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Quality By Design Driven, Development And Assessment Of A Nanoemulsion Based Gel Comprising Of Fenticonazole Nitrate And Clary Sage Oil Loaded For The Effective Treatment Of Fungal Infections

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ABSTRACT

Fenticonazole nitrate is a selective azole, antifungal agent that is only weakly soluble in water (about 0.000304 mg/ml), and it's useful when given in the right amount to treat fungal infections. This project attempted to create a nanoemulsion of Fenticonazole nitrate using clary sage oil, which is known for its antifungal properties, to help fight fungal infections. We optimized the nanoemulsion using a D-optimal design and then incorporated the same amount into a gel made with Carbopol 934P. The best nanoemulsion had about 36.44% Smix, 9.3% oil, and 54.24% water. It had a small globule size of 140 nm and a polydispersity index of 0.316. The zeta potential was measured at -14.6 mV. Its viscosity was around 36 cPs, while the gel made from it was much thicker at 2950 cPs. The pH of the nanoemulsion was 6.73. All of the nanoemulsions were oil-in-water type, which we confirmed with a conductivity measurement of 69.8 (200 µs). When tested outside the body, the nanoemulsion gel released about 69.48% of the drug after 5 hours, and in laboratory conditions, the release went as high as 89.21%. We found that combining chamomile oil with the nanoemulsion gel containing Fenticonazole nitrate boosted its antifungal activity, as shown by the larger inhibition zones. When comparing to commercial products, the 0.4% nanoemulsion based gel (NEBG) showed a bigger zone of inhibition than other formulations, even with a relatively moderate dose of Fenticonazole nitrate, indicating it's effectiveness.

KEYWORDS: *Nanoemulsion, Fenticonazole nitrate, Clary sage oil, Antifungal.*

1. INTRODUCTION

Superficial fungal infections often impact the skin, cuticles, and mucous membranes, mostly induced by Candida albicans and Trichophyton species. Typical symptoms include pruritus, erythema, desquamation, and pain, which, although not life-threatening, may impair quality of life.

Oral fenticonazole nitrate treatment is limited by adverse effects, including gastrointestinal problems and hepatotoxicity, rendering it inappropriate for extended or localized use. Traditional topical formulations (gels, creams, lotions) encounter obstacles such as inadequate retention, variable dose, and issues in administration to specific areas. Nanoemulsion-based gels provide a sophisticated drug delivery technology to overcome these constraints. Nanoemulsions, consisting of oil, water, surfactants, and co-surfactants, improve bioavailability, skin permeation, and drug stability. When integrated into gels, they guarantee extended retention at the infection site, accurate dosage, and enhanced therapeutic results. Clary sage oil has synergistic antifungal effects by obstructing

ergosterol production, whilst carrageenan increases viscosity and prolongs residence duration without causing irritation to the skin or mucosa. Consequently, Fenticonazole nitrate-loaded nanoemulsion gels provide a promising, patient-centered approach for the effective treatment of superficial fungal infections [1,2,3].

2. MATERIALS AND METHODS

2.1 Materials:

Fenticonazole nitrate was acquired as a complimentary sample from Redson Pharmaceuticals (Ahmedabad, India). In the formulation and assessment of the clary sage oil-based vehicle, the following excipients were utilized: Tween 80, Tween 20, Span 80, Labrasol, polyethylene glycol (PEG) 400, and Cremophor RH 40 (sourced from BASF, Mumbai, India); Transcutol P (obtained from Abitech Corporation); Poloxamer 147 and Poloxamer 188; carrageenan; and analytical grade methanol. All chemicals and reagents used were of pharmaceutical or analytical grade and were utilized without further purification.

2.2 Methods:

2.2.1 Quantification of Fenticonazole nitrate

The absorbance of the Fenticonazole nitrate stock result was assessed within the wavelength range of 100 to 700 nm using a UV spectrophotometer (Schimadzu UV- 1800). Methanol was use as a control. The ultraviolet immersion outside of Fenticonazole nitrate is recorded at 253 nm. A stock result was prepared by precisely importing and dissolving 10 mg of Fenticonazole nitrate in 100 ml of methanol inside a 100 ml volumetric beaker. Whatman sludge paper was use to filter the result. The produced stock result (10 µg/ ml) has an energy of 100 µg/ ml. To achieve a concentration range of 2 – 32 µg/ ml, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 ml of the result were transfer into a 10 ml volumetric beaker and adulterated with methanol to the estimation mark. The UV-Visible Spectrophotometer was use to quantify the absorbance of each result at 253 nm. The standard curve was construct for the whole diapason. The equation for the ideal line was deduce by calculating the average absorbance of the trial conducted in triplet.

2.2.2 Standard estimation curve prepared in 7.4 phosphate buffer result(PBS)

For manufacture of a one- liter, we use the following factors i.e.Prepare 800 mL of distilled water in an applicable vessel. Incorporate 8 g of NaCl, 200 mg of KCl, 1.44 g of Na2HPO, and 240 mg of KH2PO4 into the result. A stock result is created by dissolving 10 mg of Fenticonazole nitrate in 100 ml of methanol, performing in a concentration of 10 µg/ ml (stock 1). The result was prepared to a volume of 1000 ml. latterly, introduce different quantities of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 into a 10- ml volumetric beaker and blend until the target concentration is attained using a 7.4 pH phosphate buffer. The attendant concentration are 2, 4, 6, 8, 10, 12, 14, and 16 µg/ ml, independently. The UV-Visible Spectrophotometer was use to quantify the absorbance of each result at 253 nm.

