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A RESEARCH ARTICLE ON FORMULATION AND EVALUATION OF FRAXIDIN LOADED ANTIMICROBIAL NANO-EMULSION GEL

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ABSTRACT: The development of effective antimicrobial agents is critical in combating bacterial infections, particularly in the face of increasing antibiotic resistance. In this study, we explore the formulation of fraxidin-loaded antimicrobial nanoemulsion gel as a promising therapeutic approach. Fraxidin, a bioactive compound with known antimicrobial properties, has been encapsulated in a nanoemulsion system to enhance its stability, solubility, and bioavailability. The gel formulation was developed by incorporating the fraxidin-loaded nanoemulsion into a suitable gel matrix, designed for topical application. Characterization of the nanoemulsion gel, including particle size, zeta potential, encapsulation efficiency, and in vitro release studies, demonstrated the successful encapsulation of fraxidin and its sustained release profile. Antimicrobial efficacy was evaluated against common pathogens, with the fraxidin-loaded nanoemulsion gel showing significant inhibitory effects, as compared to free fraxidin, indicating an enhanced antimicrobial activity due to the nanoencapsulation. Furthermore, the gel formulation exhibited desirable rheological properties, stability, and skin compatibility, making it suitable for topical application. This research highlights the potential of fraxidin-loaded nanoemulsion gel as a novel, effective, and safe alternative for the treatment of skin infections and other microbial-related ailments. The results of this study contribute to the growing field of nanomedicine, demonstrating the therapeutic advantages of nanoemulsions in drug delivery systems for enhanced antimicrobial action.

Keywords: Nanoemulsion, Antimicrobial study, Transdermal flux, Stability study, Skin irritation study

INTRODUCTION:

The emergence of antimicrobial resistance has become one of the most pressing global health threats, rendering many conventional antibiotics less effective against a wide range of bacterial pathogens. This growing resistance has prompted the search for new antimicrobial agents with enhanced potency and fewer side effects. Fraxidin, a natural bioactive compound derived from the Fraxinus species, has shown considerable antimicrobial activity against various bacterial strains. However, despite its promising bioactivity, fraxidin clinical potential is limited by its low solubility, poor bioavailability, and instability in aqueous solutions, which restrict its effectiveness as a therapeutic agent.

To overcome these challenges, nanotechnology, particularly nanoemulsions, offers a novel solution. Nanoemulsions are colloidal systems that consist of nanoscale droplets of oil dispersed in water, and they have proven effective in enhancing the solubility and stability of hydrophobic drugs. These systems are capable of improving the bioavailability of active ingredients and allowing for controlled, in this study, fraxidin was encapsulated in a nanoemulsion and incorporated into a gel-based formulation for topical application. The resulting fraxidin-loaded antimicrobial nanoemulsion gel is designed to provide an effective, localized treatment for skin infections, wounds, and other microbial-related ailments. This formulation not only enhances the antimicrobial properties of fraxidin but also offers the added benefits of a gel matrix, which provides ease of application, improved skin compatibility, and sustained drug release. The objective of this research is to evaluate the physicochemical properties, antimicrobial efficacy, and potential clinical application of fraxidin-loaded nanoemulsion gel, contributing to the growing field of nanomedicine as a promising approach to combat skin infections and other antimicrobial challenges.

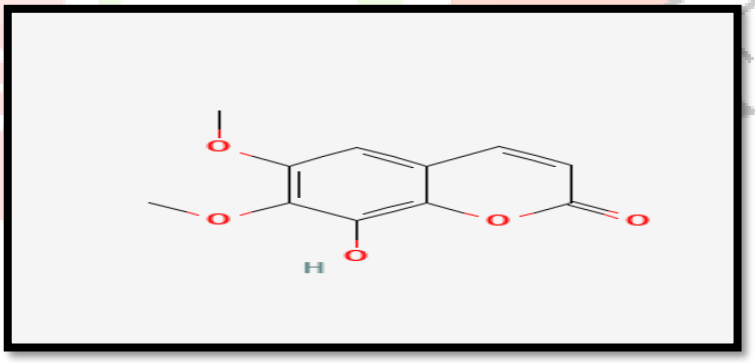


FIG.1: Chemical Structure of Fraxidin Drug

Transdermal Drug Delivery System:

