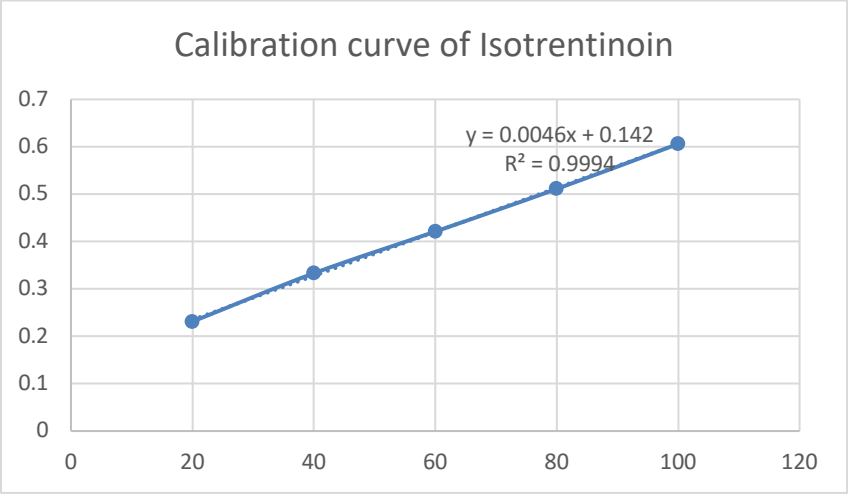


**Figure 4.1:** Calibration curve of Isotretinoin in Phosphate Buffer 6.8pH

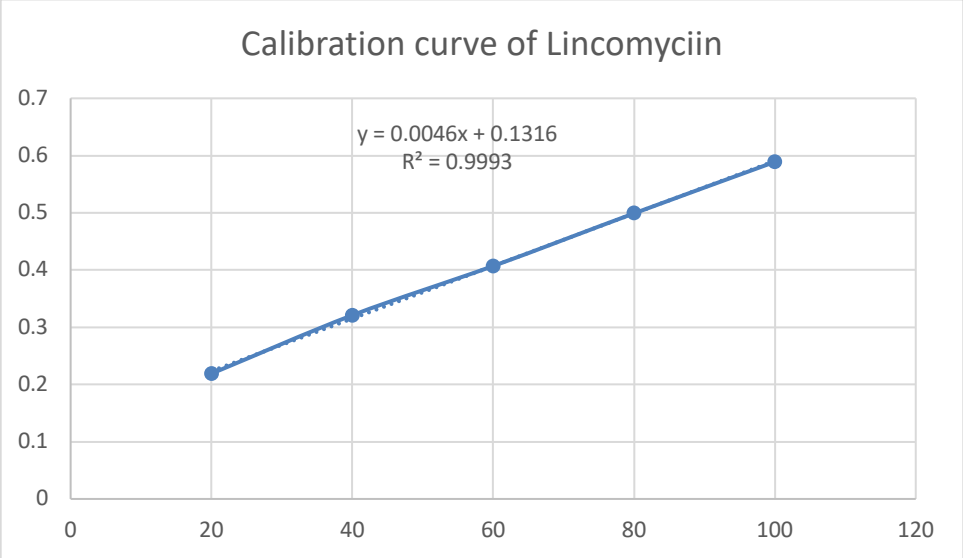


**Figure 4.2:** Calibration curve of Lincomycin in Phosphate Buffer 6.8pH

**Characterization of Isotretinoin and Lincomycin by FTIR spectroscopy:**

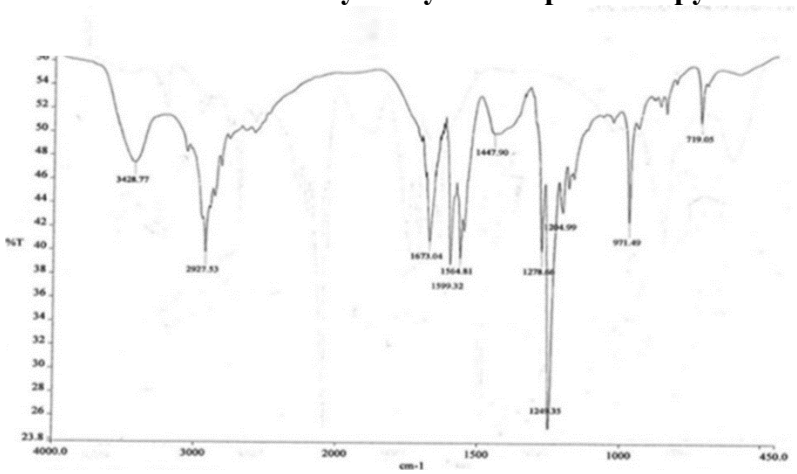


**Figure no. 4.3:** FTIR Spectra of Isotretinoin

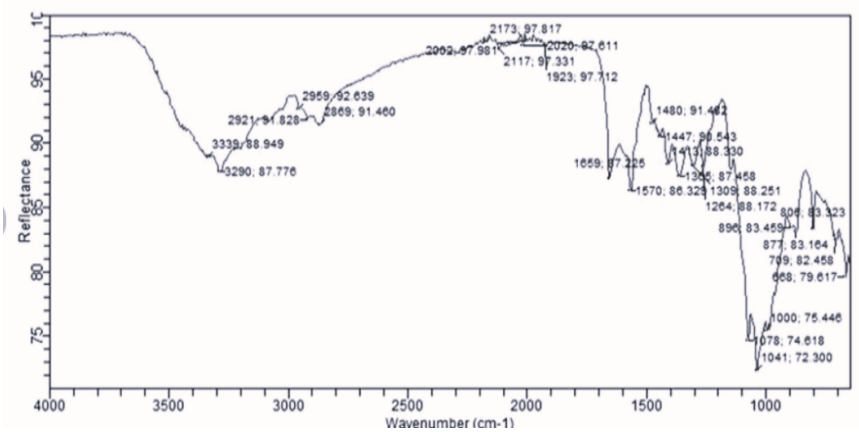


**Figure no. 4.4:** FTIR Spectra of Lincomycin

**Drug excipient compatibility**

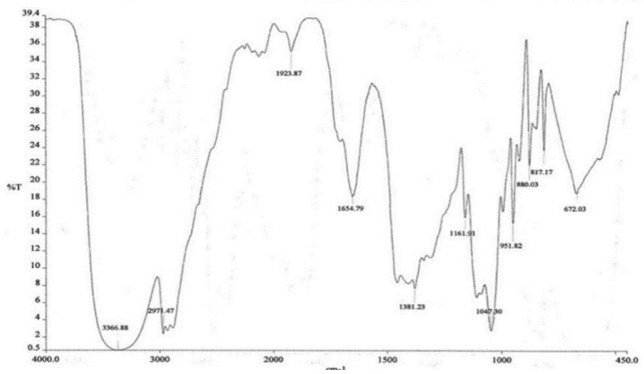


**Figure no. 4.5:** FTIR Spectra of Isotretinoin + Excipient

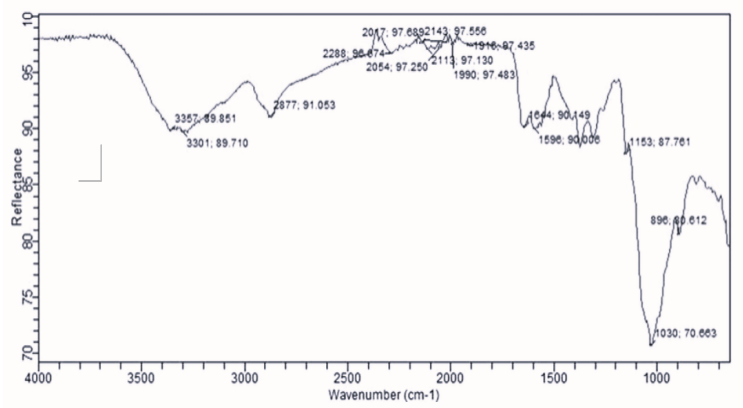


**Figure no. 4.6:** FTIR Spectra of Lincomycin+ Excipient

**Evaluation of niosomes**

**Vesicle size**:

Details of Isotetinoin and lincomycin particle sizes shows that Vesicle shaped with Span 20 is smaller than Span 80 and Span 60 shaped vesicle. When diffusion was agitated vesicle size was that. The explanation for this is the energy exerted in agitation that results in larger vesicles splitting into smaller vesicles. The size range was found to be between 67.9 nm and 121.5 nm. It was evident that due to increase in the concentration of cholesterol the vesicle is increasing due to increasing the hydrophobicity in the formulation. Among them batch F6 121.5 nm followed by F5 111.1 nm and F4 100.2 nm. Batch F1 was found to have smallest particle size of niosomes 67.9 nm.

