Section: Research Paper

Design and Characterization of Nanoemulgel for Topical Fungal Infection: Box Behnken Design Approach

Anuradha G. More[1*], Shraddha S. Satkar[2], Swati S. Mutha[3], Padmaja S. Kore[1], Swapnil S.Tarte[1], Adil M. Pathan[1], Renuka G. Zarekar[1]

[1]Progressive Education Society's Modern college of pharmacy, Nigdi, Pune, Maharashtra, India.

[2]IVM's Indrayani Institute of Pharmaceutical Education and research, Talegaon Dabhade, Pune, Maharashtra, India.

[3]School of Pharmacy, Vishwakarma University, Pune, Maharashtra, India.

*Corresponding author:
Dr. Anuradha G. More,
Department of Pharmaceutics,
PES Modern College of Pharmacy, Nigdi, Pune-411 044,
Maharashtra, India.
E-mail address: anuradhagmore2011@gmail.com
Mob. No.: 9689907667

Abstract

The current study is an innovative attempt to create nanoemulgel (NEG) that incorporates the medicinal properties of argan and ginger oil to treat potentially fatal topical fungal infections. Every year, approximately 1.5 million people worldwide are killed by fungal infections. The essential oils used in this study were argan and ginger oil, both of which have strong antibacterial properties. Argan oil and ginger oil (1:1) were chosen for antifungal activity based on the Minimum inhibitory concentration (MIC) and zone of inhibition. The goal of this study was to create an argan and ginger oil-loaded nanoemulgel for transdermal administration using a box-Behnken statistical design. A three-factor, three-level Box-Behnken design was used to optimise the nanoemulsion. The independent variables investigated were oil concentration (A), Smix concentration (B), and stirring time (C). The best nano-emulgel was made with oil, Smix, and water in a 20:10:70 ratio in 3% HPMC K15 M gel has a globule size of nanoemulsion 185±3.4 nm and a PDI of 0.352 ± 0.102. As a result of the findings, it is possible to conclude that the NEG of argan oil and ginger oil would be an effective treatment for fungal infections.

Key words – Argan oil, Ginger oil, Nanoemulsion, Nanoemulgel, Box-Behnken Design, etc.

Introduction

Fungal infections are one of the worst diseases, killing about 1.5 million people each year throughout the world. The primary factor that makes fungal infections more dangerous is that they are often overlooked by society. Even though there have been significant advancements

in the detection and treatment of fungal illness over the last 20 years, the bulk of the population has yet to reap the advantages of these advancements. Skin infection is the fourth most common fungal illness, and it also accounts for the bulk of deaths. [1]

Despite advancements in drug delivery systems and a variety of options for improved therapeutic improvement, low bioavailability is preventing a number of recently licensed medicines from moving ahead in the pipeline. A lack of solubility, permeability, or both might cause low bioavailability. As a result, enhancing the drug moiety's various pharmacokinetic characteristics, such as solubility, permeability, and therefore bioavailability, will be a major goal for increasing therapeutic efficacy. Nano lipoidal delivery systems are a one-of-a-kind delivery technique that may be used to increase both bioavailability and stability.[2]

Fatty acids, carotenoids, tocopherols, flavonoids, polyphenols, phytosterols, oil-soluble vitamins, and nutraceuticals are among the most hydrophobic bioactive compounds, flavours, and preservatives. Encapsulating these lipophilic compounds usually necessitates colloidal dispersions suitable for aquatic circumstances (i.e., oil in water type).[3]

Nanoemulgels are oil-in-water (o/w) or water-in-oil (w/o) nanoemulsions that have been transformed into nanoemulgels by the addition of a gelling agent. Nanoemulgel is excellent for transdermal application because of its gel form and enhanced nanoemulsion properties.[4] When nano-sized particles come into contact with biological systems, their physiochemical qualities have a significant impact on the nature of their interaction.

The goal of this study was to use a box-Behnken statistical design to produce and statistically optimize herbal nanoemulsion gel for transdermal administration.

Materials and Methods

Materials

Argan oil purchased from Deve Herbs and ginger oil purchased from Vihado. PEG 600 and Tween 80 were obtained from Loba Chem Pvt. Ltd. Mumbai.

Methods

Screening of Essential oil

Six oils were chosen based on the literature review to determine the MIC and zone of inhibition against P. aeruginosa and Candida albicans. Ginger oil, peppermint oil, eucalyptus oil, argan oil, lemongrass oil, and palmarosa oil were utilized in the study. With the aid of DMSO, various concentrations of essential oils were produced, including 2%, 1.5%, 1%, 0.75%, 0.5%, 0.25%, 0.2%, 0.1%, 0.075%, 0.05%, 0.025% and 0.01%.[7]

Minimum Inhibitory Concentration (MIC) determination

The MIC is defined as the lowest concentration that inhibits bacteria from developing a visible appearance. Using the agar well diffusion technique, the MIC of each essential oil was calculated. For bacterial and fungal cultures, petri plates containing nutrient agar and Saboroud's dextrose agar were produced. *P. aeruginosa* and *Candida albicans* cultures were used to inoculate the plates. Essential oils of varied concentrations were introduced into the wells using a sterile syringe and a cork borer with a diameter of 8 mm.

