International Journal of Science and Research Archive

eISSN: 2582-8185

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

Check for updates

# Formulation development, optimization, and characterization of anti-fungal topical biopolymeric film using a niosomal approach

Sukanya Patil * and Jaya Agnihotri

*Department of Pharmaceutics, H. K. College of Pharmacy, Oshiwara, Mumbai, India.*

International Journal of Science and Research Archive, 2023, 08(01), 194–209

Publication history: Received on 27 November 2022; revised on 07 January 2023; accepted on 10 January 2023

Article DOI: https://doi.org/10.30574/ijsra.2023.8.1.0031

## Abstract

**Background**: Griseofulvin is an antifungal drug that is currently available in the market only in oral dosage forms. So, the development of topical treatment could be advantageous for the treatment of superficial fungal infections. Though for superficial fungal treatment, the skin acts as a major target as well as a principal barrier for drug delivery. To overcome this the colloidal carrier system niosome was used. Niosomes being in the nanometer size range would allow the delivery of the drug at the desired site. Niosomes being non-ionic surfactant-based vehicles would facilitate the passage of the drugs through as skin is composed of both lipid and aqua, which would create problems for any other delivery system.

**Methodology**: Griseofulvin belonging to the BCS Class II was formulated in the form of niosomes to enhance the drug's solubility. In this study, optimization of niosomal formulation was done using OVAT (one variable at a time) method. Here, the CMAs (critical material attributes) such as surfactant type, conc. of charge inducer and the ratio of surfactant: cholesterol and CPPs (critical processing parameters) such as rotational speed of evaporator flask, external phase temperature, hydration time, and external phase volume (both aqueous and organic), which are independent variables influencing factors at different levels. These are said to have a potential risk on the CQAs (critical quality attributes), which are dependent variables, such as vesicle size, vesicle shape, vesicle lamellarity, niosome aggregation, and drug entrapment efficiency, for the final selection of improved optimum batch. The films were prepared from the incorporation of griseofulvin-loaded optimized niosomes in chitosan film for topical drug delivery in superficial fungal infections.

**Characterization**: The films properties were characterized by physical appearance, film thickness, weight variation, folding endurance, tensile strength, moisture, uptake, moisture content, drug content uniformity, *In-vitro* drug diffusion studies, *ex-vivo* studies, and antifungal efficacy against *Candida albicans sp*.

**Result and Discussion**: Thus, chitosan film formulation integrating griseofulvin-loaded niosomes for topical delivery enhanced the solubility of the drug and avoided the side effects associated with the orally-administered marketed formulation.

**Conclusion**: Biopolymer chitosan exhibited antifungal activity implying enhanced drug efficacy. Therefore, two concepts of using optimized vesicular carrier systems and biopolymeric films have been combined and this topical novel composite film having the potential for griseofulvin delivery to superficial fungal infections has been formulated.

**Keywords:** Niosomes; Griseofulvin; Optimization; Chitosan; Topical film

* Corresponding author: Sukanya Pati

## 1. Introduction

Superficial fungal infections are among the most common infections mainly caused by dermatophytes belonging to the genera *Epidermophyton sp*, *Microsporum sp*, and *Trichophyton sp*. Griseofulvin is FDA (Food and Drug Administration) approved and the drug of choice against the superficial fungal infection caused by dermatophytes such as tinea capitis as well as other indications such as onychomycosis [1]. But griseofulvin is currently available only in oral dosage forms [2]. The antifungal agent griseofulvin is a poorly water-soluble drug, and its absorption from the oral route is also poor [3,4], as a result, failure in providing an effective plasma drug profile on conventional oral administration [5]. The large dose and frequent administration of griseofulvin may lead to contraindicative manifestation and side effects [6]. Side effects that may arise include proteinuria, nephrosis, leukopenia, hepatitis, clotting disorders, liver enzyme elevation, hyperbilirubinemia, and bleeding in the digestive tract [7]. Since the dermatophytes infect the top layers of the skin, the topical treatment provides an alternative route to target the drug directly to the skin, also it circumvents the systemic side effects associated with oral administration, and increases patient compliance [2].

In the past few decades, considerable attention has been focused on the development of a new drug delivery system [8]. Drug delivery system using colloidal particulate carriers, such as liposomes [9] or niosomes [10], has distinct advantages over conventional dosage form. Niosomes are now widely studied as an alternative to liposomes because they alleviate the disadvantages associated with liposomes, such as chemical instability, variable purity of phospholipids, and high cost [11,12]. Niosomes attract much attention because of their advantages in many aspects, such as chemical stability, high purity, content uniformity, low cost, convenient storage of non-ionic surfactants, and large numbers of surfactants available for the design of niosomes [13]. Niosomes are promising vehicles for drug delivery. The encapsulation of drugs in niosomes can minimize drug degradation and inactivation after administration; prevent undesirable side effects [14]. Chitosan has been extensively evaluated as a drug delivery system in different forms of particles, gels, and films. The film-forming property of chitosan has made it interesting for transdermal/dermal drug delivery [15]. Furthermore, chitosan has been reported to have broad-spectrum antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and fungi [16,17].

The main goal of the study is to formulate a topical drug delivery system using a niosomal approach, in which the antifungal drug griseofulvin is embedded in a biopolymeric (chitosan) film [18]. The developed formulation would solve the solubility problem of the drug. The secondary aim is to facilitate the usage of a topical delivery to overcome systemic side effects and also provide high efficacy due to the synergistic effect of chitosan and griseofulvin. Resulting in the improved anti-fungal activity of the formulation.

## 2. Material and methods

### 2.1. Materials

Griseofulvin (98.37% purity) was purchased from Shah Enterprises via Carbanio, Mumbai. Stearylamine (Octadecylamine, 97% purity) (used as Charge Inducer) used as charge inducer was purchased from Hychem Laboratories, Hyderabad. All other chemicals and reagents used in the study were of the analytical grade and obtained from Vishal chemicals, Mumbai.

*Candida albicans sp* strain slants (ATCC 10231) were obtained from Naprod Lifesciences, Mumbai India.

### 2.2. Methods

#### *2.2.1. Preparation of niosomes*

Empty niosomes were prepared by using non-ionic surfactant Span 60, Cholesterol, and a charge inducer as Stearylamine. Using 2 different methods of preparation: The thin film hydration method reported by Jain *et al.* [19] and the Ether injection method reported by Satturwar *et al*. [20], with slight modification was followed for the preparation of niosomes. According to the morphology of vesicles observed in Results section 3.1. Ether Injection method was eliminated and then Drug loaded niosomes were prepared by the thin film hydration method by altering the CMAs and CPPs for optimization and to obtain the final improved batch of niosome.