In the context of fraxidin and its potential use in a Transdermal Drug Delivery System (TDDS), the goal would be to develop a system that improves the bioavailability and antimicrobial efficacy of fraxidin when applied topically, especially given its natural antimicrobial properties. Fraxidin, a naturally occurring coumarin compound, has shown promising antimicrobial activity, but its clinical utility is often limited by its poor solubility and bioavailability. A transdermal drug delivery system could be an ideal strategy for overcoming these limitations, providing a sustained, controlled release of fraxidin for localized treatment of infections, particularly skin-related microbial conditions. Below is a detailed study of how TDDS could be useful to fraxidin:

1. **Rationale for Transdermal Delivery of Fraxidin:**

- **Enhanced Skin Permeability:** Fraxidin, being a relatively hydrophobic compound, has low water solubility, which may limit its absorption through the skin. Using **nano emulsions** or **liposomes** to encapsulate fraxidin could improve its solubility and enable its penetration.
- **Sustained Release:** Transdermal systems, especially patches, can provide **controlled release** of fraxidin over time, maintaining a steady concentration of the compound at the site of infection, which is crucial for effective treatment.

2. **Formulation of Fraxidin-loaded Transdermal Systems:**

- **Nano emulsion Gel:** Fraxidin can be encapsulated into a nano emulsion, which would improve its solubility, stability, and permeability through the skin. The nano emulsion could be incorporated into a gel base, providing ease of application and skin compatibility.
- **Micro needles:** Another innovative approach for transdermal delivery of Fraxidin could be micro needle arrays, which are small enough to puncture the outer layer of the skin without causing pain.

3. **Mechanisms of Enhanced Penetration:**

- **Penetration Enhancers:** Formulations can be designed with **penetration enhancers** like **ethanol**, **propylene glycol**, or **surfactants** to temporarily disrupt the skin barrier, allowing fraxidin to pass through more easily.
- **Iontophoresis:** The use of electric fields in iontophoresis could be another technique to enhance the delivery of Fraxidin, particularly for charged versions of the compound, by improving its mobility through the skin layers.

4. **Antimicrobial Efficacy:**

- **Topical Antimicrobial Activity:** The Fraxidin-loaded transdermal patch or gel formulation could provide localized treatment for skin infections caused by bacterial, fungal, or viral pathogens. The antimicrobial activity of fraxidin could be enhanced by the nano encapsulation process, which ensures that the drug is delivered in a stable, providing continuous action against pathogens.
- Reduced Systemic Side Effects: By delivering fraxidin directly to the site of infection, a transdermal delivery system minimizes the risk of systemic side effects.
- **Targeted Therapy:** The TDDS approach allows for localized delivery of fraxidin directly to the skin, where it can target infections effectively without the complications associated with systemic administration.
- **Improved Patient Compliance:** A transdermal system can reduce the frequency of administration, providing more convenient treatments for patients.
- **Non-invasive:** The non-invasive nature of TDDS makes it a pain-free option compared.

6. **Challenges and Future Considerations:**

- **Skin Irritation:** Long-term use of TDDS could cause skin irritation or allergic reactions.
- **Formulation Stability:** The stability of fraxidin in the transdermal system is a critical consideration. Factors such as the choice of excipients, preservatives, and the storage conditions must be optimized to ensure the stability of the formulation.
- **Regulatory Approval:** Any new drug delivery system, obtaining regulatory approval for fraxidin-loaded TDDS requires extensive safety and efficacy testing.

Nanoemulsion:

The liquid mixture of oil (O) and water (W) that is known as a nano-emulsion is clear or translucent, thermodynamically stable, and contains a substance called surfactant. The range of nanoparticle sizes is 10 to 1000 nm. Oil-in-water (O/W) and water-in-oil (W/O) nanoemulsion are the dispersion of two immiscible liquids stabilized with the help of a suitable surfactant. When two structures are produced, each saturated with traces of a different substance, the oil and water phases are immiscible with it and cannot be distinguished from one another [7]. The dosage forms of nanoemulsion, such as liquids, creams, sprays, gels, aerosols, and foams, as well as the delivery methods, such as topical, oral, intravenous, intranasal, pulmonary, and ophthalmic, are all rather diverse. They are used in the cosmetic and pesticide industries as an aqueous foundation for organic deliverables because they have a better solubilization capacity than straight forward micellar dispersions and more kinetic stability than coarse emulsions.[8]