Characterisation of Argan and Ginger essential oils

The color, odor, and appearance of the essential oil i.e. argan and ginger oil samples were evaluated. The viscosity of both oil samples was determined using a Brookfield viscometer (Brookfield DV-II+Pro) at 25±0.5°C and 50 rpm with spindle no. D. [10] The argan and ginger oil were also evaluated for saponification value, acid value, and ester value. [11, 12]

Pseudo-ternary phase diagram construction

Tween 80 was chosen as the surfactant, PEG 400 was chosen as the co-surfactant, and water was utilized as the aqueous phase based on solubility. To estimate the optimum concentration of components in nanoemulsion (NE) formulation, the water titration method was used to build a pseudo-ternary phase diagram. Different weight ratios of surfactant and co-surfactant (Smix) were used (1:1, 2:1, and 1:2 respectively). Each Smix ratio was combined with the oil phase to provide the varied weight ratios of Smix and oil: 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 (A total of 27 formulations were prepared). Then, at room temperature, these mixtures were titrated with water and constantly swirled in a magnetic stirrer. The pseudo-ternary phase diagram was created using CHEMIX SCHOOL Version 10.

Experimental Design for optimization of nanoemulsion using factorial design

Design-Expert (Version 13, Stat- Ease Inc., Minneapolis, MN) was used to investigate the quadratic response surfaces and develop second-order polynomial models utilizing a three-factor, three-level factorial design. A design matrix with 14 trial runs was created, for which the non-linear computer-produced quadratic model is specified as:

$$Y= b_0 + b_1X_1 + b_2X_2+ b_3X_3+ b_{12}X_1X_2 +b_{13}X_1X_3+ b_{11}X_1 +b_{13}X_1X_3+ b_{11}X_1^2+ b_{22}X_2^2 + b_{33}X_3,$$

where Y is the measured response. The amount of Oil (A), Amount of Surfactant (B), and Stirring time (C) were chosen as independent variables. With limits on the formulation of nanoemulsion, the dependent variables were Y1(Globule Size), Y2 (PDI), Y3 (zeta potential), and Y4 (Viscosity). Table.1 shows the concentration ranges of the independent variables under investigation, as well as their low and high values.

Table 1: Variable in Box Behnken Experimental Design

Independent variable	**Coded Symbol**	**Levels**		
		-1	**0**	**+1**
Amount of Oil (%)	A	10	20	30
Amount of Surfactant (%),	B	10	20	30
Stirring time (sec)	C	270	300	330
Dependent Variables	**Coded Symbol**	**Levels**		
Globule Size (nm)	Y1	Minimize		
PDI	Y2	Minimize		
Zeta potential (mV)	Y3	Maximize		
Viscosity (cP)	Y4	Maximize		

Preparation of nanoemulgel

Nanoemulgel was prepared by implementing simple three steps.

Step 1: Formation of nanoemulsion- Oil and/or drug(oil itself) were put into the vial in the desired component ratio of Smix. The components were then gently stirred together to create a volume of up to 10 ml. A magnetic stirrer was used to stir this mixture for 5 minutes. The mixture was then sonicated twice, for a total of one minute.

Step 2: Formation of hydrogel- Hydrogels were prepared using carbopol-940 and HPMC K15 M separately.

Step 3: Formation of nanoemulgel- Prepared nanoemulsion was incorporated into the hydrogel to form nanoemulgel formulation (Table 8). Compare the appearance and phase separation of the various gelling agents after they've been added to the nanoemulsion. Prepare different solutions of suitable gelling agent concentrations, such as 1%, 2%, and 3%.

Characterization of optimized nanoemulsion

Globule size - The particle size analyzer was used to determine the size of the globules (Horiba nano analyzer & SZ-100). 0.1 ml of the formulation was dissolved in 100 ml of water and gently swirled in a glass beaker.

Polydispersibility index determination- The PDI measures particle diameter uniformity and may be used to visualize the size distribution of a nanoemulsion population. It is a particle homogeneity metric. The particle size analyzer was used to determine it (Horiba nano analyzer & SZ-100).

Zeta potential determination-The optimal formulation's zeta potential was measured utilizing a zeta sizer and dynamic light scattering (Horiba nano analyzer & SZ-100). A stable nanoemulsion with zeta potential values of less than -30 mV or more than 30 mV. As a consequence, the zeta potential test revealed steady findings.

% Transmittance measurement - The sample was made by vortexing 0.1 ml of nanoemulsion with 5 ml distilled water for 30 seconds to make it. The % transmittance at 650 nm was measured using a UV spectrophotometer.