#### *2.2.2. Optimization of griseofulvin niosomes*

Optimization of niosomes was carried out by applying OVAT analysis via an experimental design table. Here, the CMAs such as Surfactant type, Conc. of Charge inducer (stearylamine (SA)) and Ratio of Surfactant: Cholesterol and CPPs such as Rotational speed of evaporator flask, External phase temperature, Hydration time, External phase volume (both

Aqueous and Organic), which are independent variables as influencing factors at different levels. These are said to have a potential risk on the Critical quality attributes (CQAs), which are dependent variables, such as Vesicle size, Vesicle shape, Vesicle lamellarity, Niosome aggregation, Polydispersity Index, and Drug entrapment efficiency, for final selection of improved optimum batch. The operating ranges for the variables were optimized in a way that they pose minimum risk to the CQAs by One Variable at a Time (OVAT) optimization [21].

**Table 1** Critical Material Attributes (CMAs)

| Sr. No. | Variables (Factors) | Parameters (Levels) |
|---|---|---|
| 1. | Surfactant Type | Span 20 |
| | | Span 60 |
| | | Span 80 |
| 2. | Conc. of Charge Inducer (SA) (For 10 mg surfactant–lipid mixture) | 0.15 mg |
| | | 0.25 mg |
| | | 0.35 mg |
| 3. | Surfactant: Cholesterol | 1: 1 |
| | | 1: 2 |
| | | 2: 1 |

**Table 2** Critical Processing Parameters (CPPs)

| Sr. No. | Variables (Factors) | Parameters (Levels) |
|---|---|---|
| 1. | Speed of Evaporation Flask (RPM) | 80 |
| | | 100 |
| | | 120 |
| | | 140 |
| 2. | External Phase Temperature (°C) | 50 |
| | | 55 |
| | | 60 |
| | | 65 |
| | | 70 |
| 3. | Hydration Time (minutes) | 40 |
| | | 45 |
| | | 60 |
| | | 75 |
| 4. | Solvent/External Phase Volume (ml.) | 20 |
| | | 15 |
| | | 10 |
| | | 05 |

OVAT optimization was done for all the variables which had a medium to high risk of affecting the CQAs. In OVAT analysis, only one variable was studied at a time while the other variables are kept constant. The variables that were successfully optimized by this analysis were the surfactant type, conc. of charge inducer, ratio of surfactant: cholesterol, rotational speed of evaporator flask, external phase temperature, hydration time, and external phase volume (both aqueous and organic). Different batches of niosomes were prepared by altering the levels of CMAs and CPPs selected as per experimental design table (3) following OVAT analysis [22, 23].

**Table 3** Experimental Design Table

| | **CMA'S** | | | **CPP's** | | | |
|---|---|---|---|---|---|---|---|
| **Batch ID #** | **Surfactant Type** | **Conc. of SA (mg)** | **Surfactant: Cholesterol** | **Speed of Evaporation Flask (RPM)** | **External Phase Temperature (°C)** | **Hydration Time (minutes)** | **Solvent/External Phase Volume (ml)** |
| | (A) | (B) | (C) | (D) | (E) | (F) | (G) |
| Gr 1 | Span 20 | 0.15 | 1:1 | 120 | 60 | 45 | 10 |
| Gr 2 | Span 60 | 0.15 | 1:1 | 120 | 60 | 45 | 10 |
| Gr 3 | Span 80 | 0.15 | 1:1 | 120 | 60 | 45 | 10 |
| Gr 4 | Span 60 | 0.15 | 1:1 | 120 | 60 | 45 | 10 |
| Gr 5 | Span 60 | 0.25 | 1:1 | 120 | 60 | 45 | 10 |
| Gr 6 | Span 60 | 0.35 | 1:1 | 120 | 60 | 45 | 10 |
| Gr 7 | Span 60 | 0.25 | 1:1 | 120 | 60 | 45 | 10 |
| Gr 8 | Span 60 | 0.25 | 1:1 | 120 | 60 | 45 | 10 |
| Gr 9 | Span 60 | 0.25 | 2:1 | 120 | 60 | 45 | 10 |
| Gr 10 | Span 60 | 0.25 | 2:1 | 80 | 60 | 45 | 10 |
| Gr 11 | Span 60 | 0.25 | 2:1 | 100 | 60 | 45 | 10 |
| Gr 12 | Span 60 | 0.25 | 2:1 | 120 | 60 | 45 | 10 |
| Gr 13 | Span 60 | 0.25 | 2:1 | 140 | 60 | 45 | 10 |
| Gr 14 | Span 60 | 0.25 | 2:1 | 120 | 50 | 45 | 10 |
| Gr 15 | Span 60 | 0.25 | 2:1 | 120 | 55 | 45 | 10 |
| Gr 16 | Span 60 | 0.25 | 2:1 | 120 | 60 | 45 | 10 |
| Gr 17 | Span 60 | 0.25 | 2:1 | 120 | 65 | 45 | 10 |
| Gr 18 | Span 60 | 0.25 | 2:1 | 120 | 70 | 45 | 10 |
| Gr 19 | Span 60 | 0.25 | 2:1 | 120 | 60 | 30 | 10 |
| Gr 20 | Span 60 | 0.25 | 2:1 | 120 | 60 | 45 | 10 |
| Gr 21 | Span 60 | 0.25 | 2:1 | 120 | 60 | 60 | 10 |
| Gr 22 | Span 60 | 0.25 | 2:1 | 120 | 60 | 75 | 10 |
| Gr 23 | Span 60 | 0.25 | 2:1 | 120 | 60 | 60 | 20 |
| Gr 24 | Span 60 | 0.25 | 2:1 | 120 | 60 | 60 | 15 |
| Gr 25 | Span 60 | 0.25 | 2:1 | 120 | 60 | 60 | 10 |
| Gr 26 | Span 60 | 0.25 | 2:1 | 120 | 60 | 60 | 5 |

*2.2.3. Characterization of griseofulvin niosomes (gr 1-gr 26)*

The above batches were evaluated based on the critical quality attributes:

Vesicle Size Analysis

The size of niosome vesicles in non-sonicated formulations of all the above batches was observed by using digital microscopy [24].

Vesicle Morphology (Shape, Lamellarity, and Aggregation)

The vesicle shape, lamellar nature, and whether the aggregation has occurred or not in non-sonicated formulations of all the above batches were observed by digital microscopy under 10X power and zoomed images were captured.

Entrapment Efficiency (EE %)

Griseofulvin niosomal formulation of every individual batch was centrifuged at 15,000× speed for 60 min. at 4 °C using a refrigerated centrifuge (Eppendorf, 5415 R, Germany) to separate niosomes from the non-entrapped drug. The concentration of the free drug in the supernatant was determined by measuring absorbance at 291 nm with a UV spectrophotometer (Shimadzu, UV 1650 PC, Kyoto, Japan). The percentage of drug entrapment in niosomes was calculated using the below-mentioned formula [25].

$$\% \text{ Drug Entrapped} = [\text{Total drug} - \text{Drug in supernatant} / \text{Total drug}] \times 100$$

*2.2.4. Characterization of griseofulvin niosomes (gr 25)*

Microscopic Analysis

The Microscopic analysis was done using FE-SEM ((Field Emission Scanning Electron Microscopes) [26] and Digital Microscopy [24].