**Table 4.3 Vesicle size of niosomes**

| Formulation | Vesicle size (in nm) |
|---|---|
| F1 | 67.9 |
| F2 | 69.1 |
| F3 | 72.5 |
| F4 | 100.2 |
| F5 | 111.1 |
| F6 | 121.5 |
| F7 | 98.7 |
| F8 | 98.6 |
| F9 | 97.1 |

**Drug content**

All the batches of niosomes prepared were evaluated for yield of drug content, results shows that higher vesicle size given higher drug content.. Among all the nine batches, F5 batch was found to have maximum drug content i.e. 99.3 ± 1.1 mg isotretinoin and 98.8±1.1 lincomycin.

**Table 4.4 Drug content of niosomes**

| Formulation | Drug content (%) | |
|---|---|---|
| | Isotretinoin | Lincomycin |
| **F1** | 83.0±1.2 | 82.1±1.2 |
| **F2** | 89.5±1.5 | 88.4±1.5 |
| **F3** | 91.9±2.5 | 90.8±2.5 |
| **F4** | 90.2±1.5 | 90.5±1.5 |
| **F5** | 99.3±1.1 | 98.8±1.1 |
| **F6** | 93.8±1.4 | 92.6±1.4 |
| **F7** | 93.2±1.4 | 93.4±1.4 |
| **F8** | 89.8±1.7 | 88.8±1.7 |
| **F9** | 85.1±1.1 | 84.2±1.1 |

**SEM**

The optimized batch of niosome F-5 having maximum drug content was analyzed for surface morphology SEM. The SEM image has demonstrated the discretion of niosomes prepared using Film hydration method. SEM of formulation F-5 showed the smooth surface of niosomes formed. The results is assured by the results of another study done by Zerrin Sezgin-Bayindir & NiluferYuksel, 2012 with an aim to investigate the effects of formulation and process variables on the properties of niosomes formed from Span 40 as nonionic surfactant.

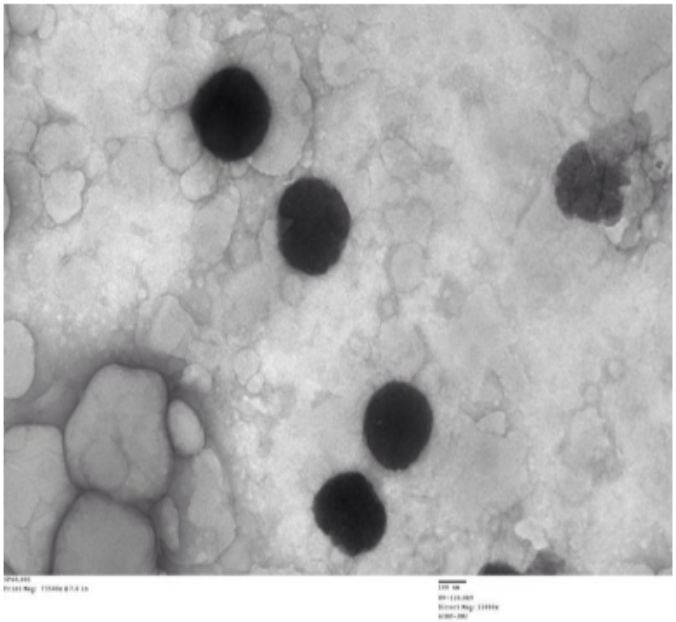

**Figure 4.7 SEM of noisome formulation (F5)**

**Drug Entrapment Efficiency:**

The entanglement productivity of the niosomal definitions were estimated by centrifugation strategy. The components, for example, HLB worth and Phase progress temperature of the surfactant influence the entanglement productivity. Low HLB and high change temperature increment the capture proficiency. Among all the details, F5 (Span 60/cholesterol S/C 0.5:1.0) indicated most extreme capture proficiency when contrasted and different definitions as appeared. It was due to its low HLB value and high transition temperature. F5 formulation corresponds to the higher drug content and entrapment efficiency 98.6%.