2.2.3 Preliminary screening for the components of nanoemulsion:

The solubility of clary sage oil, Span 20, Span 80, Tween 20, Tween 80, labrasol, PEG 200, PEG 400, Transcutol HP, ethanol, propylene glycol, and isopropyl alcohol was assess by dissolving a substantial volume of FNZ in 2 ml of each detergent. The hydrophillic layers of the composites were isolate by centrifugation of the fusions at 3000 rpm for 15 minutes after a 24- hour period. Prior to determining the concentration of (FNZ) spectrophotometrically at 253 nm, the supernatant was diluted with methanol. Every trial was perform thrice.

2.2.4 Construction of a pseudoternary phase illustration

The nanoemulsion was formulated exercising the named oil (clary sage oil), surfactant(Tween 80), and cosurfactant(PEG 400), as determined through solubility examinations. The surfactant and co-surfactant were combine in colorful several rates, specifically 1:1, 1:2, 2:1, 1:3, and 3:1. In order to ensure thorough representation of the ideal rates, a variety A series of combinations of oil and Smix were examined, specifically in

the ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. employed to construct the pseudoternary phase illustration. The aqueous titration system was employ, in which the preliminarily mentioned combination was enhanced with double- distilled water, and the endpoint was determined through turbidity assessment. The mock ternary phase illustration was strictly constructed exercising the calculated concentrations of the varied factors. The CHEMIX ® School program eased the development of the pseudo-ternary phase illustration. The stability of a specific system is illustrated by the nanoemulsion region within the phase illustration. This led to the determination of the Smix rate that yields a broader nanoemulsion zone for successive examination(4).

2.2.5 Fenticonazole nitrate -loaded nanoemulsion preparation:

By trial and error various component ratios were use to select Fenticonazole nitrate -loaded nanoemulsion phase diagrams. A magnetic stirrer dissolved Fenticonazole nitrate in clary sage oil to form a nanoemulsion. Tween 80 and PEG 400 are mixed. Water was introduce incrementally, drop by drop to the oil phase. Finally, a high shear homogenizer (IKA T25 Digital ULTRA TURRAX, Germany) at 5000 rpm for 15 minutes reduced the nanoemulsion's size. As prepared, a digital ultrasonic cleaner sonicates the nanoemulsion for an hour to remove microbubbles. After sonication, the clear nanoemulsion was stabilized for 24 hours before analysis [5,6].

2.2.6 Quality by Design nutshell

Figure-1:Quality by Design nutshell

2.2.7 Articulating the Quality Target Product Profile and Critical Quality Attributes

Quality by Design (QbD) is the foundation of contemporary pharmaceutical product development. Within the QbD framework, the Quality Target Product Profile (QTPP) functions as a strategic overview of the intended quality attributes, directing formulation design to attain maximal therapeutic effectiveness. The Quality Target Product Profile (QTPP) was designed to ensure that the fenticonazole nitrate-loaded nanoemulsion meets patientcentric requirements. In order to achieve the Quality Target Product Profile (QTPP), a number of Critical Quality Attributes (CQAs) were defined, each directly impacting the product's safety, effectiveness, and patient adherence. These encompass: medication Loading - indicates the quantity of medication integrated into the formulation, guaranteeing adequate therapeutic availability.% Transmittance – a measure of nanoemulsion clarity, associated with consistency and physical stability.Globule size is a critical factor influencing dissolution and drug release, essential for optimizing therapeutic efficacy. Drug permeation denotes the degree of drug transport across the buccal mucosa, influencing systemic absorption. Viscosity affects retention at the buccal location and regulates penetration, therefore facilitating prolonged medication administration. These CQAs together define the product's essential performance metrics. Their systematic assessment and regulation, aligned with the QbD paradigm, guarantee the creation of a resilient, efficient, and patient-focused nanoemulsion formulation[7–9].

2.2.8 Quality Target Product Profile for Nanoemulsion

Table 1: Exemplary Target Product Profile for Nanoemulsion

QTPP elements	Target	Justification
Dosage form	Nanoemulsion	The selection of phospholipidbased nanoemulsion facilitates improved penetration across the epidermal barrier.
Route of administration	Mouth	Suggested pathway for extensively metabolized medication
Ex- vivo permeation study	Higher permeation	Necessary for attaining elevated medication concentration in certain layers of buccal mucosa.
Container closure system	System qualified as suitable for this drug product	Required to get the specified duration of usability.

Table 2: Essential Characteristics of Quality for Nanoemulsion

Quality attributes of the drug products	Target	Is this a CQA?	justification
Physiological characteristics color odor appearance	Transparent No unpleasant odor Acceptable to patients	Negative	The physical features of the formulation were deemed less significant, given they are not directly associated with effectiveness and safety. security.
y and content uniformity	100%	No	Assay and content uniformity: 100%Nanoemulsions are characterized as homogeneous dispersions containing solubilized drugs within a mix of excipients, hence classified as somewhat discerning.
Size of the globule	Fewer than 100 nanometers	yes	Dimensions of globules Less than 100 nm. The reduced globule size of the nanoemulsion will enhance permeability inside the epidermal layer.
The act of spreading or flowing throughout	Elevated	Indeed	The elements of nanoemulsion is capable of engaging with the various strata of the epidermis, modifying its structural integrity, thereby diminishing the diffusion barrier and subsequently improving permeability.

Drug loading	High	yes	A substantial quantity of the medicine may be integrated owing to its elevated solubilization ability.
% Transmittance	High	yes	Elevated transmittance signifies the transparency of the formulated nanoemulsion system.