Zeta Analysis and Poly-dispersibility Index [27]

The Zeta Analysis was performed using Zetasizer and particle size analyzer Malvern Zetasizer Nano ZS, which also gave the Poly-dispersibility index.

Entrapment Efficiency (EE%) [25]

The procedure followed is same as described under 2.2.3 C.

Viscosity

The viscosity of the formulation was determined [28] using a Brookfield Viscometer (LV Brookfield DV-E) at room temperature.

Osmotic Shock

The effect of osmotic shock on niosomal formulation was investigated by monitoring the change in vesicle diameter after incubation of niosome suspensions in media of different tonicity [29]: 1.2% NaCl (hypertonic), 0.9% NaCl (normal), and 0.5% NaCl (hypotonic). Suspensions were incubated in these media for 3 h and the change in vesicle size was measured by optical microscopy with a calibrated eyepiece micrometer.

*2.2.5. Preparation of griseofulvin niosomal film*

The films were prepared using casting and solvent evaporation. Chitosan solution (Cs) (3% w/v) were prepared by dispersing chitosan in acetic acid solution (1.8% v/v). Blank chitosan films, niosomes containing a certain amount of griseofulvin, and also only griseofulvin as a pure drug at a final concentration of 0.3% w/v were mixed with Cs (v/v), vortex, and sonicated. In this Propylene Glycol was also used, as a plasticizer. About 20 mL of the mixture was placed in a glass Petri dish (9 cm diameter) and dried at 45 °C for 48 h and then peeled off [30, 31].

*2.2.6. Characterization of griseofulvin niosomal film*

Physical Appearance

Formulated films were evaluated for their physical appearance, uniformity, or entrapment of any air bubble, which on a large part determines the patient acceptability of the film.

Film Thickness

The thickness of the film was measured by using a Mitutoyo Digimatic Micrometer. The thickness of the film was determined at five different points and the average thickness was calculated.

Weight Variation

Weight variation was studied by individually weighing 10 randomly selected films and the average weight was calculated. The individual weight should not deviate significantly from the average weight [32].

Folding Endurance

Evaluation of folding endurance involves determining the folding capacity of the films. Folding endurance is determined by repeatedly folding the film at the same place until it breaks. The number of times the film could be folded at the same place without breaking is the folding endurance value [32].

Tensile Strength

Mechanical properties of the biopolymeric film were conveniently determined by measuring the tensile strength. The tensile strength of the film was determined by using an assembly consisting of a pan hanged by using a strong thread and the other end of the thread was attached to the center of the film. The whole assembly was held with a beam balance and weights were kept on the pan. Weight required to break the patch was noted. Tensile strength was then calculated using the following formula: Tensile Strength= Break Force/ a.b(1+ ΔL/L) [32].

Moisture Uptake: [33]

5 films were kept in a desiccator at room temperature for 24hrs. The film was then taken out and exposed to 84% relative humidity using saturated solution of Potassium chloride in a desiccator until a constant weight is achieved. The % moisture uptake was calculated by using following formula,

$$\% \text{ Moisture uptake} = (\text{Final wt.-Initial wt./initial wt.}) 100.$$

Moisture Content: [34, 35]

The prepared films were weighed individually and kept in a desiccator containing calcium chloride at room temperature for 24hrs. The films were weighed again after a specified interval until they show a constant weight. The percent moisture content was calculated using the following formula,

$$\% \text{ Moisture uptake} = (\text{Initial wt.-Final wt./Final wt.}) 100$$

Drug Content Uniformity

Amount of drug entrapped in a film was determined by completely dissolving a film of size 2x2 cm2 in 100ml phosphate buffer solution (pH 6.8). Complete dissolution was achieved by placing the solution containing film on an orbital shaker for about 24 hrs. The solution was then filtered and drug content was estimated spectrophotometrically at 291nm after suitable dilution.

*In-vitro* Drug Diffusion Studies: [36]

This is carried out in order to determine transition of drug from film to skin microcirculation. In this study, synthetic membrane like cellulose (pore size 0.45 um) was placed between the donor and receptor compartment of Franz diffusion cell. Receptor compartment was filled with phosphate buffer of pH 7.4. Film was placed upon the cellulose membrane facing towards the donor compartment. The other side of cellulose membrane was towards the receptor compartment having phosphate buffer. The receiver compartment was maintained at normal body temperature (37 °C) and was continuously stirred with the help of magnetic stirrer. 1 mL of receiver medium was withdrawn at 0.25, 0.5, 0.75, 1, 2, 4 and 6 h and replaced with the same volume of blank receiver medium solution.to maintain volume of

receptor compartment at a constant level. Samples withdrawn were then analysed for their absorbance at 291 nm and concentration was then calculated. Graph was then plotted between % drug release and time interval which compares % drug release from niosomal film, pure drug film and marketed formulation.

Anti-Fungal Studies: [37]

*Candida albicans sp* slant species (ATCC 10231) were obtained from the microbiology department of the Naprod lifesciences, Mumbai. 5ml of Sabouraud Dextrose Broth (SDB) was added to the slant and incubated for 24hrs for the growth of *Candida albicans sp*.

The antifungal activity of the following samples was determined:

- Pure Drug
- Niosome Suspension
- Chitosan Blank film
- Niosome-embedded film
- Marketed Formulation

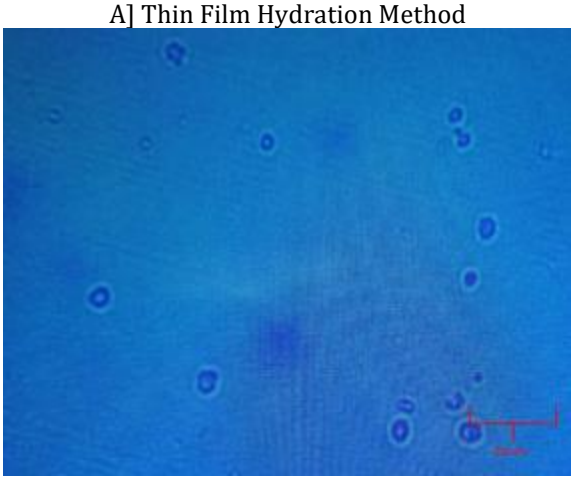
## 3. Results and discussion

### 3.1. Morphology of placebo niosomes

The Placebo niosomes were prepared by opting 2 methods viz. thin film hydration method and ether injection method. The prepared niosomes were analyzed using a digital microscope so as to eliminate one method and further proceed with the other one.