Nanoemulgel characterization- The color, appearance, odor, kind of smear, removal, phase separation, consistency, pH measurement, and viscosity of the produced nanoemulgel were all assessed.[18] Brookfield viscometer (Brookfield DV-II+Pro) was used to measure the viscosity of the optimal formula at 25 ℃ and 50 rpm using spindle C. [15,16]

Accelerated stability study

The stability test was carried out according to the ICH guideline on the topic Q1A: stability testing of new drug substance and product. The nanoemulgel was stored in air tight glass container and protected from light. Sample maintained in a stability chamber under accelerated condition (45˚C ± 2˚C, 75˚C ± 5% RH) with humidity and temperature control, was taken at 0, 1, 2 and 3 months for evaluation. The phase separation and viscosity were evaluated.[15,16]

Results and Discussions

Screening of essential oils

The data represented in Table 2 shows the results of MIC and ZOI values of various essential oils against *Candida albicans* and *P aeruginosa.*

Table 2:MIC and ZOI values various essential oils against *Candida albicans* and*P aeruginosa*

Parameter	*Candida albicans*		*P aeruginosa*	
	MIC %	**ZOI(mm)±S.D.**	**MIC %**	**ZOI (mm)±S.D.**
Argan oil	0.1	36±0.2	0.1	26±0.3
Palmarosa oil	0.5	17±0.3	0.75	17±0.1
Ginger oil	0.2	32±0.2	0.1	23±0.2
Eucalyptus oil	0.3	29±0.2	0.2	21±0.2
Peppermint oil	0.5	24±0.1	0.2	19±0.3

The antimicrobial potential of essential oils was evaluated according to their zone of inhibition against fungal and bacterial strains. Argan oil, ginger oil, and eucalyptus oil were chosen based on the before mentioned information. Because of its low MIC value and high ZOI, argan oil was kept constant. Based on the above data Argan oil and Ginger oil were considered for further formulation of nanoemulgel.Table 3 shows results of MIC and ZOI values various essential oils combination against *Candida albicans* and *P aeruginosa.*

Table 3: MIC and ZOI values of essential oil combination

Essential oil combination (1:1)	*Candida albicans*		*P aeruginosa*	
	MIC %	**ZOI(mm)±S.D.**	**MIC %**	**ZOI(mm)±S.D.**
Argan oil + Ginger oil	0.1	41±0.2	0.2	27±0.2
Argan oil + Eucalyptus oil	0.2	39±0.1	0.2	24±0.2

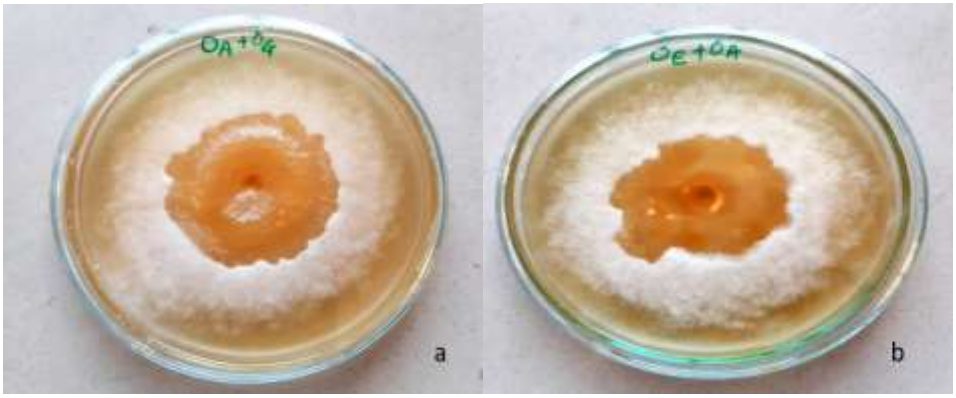

Figure 1. ZOI of a. argan oil and ginger oil combination, b. argan oil and eucalyptus oil combination against *Candida albicans*.

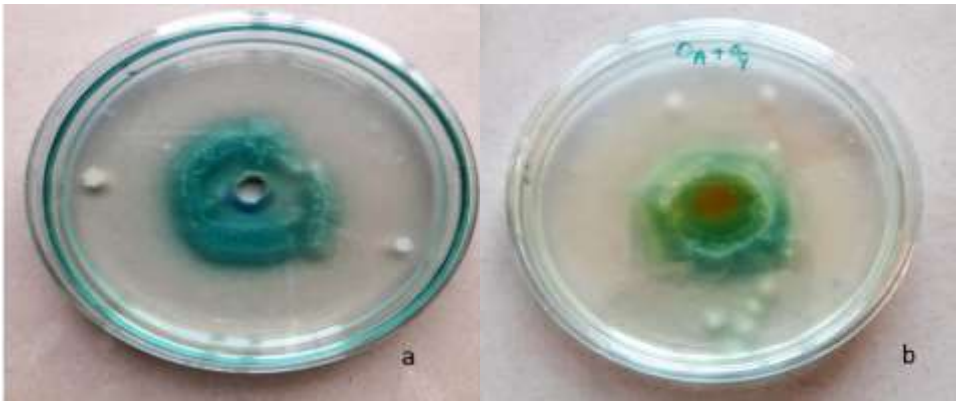

Figure 2. ZOI of a. argan oil and eucalyptus oil combination b. argan oil and ginger oil combination, against *P aeruginosa*.

