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DEVELOPMENT AND CHARACTERIZATION OF ANTI-DANDRUFF NIOSOMAL HAIR GEL CONTAINING TEA TREE OIL

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Niosomes, Anti-dandruff, Tea tree oil, Hair gel, Formulation

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ABSTRACT: Dandruff is the excessive shedding of dead skin cells from the scalp, apparently caused by a fungus called *Malassezia restricta* and *M. globosa*. Malassezia formerly called Pityrosporum is a yeast causing infection of skin and scalp. Tea tree oil (TTO) is an essential oil that is obtained by steam distillation of the leaves and terminal branches of *Melaleuca alternifolia* (Myrtales: Myrtaceae). Tea tree oil shows promise as a topical antifungal agent, with recent clinical data indicating efficacy in the treatment of dandruff and oral candidiasis. In the present research, niosomal vesicles were selected for tea tree oil delivery because of their penetration enhancing ability. Niosomes containing tea tree oil were prepared using a thin-film hydration method. A 3^2 factorial design (DOE analysis) was carried out to reduce the number of experiments. Parameters contribution was determined using a 3-D response curve. Prepared optimized batch (NB7) exhibited an entrapment efficiency of 81% and percent cumulative drug diffusion of 57.56 ± 0.092%. Transmission Electron Microscopy (TEM) revealed vesicular, spherical particles with a smooth surface in the nano range. Niosomal gel of optimized batch (NB7) was prepared to incorporate 1% w/w carbopol 974NF. The *ex-vivo* permeation study was performed in the Franz diffusion apparatus using excised porcine ear skin in phosphate buffer pH 5.5, which showed prolonged drug permeation of 30.39% and hence maximum retention on the scalp up to 69.61%. G7 exhibited good stability throughout 90 days at different temperatures and humidity.

INTRODUCTION: The skin of the scalp has several unique features that aid in its critical role in protecting the head. The higher follicular density creates a dark, warm and moist environment. This provides thermal insulation, but also creates an environment conducive to parasitic infestation.

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In adults, the high rate of sebum production along with desquamated skin cells provides a food source for microorganisms. Hence, the scalp skin is subjected to brushing and contact with other styling implements that can cause friction injury and may introduce microorganisms.

These unique features tend to make the scalp susceptible to superficial mycotic conditions (dandruff, seborrheic dermatitis, tinea capitis, *etc*.), parasitic infestation (pediculosis capitis) and inflammatory conditions (psoriasis). These disease processes of the scalp can have a significant overlap in clinical symptomatology.

Hyperkeratosis (scaling), pruritus, alopecia, and inflammatory signs (erythema, purulence) are common symptoms of scalp disorders [1].

Dermoscopy of the normal scalp shows interfollicular simple red loops, and arborizing red lines, which represents the normal vascular patterns, and honeycomb pigment in sun-exposed areas or in subjects with high phototypes. Follicular units are easily recognized and usually contain 1 to 4 hairs [2]. The pH of the scalp is 5.5 and that of the hair shaft pH is 3.67. An alkaline pH may increase the negative electrical net charge of the hair fiber surface and, therefore, increase the friction between the fibers [3]. The etiology, signs and symptoms, and treatment strategies for these common scalp conditions may vary depending upon the various disorders [4].

Dandruff (pityriasis capitis, seborrheic dermatitis confined to the scalp) is a disease that has been around for centuries even though several treatment options are available. The scaly scalp may look unhygienic and untidy. It could make the sufferer feel self-conscious and embarrassed. Dandruff affects self-esteem and confidence. Itching due to dandruff also causes great embarrassment to the sufferer in public. Dandruff causes more social and psychological problems than medical ones [5].

Dandruff, the excessive shedding of dead skin cells from the scalp, is apparently caused by a fungus called *Malassezia restricta* and *M. globosa*. Malassezia formerly called Pityrosporum is a yeast causing infection of skin and scalp. The infection of the scalp clinically represents as dandruff [6]. The pathogenesis of dandruff involves hyper-proliferation, resulting in the deregulation of keratinization. The corneocytes clump together, manifesting as large flakes of skin [7].

Causes of Dandruff: Dandruff results from at least three etiologic factors:

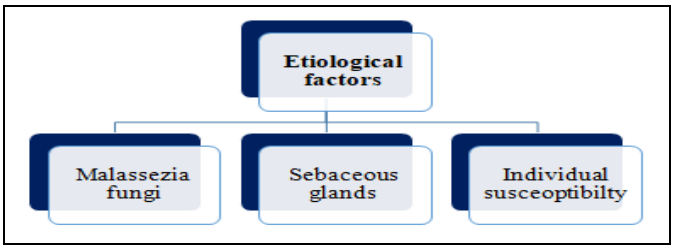


FIG. 1: THE THREE ETIOLOGICAL FACTORS RESPONSIBLE FOR DANDRUFF

Microbial Factors: Malassezia were initially identified and linked to dandruff and seborrheic dermatitis by a French scientist, Malassez, in the late 19th century. In the 1950s they were reclassified into two species: the lipid-dependent *P. ovale* and the non-lipid-dependent *P. pachydermatis*. In the 1990s, it was determined that there were multiple species of the genus Malassezia, which now consists of ten lipid-dependent species: globosa, restricta, furfur, slooffiae, sympodialis, japonica, nana, dermatitis, yamatoensis and the non-lipid dependent pachydermatis. It was proposed that dandruff is mediated by Malassezia metabolites, specifically irritating free fatty acids released from sebaceous triglycerides [8].

Human sebum is a complex mixture of triglycerides, fatty acids, wax esters, sterol esters, cholesterol, cholesterol esters, and squalene. When secreted, sebum consists of triglycerides and esters, which are broken down by microbes into diglycerides, monoglycerides and free fatty acids. The free fatty acids play a key role in the initiation of the irritant response at the base of dandruff. The role of sebaceous secretion also underlies the impact of stress and hormones on dandruff. It is well known that these are affecters of human sebum secretion and therefore impact dandruff. The Malassezia yeasts are most common in sebum-rich areas of the body and degrade sebum. Specifically, the organisms contain lipases that hydrolyze triglycerides, freeing specific saturated fatty acids that the yeast requires to proliferate. Malassezia generated free fatty acids can induce dandruff-like flaking in humans [9, 10].

The dandruff scalp presents a disrupted barrier function characterized by a decrease in hydration and an inflammatory signature [11]. However, another microorganism community composed of bacteria also inhabits the human scalp and includes facultative anaerobic bacteria, such as *P. acnes*, and aerobic bacteria, such as *Staphylococcus* [10].

Non-Microbial Factors:

- Damage to the scalp stratum corneum [12].
- Individual susceptibility to Oleic acid.
- Dry scalp
- Oily or irritated skin.

