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Research Article

FORMULATION DEVELOPMENT AND EVALUATION OF NIOSOMAL GEL OF COLLECTIVE ANTI-FUNGAL AGENTS

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ABSTRACT

Objectives: Tea tree oil (TTO) and *Eucalyptus* oil (EO) are essential oils derived from the leaves and terminal branches of *Melaleuca alternifolia* and *Eucalyptus globulus*. Both oils have narrative topical antifungal agents. Niosomal vesicles were chosen for Tea tree and EO dispersion in this investigation because of their ability to protect enclosed drugs, reduce drug dose amount, target drug delivery, increase residence time and penetration.

Methods: TTO and EO containing niosomes were made using a modified thin-film hydration process and Carbopol 934 as a gelling agent to produce a smooth antifungal niosomal gel.

Results: TTO and EO entrapment efficiency was found to be 84.89±0.19% and 86.86±0.57%, respectively, and percent cumulative drug diffusion of TTO and EO was found to be 84.21% and 85.22% in the prepared optimized batch [N9]. Transmission electron microscopy revealed vesicular, spherical particles in the nano range with a smooth surface. The optimum batch [N9] of niosomal gel was made with 1% w/w carbopol 934. TTO 84.9% and EO 86.89%, respectively, were found to have prolonged drug release in an *in vitro* release investigation utilizing the dialysis bag method. The G9 batch niosomal gel was found to be stable by performing an accelerated stability study for 3 months.

Conclusion: It was concluded that the best formulation batch G9 shows better-sustained release, enhanced residence time, and stability.

Keywords: Tea tree oil, *Eucalyptus* oil, Niosome, Span 40, Span 60, Carbopol 934.

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INTRODUCTION

Skin infections are common and often offer treatment obstacles for practitioners due to increased concerns about multidrug-resistant bacteria, viral, and fungal strains. This is mostly due to their association with a wide range of diseases, ranging from minor skin and soft tissue infections to life-threatening systemic sepsis and meningitis. Even though several antimicrobial medicines already exist, unique spectra of activity and pharmacokinetic profiles are required to treat such infections. Antimicrobial resistance and healthcare delivery improvements have created a demand for antimicrobials with novel spectrums of activity [1-4].

Physicians can choose from a variety of therapy options, including solid, semisolid, and liquid dose formulations. Clear transparent gels have been generally recognized in both cosmetics and pharmaceuticals as a topical preparation. Transparent gels have become more popular in cosmetics and pharmaceutical preparations among the principal category of semisolid preparations. Delivery of drugs to the skin is an effective and targeted therapy for local dermatological disorders. This route of drug delivery has gained popularity because it avoids first-pass effects, gastrointestinal irritation, and metabolic degradation associated with oral administration. Due to the first past effect, only 25–45% of the orally administered dose reaches the blood circulation. To avoid these disadvantages, the gel portions have been proposed as a topical application. Gels are defined as a "semi-solid system in which a liquid phase is constrained within a polymeric matrix in which a high degree of physical and chemical cross-linking is introduced [5-12].

Niosomes, which have a bilayers structure generated by the self-association of non-ionic surfactants and cholesterol in an aqueous phase, are among the most promising drug carriers. They are either adsorbed on the skin's surface, resulting in a strong thermodynamic activity gradient at the interface, facilitating drug absorption, or they penetrate the stratum corneum and act as drug reservoirs [9,13-17].

The purpose of this study was to formulate and evaluate a niosomal gel containing Tea tree and *Eucalyptus* oils (EOs) for *in-vitro* efficacy. Using a modified thin-film hydration method, niosomal formulations with variable ratios of surfactants (Span 40 and Span 60) and cholesterol were formulated. The best niosomal batch was selected based on, *in-vitro* characteristics, entrapment efficiency, stability studies, and niosomal gel were formulated, which was further studied.

MATERIALS AND METHODS

Materials

Tea tree oil (TTO) (*Melaleuca alternifolia*), EO (*Eucalyptus globulus*) was manufactured by Organix mantra, New Delhi, India. Cholesterol was manufactured by S.D. Fine chem limited, Mumbai, Maharashtra, India. Span 40 Span 60 Carbopol 934 Glycerol Triethanolamine Chloroform was manufactured by Loba Chemi, Mumbai, Maharashtra, India.

Methodology

Physicochemical test of TTO and EO

To establish the TTO and EO's authenticity, its physicochemical qualities were examined. The physicochemical properties determination result was compared to the specification in the literature. The organoleptic and other evaluations were carried out by evaluating, TTO and EO's color, odor, solubility, density, LogP, Boiling point, specific gravity, and refractive index.

Formulation of niosomes

With some modifications, the thin film hydration process was employed to make niosomes. Surfactants and cholesterol were dissolved in an organic solvent in various ratios, and 1% v/v TTO and 1% v/v EO were added to this solution. A rotary evaporator was used to extract the organic solvent under a vacuum at 60°C until a thin layer is formed. To eliminate any remaining organic solvents, more vacuum was used. This film was hydrated for 60 min at 50°C with 6 mL of phosphate buffered

saline (PBS) (pH: 7.4). The resulting niosomal suspension was sonicated 3 times at 50 Hz for 3 min each time, with a 5-min delay between each time. The niosomal suspension was kept in the refrigerator at a low temperature (4–8°C) for congealing and for further study [15,18-23]. Formulation of various batches is shown in Table 1.

Evaluation of niosomes

Transmission electron microscopy (TEM) analysis

The morphology of the niosomal vesicles was determined using TEM (Philips CM 200 super twin stem microscope, Netherlands). A transmission electron microscope was used to investigate a few drops of the improved niosomal formulation [N9] deposited on a carbon-coated copper grid [20,21].

Optical microscopy

A Digital Microscope was used to perform Optical Microscopy on the drug sample (Motic DMWB1-223ASC, Hyderabad). Niosomal batches were viewed under the optical microscope to observe the shape and lamellar nature of vesicles on the glass slide, a small amount of the niosomal sample solution was distributed. The images on this slide were obtained using various magnification lenses [20,21].

Determination of vesicle diameter, poly-dispersity index, and zeta potential

Dynamic light scattering was used to quantify the z-average diameter of sonicated vesicles using a Horiba Scientific Nanoparticle analyser (SZ-100V2, Japan). The vesicle diameter, polydispersity index (PDI), and zeta potential were assessed by diluting 100 µl of the formulation with an appropriate volume of PBS, pH 7.4, and measuring the vesicle diameter, PDI, and zeta potential [20,21].

Fourier transform infrared spectroscopy (FTIR)

On a FTIR Spectrophotometer, the infrared spectra of TTO and EO niosomal batches were determined (FT-IR Bruker-Alpha, Apexan Analytical Techniques, Vadodara). These samples were then placed in a sample holder, and scans were taken from 4000 cm^{-1} to 400 cm^{-1} at a resolution of 2 cm^{-1} [20,21].