Based on the above result,the argan oil and ginger oil was used for further study.

Characterization of argan and ginger oil

The Table 4 shows result of characterisation study of argan and ginger oil.

Table 4: Characterization study of argan and ginger oil

Parameter	Parameter	
	Argan oil	**Ginger oil**
Color	Colourless	Yellow
Odor	Aromatic	Strong spicy aroma
Density (gm/ml)	0.96	0.98
Specific gravity	0.9423	0.9134
Viscosity (cP)	6.22	3.78
Acid value	1.8	2.6
Saponification value	40.953	32.818
Esterification value	39.153	30.218

Preliminary screening of surfactants and co-surfactants:

The screening of surfactants and co-surfactants was evaluated by flask inversion method. The self-emulsification activity was evaluated by flask inversion method. It was evaluated by preparing mixtures of oil: surfactant and oil: co-surfactant which showed that tween 80 and PEG 600 had good emulsification activity since it required a smaller number of inversion and showed good % transmittance. Thus, based upon self-emulsification data Tween 80 was selected as Surfactant and PEG 600 was selected as Co-Surfactant.

Construction of Pseudo Ternary Phase diagram

The Pseudo ternary phase diagram revealed that the final combination had an excellent nanoemulsion region. The emulsification region is shown by the darkened area. It was discovered that the mixture of oil, surfactant, and co-surfactant had a 1:1 ratio.

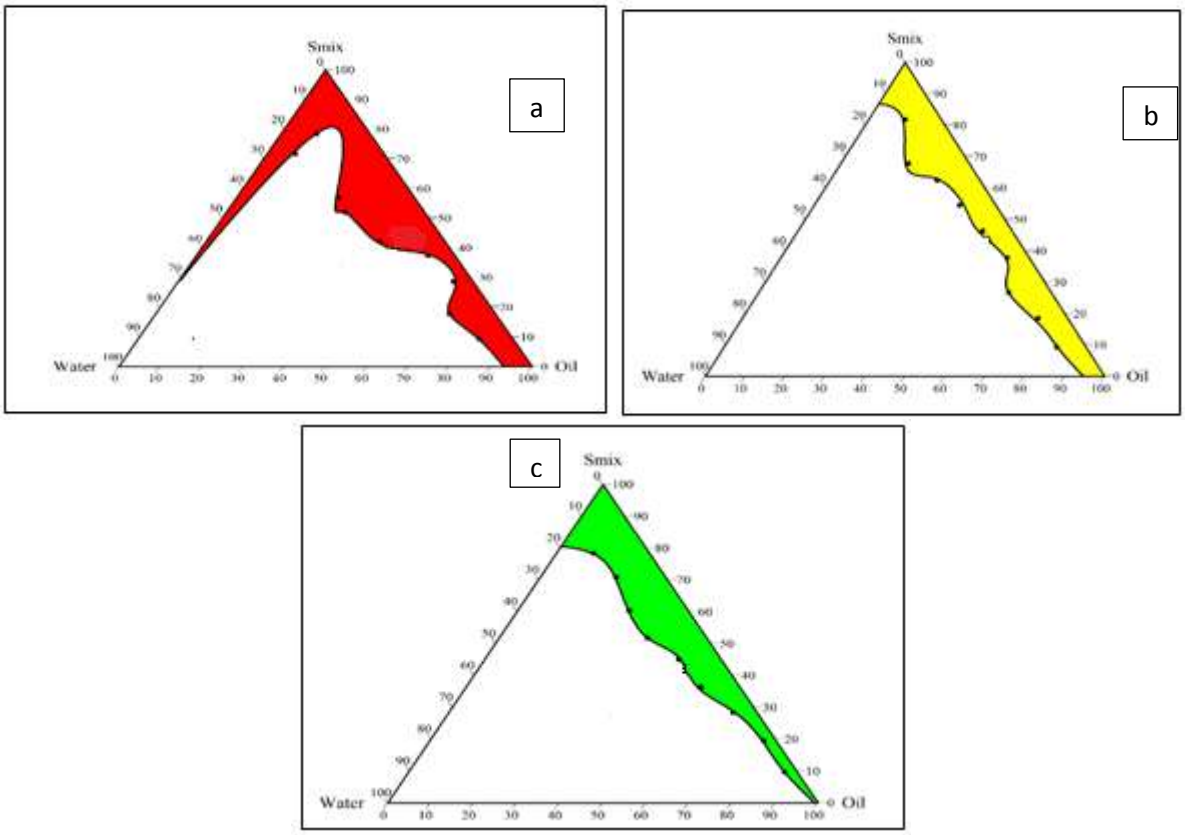

Figure 3: Ternary phase diagram; a) oil:(1:1) S_{mix}, b) oil:(1:2) Smix, c) oil:(2:1) Smix

Development and Characterization of DOE optimized (Box-Behnken design) Nanoemulsion for Argan and Ginger oil.