MATERIALS AND METHOD:

Material:

The materials used in the formulation of fraxidin-loaded antimicrobial nanoemulsion gel include fraxidin (the active ingredient), surfactants like Polysorbate 80 and Span 80 for stabilizing the nanoemulsion, and oils such as MCT oil and olive oil to enhance drug solubility and skin penetration. Ethanol is used to dissolve fraxidin, while water serves as the aqueous phase. The gel matrix is created using Carbopol 934 and HPMC, and penetration enhancers like propylene glycol help improve skin absorption.

Preformulation Studies: Preformulation studies are essential to evaluate the physicochemical properties of fraxidin and ensure the successful development of the fraxidin-loaded antimicrobial nanoemulsion gel. These studies help identify factors such as solubility, stability, and compatibility, which can influence the formulation's design and performance.

1. **Solubility Studies:**

   The solubility of fraxidin in various solvents (water, ethanol, oils, and surfactants) was tested to determine the most suitable solvent system for the nanoemulsion formulation. Solubility in oils such as MCT oil and olive oil, along with surfactants like Polysorbate 80, was evaluated to select the optimal oil-phase for the nanoemulsion.

2. **Melting Point Determination:**

   The melting point of fraxidin is approximately 178-182^{o}C with using a capillary method.

3. **Compatibility Studies:**

   Fourier Transform Infrared (FTIR) spectroscopy was conducted to evaluate the chemical compatibility between fraxidin and excipients. The FTIR spectra help identify any possible interactions that could affect the stability.

4. **Particle Size and Zeta Potential:**

   The particle size and zeta potential of the nano emulsion were measured to assess the stability and uniformity of the formulation. Smaller particle sizes are preferred for better skin penetration and enhanced antimicrobial activity.

5. **pH Determination:**

   The pH of the fraxidin-loaded gel formulation was determined to ensure it is suitable for topical application, aiming for a skin-friendly pH of approximately 5.2.

6. **Stability Studies:**

   Stability studies were conducted under different environmental conditions (temperature, light, and humidity) to evaluate the long-term stability of fraxidin in the nanoemulsion gel, ensuring its effectiveness and shelf-life.

Screening of excipients, oils, surfactants and cosurfactants:

Excipients:

The selection of appropriate excipients is a crucial step in formulating the fraxidin-loaded antimicrobial nanoemulsion gel. The excipients must ensure the stability, solubility, and optimal release of fraxidin while enhancing skin penetration and maintaining compatibility with other ingredients.

Screening of Oils:

Oils are used as the oil phase in nanoemulsions to dissolve fraxidin and enhance its skin penetration. Several oils were tested for their ability to solubilize fraxidin and improve drug absorption through the skin.

Medium Chain Triglyceride (MCT) Oil: Known for its ability to enhance skin permeability and solubility of lipophilic drugs.

Olive Oil: Alternative oil, known for its emollient properties, was also tested to ensure its compatibility with the formulation and to provide a more moisturizing effect on the skin.

1. **Screening of Surfactants:**

   Surfactants help stabilize the nanoemulsion by reducing the surface tension between the oil and water phases. Surfactants also aid in enhancing the solubility of fraxidin in the formulation. The following surfactants were screened:

   Polysorbate 80 (Tween 80): A commonly used surfactant that can stabilize the nanoemulsion and help solubilize hydrophobic drugs like fraxidin.

Span 80 (Sorbitan Monooleate): A lipophilic surfactant that is often used in combination with Polysorbate 80 to create a stable emulsion with smaller droplet sizes.

2. **Screening of Cosurfactants:**

   Cosurfactants are used in combination with surfactants to further reduce surface tension and improve the stability of the nanoemulsion. The following cosurfactants were evaluated:

   Ethanol: As a cosurfactant, ethanol enhances the solubility of fraxidin and improves the penetration of the active ingredient into the skin.

   Propylene Glycol: Used as both a cosurfactant and a skin penetration enhancer, propylene glycol helps improve the absorption of fraxidin.

   Transcutol: Known for its ability to enhance the permeation of drugs across the skin, Transcutol was evaluated as a cosurfactant for improved drug delivery.