Drug content

Drug content was determined by adding 2 ml ethanol to 1 ml niosomal buffer solution, and the remaining volume was made up with distilled water. The addition of ethanol causes the niosomes to break down, allowing the medication to dissolve freely in a solvent. Each of these solutions was then diluted with distilled water to the desired concentration. Absorbance was determined at 262 nm for EO and 273 nm for TTO using a ultraviolet (UV)-visible spectrophotometer (Shimadzo UV visible 1800, Gumtree India Analytical, Hyderabad). Drug content was determined by using the formula,

Percent drug content=(Test absorbance×Standard Conc)/(Standard absorbance×weight of drug)×Dilution factor×100 [20,21].

Determination of drug entrapment in vesicles

To separate niosomes from non-entrapped medication, niosomal formulations were centrifuged at 10,000g for 90 min at 25°C. A UV spectrophotometer was used to determine the concentration of the free drug in the supernatant by measuring absorbance for TTO at 273 nm and for EO at 262 nm (Shimadzu 1800, Gumtree India Analytical, Hyderabad). The drug entrapment % in niosomes was determined. This procedure was repeated three times to guarantee that all free drugs were eliminated. The drug entrapment efficiency was determined using the formula,

% Drug entrapment=(Total drug - Drug in supernatant)/Total drug×100 [20,21].

Anti-fungal studies

Sabouraud dextrose agar was weighed 16.25 g and transferred to a 500 ml conical flask, followed by 250 ml filtered water, which was mildly heated to dissolve the sabouraud dextrose agar and sterilized for 20 min at 121°C at 15 lb pressure. Then it was cooled to room temperature, and the fungal strain (*Aspergillus niger*)was disseminated in the medium, which was then poured into the Petri dishes and allowed to cool until it solidified at room temperature. The antifungal study was conducted using the disc diffusion method. The 6 mm sterile filter paper discs were dipped in niosomal formulation and placed evenly between each o and transferred to a 500 ml conical flask, followed by 250 ml filtered water, which was mildly heated to dissolve the sabouraud dextrose agar and sterilized for 20 min at 121°C at 15 lb pressure. Then, it was cooled to room temperature, and the fungal strain (*A. niger*) was disseminated in the medium, which was then poured into the Petri dishes and allowed to cool until it solidified at room temperature. The antifungal study was conducted using the disc diffusion method. The 6 mm sterile filter paper discs were dipped in niosomal formulation and placed evenly between each other [15,18-23].

In vitro release studies

A dialysis bag (dialysis membrane, 12,000–14,000 molecular weight cut-off) was used as a donor compartment in an *in vitro* study. After centrifugation of 2 ml of the formulation, niosomes containing entrapped medication were re-suspended in 1 ml of PBS, pH 7.4, and used for the release research. After soaking the dialysis membrane in warm water for 10 min, one end was sealed, the niosome preparation was pipetted into the bag, and the bag was shut to prevent leaking. At 37°C ± 2°C, the dialysis bag was immersed in 100 ml of pH 7.4 PBS. At 100 rpm, the medium that served as the receptor compartment was agitated. 5 ml samples were withdrawn hourly from the medium and replaced with fresh buffer and TTO and EO absorbance at 273 nm and 262 nm was measured using PBS as blank. Results were the mean values of three runs [20,21].

Kinetic release study

The mechanism of TTO and EO release from niosomal formulations was determined using the following mathematical models: Zero-order kinetics, first-order kinetics, Higuchi kinetics, and the Korsmeyer-Peppas and Hixson-Crowel models [21,24].

Zero-order release kinetics

It is the technique of a medication delivery device releasing a consistent amount of drug regardless of the concentration. Zero-order release can be written as

Table 1: Formulation table of various niosomal batches

S. No.	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1.	Tea tree oil	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v
2.	*Eucalyptus* oil	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v
Molar ratio (m.r.)										
3.	Span 40	1	1	2	-	-	-	1	2	1
4	Span 60	-	-	-	1	1	2	1	1	2
5	Cholesterol	1	2	1	1	2	1	1	2	2
6	Chloroform	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL
7	PBS (pH: 7.4)	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL

PBS: Phosphate buffered saline

$Q=Q_0+K_0$

t in its simplest form.

Where Q represents the amount of drug released or dissolved, Q_0 represents the initial amount of drug in solution (typically zero), and K_0 represents the zero-order release constantly. Cumulative drug release vs. time was plotted. The fraction of the drug dissolved will be represented graphically as a linear function of time. Zero-order release kinetics, the slope of the curve determines the value of K. This is mostly expressed via osmotic pump systems, as well as transdermal systems, matrix tablets with low soluble medicines, and coated for osmotic pump systems.

First-order release kinetics

The first-order release equation expresses the release from a system where the rate of release is concentration-dependent:

$Kt=dC/dt$

Where K denotes the first-order rate constant in units of time -1.

This equation can be written as follows:

$2.303 \text{ Log } C_t=\text{Log } C_0-k\,t$

Where C_0 represents the drug's initial concentration in solution and C_t represents the drug's concentration in solution at time t. The concentration gradient (C_0 - C_t) between the static liquid layer near the solid surface and the bulk liquid predicts a first-order dependence on the equation. The graph was made as follows: log cumulative of percent d remaining versus time, yielding a straight line with a –K/2.303 is slope. This profile is followed by dose formulations incorporating water-soluble drugs in a porous matrix, proportionate to the amount of drug released by unit time diminishing [24].

The chi created the first mathematical model to describe drug release from a matrix system in 1963. This model can be used to investigate the release of water-soluble and low soluble medicines included in semisolid and solid matrices.

The equation gives the model expression:

$Q = A\,(D\,[2C-Cs]\,Cs\,t)^{1/2}$

Where Q is the amount of medication released per unit area in time t. The drug starting concentration is A, Cs is the drug solubility in the media is Cs, and D is the drug molecules' diffusivity (diffusion coefficient) in the matrix is D.

The release of pharmaceuticals from the insoluble matrix is described by the simplified Higuchi model as a square root of a time-dependent process based on the Fickian diffusion equation.

$Q=KH\,t^{1/2}$

The Higuchi equation has a distinct and physically plausible meaning. The ability to

1. Facilitate device optimization and
2. Better understanding the underlying drug release mechanisms are two important advantages of this equation. Other forms of drug delivery systems, such as controlled release transdermal patches or films for oral controlled drug delivery, can also use the equation. To define the limits for transport and drug release, the Higuchi and zero-order models are used [24].

Hixson-Crowell cube root law

Hixson and Crowell suggested the cube root law as a way to depict the disintegration rate that is adjusted for the decrease in solid surface area as a function of time. The Hixson- Crowell cube root law discusses the release of particles or tablets from systems with a change in surface area and diameter. Assuming there is not any as suspended solid dissolves, its surface area reduces by the two-thirds power of its mass. Hixson and Crowell exploited this relationship in the development of the cube root law. When sink conditions are used, the cube root law is expressed as

$Q_t^{1/3}=Q_0^{1/3}-K_{HC}t$

$Q_0^{1/3}-Q_t^{1/3}=K_{HC}t,$

Where Q_t signifies the solid's remaining weight at time t,
Q_0 denotes the solid's initial weight at time t = 0, and
K_{HC} denotes the dissolution rate constant.