According to the results, polynomial models representing particle size, PDI, zeta potential, % transmittance were generated which are shown further in terms of coded factors. The results are shown in Table 5 and Figure 4.

Table 5: Experimental trials for factorial design

Batch no.	Factors			Responses			
	Conc. Of oil (%)	Conc. Of S_{mix} (%)	Stirring time (sec)	Particle size (nm)	PDI	Zeta potential (mV)	Viscosity (cP)
NE 1	10	30	300	135	0.256	-30.6	63.0
NE 2	30	20	270	223	0.301	-23.1	53.5
NE 3	20	30	330	223.6	0.33	-27.3	56.2

NE 4	20	10	330	232	0.277	-24	59.1
NE 5	20	20	300	211.1	0.262	-24.2	56.8
NE 6	10	10	300	227.2	0.294	-28.1	62.2
NE 7	20	20	300	196.4	0.258	-25.7	56.7
NE 8	30	20	330	226.1	0.349	-22	51.2
NE 9	20	10	270	172	0.306	-26.4	55.1
NE 10	20	30	270	202	0.296	-27.8	53.5
NE 11	10	20	330	162.8	0.272	-29	61.1
NE 12	30	10	300	240.9	0.4	-21.5	51.4
NE 13	30	30	300	223.6	0.337	-23.7	50.1
NE 14	10	20	270	148	0.26	-29.2	61.1

Figure 4. Prepared formulation batches of nanoemulsion by DOE

ANOVA

Determination of particle size- For different factor level combinations, the particle size ranges from 135 nm (formulation 1) to 240.9 nm (formulation 12). The quadratic equation below shows the influence of independent factors/variables on particle size:

Quadratic equation for Globule size = 203.75+30.07A-10.99B+12.44C+18.73AB-2.93AC-9.60BC-7.25A^2+10.18 B^2-6.53 C^2

The interaction reports showed that as oil concentration increases it also increases the globule size whereas when S_{mix} concentration increases it decreases the globule size. Thus, oil concentration has negative effect on globules size and the S_{mix} concentration plays a major role in reduction of globule size.The globule size interaction and surface response plot for particle size is shown in Figure 5.

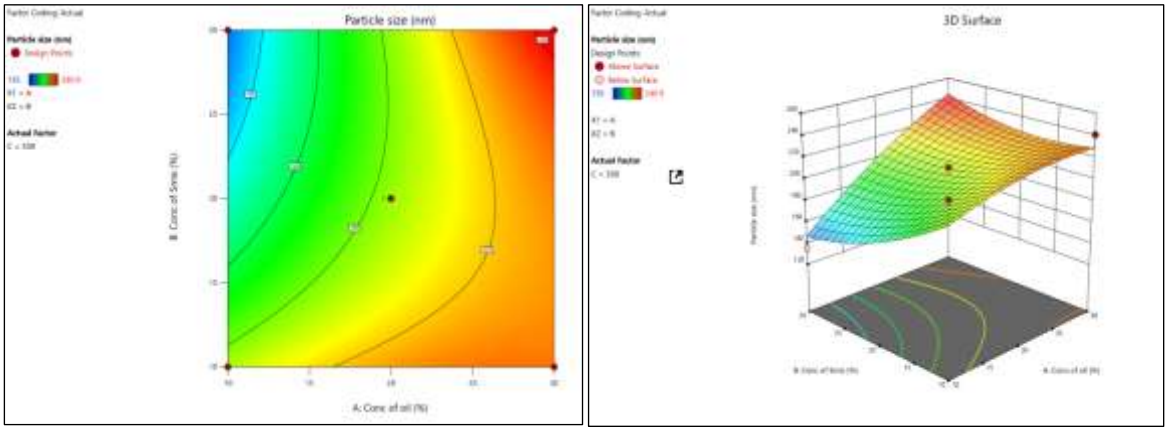

Figure 5: Counter plot &3D plot of particle Size

Determination of PDI- For different component level combinations, the PDI ranges from 0.256 to 0.4. The following quadratic equation has revealed the influence of many independent variables on PDI:

Quadratic equation for PDI = $0.2550+0.0375A-0.0075B+0.0075C-0.0075AB+0.0075AC+0.0175BC+0.0288A^2+0.0338B^2+0.008C^2$

The equation depicted positive sign for A(oil) and C(stirring time) and negative sign for B (Smix) This suggested that with an increase in the amount of oil (argan and ginger oil) and a decrease in surfactant (Tween 80) and co-surfactant (PEG 600) concentration, the PDI increases.The PDI interaction and surface response plot for particle size is shown in Figure 6.