- Dirt accumulation due to less frequent shampooing.
- Sensitivity to hair cosmetics.
- Other scalp conditions like psoriasis, eczema, *etc*.

Treatment of dandruff focuses on clear signs of the disease; ameliorating associated symptoms, especially pruritus and maintaining remission with long-term therapy [13, 14].

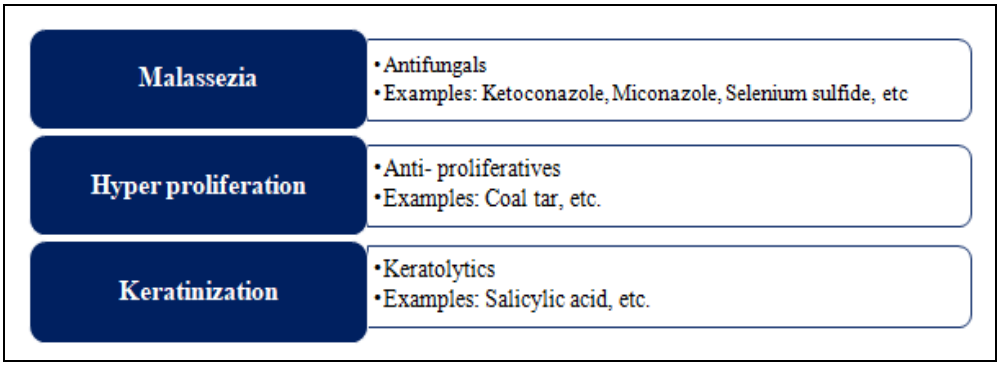


FIG. 2: EXAMPLES OF SYNTHETIC DRUGS FOR THE TREATMENT OF DANDRUFF

Tea tree oil (TTO) is an essential oil that is obtained by steam distillation of the leaves and terminal branches of *Melaleuca alternifolia* (Myrtales: Myrtaceae). Tea tree oil contains almost 100 components, Monoterpenes, sesquiterpenes, and their related alcohols are the main components of tea tree oil, providing medical benefits for antiseptic and antifungal treatments and as an antioxidant. Tea tree oil has six chemotypes, which are oils with different chemical compositions. These include a terpinen-4-ol chemotype, a terpinolene chemotype, and four 1,8-cineole chemotypes. Australian tea tree oil composition has been defined by the ISO 4730-2004 standard and the equivalent Australian Standard AS 2782-2009 for oil *Melaleuca alternifolia*, terpinen-4-ol (ISO 2004) types [7]. This standard specifies the minimum and maximum levels of concentration in percentage for 15 components of Australian tea tree essential oil as follows [15, 16].

TABLE 1: CONSTITUENTS OF TEA TREE OIL

S. no.	Components of Tea tree oil	% concentration
1	Terpinen-4-ol	30% to 48%,
2	γ-Terpinene	10% to 28%,
3	∝-Terpinene	5% to 13%,
4	∝-Terpineol	1.5% to 8%,
5	Terpinolene	1.5% to 5%,
6	∝-Pinene	1% to 6%,
7	p-Cymene	0.5% to 8%,
8	Limonene	0.5% to 1.5%
9	1,8-Cineole	trace to 15%,
10	Sabinene	trace to 3.5%
11	Aromadendrene, Lendene,	Up to 3 %
12	d-Cadinene, Globulol, Viridiflorol	Up to 3 %

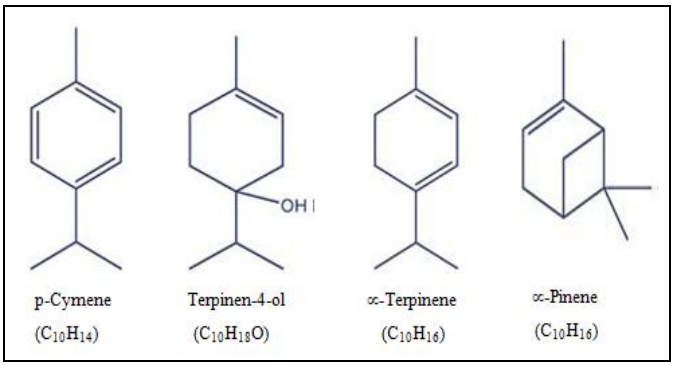


FIG. 3: CHEMICAL STRUCTURES OF P-CYMENE, TERPINEN-4-OL, ∝-TERPINENE AND ∝-PINENE [16]

Tea tree oil could play an important role in the treatment of dermatologic diseases. TTO compounds have different efficacy profiles. It is known to have antioxidant activity, anti-bacterial activity, anti-viral activity, anti-fungal activity, anti-protozoal activity, activity against *Acne vulgaris* and *Seborrhic dermatitis* and also plays a role in wound healing.

Tea tree oil shows promise as a topical antifungal agent, with recent clinical data indicating efficacy in the treatment of dandruff and oral candidiasis. Many data show that a range of yeasts, dermatophytes, and other filamentous fungi are susceptible to tea tree oil. Tea tree oil and/or components increased yeast cell permeability and membrane fluidity and inhibited medium acidification [17].

The mechanisms of antifungal action have been focused almost exclusively on *C. albicans*. TTO alters the permeability of *C. albicans* cells. TTO

also inhibits respiration in *C. albicans* in a dose-dependent manner. TTO inhibits the formation of germ tubes, or mycelial conversion, in *C. albicans* [18]. The minimum inhibitory concentration for *P ovale* is 0.25% v/v [19].

Niosomes are one of the promising drug carriers that have a bilayer structure and are formed by self-association of nonionic surfactants and cholesterol in an aqueous phase. They are either adsorbed on the surface of the skin leading to high thermodynamic activity gradient of the drug at the interface which facilitates drug permeation, or they penetrate into the stratum corneum themselves and act as drug reservoirs [20, 21].

Structural Components of Niosomes: [20, 22-24]

i. **Non-Ionic Surfactant:** Non-ionic surfactants are the most common type of surface-active agent used in preparing vesicles due to the superior benefits they impart with respect stability, compatibility and toxicity compared to their anionic, amphoteric or cationic counterpart. They are amphiphilic molecules with two distinct regions in their chemical structure, one of which is hydrophilic and the other is hydrophobic. The two portions of such molecules are linked by ether, amide or ester bonds. The most common non- ionic amphiphiles used for vesicle formation are alkyl ethers, alkyl esters, alkyl amides and esters of fatty acids.

ii. **Cholesterol:** Cholesterol influences the physical properties and structure of niosomes due to its interaction with the nonionic surfactants. Cholesterol alters the fluidity of the chains in the bilayer by abolishing the gel to the liquid phase. Several surfactants form vesicles only after cholesterol addition (up to 30-50 mol). The amount of cholesterol to be added depends on the HLB value of the surfactants. As the HLB value increases above 10, it is necessary to increase the cholesterol concentration in order to compensate for the effect of the larger head groups on the critical packaging parameter (CPP).

iii. **Charged Additives:** Charged inducers are often added in niosomal formulation because they increase the surface charge density and prevent vesicle aggregation, flocculation fusion. Diacetyl phosphate and phosphatidic acid are known as negatively-charged molecules, while stearylamine (SA) and cetyl pyridinium chloride are positively charged molecules which are both commonly used for preventing aggregation of niosomes.