3. **Selection Criteria:**

   Solubilizing Ability: The ability of oils, surfactants, and cosurfactants to solubilize fraxidin.

   Particle Size Reduction: The ability of surfactants and cosurfactants to reduce the droplet size of the nanoemulsion, which is important for enhancing skin penetration and improving the antimicrobial activity.

   Stability: The stability of the nanoemulsion, including physical stability (no phase separation) and chemical stability (preserving fraxidin's potency), was assessed under different conditions (e.g., temperature).

   Skin Compatibility: The compatibility of the formulation with the skin was evaluated to avoid irritation or allergic reactions.

UV-Visible Spectroscopy of Fraxidin:

UV-Visible spectroscopy is used to analyze fraxidin by measuring its absorption of ultraviolet or visible light. The technique helps identify the compound's characteristic absorption peaks, particularly its **λmax** (maximum absorption wavelength), which is typically around 290-310 nm for fraxidin.

This method allows for the quantification of fraxidin in formulations by creating a calibration curve. It also helps monitor fraxidin stability over time and notice any potential chemical interactions with other excipients in the formulation, ensuring the compound remains effective in the final product.

HPLC Analysis of Fraxidin:

Column: C18 (octadecylsilane), 5µm particle size, 10 cm

Mobile Phase: A mixture of solvents (commonly acetonitrile and **water** with or without a small percentage of an acid like phosphoric acid to ensure proper separation of the compound.

Flow Rate: 1 mL/min

Detection: 290-310 nm.

Run time: 10min.

Retention time: 5.345 min.

In Methanol: 10 mg of Fraxidin was dissolved in 100 ml of methanol (100µg/ml) and different dilutions of 1-10 µg/ml was prepared after appropriate dilution with methanol. The samples were filtered using a 0.45µm filter and analyzed using HPLC at 249 nm.[13]

Fourier Transform Infrared spectroscopy (FTIR):

Fourier Transform Infrared Spectroscopy is an analytical technique used to identify and characterize the chemical structure of compounds by measuring the absorption of infrared light. In the case of fraxidin, FTIR can be used to detect its characteristic functional groups, such as the carbonyl and furanocoumarin group, by analyzing the absorption peaks in the infrared spectrum. FTIR is also useful for assessing the compatibility of fraxidin with other excipients in the formulation, as any chemical interactions between fraxidin and excipients will result in shifts or changes in the absorption peaks, helping to ensure the stability and integrity of the formulation.

Development of Pseudo Ternary Phase Diagram: A pseudo ternary phase diagram is a graphical representation used to optimize the formulation of nanoemulsions by illustrating the relationship between the oil, surfactant, and co-surfactant at varying concentrations. The diagram helps identify the optimal composition for achieving a stable nanoemulsion with the desired properties, such as small particle size and good stability.

Steps to Develop the Pseudo Ternary Phase Diagram:

Selection of Components:

1. **Oil Phase**: Choose an oil, such as MCT oil or olive oil, which will serve as the primary phase for the nano emulsion.
2. **Surfactant**: A surfactant like Polysorbate 80 (Tween 80) is selected to stabilize the oil-water interface.
3. Co-surfactant: A co-surfactant like Span 80 or propylene glycol is included to reduce the interfacial tension and enhance the formulation's stability.

1. **Preparation of the Phase Diagram:**

- Vary the ratios of oil, surfactant, and co-surfactant while keeping the total percentage (oil + surfactant + co-surfactant) constant, typically around 100%.
- The water phase is gradually added to the oil-surfactant-co-surfactant mixture, and each mixture is visually examined for clarity, stability, and homogeneity.

2. **Identification of Regions:**

- The diagram helps identify different regions corresponding to the nanoemulsion, microemulsion, gel, and free oil phases.

- The nanoemulsion region is typically a clear or translucent area in the phase diagram, indicating a stable and homogeneous mixture with small droplet sizes suitable for drug delivery.

3. **Optimization:**

   The goal is to find the optimal combination of oil, surfactant, and co-surfactant concentrations that provide a stable nanoemulsion. This is typically achieved when the mixture exhibits low viscosity, small droplet size (usually <100 nm), and good stability over time.

   By examining the pseudo ternary phase diagram, the best formulation range of each component (oil, surfactant, co-surfactant, and water) is identified, ensuring the stability of the fraxidin-loaded nanoemulsion.