**Table 4.5 Entrapment efficiency of niosomes**

| Formulation | Entrapment Efficiency (%) |
|---|---|
| F1 | 80.5±1.2 |
| F2 | 85.9±1.3 |
| F3 | 90.9±2.1 |
| F4 | 93.1±1.2 |
| F5 | 98.6±2.1 |
| F6 | 96.2±2.1 |
| F7 | 88.3±1.6 |
| F8 | 87.8±1.3 |
| F9 | 86.1±1.5 |

**Formulation of niosomal gel**

From the results of evaluation of batches of niosomal formulations, batch F5 is found to have with optimized results and suitable found suitable for further process. Niosomes of batch F5 is used for the formation of gel using different compositions.

**4.3 Evaluation of niosomal gel**

**Clarity, Spreadibility and Homogeneity**

Gel preparations were than evaluated as per the clarity, its spreadability and homogeneity. Among all the formulations the gel formed G5 and G6 were found to be clearest with better spreadability and with excellent homogeneity.

**Table 4.6 Evaluation of niosomes gel (Clarity, Spreadability, Homogeneity)**

| Formulation | Clarity | Spreadability | Homogeneity |
|---|---|---|---|
| G1 | Good | better | excellent |
| G2 | Good | better | excellent |
| G3 | Good | good | good |
| G4 | Good | good | good |
| G5 | very clear | better | excellent |
| G6 | very clear | better | excellent |
| G7 | Turbid | average | good |
| G8 | Turbid | average | good |
| G9 | Turbid | poor | good |
| G10 | Turbid | poor | good |

**Viscosity:**
Viscosity of all the niosomal gel formulation was evaluated. Formulation F6 and F5 were found to be highest viscosity among all the other formulation batches i.e. 8165 cPs and 8022 cPs respectively. This proves the better retention of gel formulation on the skin. Formulation F3 and F4 found to be least viscous with viscosity 5105 cPs and 5156 cPs respectively. F6 was reported with highest viscosity and would have higher retention time on the skin.

**Extrudability**
Through the test it was evident though all the formulations showed good extrudability properties but among them formulations F6, F5, F4 and F3 showed excellent extrudability property.

**pH:**
Found in the range of 6.1-6.3.

**Table 4.7 Evaluation of niosomes gel (Viscocity, Extrudability, pH)**

| Formulation | Viscocity (in cps) | Extrudability | pH |
|---|---|---|---|
| G1 | 7871 | ++ | 6.2 |
| G2 | 6974 | ++ | 6.2 |
| G3 | 5105 | +++ | 6.1 |
| G4 | 5156 | +++ | 6.1 |
| G5 | 8022 | +++ | 6.3 |
| G6 | 8165 | +++ | 6.3 |
| G7 | 6581 | ++ | 6.2 |
| G8 | 5920 | ++ | 6.2 |
| G9 | 5235 | ++ | 6.2 |
| G10 | 5236 | ++ | 6.2 |

Good= ++, Excellent = +++

***In vitro* drug release**
Niosomal gel G6 is released in vitro and shows the release profiles. Gel, however, demonstrated a controlled release profile of 94.6% isotretinoin and 93.7% lincomycin over the 48 h period. Controlled release profile of niosome gel may be due to slow diffusion of isotretinoin and lincomycin in the Carbopol matrix from lipid niosome. Controlled release phenotype of Niosome Gel will avoid the fast delivery of the drug to the exfoliated psoriatic skin, which is sensitive to accelerated absorption of drugs and can thus avoid toxicity.

**Table 4.8 *Invitro* drug release of Isotretinoin from niosomes gel**

| Formulation | Isotretinoin Drug release (in %) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 45 min | 90 min | 3 hr | 6 hr | 12 hr | 24 hr | 36 hr | 48 hr |
| G1 | 12.2 | 18.2 | 26.4 | 28.2 | 32.4 | 36.2 | 54.2 | 68.4 |
| G2 | 13.2 | 16.4 | 26.8 | 29.1 | 34.6 | 40.4 | 58.2 | 75.2 |
| G3 | 11.2 | 15.2 | 22.3 | 27.8 | 31.6 | 35.2 | 51.2 | 64.8 |
| G4 | 11.1 | 15.3 | 23.2 | 27.9 | 31.8 | 34.6 | 45.8 | 65.2 |
| G5 | 13.2 | 16.5 | 22.5 | 31.2 | 34.6 | 36.8 | 44.1 | 78.8 |
| **G6** | **14.5** | **18.1** | **24.5** | **34.5** | **38.8** | **44.5** | **75.2** | **94.6** |
| G7 | 12.1 | 18.3 | 26.4 | 27.9 | 32.8 | 34.7 | 59.4 | 68.2 |
| G8 | 11.3 | 15.2 | 24.1 | 26.8 | 31.5 | 32.6 | 57.2 | 65.7 |
| G9 | 10.8 | 12.5 | 22.1 | 24.2 | 28.5 | 34.1 | 58.3 | 62.8 |
| G10 | 11.1 | 12.3 | 22.5 | 24.8 | 27.1 | 31.2 | 50.2 | 64.2 |