As per the above observations of niosomes under the digital microscope; the Thin Film Hydration produced smaller multilamellar vesicles and gave a higher yield, whereas the Ether Injection produced larger unilamellar vesicles and gave a lower yield as compared to niosomes produced by the Thin Film Hydration. As the Thin Film Hydration gave a smaller vesicle size and higher yield which was desired for the formulation; it was selected for further preparation and optimization of drug-loaded niosomes.

A] Thin Film Hydration Method



B] Ether Injection Method

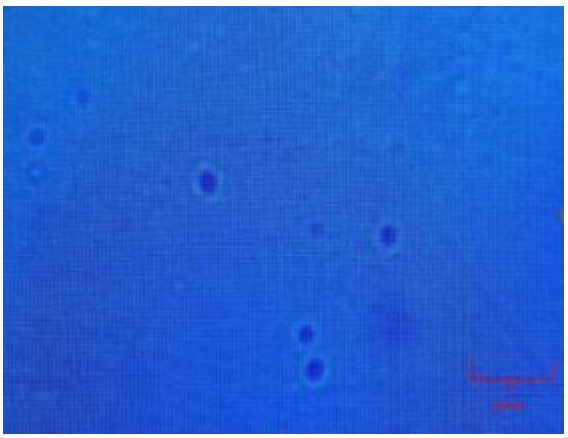


**Figure 1** Digital Microscopic images of Niosomes prepared by A) Thim Film Hydration and B) Ether Injection Method

### 3.2. Optimization of griseofulvin niosomes

**Table 4** OVAT Optimization of Niosomes (S-Spherical, D-Distorted, M-Mutilamellar, U-Unilamellar)

| Batch ID # | Niosomes Formed | Vesicle Size (diameter in m) (Mean ±S.D.) | Vesicle Shape (Spherical-S, Distorted-D) | Vesicle Lamellarity (Multimeter-M, Unilamellar-U) | Niosome Aggregation | Entrapment Efficiency (EE%) (Mean ± S.D.) |
|---|---|---|---|---|---|---|
| Gr 1 | Yes | 3.9513± 0.28 | S | M | No | 71.745± 0.505 |
| Gr 2 | Yes | 3.27±0.20 | S | M | No | 76.805± 0.305 |
| Gr 3 | Yes | 3.6002± 0.33 | S | M | No | 74.0695± 0.2305 |
| Gr 4 | Yes | 3.2574± 0.23 | S | M | Yes | 75.12±0.82 |
| Gr 5 | Yes | 3.2568± 0.27 | S | M | No | 74.46±0.36 |
| Gr 6 | Yes | 3.3429±0.20 | S | M | No | 73.27±0.175 |
| Gr 7 | Yes | 3.6485± 0.10 | S | M | No | 75.35±0.050 |
| Gr 8 | Yes | 4.0052± 0.27 | S | M | Yes | 73.75±0.55 |
| Gr 9 | Yes | 3.2524± 0.1009 | S | M | No | 78.45±0.049 |
| Gr 10 | Yes | 3.2271± 0.75 | S | M and U | Yes | 77.080±0.03 |
| Gr 11 | Yes | 3.2897± 0.81 | S | M | No | 76.815± 0.195 |
| Gr 12 | Yes | 3.2163± 0.70 | S | M | No | 77.305± 0.135 |
| Gr 13 | Yes | 3.4293± 0.83 | S | M | Yes | 76.685±0.46 |
| Gr 14 | Yes | 4.1249± 0.42 | S and D | Less M and moreU | No | 66.845± 1.005 |
| Gr 15 | Yes | 3.8805± 0.29 | S | More M and less U | No | 74.035± 0.085 |
| Gr 16 | Yes | 3.3148± 0.44 | S | M | No | 77.260±1.11 |
| Gr 17 | Yes | 2.5705± 0.41 | S and D | M | No | 83.895± 0.265 |
| Gr 18 | No | - | - | - | - | - |
| Gr 19 | Yes | 3.7836± 0.14 | S | M | No | 68.885± 0.345 |
| Gr 20 | Yes | 3.2714± 0.30 | S | M | No | 75.435± 0.965 |
| Gr 21 | Yes | 2.7470± 0.02 | S | M | No | 82.475± 2.335 |
| Gr 22 | Yes | 2.5785± 0.29 | S | M | No | 87.095± 1.715 |
| Gr 23 | Yes | 2.4872± 0.37 | S | M | No | 77.95±0.09 |
| Gr 24 | Yes | 2.4586± 0.34 | S | M | No | 80.535±0.01 |
| Gr 25 | Yes | 2.4044± 0.48 | S | M | No | 84.555±0.10 |
| Gr 26 | No | - | - | - | - | - |

#### *3.2.1. One Variable at a Time Optimization*

Effect of surfactant type

As from the above results it can be concluded that Span 20 gave the largest vesicles, whereas Span 60 gave the smallest vesicles. Also, surfactant type used affected the EE% of the formed vesicles, EE% was found increasing as Span 20 < Span 80 < Span 60. So, Batch Gr. 2 prepared using Span 60 yielding smallest vesicles with highest EE% was selected for further optimization.

Effect of charge inducer conc

It was observed that as there was increase in the conc. of charge inducer (Stearylamine) no as such effect was seen on critical quality attributes. Only Batch Gr. 4 showed aggregation of niosomes as it contained the lowest conc. of charge inducer. Whose purpose was to prevent the aggregation of vesicles. So, as the conc. was increased no aggregation was seen. Hence Batch Gr. 5 with 0.25 mg SA was selected for further optimization.

Effect of surfactant: cholesterol ratio

When the concentration of cholesterol was increased in comparison to surfactant used it led to increase in vesicle size. So, Batch Gr. 8 with Surf.: Chol. ratio as 1:2 had the highest vesicle size. It also affected the EE% of the vesicles, Batch Gr. 9 with Surf.: Chol. ratio as 2:1 showed highest EE% and lowest vesicle size, hence, was selected for further optimization.

Effect of evaporating flask speed

Speed of evaporating flask as such did not affect majorly. But Gr. 10 and 13 were eliminated, as aggregation of niosomes was observed at lowest and highest RPM. Also, in Gr. A mixture of multilamellar as well as unilamellar niosomes was seen. Leaving both 100 and 120 RPM, Gr. 11 and 12 both can be used. Batch Gr. 12 with 120 RPM was selected for further optimization.