2.2.9 Analyses of potential hazards

A cause-and-effect (Ishikawa/fishbone) diagram was created systematically. assay the interconnections among implicit material characteristics, process variables, and their awaited influence on the Essential Characteristics of the Nanoemulsion formulation. The illustration (created with Microsoft Visio ®, 2007) offers a methodical representation of the interplay between formulation factors and process conditions, emphasizing their collaborative impact on product qualityA Risk Estimation Matrix (REM) was established to prioritize components based on their inherent influence on the Essential Quality Characteristics (EQCs) of the fenticonazole nitrateloaded nanoemulsion. Each material point and process parameter was assessed for its threat contribution degree — distributed as high, medium, or low — concerning critical attributes similar as medicine lading, chance transmittance, drop size, medicine penetration, and density. The REM served as a quantitative tool to identify and prioritize high- threat elements. High- threat elements were subordinated to Design of Experiments(DoE) for methodical examination, enabling precise assessment of their influence on product performance. Medium- and low- threat factors were noted but considered less impressive on variability. The table below presents the threat Estimation Matrix(REM) for the fenticonazole nitrate- loaded nanoemulsion, emphasizing the interplay of material properties, process factors, and their associated threat situations on CQAs.

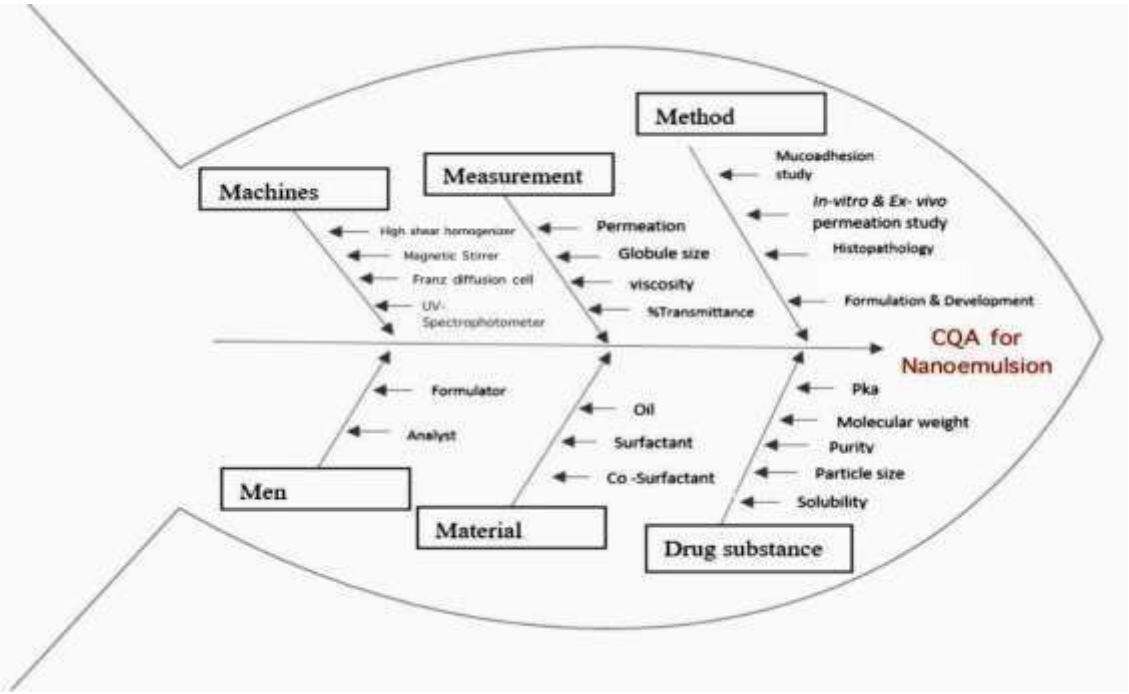


Figure-2:Ishikawa fish bone diagram

Table 3:Risk estimation matrix of drug loaded nanoemulsion

Drug loaded	Risk estimation matrix

Nanoemulsion CQAs	Conc. Of Oil	Conc. Of Surfactant	Conc. Of Co-Surfactant	ıount of water	Stirring speed	Temprature	tirring Time
Globule size	High	High	High	High	Medium	Low	Low
Permeation	High	High	High	Medium	Low	Low	Low
Viscocity	Medium	Medium	Medium	Medium	Low	Low	Low
Transmittance	High	High	High	High	Medium	Low	Low

The green color shows that all risk factors are under control (low risk)

Initial risk assessment for nanoemulsion Optimization
of Nanoemulsion:
D-optimal design:
The formulation of experimental design (DoE) was employed to enhance the nanoemulsion, and the D- Optimal design(admixture) was chosen. This design's features include multiple objective coextensive optimizations, high predictive precision, and simplicity. When experimental results calculate solely on the rates of the admixture's constituent parts, this kind of design is used to optimize the variables. Design factors are admixture constituents, and responses are functions of their proportions in this kind of response surface optimization approach. The constituents of the admixture can not vary singly because their sum must equal 100. In order to ascertain the primary goods and commerce goods among the independent variables within an experimental framework, the D-optimal admixture design is constantly employed(77). Expert in design The software used for the experimental design in the current studies was interpretation 13(State- Ease Inc., Minneapolis, USA). Different situations of the design constraints X1(Smix), X2(oil), and X3(water) were taken. The constituent ranges for nanoemulsion optimization were as follows (11,12).

Thermodynamic stability study
The formulation of the nanoemulsion was visually examined for phase separation after it was centrifuged for 10 minutes at 10,000 rpm. Three or four snap- thaw cycles were conducted, involving a freezing period Maintained at -4 °C for a duration of 24 hours, thereafter exposed to a thawing phase at 40 °C for an additional 24 hours. This procedure was applied to the formulation that demonstrated no phase separation after centrifugation. The centrifugation process was conducted for a duration of five minutes at a speed of 3,000 revolutions per nanosecond. Phase separation was also observed using the formulation. For further study, only formulations that remained stable during phase separation were chosen.