Normally the charged molecule is added in niosomal formulation in an amount of 2.5-5 mol% because the high concentration of charged molecules can inhibit the formation of niosomes.

Factors Affecting Formulation of Niosomes:

1. **Nature of Encapsulated Drug:** Entrapment of drug in niosomes increases vesicle size, probably by the interaction of the solute with surfactant head groups, increasing the charge of mutual repulsion of the surfactant bilayers, thereby increasing vesicles size. In poly-oxyethylene glycol (PEG) coated vesicles; some drug is entrapped in the long PEG chains, thus reducing the tendency to increase the size. The hydrophilic-lipophilic balance of the drug affects the degree of entrapment

2. **Amount and Type of Surfactant:** A surfactant used for the preparation of niosomes must have a hydrophilic head and a hydrophobic tail. The hydrophobic tail may consist of one or two alkyl or perfluoroalkyl groups or, in some cases, a single steroidal group. The ether-type surfactants with single-chain alkyl tail are more toxic than the corresponding dialkyl ether chain. The ester-type surfactants are chemically less stable than ether-type surfactants and the former is less toxic than the latter due to ester-linked surfactant degraded by esterases to triglycerides and fatty acid *in-vivo*. The surfactants with alkyl chain length from C_{12} to C_{18} are suitable for the preparation of noisome. Span series surfactants having HLB number between 4 and 8 can form vesicles.

3. **Cholesterol Content and Charge:** Inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency. Inefficiency, the action of

cholesterol is two folds; on one hand, cholesterol increases the chain order of liquid state bilayers and on the other, cholesterol decreases the chain order of gel state bilayers. At a high cholesterol concentration, the gel state is transformed to a liquid-ordered phase.

4. **Temperature of Hydration:** Hydration temperature influences the shape and size of the noisome. Ideally, it should be above gel to the liquid phase transition temperature of the system. A temperature change of niosomal system affects the assembly of surfactants into vesicles and also induces vesicles to shape trans-formation.

5. **Resistance to Osmotic Stress:** The addition of a hypertonic salt solution to a suspension of niosomes brings about a reduction in diameter. In hypotonic salt solution, there is initial slow release with a slight swelling of vesicles probably due to inhibition of eluting fluid from vesicles, followed by faster release, which may be due to mechanical loosening of vesicles structure under osmotic stress.

6. **Membrane Additives:** The membrane stability, morphology, and permeability of vesicles are affected by numbers of additives *e.g.* cholesterol, diacetyl phosphate, phospholipid. The most common additive used is cholesterol. Cholesterol is used to eliminate gel to liquid phase 1 transition of liposome resulting in noisome which increases the rigidity and reduces the leakage of drugs from noisome.

Advantages of Niosomes:

- ✓ Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drugs and administer normal vesicles in the external non-aqueous phase.
- ✓ The vesicle suspension is water-based vehicle. This offers high patient compliance in comparison with oily formulations.
- ✓ Handling and storage of surfactants do not require any special conditions. Niosomes are more stable than liposomes.
- ✓ These improve the therapeutic performance of the active agent by prolonging its release rate.
- ✓ Also, niosomes serve as a drug reservoir in the upper skin layers and avoid systemic absorption of the active agent. This helps prevent systemic side effects of dug. On topical application. Niosomes restrict drug effects to target cells.

The present study was focused on formulating and evaluating Tea tree oil containing niosomal gel for *in-vitro* and *ex-vivo* efficacy. Niosomal formulations were prepared by using different ratios of surfactant (Span 60) and cholesterol by thin-film hydration method and were evaluated for *in-vitro* characteristics, stability studies and *ex-vivo* study.

MATERIALS AND METHODS:

Materials: Tea tree oil was purchased from Aromex Industry, Mumbai. P-Cymene was purchased from TCI chemicals. Cholesterol was procured from LobaChemie Pvt. Ltd. *Sorbitan monostearate* (Span 60) and *Polyoxyethylene-sorbitan monooleate* (Tween 80) were received from Mohini Organics Pvt. Ltd., Mumbai. Carbopol 980, Carbopol 971NF, Carbopol 974P and Carbopol ETD 2020 were received from Lubrizol Advanced Materials India Pvt. Ltd. All materials used in the study were of analytical grade.

Methods:

Formulation of Niosomes: Multilamellar niosomes were prepared by the thin-film hydration method. Accurately weighed quantities of the drug, surfactants (Tween or Span), and cholesterol was dissolved in chloroform in a round-bottom flask. The chloroform was evaporated at 60 °C under reduced pressure using a rotary evaporator (Equitron Roteva). After chloroform evaporation, the thin films were hydrated with 10 ml of phosphate buffer pH 7.4, and the flask was kept rotating at 60 °C at various revolutions per minute (rpms). Formulations were sonicated three times at 50 Hz in a bath-sonicator (OSCAR Ultrasonic cleaner- Microclean 103) for 15 min with a 5-min interval between successive times.

Formulation Optimization: The formulations were prepared as per 3^2 factorial design. The

selection was done by taking different concentrations of cholesterol and surfactant.

TABLE 2: FACTORS FOR DESIGNING OF FORMULATION

S. no.	Coded factors	Factors	
		Concentration of Cholesterol	Concentration of surfactant
1	-1	2% w/w	2% w/w
2	0	3% w/w	3% w/w
3	+1	4% w/w	4% w/w

TABLE 3: VARIOUS BATCHES AS PER DOE WITH DIFFERENT CONCENTRATIONS OF CHOLESTEROL AND SURFACTANT

Batch no.	Cholesterol	Surfactant
1	-1	-1
2	-1	0
3	-1	+1
4	0	-1
5	0	0
6	0	+1
7	+1	-1
8	+1	0
9	+1	+1

Determination of Drug Entrapment in Vesicles:

Entrapment Efficiency (EE): To estimate the amount of drug-loaded in niosomes, the niosomes were first separated by centrifugation. After separation, the pellet obtained was dissolved in ethanol and the encapsulated amount was estimated by UV-Visible spectroscopy at 273 nm. Tea tree Oil loaded niosomes were first separated by centrifugation at 5,000 rpm for 30 min. Niosomes were then washed twice with distilled water to remove any drug in the supernatant. The amount of drug present in supernatant was estimated by UV-Visible spectroscopy (Perkin Elmer UV/Vis spectrometer Lambda 25).