Effect of external phase temperature

As the external phase temperature was increased the vesicle size tend to decrease in diameter. Gr. 18 with highest temp. was eliminated from the process as no niosomes were formed. Temp. of solvent also affected the shape of the vesicles at lowest and highest temp., 50 and 65 respectively showed distorted vesicles along with spherical. Its effect was also seen on lamellarity of the vesicles, lowest temp. showed more unilamellar vesicles which decreased as temp. was increased thus, Gr. 16 and 17 showed only multilamellar vesicles. Also, EE% was for increasing as temp. was increased. But, as Gr. 17 showed the presence of distorted vesicles as well, hence Gr. 16 with 60-degree Celsius temp. was selected for further optimization.

Effect of hydration time

Hyd. time showed its measure effect on vesicle size and EE%. With increase in time decrease in vesicle size and increase in EE% was observed. Resulting in Gr. 22 with smallest vesicles with highest EE%. But Gr. 22 showed distorted vesicles, so Gr. 22 was eliminated and Gr. 21 with 60 min. as hyd. time was selected for further optimization.

Effect of solvent/external phase volume

Batch Gr. 26 with 5 ml solvent used showed no niosome formation, hence was eliminated. Leaving 3 batches on which effect of solvent vol. was observed on the EE% of the vesicles. As there was decrease in solvent vol. increase in EE% was observed. Hence, batch Gr. 25 was selected as a final optimized batch.

Thus, based on the above observations and analysis an improved optimum batch of Griseofulvin niosomes with the smallest particle size, spherical in shape, and with the highest entrapment efficiency without aggregation was found to be Batch Gr. 25. This optimum batch was further sonicated using an ultrasonic bath sonicator for 2 min. and 5 min. respectively in on and off manner (10 sec. on and 10 sec. off), to avoid disruption of niosome vesicles and to obtain the niosomes of the desired nm size range. This final optimized batch was further selected for final characterization.

### 3.3. Characterization of griseofulvin niosomes (final optimized batch)

The above batches were further evaluated based on the critical quality attributes: [Refer 2.2.4 for all procedures used for characterization]

*3.3.1. Microscopic Analysis*

FE-SEM

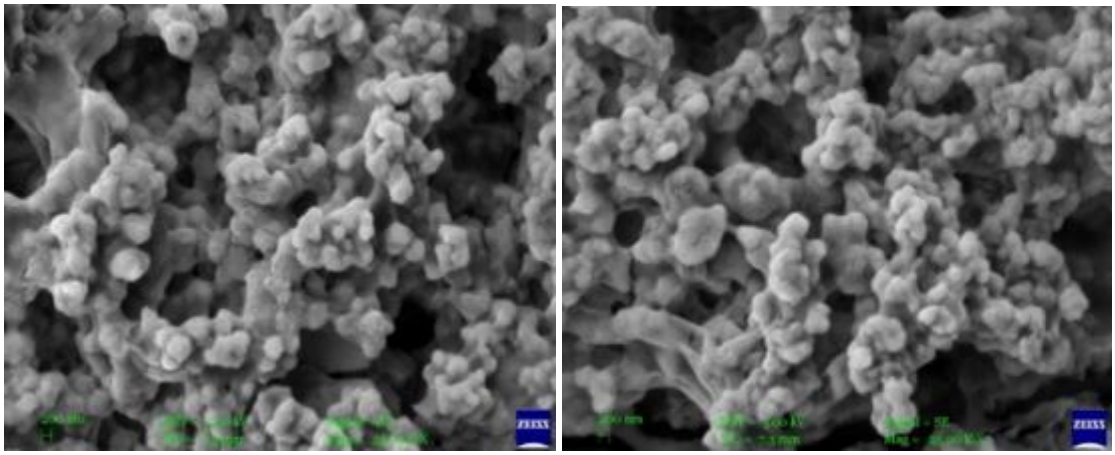


**Figure 2** FE-SEM Images of Optimized Niosome Batch

Digital Microscopy

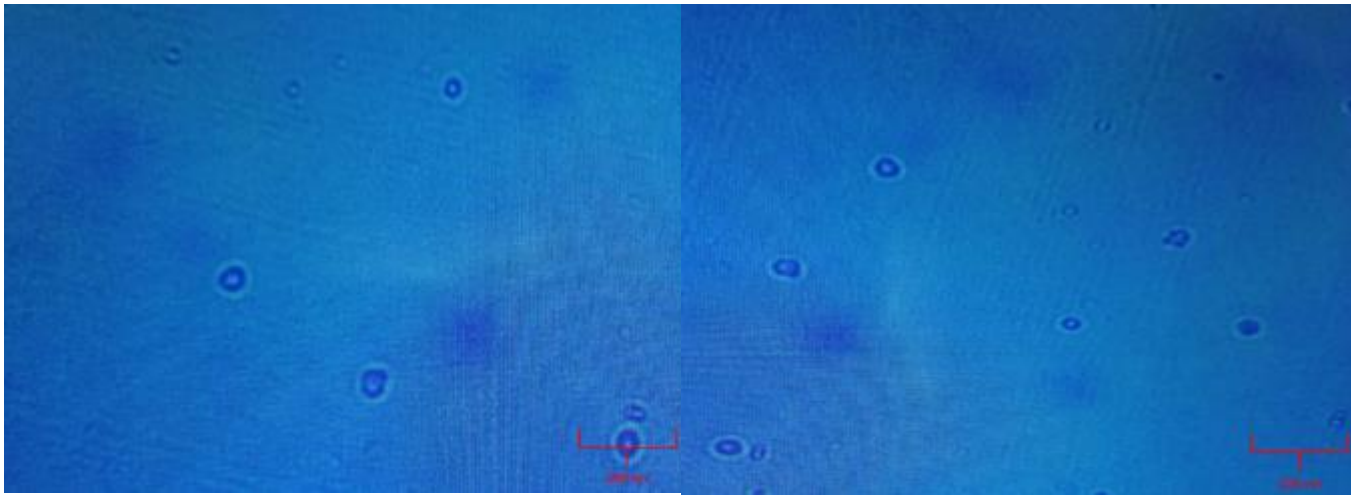


**Figure 3** Digital Microscopic Images of Optimized Niosome Batch

The microscopic analysis done using FE-SEM and Digital microscopy put forth the images, which revealed that the optimized niosomes were spherical in shape and within a size range of 150-250 nm. The results obtained were as per desired niosome specifications.