TEM(transmission electron microscopy)
The formulation's nanostructure was characterized using TEM. A single drop of a roughly diluted nanoemulsion sample was directly applied to a holey carbon-covered copper grid. A solitary drop of phosphotungstic acid was introduced for the purpose of negative staining. A filtration process was employed to exclude any extraneous phosphotungstic acid from the sample, which was latterly permitted to desiccate.

Preparation of nanoemulsion grounded gel
In order to achieve a smooth dispersion, the gel- forming agent Carbopol 934P was first agitated at about 150 rpm using a magnetic stirrer for two hours in 100 ml of Fenticonazole nitrate- loaded nanoemulsion. For fifteen minutes, it was left to stand in order to release any trapped air. By gradual stirring, the resulting viscous gel was neutralized with citric acid to a pH of 7. 1, 1.5, and 2 carbopol concentrations were used to produce the gel formulation.

In vitro pharmacological liberation

The release profile of a medicine offers critical perception into its in vivo behavior and anticPEG 400tes the functionality of a delivery system. The assessment of Fenticonazole nitrate incorporated with clary sage oil was conducted in vitro through a gel release profile exercising a nanoemulsion, employing a Franz diffusion cell for the analysis. The receptor compartment was filled with a methanol result in a 6040 rate. The donor compartment was populated with gels formulated from nanoemulsions, which incorporated Fenticonazole nitrate infused with clary sage oil. A 0.2 µm cellulose membrane separated the donor and receptor chambers. The recently prepared medium was replenished, and a 1 ml sample was uprooted at destined intervals. The quantification of medicine release was conducted exercising a UV Spectrophotometer, with measures taken at 253 nm subsequent to applicable dilution with the admixture. PBS 7.4 Methanol(6040)(14,15).

Permeation analysis

The abattoir supplied goat hide. The mucosal part was removed along the Franz diffusion cell diameter. drug release from the enhanced Fenticonazole nitrate with clary sage oil in a nanoemulsion- grounded gel was studied using the Franz diffusion cell. PBS 7.4 The receptor cube was filled with 6040 methanol. The patron and receptor chambers were separated by goat skin. At regular intervals, one millilitre of material was withdrawn and replaced with new medium. After dilution with PBS 7.4 Methanol(6040), The drug concentration was measured using a UV Spectrophotometer at a wavelength of 253 nm.. The goat tissue was also separated and purified in PBS 7.4 Methanol(6040) and water to remove any leftover medicine. The tissue was also cut into little pieces. Slices were homogenized in PBS 7.4 Methanol(6040) using a high shear homogenizer. Goat tissue was homogenized and filtered using a high Tissue Homogenizer(Mac, Mumbai, India). To homogenize tissue, methanol was applied again. After filtering and 20 minutes at 5000 rpm centrifugation, the supernatant was recovered. A UV spectrophotometer measured medicine content in the supernatant after dilution. Mass balance was calculated from drug staying on and circulated within tissue.

Studies on antifungals:

The cup plate method of the agar diffusion test was used to accomplish this. The usual dosage of 400 mg of Fenticonazole nitrate was given along with the new formulations. Prior to being put on plates containing Candida albicans test organisms and potato dextroOSE agar, all of the previously mentioned preparations were sterilized. Agar plates were left in incubators set to 37°C for 24 hours after the solutions had solidified. The zone of inhibition (ZOI) of each plate was identified and contrasted with the control. Except for incubation, every step of the process was completed in a laminar airflow unit 16 [16–18].

3. RESULTS AND DISCUSSION

3.1 Method of analysis:

The range of Fenticonazole nitrate 's absorbance in methanol is reported to be between 0.2-0.8 at concentrations between 100 and 350 g/mL. The calibration equation, y=0.0305x+0.0075 has R^2 value of 0.9991.

3.2 Screening of all components that constitute nanoemulsion:

Solubility of Fenticonazole nitrate in various oil, surfactant and co-surfactants was estimated for screening the component for nanoemulsion containing Fenticonazole nitrate . Solubility of Fenticonazole nitrate was highest in clary sage oil among all different oils. Fenticonazole nitrate showed highest solubility in Tween80. Among co-surfactant, PEG 400 had higher solubilizing power compared to other co-surfactants so it was selected as co-surfactant. Among surfactants, Fenticonazole nitrate showed higher solubility in Tween-80. As Tween-80 gave clear nanoemulsion so it was selected as surfactant.