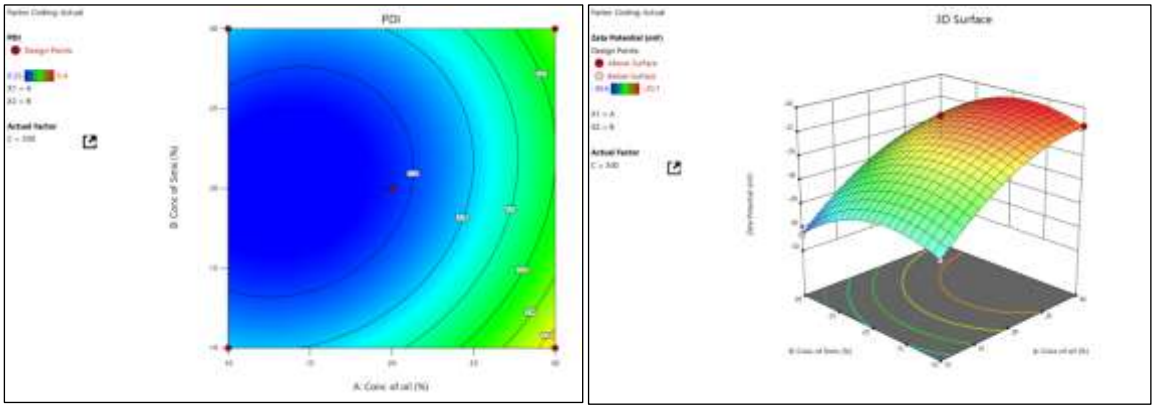

Figure 6: Counter plot &3D plot of PDI

Determination of zeta potential- For different component level combinations, the zeta potential ranges from -23.1 to -30.6. The following quadratic equation has revealed the influence of many independent variables on zeta potential:

Quadratic equation for Zeta potential = -22.45+ 3.33A -1.17B +0.5250C +0.0750AB +0.2250AC -0.4750BC -1.49A^2-2.04B^2 -1.89C^2

The Zeta potential interaction and surface response plot for particle size is shown in Figure7.

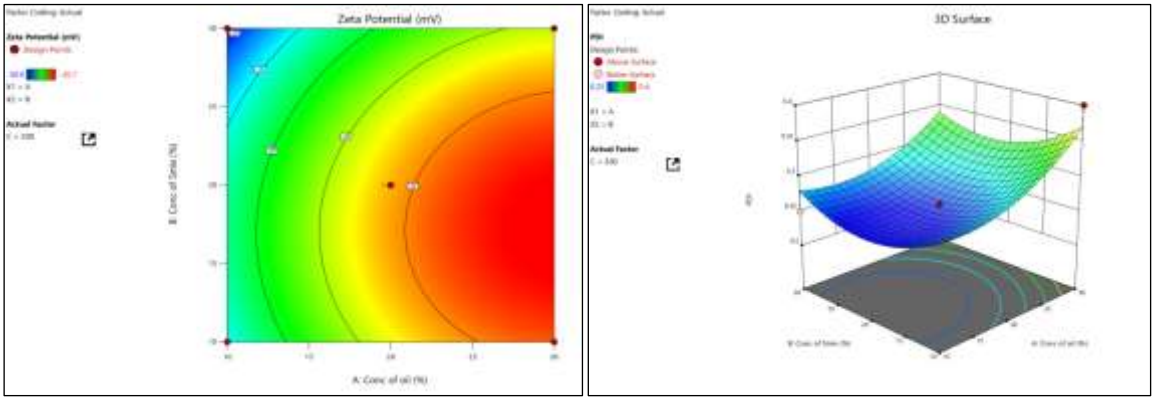

Figure 7: Counter plot & 3D plot of Zeta potential

Determination of viscosity- For different component level combinations, the viscosity ranges from 0.256 to 0.4. The following quadratic equation has revealed the influence of many independent variables on viscosity:

Quadratic equation for viscosity = 56.75 -5.15A -0.6250B+ 0.5500C -0.5250AB -0.5750AC -0.3250BC +0.3375A^2 -0.4125 B^2 -0.3625C^2

The decrease in oil and Smix concentration, the viscosity increases. The viscosity interaction and surface response plot for particle size is shown in Figure8.