*3.3.2. Zeta Analysis*

**Table 5** Result of Zeta Analysis

| Peak No. | Mean | S. D. | Mode |
|---|---|---|---|
| 1 | 180.9 nm | 4.6 nm | 181.4 nm |
| 2 | - nm | - nm | - nm |
| Total | 180.9 nm | 4.6 nm | 181.4 nm |

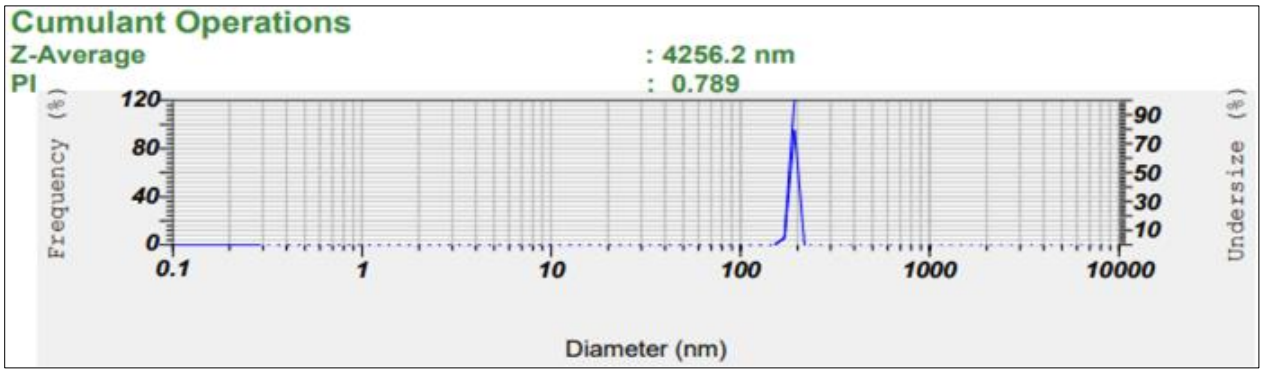



**Figure 4** Zeta Analysis Report

The above Zeta analysis report specified below results of optimized niosomes batch,

- Mean Particle Size: 180.9 nm ± 4.6 nm
- Distribution Form: Polydisperse
- Polydispersity Index (PI): 0.789
- Z-Average: 4256.2 nm

As we are targeting Superficial fungal infection, which mostly occurs to be in the Epidermis layer of the skin. For the drug to act on the layer the niosomes should be below 1 micrometer, the above result was found to be in accordance to the desired specification.

*3.3.3. Entrapment Efficiency (EE%)*

As per the optimization results, it can be said that the Entrapment Efficiency of Griseofulvin was highly influenced by CMAs (Surfactant type and Surfactant: Cholesterol ratio) and by CPPs (external phase temperature, hydration time, and external phase volume (both aqueous and organic)). The Entrapment Efficiency of the optimized batch with Span 60: Cholesterol (2:1); external phase temperature 60 °C; hydration time 60 min.; and external phase volume (both aqueous and organic) 10 ml was found to be 84.555 ± 0.10% (Mean ± S.D.). This entrapment efficiency was highest taking into consideration of CMAs and CPPs on other CQAs as well.

*3.3.4. Viscosity*

The Viscosity of the optimized batch was found to be 2.3 ± 0.2943CP (Mean ± S.D.). This viscosity indicates the suspension consistency. The prepared niosomes were suspended in phosphate-buffered saline (PBS; pH 6.8) aqueous solution which was used for hydration of the niosomes as a final preparation step.

*3.3.5. Osmotic Shock*

**Table 6** Effect of Osmotic shock on optimized niosome batch

| Sr. No. | Formulation | Average Vesicle size (nm) ± S.D. after incubation with | | | |
|---|---|---|---|---|---|
| | | PBS pH 6.8 | Hypertonic 1.2% NaCl | Normal 0.9% NaCl | Hypotonic 0.5% NaCl |
| 1 | Optimum batch | 180.9 ± 4.6 | 141.05 ± 4.12 | 181.02 ± 6.92 | 221.7 ± 2.27 |

Formulations were treated with hypotonic (0.5% NaCl), hypertonic (1.2% NaCl), and normal saline (0.9% NaCl) solutions. In the hypertonic solution, all the formulations shrank uniformly. Formulations incubated with Hypotonic saline showed a slight increase in vesicle size when compared to normal saline media (Table. No. 5). This demonstrates that griseofulvin niosomes could be diluted with normal saline for parenteral use.

### 3.4. Characterization of griseofulvin niosomal film

[Refer 2.2.6 for all procedures used for characterization]

**Table 7** Results of Griseofulvin Niosomal Film Evaluation

| Sr. No. | Parameters | Observation |
|---|---|---|
| 1 | Physical Appearance | Smooth, Uniform, and Flexible |
| 2 | Film Thickness (Average) | 0.17766 ± 0.00101 mm |
| 3 | Weight Variation (Average) | 1.6629 ± 0.0553 gm |
| 4 | Folding Endurance (Average) | 95 ± 2 times |
| 5 | Tensile Strength (Average) | 3.64676 ± 0.7632 |
| 6 | Moisture Uptake (Average) | 20.5181 ± 3.4311% |
| 7 | Moisture Content (Average) | 14.6412 ± 0.5966% |
| 8 | Drug Content Uniformity (Average) | 99.2818 ± 0.23% |

*3.4.1. In-vitro Drug Diffusion Studies*

**Table 8** *In-vitro* drug diffusion study report of Griseofulvin niosomal film, Griseofulvin Pure Drug Film and Griseofulvin Marketed Formulation

| Time (hr) | %CDR (Niosomal film) | %CDR (Pure drug film) | %CDR (Marketed Formulation) |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 0.50 | 15.41 | 23.55 | 20.89 |
| 0.75 | 26.85 | 30.51 | 22.51 |
| 1 | 33.71 | 70.79 | 42.48 |
| 2 | 47.17 | 66.96 | 58.15 |
| 3 | 68.11 | 55.23 | 73.18 |
| 4 | 79.35 | 47.23 | 69.65 |
| 5 | 89.84 | 31.16 | 57.31 |
| 6 | 83.46 | 17.77 | 50.89 |

The pure drug film showed high release of drug within 1 hr., this resulted in the phenomenon of dose dumping. As, 70% CDR was seen in 1 hr. time period. Marketed formulation of griseofulvin used for drug release comparative study showed a significant drug release after 3 hr., which was the maximum drug release of the formulation observed. Whereas, niosomal griseofulvin film showed significant drug release for a period of 5 hr. This was because the film acted as a depot for drug release in the form of niosomes. An extended drug release was seen due to the formulation of drug in the form of niosomes embedded in the biopolymeric film.