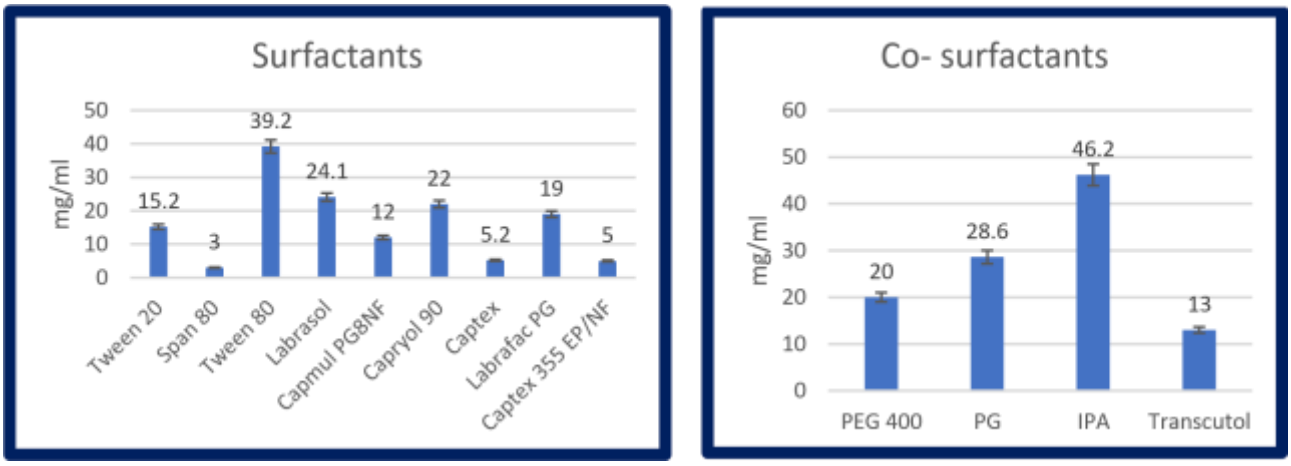


Figure-3 : Screening of oil, surfactant and co-surfactant

3.3 Development of pseudoternary phase diagrams:

By comparing the results of the present studies, compositions of 1:1 and 1:2 revealed that the number of phase diagrams with the highest share of the area of the nanoemulsion increased at the S/CoS weight ratio of 2:1. This is due to enhanced micelle formation as a result of an increase in the S/CoS ratio which enhances the solubilization ability of the nanoemulsion. Additionally, at a S/CoS weight ratio of 2:1, On the same wave length, the results revealed that Clary sage oil produced the largest nanoemulsion.

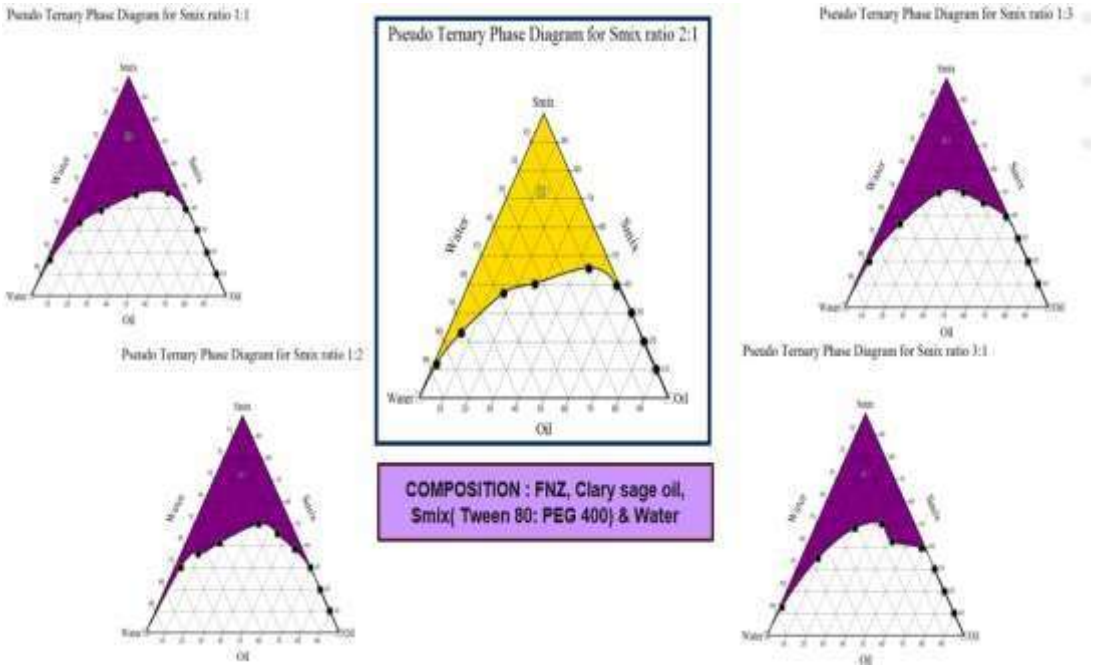


Figure - 4: Pseudoternary Phase Diagram of 2:1 Smix ratio

The pseudo ternary phase diagrams of the Smix (Tween 80: PEG 400) ratio 1:1, 1:2, 1.3, 2:1. and 3:1 of nanoemulsion was constructed. The nanoemulsion area was represented by the dark colored region in the phase diagras. As 2:1 has larger nanoemulsion area it was selected for further optimization.

3.4 Optimization of nanoemulsion:

Figure-5: Batches of Nanoemulsion

Table-4:Results of D-optimal design for nanoemulsion

Sr. no	Batch no.	Smix	Oil	Water	Globule size	Zeta Potential	PDI
1	A1	30	5	64.99	27.1	-20.4	0.405
2	A2	35.04	5	59.95	17.5	-27.3	0.215
3	**A3**	**36.44**	**9.3**	**54.24**	**140.4**	**-14.6**	**0.315**
4	A4	38.6	13.66	47.65	286	-16	0.495
5	A5	43.1	9.42	47.47	148.6	-12.4	0.41
6	A6	46.04	13.95	40.00	435.9	-14.7	0.624
7	A7	54.99	5	40.00	44.8	-17.1	0.425
8	A8	48.07	6.98	44.94	110.1	-10.5	0.52
9	A9	30.65	15	54.34	206.1	-15.7	0.409
10	A10	43	5	51.99	22.9	-23.9	0.394
11	A11	30	10.04	59.95	140.1	-13.9	0.239

An overlay plot represents a region within a design space where all factors are concentrated within their effective or desired ranges. This means that any combination of factor ranges chosen within this region will yield formulations that deliver the desired outcome while maintaining robustness. In essence, it's a visual representation that helps identify the optimal parameter ranges for achieving desired outcomes in a formulation process.