The amount of drug encapsulated in niosomes was directly calculated by subtracting the amount present in the supernatant (*i.e.* loss) from the original drug solution. All experiments were performed in triplicate. The % EE was calculated by the following equation:

% EE = Amount of drug-loaded in the noisome / Initial amount of the drug × 100

Reproducibilty: One formulation of each of niosomes was prepared six times and studied to validate the reproducibility of the formulation. The statistical analysis was avoided, as the results were highly reproducible each time.

Microscopy: All the batches were viewed under the optical microscope to observe the shape and lamellar nature of vesicles, as shown in the photomicrographs in **Fig. 4**. A drop of the niosomal dispersion was placed on a clean glass slide and observed under high power 40x of the optimal microscope (Motic microscope).

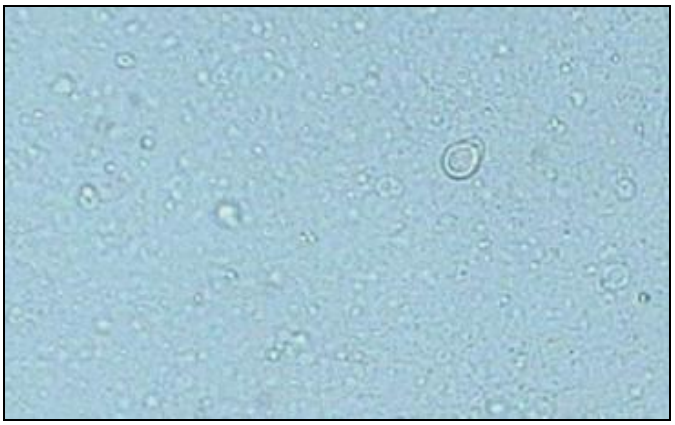


FIG. 4: PHOTOMICROGRAPH OF TEA TREE OIL LOADED NIOSOMES

Determination of Vesicle Size: Vesicle size was determined by dynamic light scattering using a computerized inspection system (Malvern particle size analyzer). Freshly prepared batches of vesicles were diluted tenfold with filtered distilled water and then analyzed. Vesicle size and polydispersity index were measured for the niosomal dispersion.

Polydispersity index is a parameter that gives an estimate of the width of the distribution of the vesicles. The higher the polydispersity index, the wider is the distribution. Optimized niosomal dispersion batches were selected and were diluted with filtered distilled water and zeta potential of the optimized batches was measured with the help of Malvern zeta sizer.

Surface Morphology: The morphological study of niosomes was carried out using TEM (JEM 1400). The TEM consists of a 200 KV TEM, with a high brightness field-emission gun (FEG) source which produces improved sensitivity and resolution compared to more traditional thermionic sources like LaB6 or Tungsten filaments. This technique involves freezing biological material quickly in vitreous ice and imaging at temperatures less than -180°C. TEM analysis was carried out of the final optimized batch of drug-loaded niosomes as shown in **Fig. 5**.

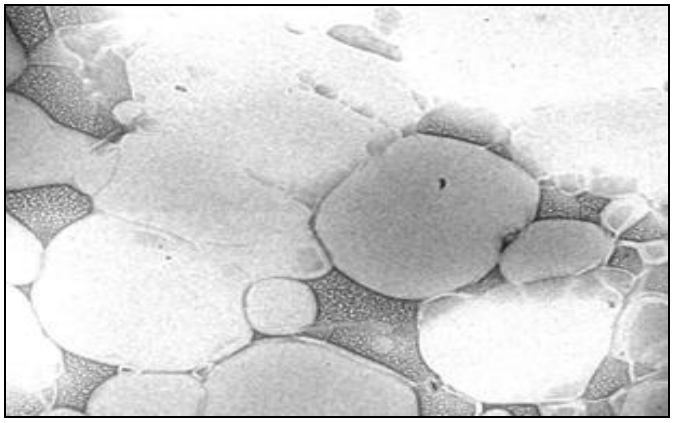


FIG. 5: TEM IMAGES OF OPTIMIZED TEA TREE OIL LOADED NIOSOMES

TABLE 4: CHARACTERISATION OF TEA TREE OIL LOADED NIOSOMES

Batch code	Cholesterol: Surfactant ratio	Particle size (nm)	EE (%)
NB1	1:1	172.1	72%
NB2	1:1.5	400.8	56%
NB3	1:2	581.4	42%
NB4	1.5:1	254.4	74%
NB5	1.5:1.5	477.4	61%
NB6	1.5:2	512.8	55%
NB7	2:1	166.4	81%
NB8	2:1.5	361.8	69%
NB9	1:1	641.9	57%

Selection of Optimized Niosomal Formulation: On the basis of particle size and entrapment efficiency parameters of all the nine niosomal formulations prepared as per 3^2 factorial design, where the values corresponding to NB1- NB9 batches represented particle size and entrapment efficiency.

Preparation of Gel Containing Niosomes: Gel containing Tea tree oil loaded niosomes equivalent to 2% w/w of the drug was prepared. The gel phase of formulations was prepared by soaking selected gelling agents (Carbopol 974NF) in distilled water and niosomes were dispersed into it with constant stirring at a moderate speed using mechanical stirrer, then pH was adjusted to 5.5-6.5 using triethanolamine. The developed gels were observed for their appearance, texture and spreadability.

Rheological Study: Rheological study of the prepared niosomal gels was performed using a Brookfield digital viscometer (DV III Ultra-model D 220) at 37 °C. Measurements were done at different shearing rates and the rheogram was constructed. Each experiment was performed in triplicates and mean values were calculated.

Fourier Transform Infrared Spectroscopy (FTIR): The FTIR spectra of the drug, the individual solid components of niosomes (span 60 and cholesterol) were compared to that of their physical mixture (1:1 ratio) to detect any interaction. Spectra were recorded using the FTIR spectrophotometer (Shimadzu IR Infinity). Samples were mixed with potassium bromide (spectroscopic grade) and compressed into disks using hydraulic press before scanning from 4000 to 600 cm^{-1}. However interaction of the drug with gel-forming polymers using FTIR analysis has been previously reported, so it was not carried out in the current study.

***In-vitro* Diffusion Studies:** *In-vitro* release of the drug from different niosomes and gel formulation was studied using a semi-permeable membrane. Medium prepared was buffer pH 5.5: Ethanol AR (1:1). Placebo and drug-loaded batches were prepared. Cells in the diffusion apparatus were filled with the medium. The dialysis bag method was used to evaluate the drug diffusion profile of tea tree oil loaded niosomes. 1 ml of tea tree oil loaded niosomal formulation was placed in a dialysis bag which was suspended in a beaker containing medium Phosphate buffer 5.5: Ethanol (1:1). The receptor medium was maintained at 37 ± 2 °C and stirred magnetically at 500 rpm. Aliquots were withdrawn at the predetermined time intervals and analyzed by UV spectrophotometer at 273 nm.