4. **Final Selection:**

   Once the appropriate region for nanoemulsion formation is identified, the formulation is finalized, and further testing can be conducted, such as determining the particle size, zeta potential, and viscosity to ensure the desired properties for drug delivery.

Formulation Development:

Step 1: Screening of different oils on the basis of inherent solubilizing property.

Step 2: Screening Surfactant and Co-surfactant by miscibility.

Step 3: Preparation of Smix (Surfactant and Cosurfactant) ratio 1:1, 1:2 and 2:1.

Step 4: Mixing oil with particular surfactant mixture ratio (Smix) indifferent ratios1:9,2:8,3:7, 4:6,5:5, 6:4, 7:3, 8:2, 9:1(oil: Smix).

Step 5: Titrating each (oil: Smix) with the aqueous phase from 5% to 95% of total volume, in 5% increments. Visual observation to be done on each addition.

Thermodynamic Stability Study:

Freeze thaw Cycle: Placebo formulations were carefully kept at -20^{0}C for 24 hours in a deep freezer. The NE was then removed out of the deep freezer and placed in a room temperature storage container. These formulas returned to their original condition within 5-8 minutes. 3-5 cycles were completed in total.

Centrifugation Research Analysis: Centrifugation tests were carried out after the thaw cycle method. They were spun for 15 minutes at 10000 rpm in a centrifuge in this study, and the formulations were determined to be stable and free of turbidity [16].

Characterization of Nanoemulsion:

Percentage Transmittance: A Shimadzu UV visual spectrophotometer was used to determine the percent transmittance of the generated nanoemulsion formulations. Before testing at 249 nm, the formulation was diluted 100 times in water.

Determination of Zeta Potential: Electrophoretic mobility was used to analyse the surface charge of the emulsions (EM). A mixture of 20 liters of sample and 40 liters of DDW was used for all measurements. The combination was then adjusted with a NaCl solution to a conductivity of 50 S/cm and evaluated using a zeta potential measuring equipment (Zetasizer-1000 HAS from Malvern Instruments in the UK: PCS) [17].

Viscosity Determination: The viscosity of the generated nanoemulsion compositions without dilution was evaluated using an R/S CPS plus Rheometer spindle # C 50-1 at 250.5°C. The programme that was used was RHEO3000. One cc of the formulation was used for the viscosity test. For a single run, the spindle speed was adjusted at 70 rpm and the temperature to 250.5°C. It took 50 minutes to perform the surgery. The spindle diameter was 50 mm, and the shear rate was 413per minute [18]. The following parameters were set once the procedure had been improved:

TABLE 1: Specification Set in Viscometer for Determining Viscosity

Sample	**0.5 gm**
Probe speed(rpm)	30 rpm
Data Interval(min)	60 second
Loop Start	60 second
Wait period	30 minutes
Temperature	25±0.5°C
Shear rate(1/sec)	60

Refractive Index: The refractive index of chosen formulations was measured using an Abbe refracto meter. To standardize it, Castor oil was used.

Determination of Partition Coefficient: Excess fraxidin was dissolved in a 10 mL combination of n-octanol and water (1:1) in a separating funnel to measure the partition coefficient. To establish equilibrium, the system was agitated intermittently for 30 minutes and then left alone overnight. The two phases were then separated and centrifuged for 15 minutes at 10000 rpm. After centrifugation, using a UV-Visible spectrophotometer to measure absorbance, the concentration of fraxidin in both phases were determined [19]. The partition coefficient is usually estimated using the shaking flask method using the following formula:

$$P_{(O/W)} = C_1(\text{oil})/C_2(\text{aqueous})$$

Where, C_1=concentration of solute in organic phase

C_2=concentration of solute in aq. Phase

P (o/w) = Partition coefficient

Log =$\log_{(O/W)}$

***In-vitro* Release Study:**

The dialysis membrane employed in this study was cellulose membrane (Sigma, USA).It had a 60mL/ft capacity, an Average flat width of 2.5mm, and a diameter of 16

mm. The molecular weight limit was set at 12000 g/mol.

The *in-vitro* release of Fraxidin from the Fraxidin solution was determined using the dialysis method.