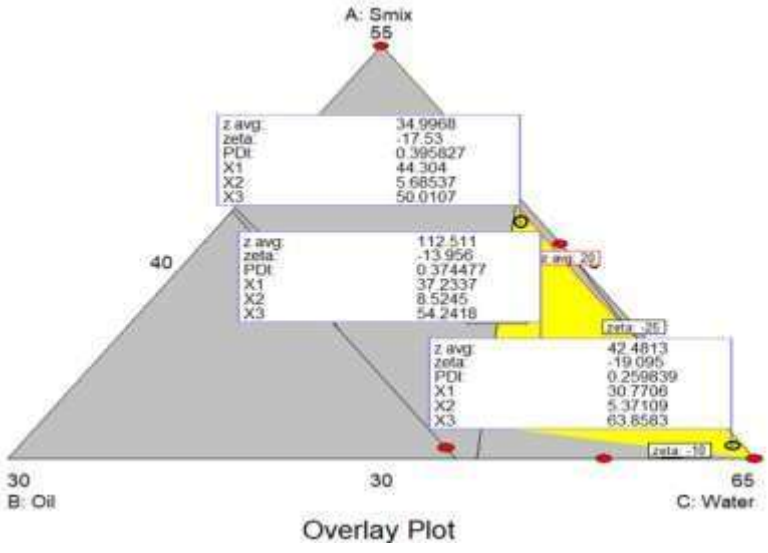


Figure-6:Overlay plot of Fenticonazole nitrate nanoemulsions

A flag batch was generated and formulation was prepared and further evaluation was done.

3.5 Validation of checkpoint batch

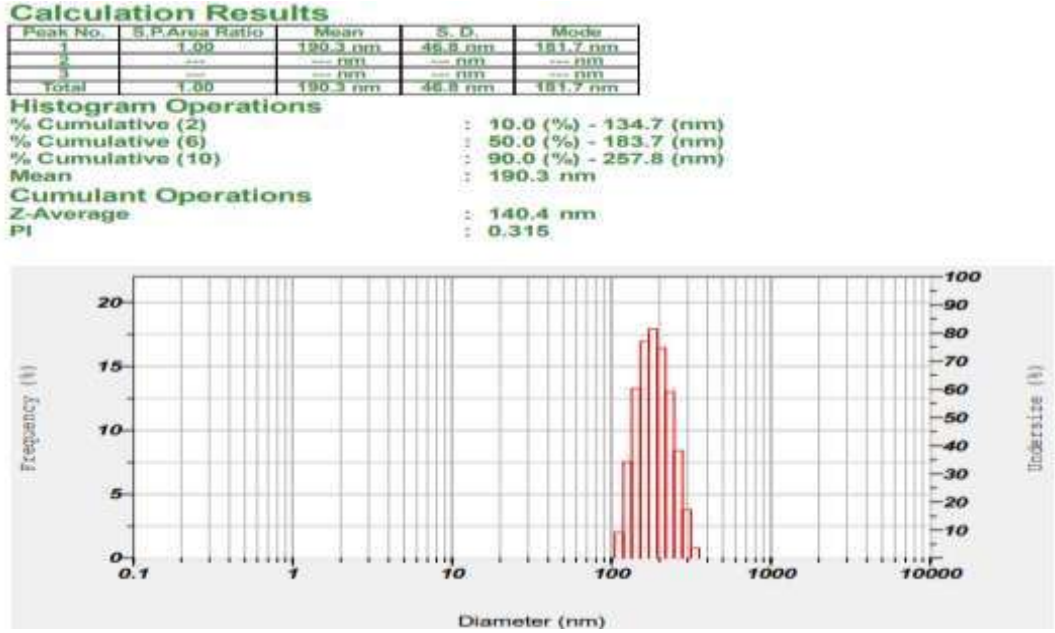
To evaluate the dependability of the equations that articulate the impact of the factors on the responses check point batch were formulated. Composition of check point batch is shown in

Table-5:Characterization of optimized nanoemulsion

Physicochemical characteristicsn	of clary sage oil based Nanoemulsion
% Transmittance	96.4%
Conductivity	69.8(200 µs)
pH	7.25
Refractive index	1.436
Viscosity	5.24
Drug content	O/W
Type of nanoemulsion	96.4%

3.6 Globule size distribution and Zeta potential

A correlation exists between the dimensions of the globules and the level of the surfactant employed. In certain instances, elevating the surfactant concentration may result in droplets exhibiting a reduced mean globule size. The stabilization of the oil globules can be elucidated by the the positioning of the surfactant molecule at the oil-water interface. The size of the globule is noted to influence the permeation of drugs. The reduction in globule size results in an increase in the interfacial area available for drug permeation.



Globule size (nm) of formulation was found to be **140.3 nm**, **PDI** as **0.367** and **-14.6 mV as Zeta potential**.