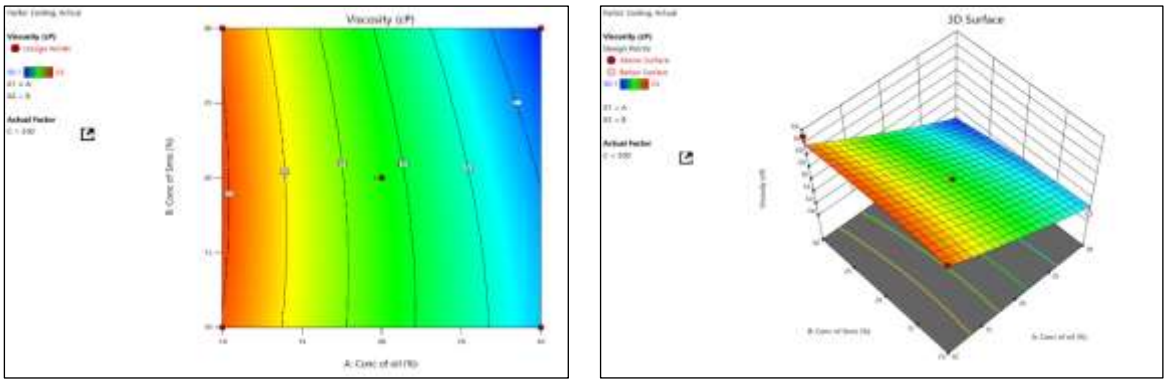

Figure 8: Counter plot & 3D plot of viscosity

Characterization of Optimised Nanoemulsion

Organoleptic characterization- The optimized nanoemulsion was milky white in colour and aromatic in odour.

Globule size and PDI value- The globule size was found to be 185.5 nm i.e. formulation is in the range specified for nanoemulsion and PDI of the formulation was found to be 0.352. The plot of globule size is shown in Figure9.

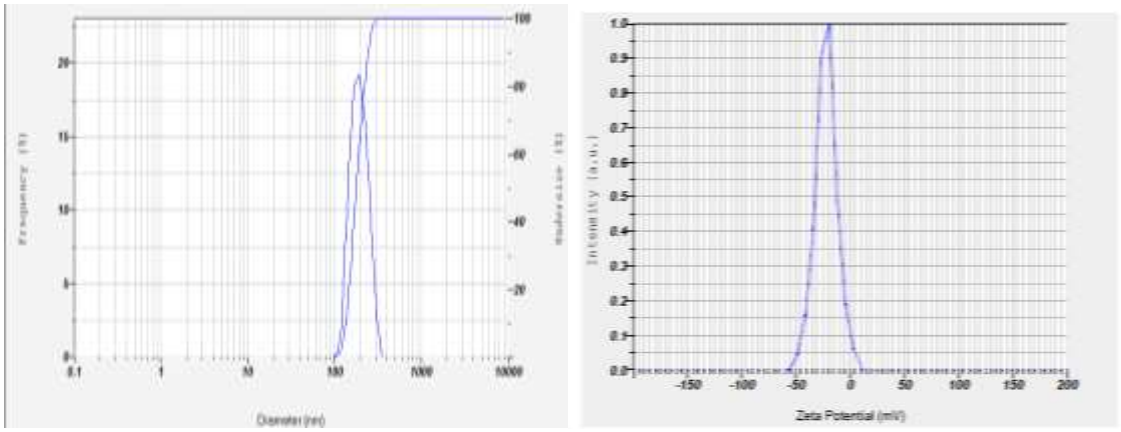

Figure 9: Globule size and Zeta potential of optimised batch

Zeta potential- Zeta potential is a physicochemical characteristic of a medication, polymer, or vehicle that is used to predict dispersion stability. Its value is determined by the presence of electrolytes and their adsorption. With a value of -24.0 mV, the formulation was stable.The plot of zeta potentialis shown in Figure9.

% Transmittance- A transparency metric of percent transmittance was used to assess the clarity of the nano emulsion. The chosen formulation has a 94.86 percent transmittance value.

Viscosity- Viscosity is a measure of a fluid's resistance to flow. The viscosity of the formulation was measured at 112.5 cps at 50 rpm using spindle C.

Nanoemulgel Development and Characterization

Formulation of Nanoemulgel

In the preliminary trials, Carbomer 940 and HPMC K15 M gels were prepared by using various concentrations in water. The gels were inspected visually for their colour, clarity, transparency, homogeneity, clogs, grittiness and consistency. Only HPMC K15 M gel was found to be more transparent, clear, homogeneous without grittiness, while gel with Carbomer 940. HPMC K15 M was then selected for further optimization. Total 9 formulations were prepared by dispersing each selected nanoemulsion formulations into various concentration range of HPMC K15 M (1%, 2%, 3%). All the 9 formulation were kept and examined visually for its colour, Appearance, Odour, Type of smear, Removal, Phase separation, Consistency. Fromthestudy,it was revealed that the formulations prepared with3%HPMC K15 Mhave passed all visual test and it was used for preparation of nanoemulgel. Based on the conducted study, the final composition of optimized nanoemulgel were given in below Table6.

Table 6: Composition of optimized Nanoemulgel

Sr.no.	Content	Quantity
1	Oil (Argan oil: Ginger Oil)	20%
2	S_{mix}(Tween 80: PEG 600)	10%
3	HPMC K15 M	3%
4	Water	Q.S

Accelerated stability studies

The stability study followed ICH recommendations. Table 7 displays the findings of the stability study. It was discovered that there was no significant difference in viscosity and phase separation for formulation after three months of storage.