After each sampling, the fresh buffer solution was replenished into the receptor chamber. Percent cumulative release of drugs was then plotted as a function of time. All experiments were carried out in triplicates and the mean values were calculated.

***Ex-vivo* Diffusion Studies:** Porcine ear skin was freshly excised. Fat was scraped off the skin and was immersed in the medium overnight. 1g niosomal gel was placed on top of the skin with dermal side facing donor compartment and studies were carried out using Franz diffusion cells. Receptor compartment was filled with 22 ml medium (5.5 pH phosphate buffer: Ethanol = 1:1) and allowed to stir at 500 rpm using a magnetic bead. 1ml aliquot was taken at regular intervals for 8 h and at 24^{th} h at 37 ºC ± 0.5 ºC and was replaced with the medium. Samples were analyzed using UV- Visible spectrophotometer at 273 nm.

Drug release of conventional gel, marketed gel, and placebo gel was also carried out for comparative studies.

Kinetic Treatment of the Release Data: The *in-vitro* release data of the drug from the investigated gel formulations was analyzed by curve fitting method to different kinetic models of zero-order, first-order and Higuchi models.

Zero-order release: Mt/M = kt

First-order release: Ln(1- Mt/M) = -kt

Higuchi model: $(Mt/M)^2 = kt$

The Korsmeyer-Peppas equation: $Mt = M = kt^n$

The Korsmeyer-Peppas equation was used to study the drug release mechanism by analyzing (n) as the diffusion exponent. According to this equation if $n \leq 0.45$, the release of drug follows Fickian mechanism, if $0.5 \leq n \geq 0.8$ the release of drug is Non-fickian and if $0.8 \leq n \geq 1$, a zero-order mechanism is governing the drug release mechanism from the gel.

Antifungal Testing: Antifungal testing was carried out using a cup and plate method. Saboraud dextrose agar was prepared and autoclaved for 15 min at 121 ºC. The media was poured into plates and allowed to solidify. A cup was made in the center of the plate with a cork borer, the formulation was added in the cup and culture (Candida albicans and Malassezia furfur) was inoculated. Prepared plates were placed in an aerobic condition in an incubator for 24 h. 2% niosomal gels, conventional gel and placebo niosomal gels were compared.

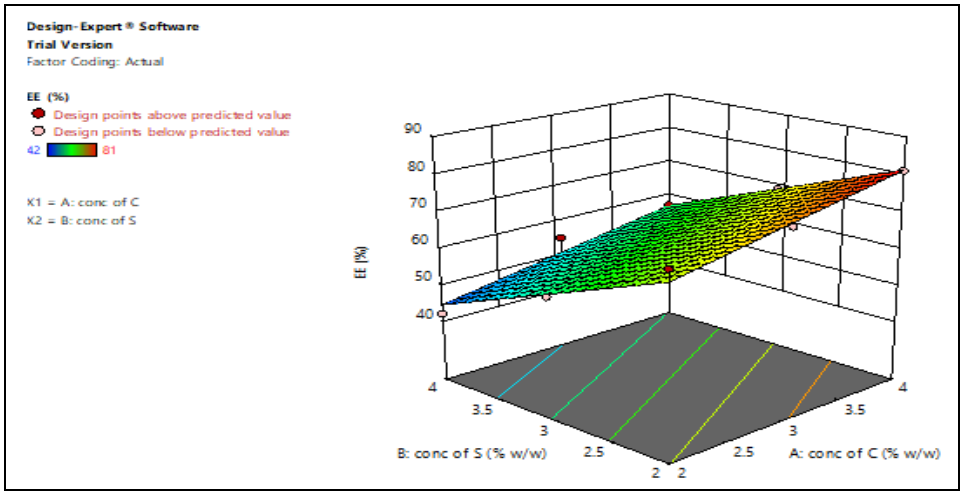


FIG. 6: RESPONSE SURFACE PLOT SHOWING THE INFLUENCE OF SURFACTANT (SPAN 60) AND CHOLESTEROL ON PERCENTAGE ENTRAPMENT EFFICIENCY OF THE NIOSOMES OF TEA TREE OIL

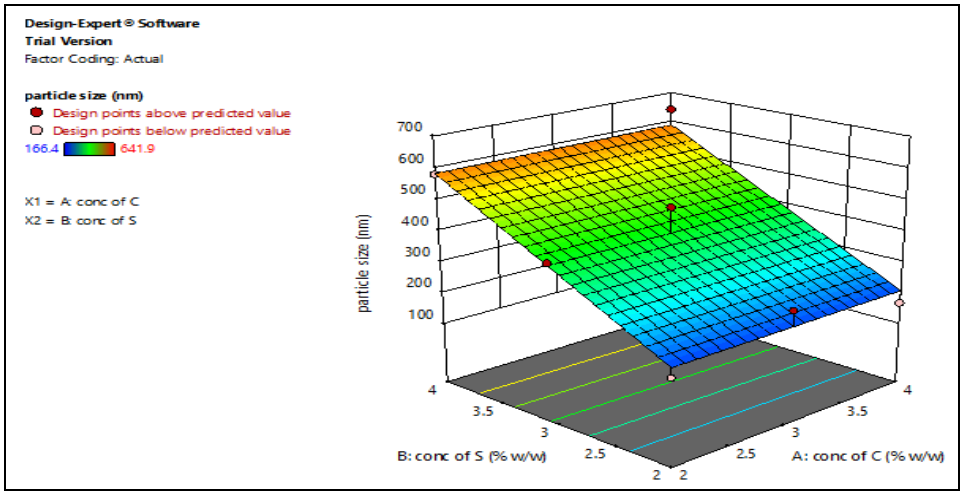


FIG. 7: RESPONSE SURFACE PLOT SHOWING THE INFLUENCE OF SURFACTANT (SPAN 60) AND CHOLESTEROL ON PARTICLE SIZE OF THE NIOSOMES OF TEA TREE OIL

RESULTS AND DISCUSSION: Niosomes were prepared by a thin-film hydration method using a rotary evaporator. All the nine batches as specified by 3^2 factorial design were prepared and data were further analyzed for all the batches as shown in **Table 3**. The particle size and polydispersity index of all 9 batches ranged from 166.4 to 641.9 nm, 0.171 to 0.503 respectively. Particle size and polydispersity index for the optimized batch (NB7) were found to be 166.4 nm and 0.503 respectively.

The entrapment efficiency of Tea tree oil niosomes ranged from 42% to 81%. The entrapment efficiency for optimized batch (NB7) was found to be 81%.