Figure-7: Globule size distribution and PDI

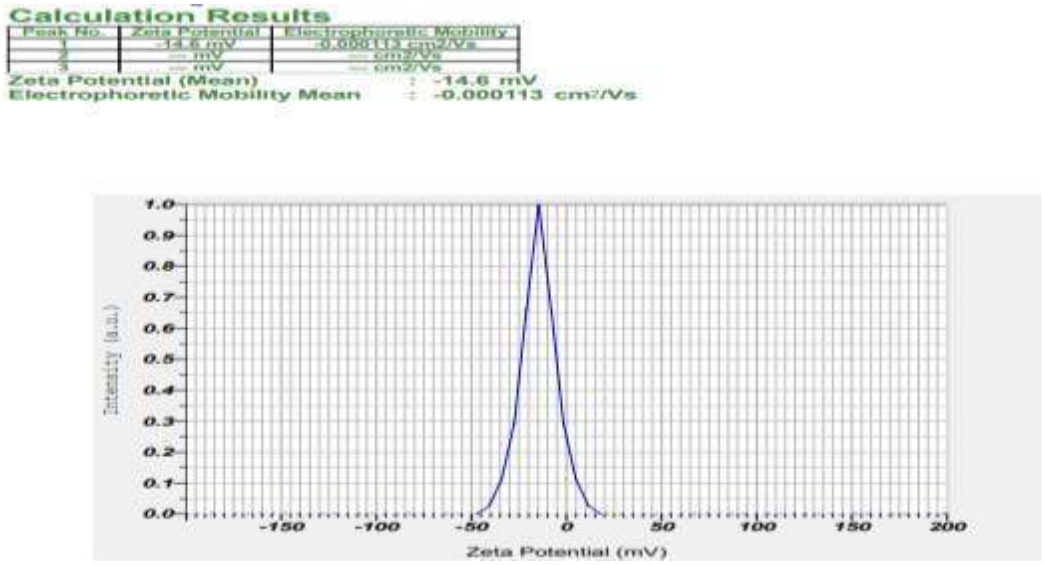


Figure-8: Zeta potential

3.7 Characterization of CTM Nanoemulsion System:

The optimised nanoemulsion, which had a PDI of 0.458 and the smallest globule size of 208 nm, contained 70% Smix, 5% oil, and 20% water. The measured zeta potential was -0.9 mV. Nanoemulsion and in-situ gel were discovered to have viscosities of 33 cP and 181 cP, respectively, and a pH of 6.71. According to the results of conductivity, which is 0.343 ms/cm, all nanoemulsions were of the o/w type. [14]

3.8 Transmission electron microscopy (TEM)

The prepared formulation of nanoemulsion was evaluated for globule size and aggregation. The globule of optimized Fenticonazole nitrate loaded nanoemulsion appeared to be almost round in shape, distributed uniformly and do not showed aggregation in transmittance electron microscope. Image of formulation is as such and magnified, showed in below figure.

Figure-9:TEM images of optimized nanoemulsion

3.9 Investigation of drug release in vitro

The peak drug release observed was 90.0% from Fenticonazole nitrate loaded nanoemulsion based gel was achieved within 4 hours while in case of nanoemulsion 89.21% of drug release was achieved.[10]

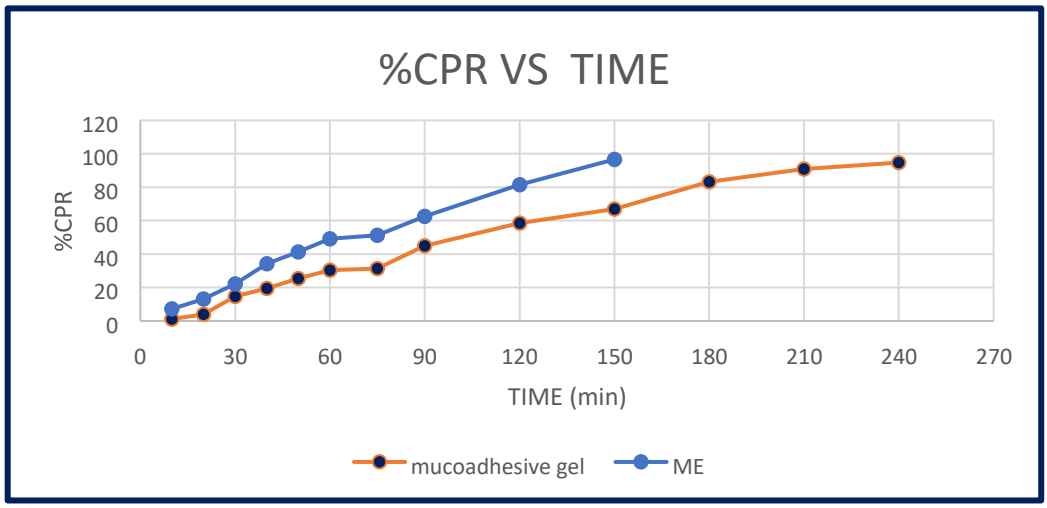


Figure-10: A study on the release of drugs in vitro

3.10 Investigation of drug permeation in an ex-vivo setting

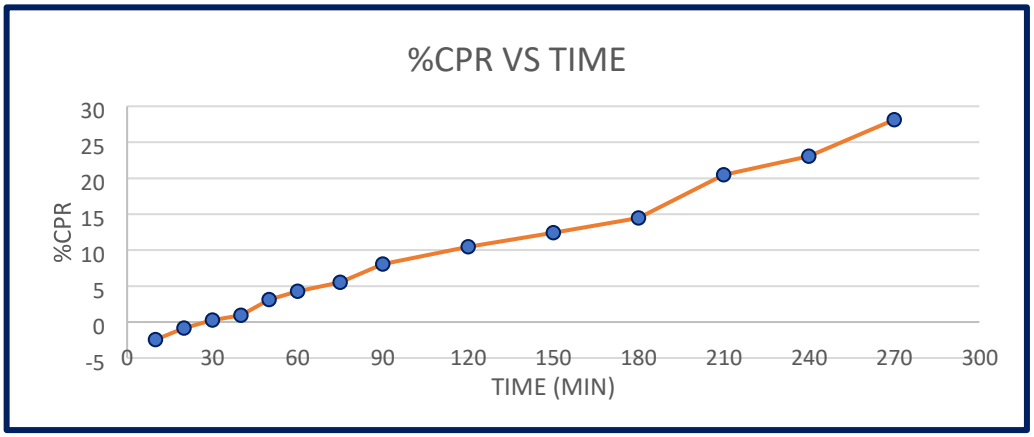


Figure-11: Investigation of skin permeation in an ex vivo context

Ex vivo investigation of skin penetration goat skin was carried out for nanoemulsion based gel. Table 49 shows the %CPR of NEBG formulations. The permeation profile of NEBG formulations through goat buccal mucosa is shown in figure 57. After 5 hours, the NEBG exhibited a permeation of 65.48%. Additionally, the retention of Fenticonazole nitrate within the skin was 23.6% for the NEBG. These results shows that the NEBG formulation enhances both the permeation and retention of Fenticonazole nitrate.