Table 7. Accelerated stability study data (mean ±SD, n=3)

Month	Phase separation	Viscosity (at 50 RPM)
0 month	No phase separation	254 cP ± 0.19
1 month	No phase separation	253.5 cP ± 0.31
2 months	No phase separation	253.4 cP ± 0.009
3 months	No phase separation	252.9 cP ± 0.71

Conclusion

Dermatophytes are fungi that infect the top layer of the skin, nails, and hair. Nano emulsion is a novel carrier that releases active pharmaceutical ingredients in a sustainable manner and is used to develop a transdermal delivery system for fungal infection. Following that, an emulgel was created to improve the topical applicability of the Nano emulsion in order to localise the API at the target site of action. Optimization of Nano emulsion is one of the critical processes in formulation development that requires consideration of a number of factors and ingredient interactions. We used the 3-factor, 3-level BBD design approach. As a result, the developed formulation demonstrated good antifungal activity against Candida albicans. As a result, it can be concluded that a safe, efficacious, non-irritant surfactant-based topical nanoemulgel formulation for fungal disease treatment was successfully developed.

References

1. Martin KW and Ernst E (2004) Herbal medicines for treatment of fungal infections: a systematic review of controlled clinical trials. Blackwell Publishing Ltd, Mycoses,47: 87–92.
2. Anand K, Ray S, Rahman M, Shaharyar A, Bhowmik R, Bera R, et al (2019) Nano-emulgel: Emerging as a Smarter Topical Lipidic Emulsion-based Nanocarrier for Skin Healthcare Applications. Recent Patents on Anti-Infective Drug Discovery, 2019; 14.

3. Tamjidi F, Shahedi M, Varshosaz J, Nasirpour A () Nanostructured lipid carriers (NLC): A potential delivery system for bioactive food molecules.0 Innovative Food Science and Emerging Technologies,19; 29–43.
4. Choudhury H, Gorain B, Pandey M, Chatterjee LA, Sengupta P, Das A, Molugulu N, Kesharwani P (2017) Recent update on nanoemulgel as topical drug delivery system. Journal of Pharmaceutical Sciences.
5. Singh RP, Parpani S, Narke R, Chavan R (2014) Emulgel: A Recent Approach For Topical Drug Delivery System. Asian Journal of Pharmaceutical Research and Development, Vol. 2: 112-123.
6. Mou D, Chen H, Du D, Mao C, Wan J, Xu H, et al (2008) Hydrogel-thickened nanoemulsion system for topical delivery of lipophilic drugs. International Journal of Pharmaceutics, 353: 270–276.
7. Ebani VV, Nardoni S, Bertelloni F, Pistelli L, Manciant F (2018) Antimicrobial Activity of Five Essential Oils against Bacteria and Fungi Responsible for Urinary Tract Infections. Molecules, 23(7): 1668.
8. Agarwal V, Lal P, Pruthi V(2010) Effect of Plant Oils on Candida albicans. J Microbiol Immunol Infect, 43(5): 447–51.
9. Devkatte AN, Zore GB, Karuppayil SM (2005) Potential of plant oils as inhibitors of Candida albicans growth. FEMS Yeast Research,5: 867–873.
10. Tungadi R, Susanty W, Wicita P, Pido E (2018) Transdermal Delivery of Snakehead Fish (Ophiocephalus striatus) Nanoemulgel Containing Hydrophobic Powder for Burn Wound. Pharmaceutical Sciences, 24: 313-323.
11. Mustapha A (2018) Comparative Analysis on the Extraction of Essential Oil from Lemongrass and Basil Leaves. International Journal of Innovative Science, Engineering & Technology, 5 (11): 114-118.
12. Manuranjan G, Lalduhsanga P, Lalhlenmawia H, et al (2019) Physicochemical, Antibacterial and Antioxidant Properties of Fixed and Essential Oils Extracted from the Peels of Citrus macroptera Fruit. Indian J Pharm Science, 81(1):82-88.
13. Patel J, Patel A, Raval M, Sheth N (2011) Formulation and development of a self-nanoemulsifying drug delivery system of irbesartan. Journal of Adv Pharm Technol Res, 2(1): 9–16.
14. Shah N.H., Carvajal M.T., Patel C.I., Infeld M.H, Malick A.W. (1994) Self-emulsifying drug delivery systems (SEDDS) with polyglycolyzed glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs. Int J Pharm., 106(1):15-23.
15. Chandra A, Arya RK, Pal GR, Tewari B (2019) Formulation and Evaluation of Ginger Extract Loaded Nanoemulgel for the Treatment of Rheumatoid Arthritis. Journal of Drug Delivery & Therapeutics, 9(4):559-570.
16. Paliwal S, Kaur G, Arya RK K (2018) Formulation and characterization of topical nanoemulgel of terbinafine. Universal Journal of Pharmaceutical Research, 3(6): 28-37.