**Table 4.9 *Invitro* drug release of Lincomycin from niosomes gel**

| Formulation | Lincomycin Drug release (in %) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **45 min** | **90 min** | **3 hr** | **6 hr** | **12 hr** | **24 hr** | **36 hr** | **48 hr** |
| G1 | 11.1 | 17.3 | 25.6 | 27.4 | 31.6 | 35.4 | 53.3 | 67.5 |
| G2 | 12.1 | 15.5 | 26 | 28.3 | 33.8 | 39.6 | 57.3 | 74.3 |
| G3 | 10.1 | 14.3 | 21.5 | 27 | 30.8 | 34.4 | 50.3 | 63.9 |
| G4 | 10 | 14.4 | 22.4 | 27.1 | 31 | 33.8 | 44.9 | 64.3 |
| G5 | 12.1 | 15.6 | 21.7 | 30.4 | 33.8 | 36 | 43.2 | 77.9 |
| **G6** | **13.4** | **17.2** | **23.7** | **33.7** | **38** | **43.7** | **74.3** | **93.7** |
| G7 | 11 | 17.4 | 25.6 | 27.1 | 32 | 33.9 | 58.5 | 67.3 |
| G8 | 10.2 | 14.3 | 23.3 | 26 | 30.7 | 31.8 | 56.3 | 64.8 |
| G9 | 9.7 | 11.6 | 21.3 | 23.4 | 27.7 | 33.3 | 57.4 | 61.9 |
| G10 | 10 | 11.4 | 21.7 | 24 | 26.3 | 30.4 | 49.3 | 63.3 |

**Stability Studies:**

The optimized Niosomal Gel formulation G6 was subjected to stability studies over the period of 6 months. The formulation was evaluated for pH and % drug release over the period. The formulation was attributed to have average 94.6 and 93.7% drug release at the starting 0 month, which found to be 94.1 and 93.1 % at the 6th month. There is no significant difference between the drug releases in mean time interval. In the duration of the study the pH of the formulation remained constant.

**Table 4.10 Stability studies of optimized formulation G6**

| **Parameter** | **Drug** | **0** | **1** | **2** | **3** | 6 |
|---|---|---|---|---|---|---|
| **pH** | Isotretinoin | 6.1 | 6.1 | 6.2 | 6.3 | 6.3 |
| **Drug release** | Isotretinoin | 94.6 | 94.2 | 94.2 | 94.1 | 94.1 |
| | Lincomycin | 93.7 | 93.7 | 93.5 | 93.4 | 93.1 |

**Conclusion**

Depending the evidences given in literature drug Isotretinoin and Lincomycin were selected for the study. The drug was procured and subjected to physical characterisation and through spectral methods like UV and IR.

Drug was standardised to check the purity and found that the calibration curve of UV absorption ranges in 344 and 196 nm for Isotretinoin and Lincomycin respectively. The drug solution of different concentration showed linear relationship with an R2 value of 0.999 for both drugs. Characterization of the drug through FTIR showed corresponding peaks structural elements of for Isotretinoin and Lincomycin, which also confirmed them.

Isotretinoin and Lincomycin mixture with polymers like Carbopol, HPMC and SPAN were subjected to the analysis by FTIR for their interaction with drug. The results showed that there was no interaction found between them.

Niosomes of the drug were prepared using various surfactant: cholesterol ratio and total 9 batches of niosomes were prepared. The prepared batched were than subjected to characterization in order to identify the optimized batch for further use of preparation of Niosomal gel. And as per the results of particle size, drug content and entrapment efficiency the F-5 batch was found most suitable and further used for the preparation of gel batches.

Total 10 batches of the niosomal gel were formulated and characterized. As per the characterization parameters batch G-6 was found as optimized formulation, which is further subjected to stability studies and pharmacological studies. The stability studies showed a non-changing parameters over the

course of study of 6 months. There was no significant change observed.

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