If the equilibrium conditions are not met and the geometrical shape of the dose form reduces proportionally with time, the graphical plot of the cubic root of the unreleased fraction of the drug versus time should provide a straight line. This model is employed by assuming that the rate of release is restricted by the dissolution rate of the drug particles rather than diffusion. Hixson and Crowell's assumptions about the law's validity can be expressed as follows:

1. The law is said to be better suited to mono-dispersed, primarily spheroidal materials, in which the solid is in the form of a single unit or units with similar size, shape, surface, and volume attributes
2. The disintegration occurs in a plane perpendicular to the surface. The variation in rates between crystal faces is much smaller, and agitation of the liquid against all areas of the surface has the same impact
3. The liquid is vigorously stirred to minimize stagnation in the vicinity of the dissolving particle, resulting in a sluggish diffusion rate [24].

Korsmeyer-Peppas Model

Korsmeyer (1983) developed a simple equation to characterize drug release from a polymeric system. Ritger Peppas and Korsmeyer Peppas devised an empirical equation to model both Fickian and non-Fickian drug release from swelling and non-swelling polymeric delivery methods. To figure out how drugs are released, the first 60% of drug release data were fitted into the Korsmeyer – Peppas model.

$M_t/M^{\alpha}=K\,t^n$

Where, M_t/M_{α} is the fraction of medicine released at time t, and k is the rate constant (in units of t^n) that incorporates the delivery system's structural and geometric properties. n is the release exponent, which indicates how the medication is transported through the polymer. The n value is used to distinguish between various release techniques [24].

Stability study

Niosomal formulation was selected based on entrapment efficiency, *in-vitro* release studies and FTIR. Stability studies were assessed by keeping niosomal batches in sealed glass vials and storing them at 40°C±2°C/75% RH±5% RH for 3 months (Accelerated stability study). The samples were withdrawn at different time intervals over a period over which was studied [25].

Preparation of gel containing niosomes

In a beaker, carbopol 934 and purified water were combined and left to soak for 24 h. To do so, a suitable amount of niosomal batch [N9] containing 1% TTO and 1% EO was distributed in water, and then carbopol 934 was neutralized with enough triethanolamine (pH: 6.5). As a moistening agent, glycerine was slowly added with gentle stirring until a homogeneous gel was created (using a homogenizer, Omni International, Kennesaw Georgia) [8-13].

Evaluation of niosomal gel

Appearance

The gels' appearance was scrutinized for clarity. Visual assessment against a black and white background was used to assess the clarity of various formulations [8,10,21].

Percentage yield

The empty container was weighed, then the container in which the gel formulation was stored, then weighed again with the gel formulation. The practical yield is calculated by subtracting the empty container's weight from the weight of the container containing the gel mixture. Then the percentage yield was calculated by the formula.

Percentage yield=Practical yield/Theoretical Yield×100 [8,10,21].

pH measurements

A digital pH meter (Hanna instruments, Singapore) was used to determine the pH of the gel compositions. The pH meter was calibrated before each measurement, and readings were taken by dipping the glass rod into the gel compositions [8,10,21].

Drug content of the gel

Each formulation (1 ml) was placed in a 100 ml volumetric flask and diluted with distilled water to the desired concentration. The amount of medication in the formulation was assessed using ultraviolet spectroscopy at 262 and 273 nm for EO and TTO, respectively, after appropriate dilutions [8,10,21].

Viscosity measurement

A Brookfield viscometer (DV-I+, Thane, Maharashtra) was used to determine the viscosity of gel compositions. A 25.0 g gel was placed in a beaker and rotated on spindle number 4 to evaluate the sample's viscosity [8,10,21].

Spreadability

The spreadability of gel formulations was determined by using spreadability apparatus. 1.0 g of gel sample was placed on the lower slide and an upper slide was placed on the top of the sample. The spreadability was determined by the formula,

$$S = (m \times l)/t$$

Where, S = Spreadability, m = weight tied to upper slide, l = length travelled by upper slide, t = time taken by slide to travel [8,10,21].

Table 2: Release kinetics [24]

Release Exponent (n)	Drug transport mechanism	Drug release mechanism
n<0.5	Quasi-Fickian diffusion	Non-swellable matrix- diffusion
n=0.5	Fickian diffusion	
0.5<n < 1.0	Anomalous (Non - fickian transport)	both diffusion and relaxation (erosion)
1.0	Case II transport	Zero-order release
n>1.0	Super case II transport	(relaxation/erosion)

Anti-fungal studies

The anti-fungal studies procedure for niosomal gel is performed similarly to of niosomes antifungal study [8,10,21].

In vitro release studies

Niosomal gel containing entrapped drug obtained after centrifugation of 2 ml of the formulation was resuspended in 1 ml of PBS, pH 7.4, and used for the release study. The rest of the procedure is the same as the niosomes *in-vitro* release study [8,10,21].

Stability studies

Niosomal gel stability study was performed the same as of niosomal stability study [25,26].

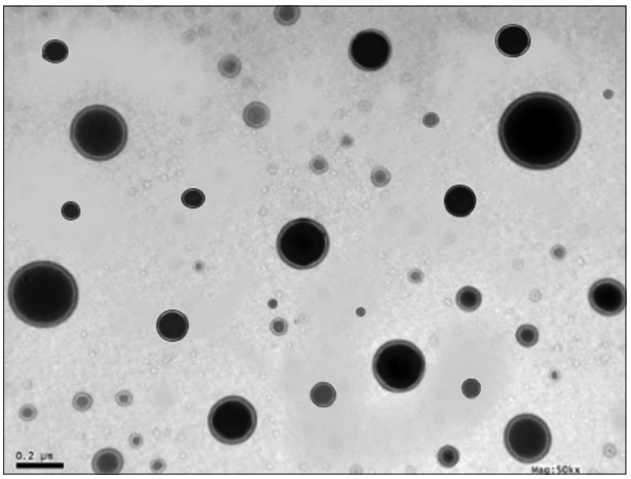

Fig. 1: Transmission electron microscopy image of N9 batch

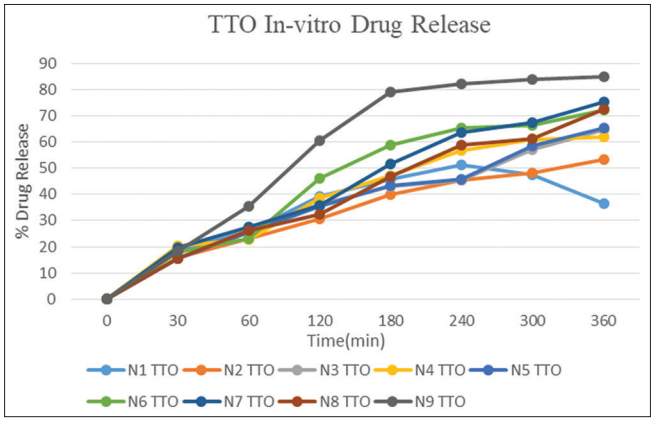

Fig. 2: Tea tree oil *in-vitro* drug release

Table 3: Physicochemical properties results of TTO and EO

Physicochemical properties	Specification		Result	
	TTO	EO	TTO	EO
Organoleptic	Colorless to pale yellow, clear, mobile liquid, Earthy, spicy odor	Colorless mobile liquid; camphor-like aroma	Colorless, clear, mobile liquid, Earthy, spicy odor	Colorless mobile liquid; camphor-like aroma
Solubility	Insoluble in water, soluble in non-polar solvents	Insoluble in water, soluble in non-polar solvents	Insoluble in water, soluble in non-polar solvents	Insoluble in water, soluble in non-polar solvents
LogP	2.82–6.64	2.74	6.63	2.74
Density (g/ml)	0.8950–0.9050	0.921–0.923	0.9010	0.923
Boiling point (°C)	165	176-177	165	177
Specific gravity (T=25°C) (g/cm^3)	0.885–0.906	0.921–0.923	0.894	0.922
Refractive index (T=25°C)	1.475–1.482	1.455–1.460	1.479	1.479