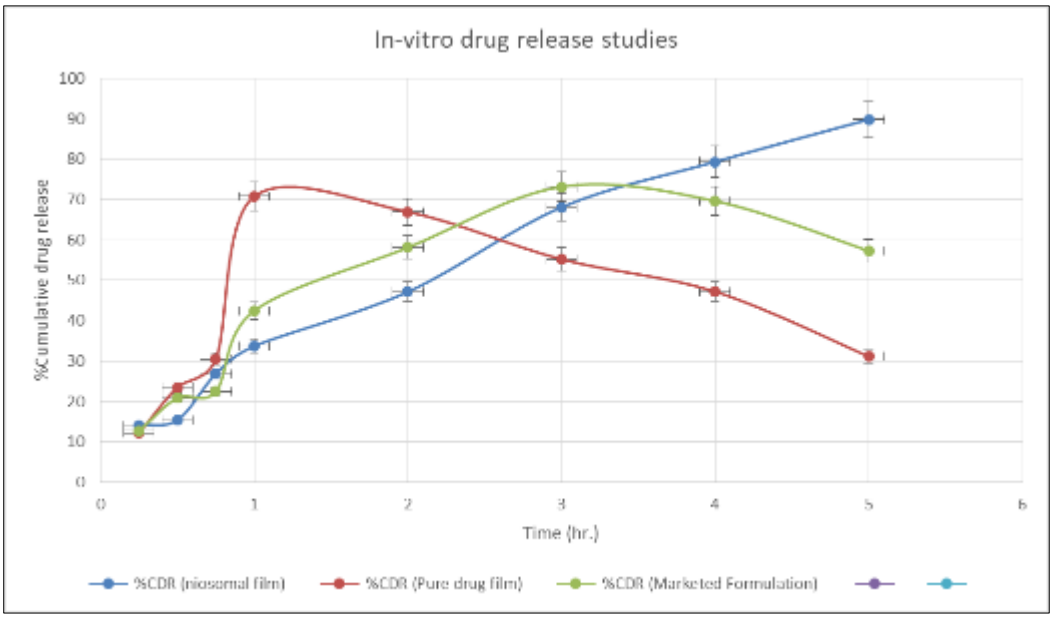



**Figure 5** *In-vitro* drug release graph of niosomal film, Pure drug film and Marketed formulation of Griseofulvin

*3.4.2. Anti-Fungal Studies*

**Table 9** Anti-Fungal Studies Observation

| Formulation | Pure Drug | Griseofulvin Niosomal Suspension | Chitosan Blank film | Griseofulvin Niosomal film | Marketed formulation |
|---|---|---|---|---|---|
| Zone of inhibition (mm) ± SD | 7 ± 0.81 | 8.67 ± 0.94 | 3 ± 0.81 | 13 ± 0.81 | 6 ± 0.81 |


**Figure 6** Antifungal Effect of Pure Drug, Niosome Suspension, Chitosan Blank film, Niosome-embedded film, Marketed Formulation on *Candida albicans sp*

From the above observation, it can be concluded that; Niosome, as well as niosomal film, exhibited higher antifungal activity as compared to pure drugs. Chitosan also exhibits antifungal activity thereby increasing the activity of niosomal

film. So, it can be said that chitosan also exhibits antifungal activity. This would result in a synergistic effect, thereby enhancing the efficacy of the antifungal drug griseofulvin.

## 4. Conclusion

Griseofulvin is a BCS class 2 antifungal drug that is currently available in the market only in oral dosage form. But as fungal infection mainly occurs on the superficial layer of the skin. So, the goal of this study was to formulate griseofulvin in topical formulation. Also, as the drug belongs to BCS class 2 it has low solubility. So, lipid vesicles were formulated to enhance the solubility of griseofulvin. Here, Chitosan was used as a biopolymer for film formulation which enhanced the overall efficacy of the drug, as chitosan also exhibited anti-fungal activity. The aim of the present work was to formulate and characterize chitosan film formulation integrating of griseofulvin-loaded niosomes for topical delivery. To have a dosage form that solves the instability problem and facilitates the usage of a topical drug for nearly long-term. From the present study it can be concluded that chitosan film formulation integrating griseofulvin-loaded niosomes for dermal (topical) delivery enhanced the solubility of the drug, avoided the first-pass metabolism, avoided the side effects associated with the orally-administered marketed formulation. Finally giving improved efficacy of the drug. Resulted in quality drug product and improved patient compliance.

## Compliance with ethical standards

### *Acknowledgments*

I would simply humble for words to express my heavy depth of gratitude towards my guide Dr. Jaya Agnihotri for guiding and encouraging me throughout the research work. I would also extend my gratitude to Dr. Indira Parab for sparing time out of her busy schedule and providing the necessary guidance. I would also thank Carbanio, Mumbai; Hychem Laboratories, Hyderabad; Vishal chemicals, Mumbai for providing drugs and chemicals and Naprod Lifesciences, Mumbai India for providing *Candida albicans* strain slants (ATCC 10231).

### *Disclosure of conflict of interest*

The authors declare that they have no conflicts of interest.

## References

[1] Elghblawi E. Tinea Capitis in Children and Trichoscopic Criteria. Int J Trichology. 2017 Apr-Jun, 9(2):47-49. [PMC free article] [PubMed].

[2] Aggarwal N, Goindi S. Preparation and evaluation of antifungal efficacy of griseofulvin loaded deformable membrane vesicles in optimized guinea pig model of Microsporum canis – Dermatophytosis. Int J Pharm. 2012, 437:277–87. [PubMed] [Google Scholar]

[3] Arida AI, Al-Tabakha MM, Hamoury HAJ. Improving the high variable bioavailability of griseofulvin by SEDDS. Chem Pharm Bull. 2007, 55:1713–1719. doi: 10.1248/cpb.55.1713. [PubMed] [CrossRef] [Google Scholar]

[4] Wong SM, Kellaway IW, Murdan S. Enhancement of the dissolution rate and oral absorption of a poorly water soluble drug bywater-soluble surfactathe nt-containing microparticles. Int J Pharm. 2006, 317:61–68. doi: 10.1016/j.ijpharm.2006.03.001. [PubMed] [CrossRef] [Google Scholar]

[5] Dangi JS, Vyas SP, Dixit VK. Effect of various lipid-bile salt mixed micelles on the transfer of amphoterithe cin-B across the everted rat intestine. Drug Devel Ind Pharm. 1995, 21:2021–2027. doi: 10.3109/03639049509065886. [CrossRef] [Google Scholar]

[6] Williams DI, Marten RH, Sarkany I. Griseofulvin. Br J Dermatol. 2006, 71:434–443. doi: 10.1111/j.1365-2DOI.1959.tb13380.x. [PubMed] [CrossRef] [Google Scholar].

[7] U.S. National Library of Medicine. Antifungal drug-Antifungal drug Tablet, 2016. Available from: http://www.dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=6149c044-58bb-402c-a4b5-18b96dd9b3b9. [Last accessed on 2016 Jan 11].

[8] Choi MJ, Maibach HI. Liposomes and niosomes as topical drug delivery systems. Skin Pharmacol Physiol. 2005, 18:209–19. [PubMed] [Google Scholar].

[9] Narang T, Dogra S, Kaur I, Katare OP, Khurana R. Ciclosporin 1% liposomal gel in limited plaque psoriasis: a prospective randomized double-blind placebo-controlled study in stable plaque psoriasis. Br J Dermatol. 2007, 157:53. [Google Scholar].