The ex vivo skin permeation investigation of NEBG demonstrated a satisfactory permeation profile through goat skin. The prepared nanoemulsion may function as a drug reservoir, facilitating the dissemination of the drug from the internal phase to the exterior phase and enhancing permeability by modifying or compromising the tight junctions of the mucosal epithelium. The NEBG demonstrated enhanced penetration owing to its inherent properties that promote adhesion to mucosa, hence prolonging contact duration and facilitating improved diffusion. [8]

4. RESULT OF COMPARATIVE ANTIFUNGAL STUDIES

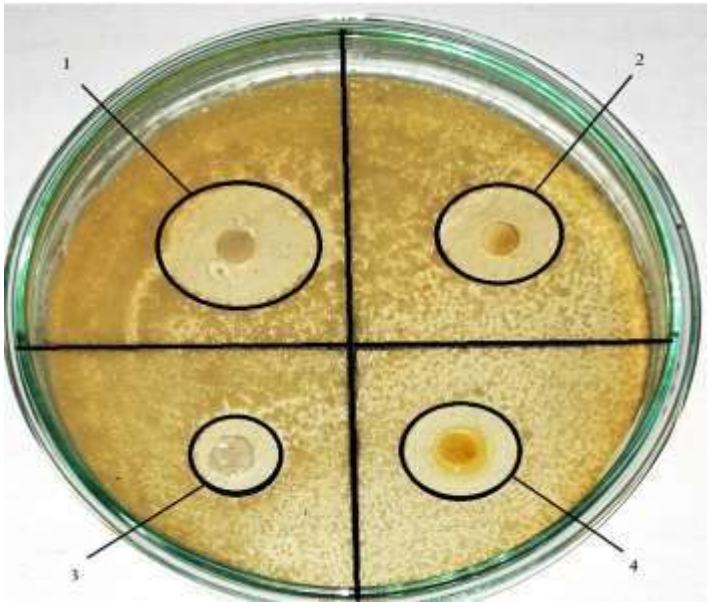


Figure-12:Comparative Antifungal Study (SET 1)

Table-6:Comparative Antifungal Study (SET 1)

Sr. No	Formulation	ZOI (mm) Diameter SET 1	ZOI (mm) Diameter SET 2	ZOI (mm) Diameter SET 3	MEAN	SD
1	Drug loaded nanoemulsion based gel	21	24	22	22.33	1.527525
2	Drug loaded oleic acid based nanoemulsion	16	18	16	16.66	1.154701
3	Marketed formulation	14	13	14	13.66	0.57735
4	Blank nanoemulsion	15	17	16	16.00	1.00

Antifungal activity of Fenticonazole nitrate loaded nanoemulsion gel was compared with different prepared formulation and marketed formulation through its zone of inhibition. The antifungal activity based on zone of inhibition of clary sage oil comprising nanoemulsion based gel was found to be more effective than other prepared formulation as well as marketed formulation. Based on observations, it was found that zone of inhibition of 0.4% of NEBG was higher than marketed formulations.

5. CONCLUSION – A QBD BASED APPROACH:

Fenticonazole nitrate, an antifungal medicine, doesn't dissolve veritably well in water, which makes it tricky to formulate as a gel (0.4%) for operation on the mouth's mucous membranes. To break this, Tween 80 was used as a surfactant and PEG 400 as a co-surfactant. Carbopol 934P served as the gelatinizing agent in combination with HPMCK100M as mucoadhesive polymer. When assaying the mock ternary phase illustration across different Smix rates, it turned out that a 2:1 rate offered the effective nanoemulsion region. We optimized the nanoemulsion formulation using D- optimal design, fastening on drop size, polydispersity indicator, and zeta eventuality as crucial parameters. The designed batch, called Nanoemulsion BATCH CP2, was named because it met all the target responses and was emphasized in the overlay plot. The perfected nanoemulsion contained about 36.44% Smix, 9.3% oil and 54.24% water. The drop size was roughly 140.4 nm, with a polydispersity indicator (PDI) of 0.316. The measured zeta eventuality was -14.6 mV. Its density was 36 cP, while the nanoemulsion grounded gel was thicker at 2950cP. The pH of the nanoemulsion was 6.73, compared to 5.82 for the gel. All the

nanoemulsions were oil- in- water (O/ W) types, verified by a conductivity dimension of 69.8 μs/ cm. We also tested the nanoemulsion's stability under thermodynamic conditions, and it showed no signs of phase separation, attesting its good stability. In vitro, we set up that the nanoemulsion without a gel released the medicine briskly than the nanoemulsion in gel form. The gradual release from the gel could be attributed to the incorporation of the gelatinizing polymer. Further ex vivo testing revealed that after 5 hours, approximately 65.48% of the medication was released from the nanoemulsion-based gel.The combination of clary sage oil with fenticonazole nitrate in the gel appears to broaden its antifungal exertion. When tested against a marketable product, the nanoemulsion gel displayed a larger zone of inhibition, indicating stronger antifungal goods. specially, the 0.4% nanoemulsion gel outperformed other products, indeed with a lower cure of fenticonazole nitrate, suggesting a reduced chance of developing resistance.

Ex-vivo liberation was also conducted for Nanoemulsion based gel. At 5 hours 65.48% drug release was observed. Combination of Clary sage oil and Fenticonazole nitrate containing nanoemulsion based gel was increase the spectrum of antifungal activity. Antifungal activity of nanoemulsion based gel was compared with marketed formulation through its zone of inhibition. Based on observations, it was found that zone of inhibition of 0.4% of NEBG was higher than other marketed formulations. 0.4% of NEBG was hence proved to be more effective then other marketed formulation, that too with relatively lower dose of Fenticonazole nitrate. Thus, the chances of drug resistance shell be minimal.

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