TTO: Tea tree oil, EO: *Eucalyptus* oil

Table 4: Drug content, entrapment efficiency, the production yield of TTO and EO

Batch	DC (%)*TTO	DC (%)*EO	EE (%)*TTO	EE (%)*EO	% Production yield
Placebo	-	-	-	-	91.15±0.27
N1	53.21±0.71	54.47±0.67	51.23±0.54	56.47±0.28	79.85±0.74
N2	54.63±0.57	57.81±0.57	53.48±0.36	55.82±0.46	88.7±0.82
N3	66.78±0.66	62.95±0.98	64.91±0.85	61.5±0.29	90.94±0.73
N4	68.11±0.91	70.92±0.80	66.72±0.25	64.2±0.18	81.15±0.51
N5	68.93±0.6	64.52±0.47	65.5±0.82	62.81±0.38	84.6±0.18
N6	74.13±0.8	76.47±0.47	72.33±0.76	74.64±0.26	86.34±0.38
N7	77.73±0.88	80.19±0.73	75.56±0.28	78.93±0.51	83.92±0.24
N8	74.13±0.82	77.56±0.38	72.59±0.67	75.88±0.72	86.71±0.66
N9	85.49±0.32	87.66±0.94	84.89±0.19	86.86±0.57	93.4±0.85

*Mean±SD, n=3, TTO: Tea tree oil, EO: Eucalyptus oil

Table 5: Release kinetics of Tea tree oil drug

Batch Code	Zero-order model	First-order model	Higuchi/Matrix model		Hixson-Crowell model	Korsmeyer-Peppas model	Best fit kinetic model
	R	R	R	n	R	R	
N1	0.8095	0.8545	0.9602	3.0100	0.8413	0.917	HM
N2	0.9027	0.9504	0.9966	2.8478	0.9362	0.8946	HM
N3	0.8875	0.9423	0.9876	3.1022	0.9281	0.9062	HM
N4	0.9295	0.9787	0.9914	3.5964	0.966	0.8570	HM
N5	0.9199	0.9598	0.9880	3.2537	0.9508	0.8626	HM
N6	0.9071	0.9550	0.9721	4.2397	0.9420	0.8252	HM
N7	0.9471	0.9832	0.9842	4.0008	0.9766	0.8282	HM
N8	0.9456	0.9773	0.9808	3.6836	0.9706	0.8196	HM
N9	0.8802	0.9529	0.9639	5.4555	0.9351	0.8311	HM

Table 6: Release kinetics of *Eucalyptus* oil drug

Batch Code	Zero-order model	First-order model	Higuchi/Matrix model		Hixson-Crowell model	Korsmeyer-Peppas model	Best fit kinetic model
	R	R	R	n	R	R	
N1	0.5946	0.5652	0.7860	2.6666	0.5779	0.7772	HM
N2	0.9288	0.9554	0.9518	2.849	0.9499	0.8110	FO
N3	0.9034	0.9506	0.9872	2.7994	0.9377	0.8907	HM
N4	0.9780	0.9833	0.9894	3.1578	0.9855	0.7734	HM
N5	0.9678	0.9787	0.9639	3.0194	0.9753	0.7699	FO
N6	0.9758	0.9157	0.9304	4.0296	0.9257	0.6849	HM
N7	0.9795	0.9821	0.9886	3.8256	0.9872	0.7478	HM
N8	0.9698	0.9870	0.9549	3.8335	0.9849	0.7348	FO
N9	0.9207	0.9604	0.9631	5.4593	0.9561	0.7924	HM

RESULTS AND DISCUSSION

Physicochemical test of TTO and EO

All of the physicochemical parameters of the TTO and EO utilized in this investigation met the specifications, as shown in Table 3. One of the most essential parameters for measuring the quality and purity of oil content is specific gravity. The degree of unsaturation and the molecular weight of the oil influence specific gravity. The bigger the average number of fatty acid molecules in the oil, the higher the specific gravity. The refractive index is used to determine the purity of the oil. The fatty acid concentration of vegetable oils, the free fatty acid content, the oxidation process, and the temperature all influence the refractive index values. The refractive index will grow in value as the carbon chain lengthens and the number of double bonds increases.

The physicochemical of TTO and EO was found to meet the literature specifications in all of the tests.

TEM images of niosomes

The development of niosomes was confirmed by TEM pictures of the N9 Batch. The vesicles were spherical and resembled typical niosome micrographs found in previous research. The niosomes were almost the same size as the average particle size detected by Zetasizer (1114.1±0.631 nm). The bimodal size distribution of the N9 formulation was revealed by the particle size distribution histogram. The TEM study, which revealed numerous niosomes around 1 μm, corroborated this. However, when compared to the overall size distribution, the fraction of larger niosomes was found to be quite low.

Table 7: Antifungal study of Tea tree oil, *Eucalyptus* oil, and N9 batch

Sr. no.	Name	Zone of inhibition observed (mm)
1.	Positive control	-
2.	Negative control	-
3.	Tea tree oil	10
4.	*Eucalyptus* oil	9
5.	Tea tree oil+*Eucalyptus* oil	15
6.	N9	13

Production yield

The percentage production yield of F1 to F9 batches was observed in a wide range from 79.85% to 93.4%. This F9 batch hasa good % production yield of 93.4%. It was concluded that as Surfactant concentration

increases the production yield of niosomes increases. The percentage production yield of nine batches of niosome is listed in Table 4.

Entrapment efficiency

The entrapment efficiency of drug-loaded niosomal formulations was observed to rise as the cholesterol ratio was increased to 2, but it decreased as the cholesterol ratio decreased to 1. This could be attributed to two things. First, as the cholesterol ratio rises, the hydrophobicity and stability of bilayer vesicles rise while permeability falls, potentially allowing the hydrophobic medicine to be trapped efficiently in bilayers as the vesicles form. Second, a larger level of cholesterol may compete with the oils for packing space within the bilayer, preventing the drug from being formed as amphiphiles. In addition, evaluating different niosomal formulations including different grades of Span (Span 40, Span 60) at different ratios of surfactant to cholesterol. In comparison to Span 40 formulations, the Span 60 incorporating niosomal formulation had the better entrapment efficiency. This could be because Span 60 has the largest alkyl chain length of any of the Span series. The combination of both surfactants i.e showed the best entrapment efficiency as compared to other formulations. The percentage entrapment efficiency of batches N1 to N9 was in the range from 51.23% to 84.89% of TTO and for EO 56.47% to 86.86%. The highest % entrapment efficiency shown in the N9 batch was 84.89% and 86.86%, respectively, listed in Tables 3, 4, 8, it was concluded that an increase in span 60 and Cholesterol concentration increases percentage entrapment efficiency. Based on the above information, the niosomal formulation with the best entrapment efficiency across all Span series was chosen for further research.