Selection of Optimized Formulation: After evaluating the parameters such as entrapment efficiency and particle size using Design expert® software version 11.0. On the basis of obtained parameters batch NB7 was chosen for further study. The selection of optimized formulation among all the 9 formulations was better explained by the 3D response plot.

Fig. 6 Entrapment efficiency decreased with an increase in the concentration of span 60 and increased with an increase in the concentration of cholesterol.

Fig. 7 confirmed that at a higher level of cholesterol, particle size got reduced and *vice versa*. Similarly, at the lower concentration of surfactant particle size decreased.

Surface Morphology: This was studied by TEM. The surface morphology displayed through TEM revealed vesicular, spherical particles with a smooth surface in the nano range as shown in **Fig. 2.**

Particle Size and Zeta Potential: Particle size of niosomes was found to be in nano range as shown in **Fig. 8(A, B)** Surface zeta potential was determined using zeta sizer. The zeta potential of optimized batch (F4) was found to be -20.5 mV **Fig. 5**. Although stabilizing agent *i.e.* surfactants used were non-ionic in nature, the zeta potential is greater in its digital value. Some charges were also generated due to cholesterol.

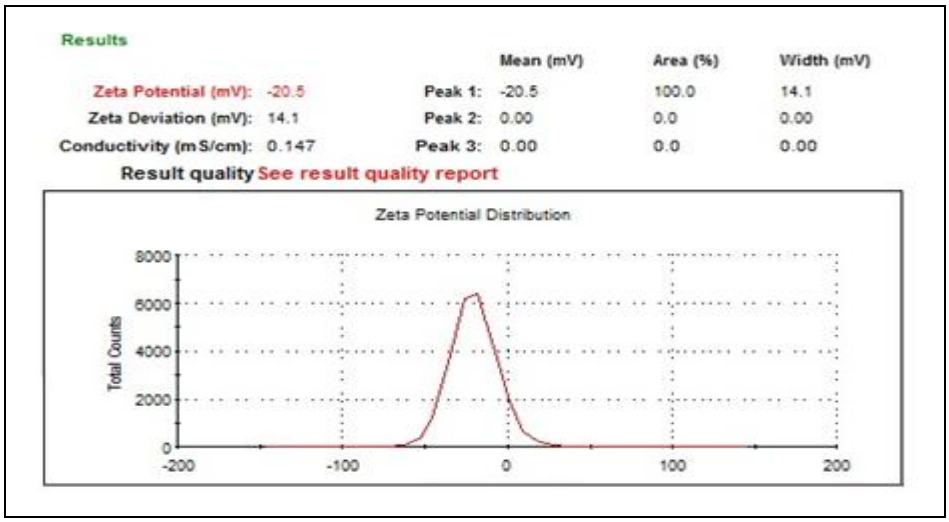


FIG. 8: ZETA POTENTIAL OF THE OPTIMIZED NIOSOMAL BATCH CONTAINING TEA TREE OIL

***In-vitro* Drug Diffusion Test:** *In-vitro* drug diffusion studies were carried out for two niosomal batches NB4 and NB7.

The *in-vitro* drug release was carried out for two optimized niosomal batches NB4 and NB7. It was observed that the drug released from NB4 was more as compared to NB7. Hence, NB7 was selected as the optimized batch since it showed the sustained release of drug from the vesicular formulation.

TABLE 5: *IN-VITRO* DRUG RELEASE OF NIOSOMAL BATCHES

Time (h)	NB4 (mean ± SD)	NB7 (mean ± SD)
0.5	5.49 ± 0.067	0.87 ± 0.030
1	14.04 ± 0.091	6.20 ± 0.040
2	19.6 ± 0.077	15.28 ± 0.052
3	27.22 ± 0.090	23.36 ± 0.040
4	30.68 ± 0.072	30.08 ± 0.033
5	33.34 ± 0.060	44.46 ± 0.055
6	37.16 ± 0.076	51.83 ± 0.045
7	46.10 ± 0.091	63.65 ± 0.016
8	52.68 ± 0.096	71.91 ± 0.040
24	57.56 ± 0.092	78.82 ± 0.030

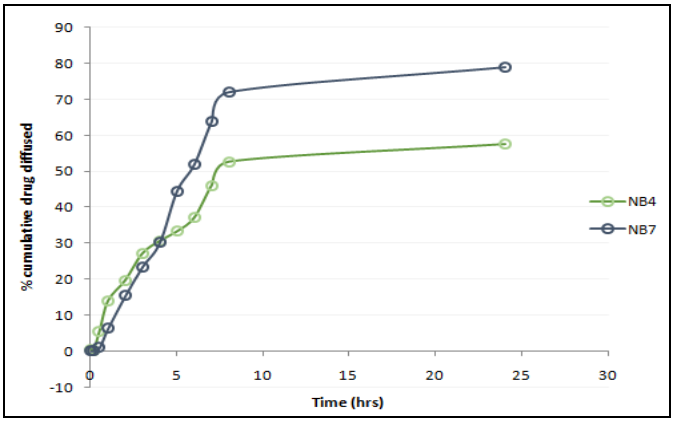


FIG. 9: *IN-VITRO* DRUG RELEASE OF NB4 AND NB7 NIOSOMAL BATCHES

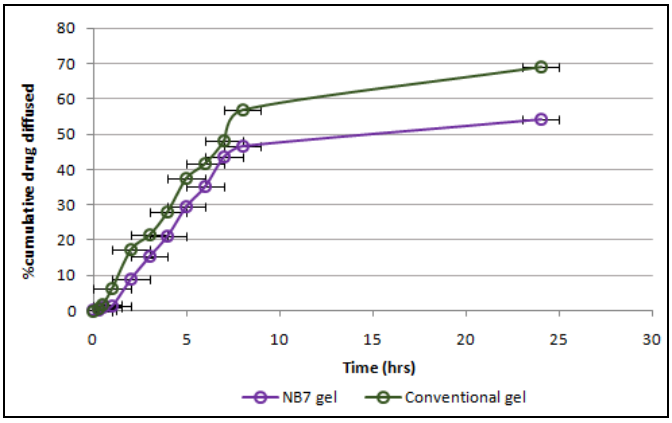


FIG. 10: *IN-VITRO* DRUG DIFFUSION STUDY OF G7 AND CONVENTIONAL GEL

Evaluation of Niosomal Gel G7: The prepared niosomal gels were found to be opaque, creamish with characterstic odor. The drug content of niosomal gel G7 was found to be 98.89 ± 0.01%. a pH of niosomal gel G7 was found to be 5.9 with spreadability of 67.91 g/cm. *In-vitro* release of niosomal gel was performed at different time period.

Cumulative drug release of niosomal gel was compared with conventional gel and the niosomal gel showed higher release (79.81 ± 0.01%) than the standard drug (51.91 ± 0.02%) **Fig. 10, B**.