[10] Sultana Y, Aqil M, Ali A, Samad A. Advances in the topical ocular drug delivery. Expert Rev Ophthalmol. 2007, 2:309–323. doi: 10.1586/17469899.2.2.309. [CrossRef] [Google Scholar].

[11] Vora B, Khopade AJ, Jain NK. Proniosome based transdermal delivery of levonorgestrel for effective contraception. J Control Release. 1998, 54:149–165. doi: 10.1016/S0168-3659(97)00100-4. [PubMed] [CrossRef] [Google Scholar].

[12] Nasr M, Mansour S, Mortada ND, Elshamy AA. Vesicular aceclofenac systems: a comparative study between liposomes and niosomes. J Microencapsul. 2008, 25:499–512. doi: 10.1080/DOI52040802055411. [PubMed] [CrossRef] [Google Scholar].

[13] Hao YM, Li K. Entrapment and release difference resulting from hydrogen bonding interactions in niosome. Int J Pharm. 2011, 403:245–53. [PubMed] [Google Scholar].

[14] Di Marzio L, Marianecci C, Petrone M, Rinaldi F, Carafa M. Novel pH-sensitive non-ionic surfactant vesicles: Comparison between Tween 21 and Tween 20. Colloids Surf B Biointerfaces. 2011, 82:18–24. [PubMed] [Google Scholar].

[15] Ahmed S, Ibrahim M, Sarhan H, Amin M. Formulation and characterization of biodgradable biodegradables for topical application of terbinafine HCL. Bull Pharm Sci Assiut Univ. 2007, 30:111–29. [Google Scholar].

[16] Kong, M., Chen, X. G., Xing, K., and Park, H. J. (2010). Antimicrobial properties of chitosan and mode of action: a state of the art review. Int. J. Food. Microbiol. 144, 51–63. doi: 10.1016/j.ijfoodmicro.2010.09.012.

[17] Cheung, R. C., Ng, T. B., Wong, J. H., and Chan, W. Y. (2015). Chitosan: an update on potential biomedical and pharmaceutical applications. Mar. Drugs 13, 5156–5186. DOI: 10.3390/md13085156, PubMed Abstract | CrossRef Full Text | Google Scholar.

[18] Bhalekar rohini. V, nagoba shivappa n2, Shaikh nasheer s3 & swami avinash. B4, Formulation and evaluation of nanosponges hydrogel for topical drug delivery containing griseofulvin, International Journal of Medicine and Pharmaceutical Sciences (IJMPS) ISSN (P): 2249–6890, ISSN (E): 2249–8001 Vol. 10, Issue 2, Apr 2020, 57–70.

[19] Jain CP, Vyas SP. Preparation and characterization of niosomes containing rifampicin for lung targeting. J Microencapsul. 1995, 12:401–407. doi: 10.3109/02652049509087252. [PubMed] [CrossRef] [Google Scholar].

[20] Satturwar PM, Fulzele SV, Nande VS, Khandare JN. Formulation and evaluation of ketoconazole niosomes. Indian J Pharm Sci. 2002, 64:155–158. [Google Scholar].

[21] Murray, P. M. et al. The application of design of experiments (DoE) reaction optimisation and solvent selection in the development of new synthetic chemistry. Org. Biomol. Chem. 14, 2373–2384 (2016).

[22] Murray, P. M., Tyler, S. N. G. & Moseley, J. D. Beyond the numbers: Charting chemical reaction space. Org. Process Res. Dev. 17, 40–46 (2013).

[23] Tye, H. Application of statistical 'design of experiments' methods in drug discovery. Drug Discov. Today 9, 485–491 (2004).

[24] Agarwal R, Katare OP, Vyas SP. Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol. Int J Pharm. 2001, 228:43–52.

[25] Alsarra IA, Bosela AA, Ahmed SM, Mahrous GM. Proniosomes as a drug carrier for transdermal delivery of ketorolac. Eur J Biopharm. 2005, 59:485–90.

[26] Jain S, Umamaheswari B, Bhadra D, Jain NK. Ethosomes: a novel carrier for enhanced transdermal delivery of an anti-HIV agent. Indian J Pharm Sci. 2004, 66(1):72.

[27] Perrie Y, Barralet JE, McNeil S, Vangala A. Surfactant vesiclemediated delivery of DNA vaccines via the subcutaneous route. Int J Pharm. 2004, 284:31–41.

[28] Arunothayanun P, Uchegbu IF, Craig DQM, Turton JA, Florence AT. In vitro/in vivo characterisation of polyhedral niosomes. Int J Pharm. 1999, 183:57–61.

[29] Vyas SP, Venkatesan N. Poly (phthaloyl-L-lysine)-coated multilamellar vesicles for controlled drug delivery: in vitro and in vivo performance evaluation. Pharm Acta Helv. 1999, 74:51–8.

[30] Srinivasa P, Ramesh M, Tharanathan R. Effect of plasticizers and fatty acids on mechanical and permeability characteristics of chitosan films. Food Hydrocoll. 2007, 21:1113–22. [Google Scholar].

[31] Kouchak M, Ameri A, Naseri B, Kargar Boldaji S. Chitosan and polyvinyl alcohol composite films containing nitrofurazone: Preparation and evaluation. Iran J Basic Med Sci. 2014, 17:14–20. [PMC free article] [PubMed] [Google Scholar].

[32] Nitayaphat W, Jiratumnukul N, Charuchinda S, Kittinaovarat S. Mechanical properties of chitosan/bamboo charcoal composite films made with normal and surface oxidized charcoal. Carbohydr Polym. 2009, 78:444–8. [Google Scholar].

[33] Wittaya-areekul S, Prahsarn C, Sungthongjeen S. Development and in vitro evaluation of Chitosan-Eudragit RS 30D composite wound dressings. AAPS PharmSciTech. 2006, 7:E30. [PMC free article] [PubMed] [Google Scholar].

[34] Grant J, Blicker M, Piquette-Miller M, Allen C. Hybrid films from blends of chitosan and egg phosphatidylcholine for localized delivery of paclitaxel. J Pharm Sci. 2005, 94:1512–27. [PubMed] [Google Scholar].

[35] Tang C, Guan YX, Yao SJ, Zhu ZQ. Preparation of ibuprofen-loaded chitosan films for oral mucosal drug delivery using supercritical solution impregnation. Int J Pharm. 2014, 473:434–41. [PubMed] [Google Scholar].

[36] Hu CJ, Rhodes DG. Proniosomes: a novel drug carrier preparation. Int J Pharm. 1999, 185:23–35. doi: 10.1016/S0378-5173(99)00122-2. [PubMed] [CrossRef] [Google Scholar].

[37] Nweze EI, Mukherjee PK, Ghannoum MA. Agar-based disk diffusion assay for susceptibility testing of dermatophytes. J Clin Microbiol 2010, 48:3750-2.