***In-vitro* drug release study**

In-vitro drug release studies were carried out for niosomal batches (Figs. 2 and 3). It was observed that the drug released from the niosomal N9 batch was more and sustained release as compared to other batches. This could be because Span 60 has the longest alkyl chain length than Span 40 and hence Span 60 has a better release profile than Span 40. The formulation containing both the surfactant showed slow release. Hence, N9 was selected as the optimized batch since it showed the sustained release of TTO and EO from the vesicular formulation.

Table 8: Accelerated stability study of N9 batch

Period	Particle size (nm) of N9 batch	Entrapment efficiency (%) of N9 batch	
		TTO	EO
Initial	389.6±0.31	84.89±0.19	86.86±0.57
After 1 month	390.8±0.64	83.72±0.91	85.28±0.16
After 2 month	391.2±0.78	82.82±0.17	83.81±0.73
After 3 month	392.1±0.53	80.52±0.42	81.63±0.34

*mean±SD, n=3, TTO: Tea tree oil, EO: *Eucalyptus* oil

Table 9: Evaluation parameters of G9 Batch

S. No.	Formulation	G9
1	Appearance	Translucent
2	pH*	6.5±1
3	Drug Content (%)*	90.49±0.36
4	Spreadability (cm)*	2.2±0.32

*Mean±SD, n=3

Optical microscopy

From the microscopy (Figs. 4-6), it was concluded that niosomes containing both surfactants i.e. Span 40 and Span 60 were slightly bigger as compared to individual surfactants. There was not any significant difference between Span 40 niosomes and Span 60 niosomes. N3, N6, N9 batches niosomes were observed to be spherical in shape and multilamellar in nature.

Zeta potential determination

The values of N9 Niosomal batch as per Fig. 7, was found out to be Zeta potential (mV): −45.1 Conductivity (mS/cm): 5.923 Electrophoretic Mobility (cm^2/vs): 0.000035. Even though the stabilizing agents, such as surfactants, were non-ionic, the zeta potential has a higher digital value. The negative charge is contributed by the negatively charged sulfonate groups present in cholesterol. The particles with zeta potential more positive than +30 or more negative than -30 are considered stable [21]. Thus, it was concluded that the N9 niosomal batch has good stability and particle size will not increase due to aggregation or coagulation even after the formulation is kept for a long time.

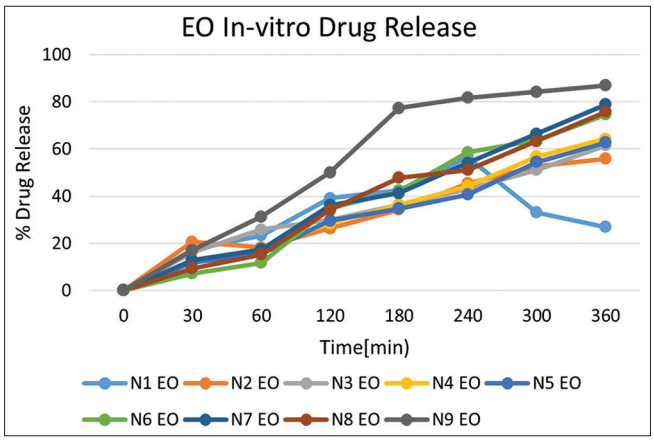

Fig. 3: *Eucalyptus* oil *in-vitro* drug release

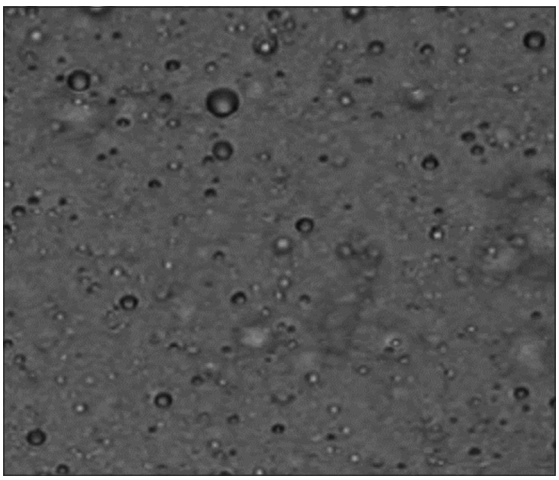

Fig. 4: Microscopy of N3 batch

Table 10: Release kinetic study of G9 batch

Batch Code	Zero-order model	First-order model	Higuchi/Matrix model		Hixson-Crowell model	Korsmeyer-Peppas model	Best fit kinetic model
	R	R	R	n	R	R	
G9 TTO	0.8619	0.9425	0.9757	5.1206	0.9237	0.8843	HM
G9 EO	0.8802	0.9544	0.9810	5.0304	0.9391	0.8826	HM

TTO: Tea tree oil, EO: *Eucalyptus* oil

Particle size and PDI

From Figs. 8-10, it was concluded that niosomes containing a combination of surfactants were slightly bigger as compared to individual surfactant niosomes. This may be due to the number of alkyl chains present in the surfactants. As the number of alkyl chains increases, the size of the niosomes increases. The particle size of the niosomes was found to be in the nano range as mentioned in Fig. 8-10.

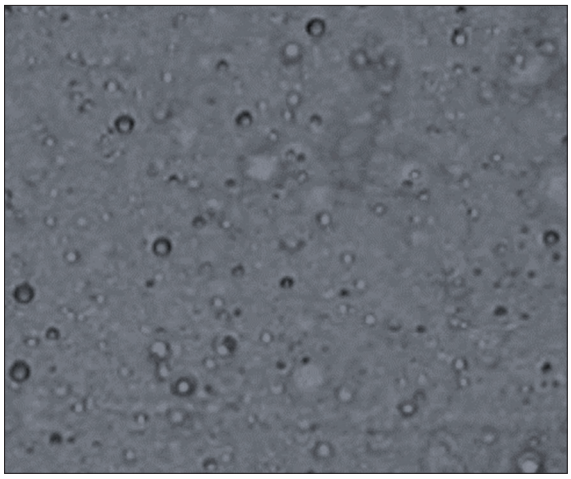

Fig. 5: Microscopy of N6 batch

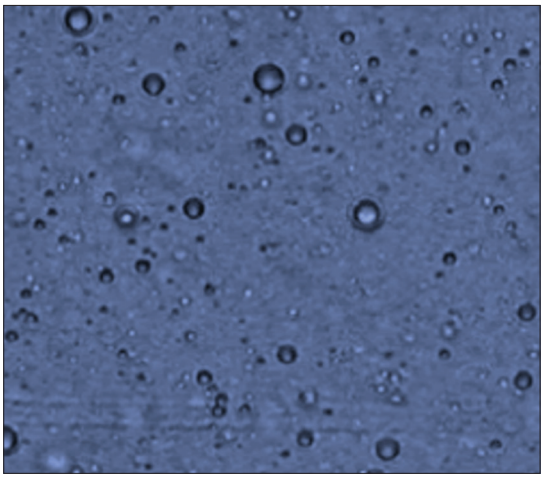

Fig. 6: Microscopy of batch N9

Release kinetics of drug

From the kinetic release study, it was concluded that the best fit model for both the drugs was found to be the Higuchi model. The best batch i.e. N9 batch, n value for TTO and EO was found to be 5.4593 and 5.4555 respectively. This means both the drugs show Anomalous (Non-fickian transport) i.e. both non-swellable matrix diffusion and relaxation mechanism [24].