***In-vitro* Drug Diffusion Studies:** *In-vitro* drug diffusion studies were carried for the optimized gel batch G7 and the conventional gel. The *in-vitro* drug diffusion of optimized gel batch G7 was compared with the diffusion of conventional gel. It was observed that drug diffusion from G7 was at a slower rate as compared to the conventional gel. Hence, the optimized gel batch gave a sustained release of drug as required.

TABLE 6: *IN-VITRO* DRUG DIFFUSION OF G7 AND CONVENTIONAL GEL

Time (h)	NB7 gel (mean ± SD)	Conventional gel (mean ± SD)
0.5	0.87 ± 0.0076	1.89 ± 0.0373
1	1.20 ± 0.0032	6.27 ± 0.0157
2	8.96 ± 0.015	17.25 ± 0.0118
3	15.26 ± 0.0217	21.62 ± 0.0248
4	21.23 ± 0.0173	28.15 ± 0.0401
5	29.54 ± 0.0291	37.54 ± 0.0173
6	35.29 ±0.0439	41.79 ± 0.0157
7	43.44 ± 0.03	47.97 ± 0.0338
8	46.50 ± 0.0278	56.96 ± 0.0261
24	54.21 ± 0.0199	68.96 ± 0.0301

***Ex-vivo* Drug Diffusion Studies:** *Ex-vivo* drug diffusion studies were carried for the optimized gel batch G7 and the conventional gel. The *ex-vivo* drug diffusion of optimized gel batch G7 was compared with the diffusion of conventional gel. It was observed that drug diffusion from G7 was at a slower rate as compared to the Conventional gel. Hence, the optimized gel batch gave a sustained release of drug as required.

TABLE 7: *EX-VIVO* DIFFUSION STUDIES OF G7 AND CONVENTIONAL GEL

Time (h)	NB7 gel (mean ± SD)	Conventional gel (mean ± SD)
0.5	0.27 ± 0.02	0.36 ± 0.021
1	0.58 ± 0.016	2.17 ± 0.006
2	4.80 ± 0.030	10.97 ± 0.023
3	11.72 ± 0.019	16.07 ± 0.030
4	15.74 ±0.030	19.37 ± 0.020
5	20.82 ± 0.028	26.39 ± 0.030
6	22.30 ± 0.027	31.19 ± 0.024
7	24.91 ± 0.040	36.96 ± 0.016
8	26.19 ± 0.013	47.54 ± 0.020
24	30.39 ± 0.022	54.23 ± 0.029

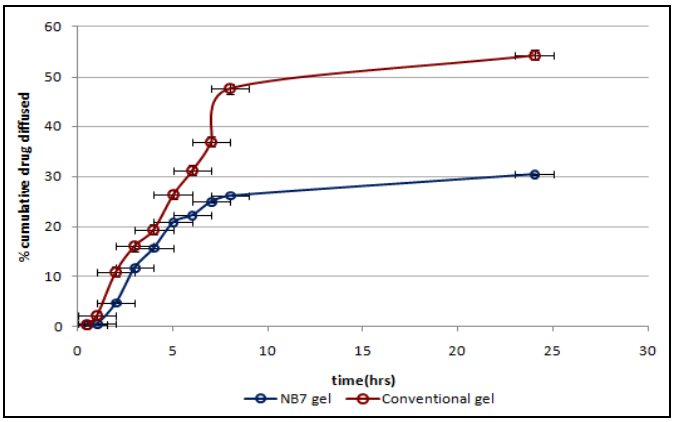
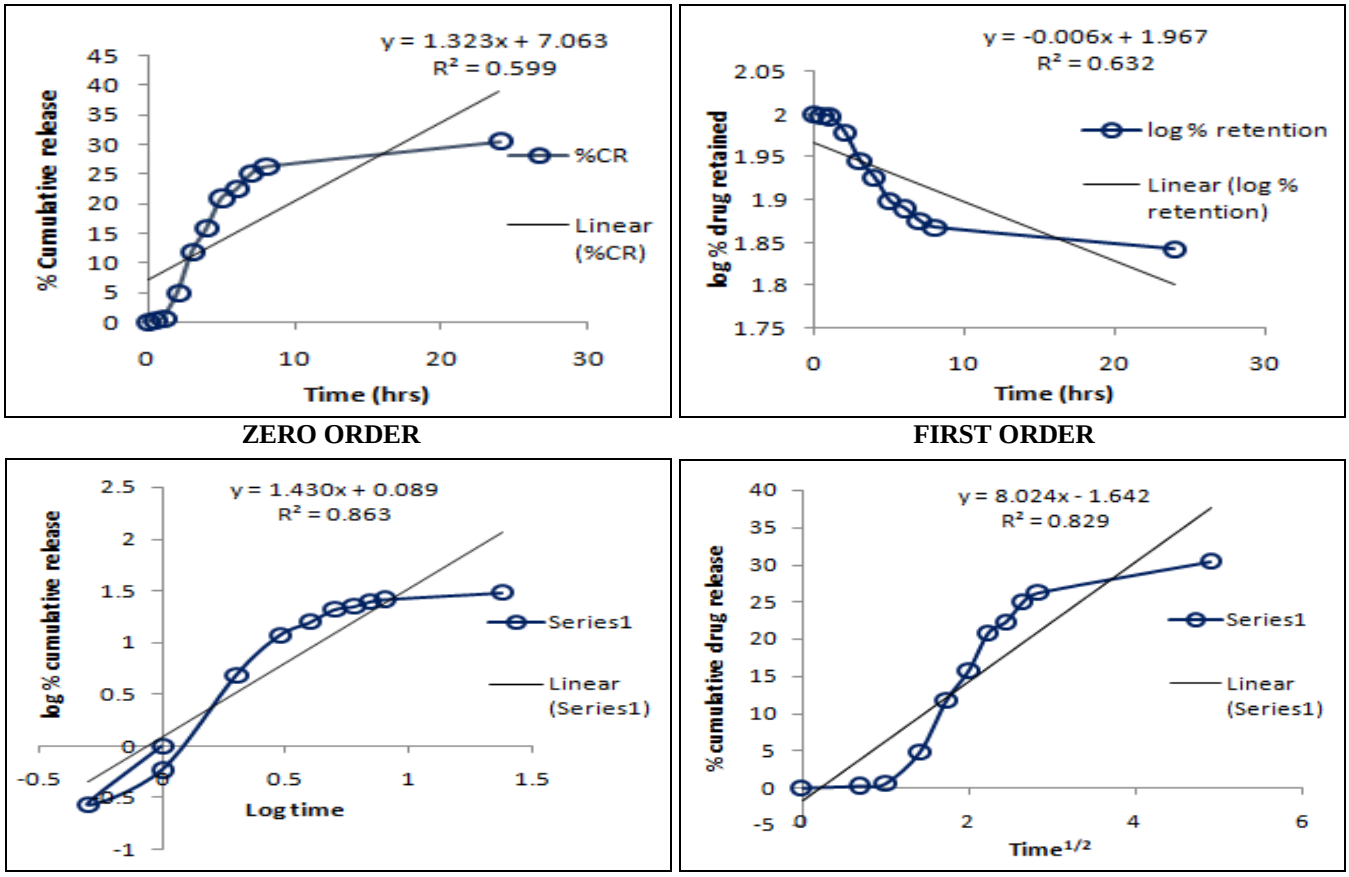