Antifungal study

From the antifungal study Figs. 11-14 and Table 7, it was concluded that the zone of inhibition of the drug was more when both drugs were used in combination as compared to individual drugs. The zone of inhibition of N9 formulation was slightly less as compared to the zone of inhibition of a pure combination of drugs. This may be due to the entrapment efficiency of drugs in the niosomes being <100%.

Stability study of niosomes

Accelerated stability study period.

FTIR of niH2osomes and acceleratedstability study

From the IR studies Figs. 15 and 16 acceleratedstability and Table 8, it was concluded that after 3 months of accelerated stability study the N9 niosomal batch there was no chemical instability between drug and excipients. N9 niosomal batch was found to be stable.

EVALUATION OF NIOSOMAL GEL

Appearance, pH, drug content, gelation temperature, gelation time, gelation strength, spreadability

From Table 9, the appearance of G9 gel was found to be translucent and clear. The pH of the gel was found to be suitable for skin pH. The drug content, gelation temperature, gelation time, and gelation strength, Spreadability of G9 was found to be good.

Viscosity measurement

From Fig. 17, it was concluded that as the shear rate increases viscosity decreases up to a certain point, and after that viscosity remains constant. The viscosity of the G9 batch at 50 rpm was found to be 2359 cps and the behaviour of gel was found to be pseudo plastic [21].

***In vitro* drug release study**

From the Fig. 18, it was concluded that there is 82.54% release of TTO and 83.61% release of EO from the G9 batch. The G9 Batch shows the sustained release for both the drugs.

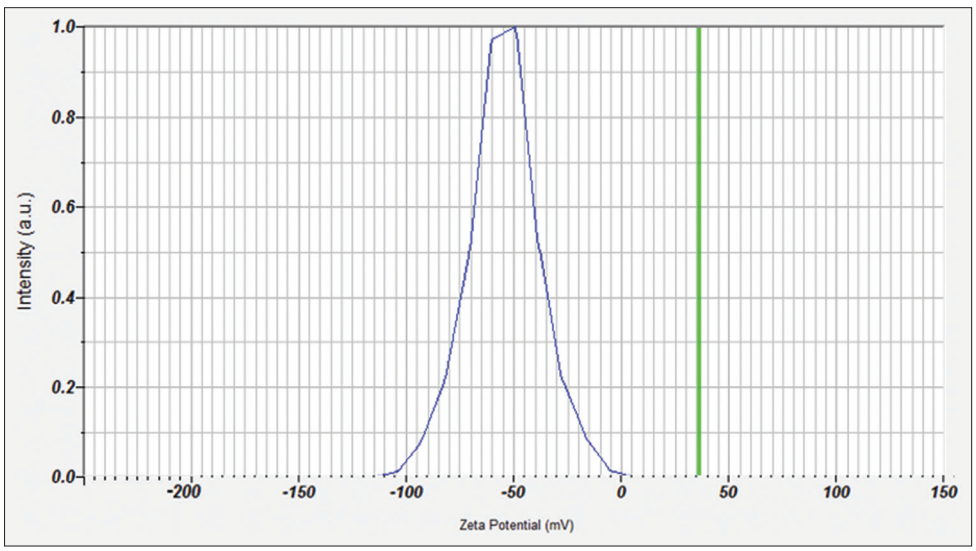

Fig. 7: Zeta potential of N9 niosomal batch

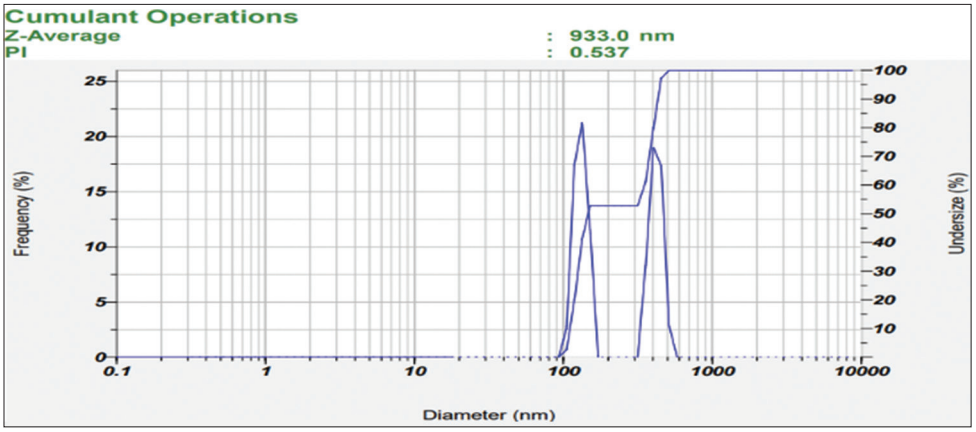

Fig. 8: Particle size and polydispersity index of N3 batch

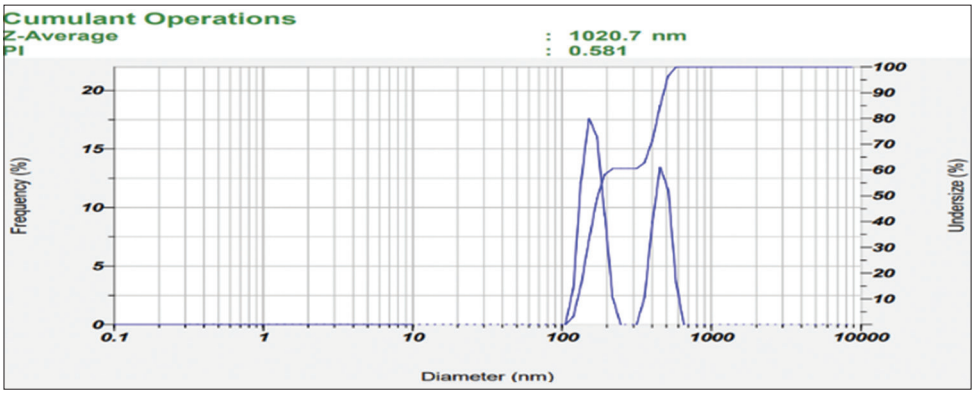

Fig. 9: Particle size and polydispersity index of N6 batch

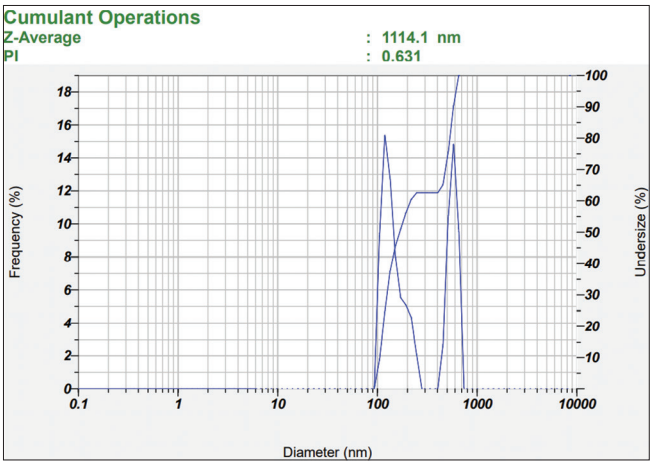

Fig. 10: Particle size and polydispersity index of N9 batch

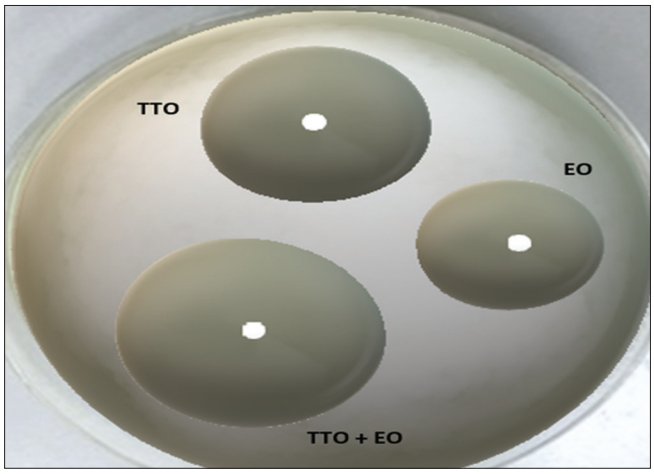

Fig. 11: Zone of Inhibition of tea tree oil, *Eucalyptus* oil

Study of release kinetics for G9 batch

From this kinetic study Table 10, it was concluded that, the best fit model for the G9 batch is the Higuchi model and that it follows Anomalous (Non - fickian transport) i.e. both non-swellable matrix diffusion and relaxation mechanism based on the n values of both the drugs [24].

Antifungal study

From the antifungal study Figs. 11-14, and 19, and Table 11, it was concluded that the zone of inhibition of the G9 niosomal gel was the same as the N9 niosomal batch. It was because the N9 niosomal batch was used in the preparation of G9 niosomal gel.

Stability study period

From the FTIR study Figs. 20 and 21 accelerated stability study Table 12 of G9 niosomal gel batch, it concludes that after 3 months of accelerated stability study the G9 niosomal gel there was no chemical instability between drug and excipients. Hence, G9 Niosomal Gel Batch was found to be stable.

CONCLUSION

The optimized Niosomal gel was successfully developed with high encapsulation efficiency and sustained release. The TTO and EO N9 Niosomal suspension showed sustained release as compared to other

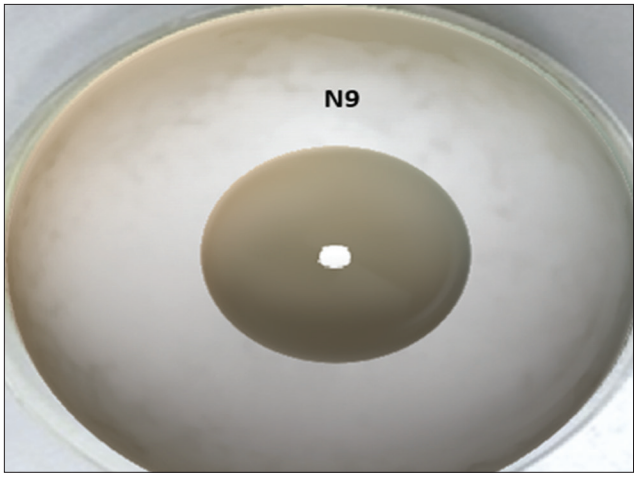

Fig. 12: Zone of inhibition of N9 batch

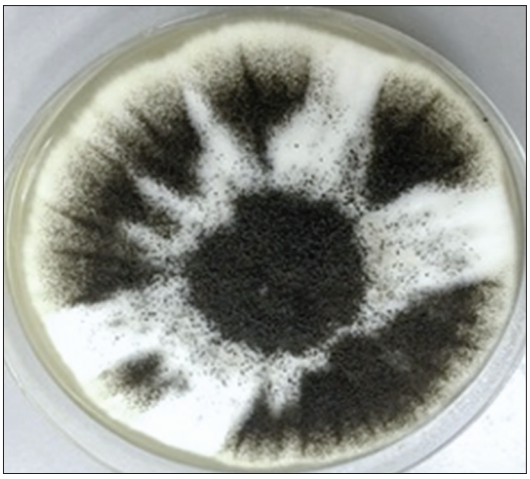

Fig. 13: Negative control

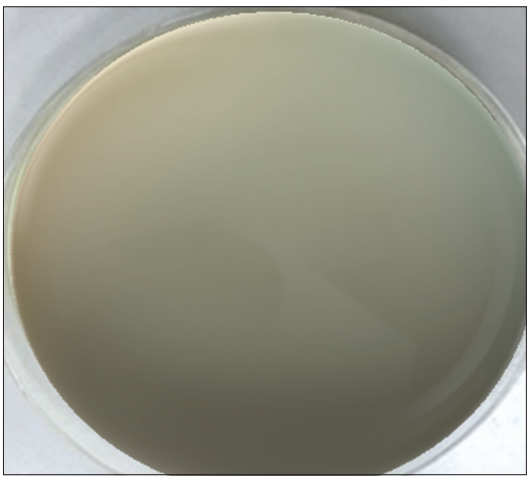

Fig. 14: Positive control

batches and entrapment efficiency of the N9 batch was found to be 84.89±0.19% and 86.86±0.57%. Hence, it was selected for further study. Batch N9 niosomes showed for TTO 84.21% and EO 85.22% release and best fit model for this batch was found to be Higuchi model and that it follows Anomalous (Non - fickian transport) i.e. both non-swellable matrix diffusion and relaxation mechanism. Niosomal gel of batch G9 showed *in-vitro* release of TTO 84.9% and release of EO was found to be