FIG. 11: *EX-VIVO* DIFFUSION STUDIES OF G7 AND CONVENTIONAL GEL

Kinetic Models:

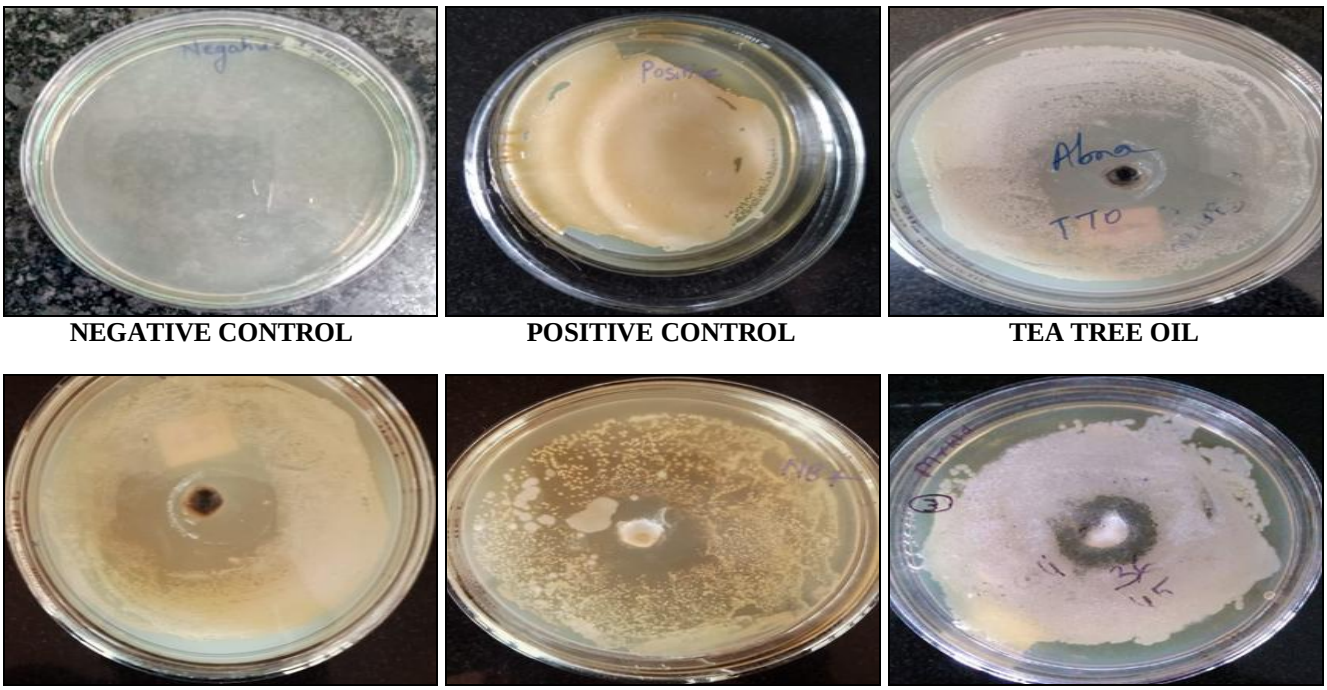


ZERO ORDER **FIRST ORDER**

KORSEMEYER PEPPAS **HIGUCHI**

FIG. 12: GRAPHICAL REPRESENTATION OF KINETIC MODELS FOR *EX-VIVO* DRUG RELEASE OF OPTIMIZED GEL BATCH G7

Antifungal Testing: The zone of inhibition was measured for each plate to compare the activity.



NEGATIVE CONTROL **POSITIVE CONTROL** **TEA TREE OIL**

NB7 **G7** **CONVENTIONAL GEL**

FIG. 13: AGAR DIFFUSION PLATES OF ANTI-FUNGAL TESTING AGAINST CANDIDA ALBICANS

TABLE 8: RESULTS OF ANTIFUNGAL TESTING AGAINST CANDIDA ALBICANS

S. no.	Plates prepared	Zone of inhibition observed (mm)
1	Negative control	-
2	Positive control	-
3	TTO	9
4	NB7	11
5	G7	7
6	Conventional gel	5

FIG. 14: AGAR DIFFUSION PLATES OF ANTI-FUNGAL TESTING AGAINST MALASSEZIA FURFUR

TABLE 9: RESULTS OF ANTIFUNGAL TESTING AGAINST MALASSEZIA FURFUR

S. no.	Plates prepared	Zone of inhibition observed (mm)
1	NB7	3
2	G7	2
3	Conventional	2

The zone of inhibition was calculated for both Tea tree oil loaded niosomal formulation and niosomal gel (G7). The zone of inhibition was also calculated for the conventional formulation for comparison. It was observed that G7 showed better inhibition as compared to the conventional gel against *Candida albicans* and G7 showed almost similar inhibition as compared to conventional gel against Malassezia furfur.

CONCLUSION: Tea tree oil niosomes were prepared by the thin-film hydration method. To obtain optimum entrapment efficiency and particle size, various parameters were modified including the concentration of cholesterol and concentration of surfactant. Entrapment efficiencies obtained for the optimized batches NB7 dispersions were found to be *in-vitro* drug release test results for NB7 gels was found 46.50% and 54.21% at 8^{th} h and 24^{th} of diffusion.

In-vitro drug release of niosomal dispersion showed enough sustained effect at the end of 8 h, which was decreased further after incorporation into the gel base. Incorporation of Tea tree oil in niosomes, helped to retain the drug on the skin which was supported by *ex-vivo* skin permeation and skin retention studies; where drug retention in case of the prepared formulation was better as compared to conventional gel.

The antifungal study was performed by the agar diffusion technique by the cup plate method and disc plate method. A final gel containing 2% Tea tree oil in niosomal dispersion (NB7) showed a higher zone of inhibition as compared to the conventional gel against *Candida albicans* and showed an equivalent zone of inhibition as compared to the conventional gel against Malasseiza furfur. So, it can be concluded that niosomes containing 2% Tea tree oil in a gel base may provide a longer duration of action, reduced frequency of application and may give effect equivalent to conventional gel.

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CONFLICTS OF INTEREST: Nil

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