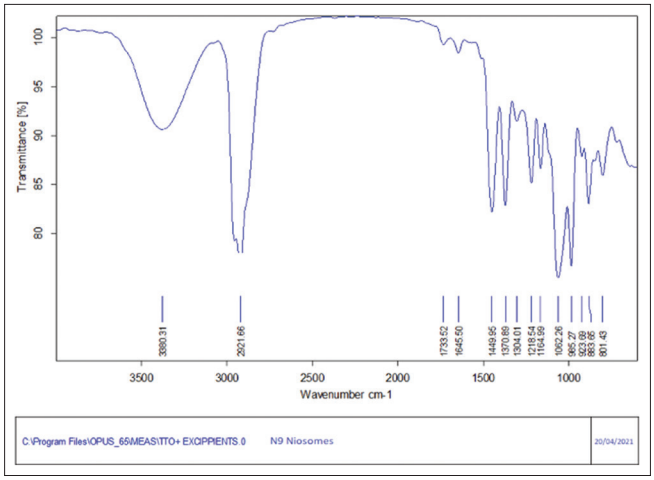

Fig. 15: Fourier transform infrared of N9 niosomal batch at 0 days

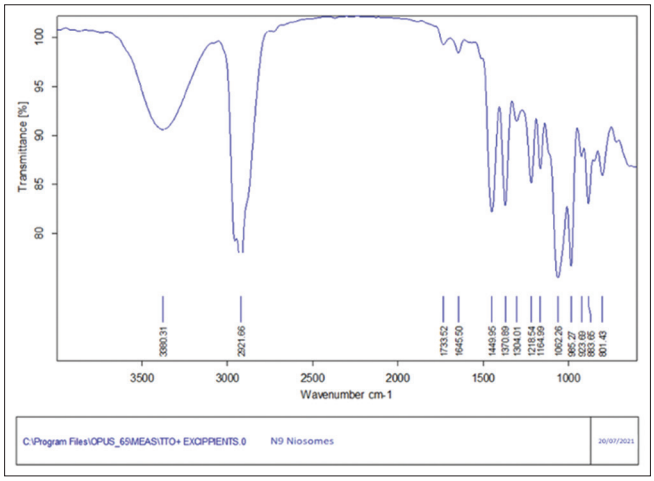

Fig. 16: Fourier transform infrared of N9 niosomal batch after 90 days

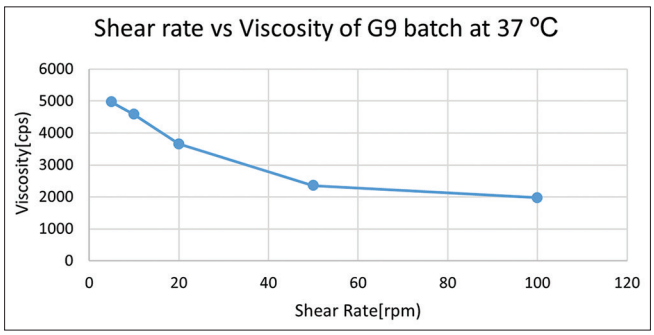

Fig. 17: Viscosity measurement of G9 batch

Table 11: Antifungal study of G9 Batch

Sr. no.	Name	Zone of inhibition observed [mm]
1.	Positive control	-
2.	Negative control	-
3.	G9	13

86.89%. G9 Niosomal gel batch follows Higuchi model i.e. Anomalous (Non - fickian transport) i.e. both non-swellable matrix diffusion and relaxation mechanism. There was significantly improved stability with

Table 12: Stability study of G9 niosomal gel batch

Time period	The appearance of the G9 batch	% Drug content of G9 Batch	Gelation temperature (°C) of G9 batch
Initial	Translucent	90.49±0.36	37.3±0.17
After 1 month	Translucent and stable	90.23±0.65	37.3±0.11
After 2 month	Translucent and stable	89.53±0.84	37.2±0.53
After 3 month	Translucent and stable	88.31±0.53	37.1±0.42

*mean±SD, n=3

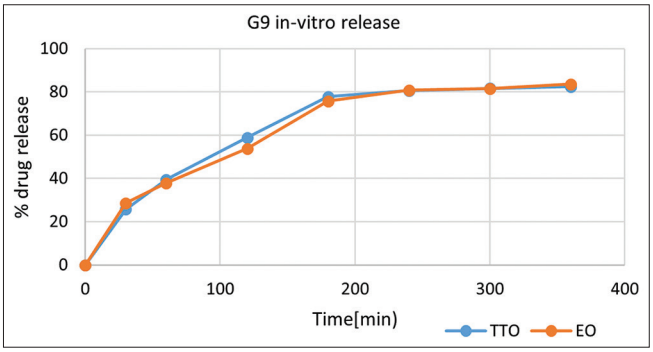

Fig. 18: G9 *in-vitro* release study

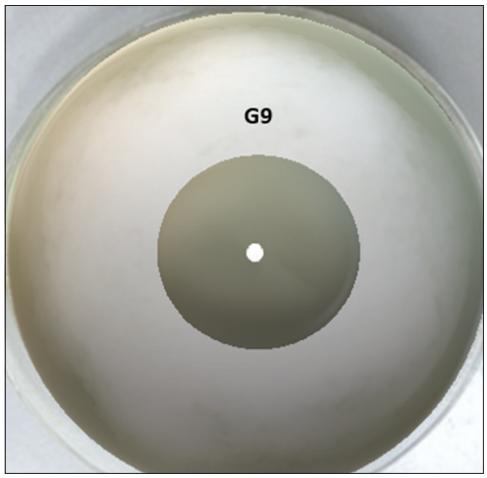

Fig. 19: Antifungal study of G9 batch

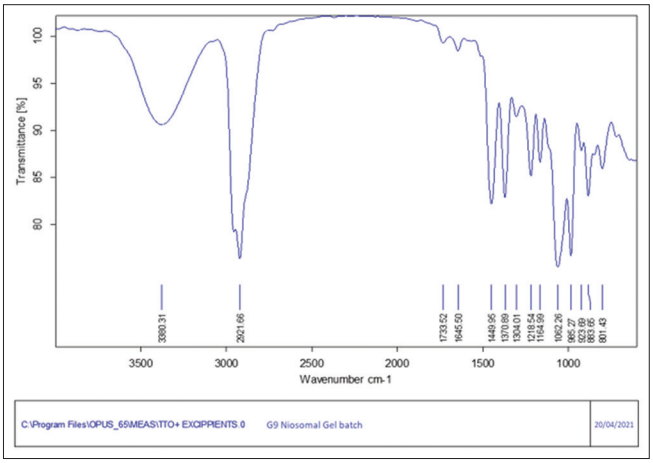

Fig. 20: Fourier transform infrared of G9 niosomal gel batch at 0 days

better control over drug release for a longer period through *in-vitro* release study and stability study this was observed. Therefore, it can be

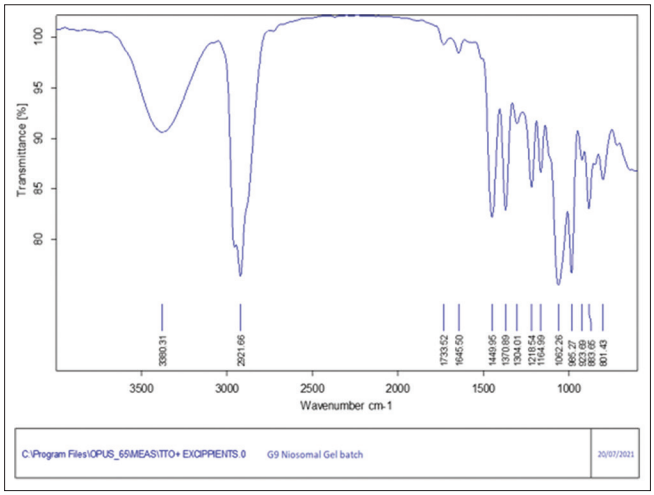

Fig. 21: Fourier transform infrared of G9 Niosomal Gel batch at 90 days

concluded that TTO and EO Niosomal gel through transdermal delivery could be a promising platform with prolonged transdermal retention time to improve its effective period. Based upon the evaluations, optimized niosomal gel showed better results with batch G9.

AUTHORS CONTRIBUTIONS

All authors have read and approved the manuscript. Vikrant Nikam is the first author to contribute to the writing of the manuscript and give scientific suggestions. Simran Maniyar is the second author contributing to the collection of information and writing of manuscripts.

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