4 mL of each formulation in a dialysis bag (MWCO 3500 Da) were dissolved in 40 mL of PBS with 0.1% Cween-80.

The dialysis bag was swirled at 100 rpm at 37 °C. At the predetermined intervals (0.25,0.5,1,2,4,6,

8, 24, 48, and 72 hours), 1 mL of the sample was taken out and the same volume of fresh medium was added. The concentration of curcumin was determined using the HPLC method.

Ex-vivo Permeation Study**:** For the permeation studies, a vertical type Franz diffusion cell was manufactured by a local fabricator. It had a water jacket that kept the assembly temperature at 37 ± 0.5^{o}C. It was made up of two half cells, the donor compartment on top and the receiver compartment on the bottom (body). The diffusion area between 2 half cells was 3.0096 cm^2 (3 cm^2), and the receiver compartment had a capacity of 20 ml.

The entrance of the diffusion cell's receiver compartment was connected to a water bath, and the jacketed receiver compartment's output was placed in the sink. The flow of the water was adjusted to maintain a temperature of 37±0.5°C in the receiver chamber. The stratum layer corneum faced the giver partition and was wedged between the 2 compartments with the preparation [20].

% Drug release= (conc.(µgml^{-1}) × Dilution factor × Vol. of release medium (ml)) / (Initial dose (µg)) × 100

***In-vitro* Kinetics Analyses of Nano emulsion:** The rate constants for zero order and first order were calculated, along with their standard deviation (SD) and coefficient of variation (Cv), for *in-vitro* skin permeation studies after each time interval. The calculations were based on the cumulative percentage of drug that permeated from the skin permeation study.

Zero order rate constant, K_0

K_0=X/t=(Percentage drug permeated)/(Time(h)

First order rate Constant, K_1

K_1=2.303/Talgo (Co/C)

Whereof=Initial drug concentration.

C=Amount of drug remaining at time(t).

Skin Irritancy Test: The dermis irritancy test was carried out to verify the topical preparation efficacy. The mouse's left ear was treated with a single dose of Nano emulsion, and its right ear with control. The evolution of erythema, wheel or any allergic reaction was thoroughly investigated [21].

RESULTS:

Authentication of Fraxidin: Fraxidin was characterized using physicochemical characteristics such as color, smell, taste, and solubility in water and other organic solvents.

TABLE2: Solubility of Fraxidin in Different Oils, Surfactant and Co-Surfactant

S.no.	Oil	Solubility		
		24 hrs	48 hrs	72 hrs
1	Castor oil	In	Soluble	-
2	IPM	In	Slightly soluble	Slightly soluble
3	Oleic acid	In	Slightly soluble	Slightly soluble
4	Olive oil	In	Soluble	-
5	Glycerol	In	In-soluble	In-soluble

TABLE3: Solubility of Fraxidin in Surfactants and Co-Surfactants

S.no.	Surfactant and Co-surfactant	Solubility		
		24 hrs	**48 hrs**	**72 hrs**
1	Tween20	In-soluble	Soluble	-
2	Span 20	In-soluble	Soluble	-
3	Cween80	In-soluble	Soluble	-
4	Ethanol	Soluble	-	-
5	Methanol	Soluble	-	-
6	Benzene	In-soluble	In-soluble	In-soluble
7	DMSO	Soluble	-	-
8	Toluene	In-soluble	In-soluble	In-soluble
9	Propanol	Soluble	-	-
10	Butanol	Soluble	-	-
11	Octanol	Soluble	-	-
12	Carbitol	Soluble	-	-

Preformulation tests were carried out to establish the drug's solubility and partition coefficient in order to determine its appropriateness for use in a topical system.

The drug's solubility in several oils was also investigated in order to choose the best oil phase for Nano emulsion formulations.

After evaluating the oils for Fraxidin solubility, it was discovered that Castor oil had the highest solubility. As a result, castor oil was selected as the oil phase. Studies have demonstrated that Castor oil can enhance transdermal delivery by creating distinct domains that disrupt the continuity of the multi lamellar stratum corneum, resulting in highly

Melting point (DSC), UV- spectrophotometry, FTIR, and mass spectrum analysis (m/z value) were performed on the obtained sample and compared to those reported in the literature.

Permeable pathways. This is achieved by increasing the fluidity of the intercellular lipid barriers in the stratum corneum.

Because there is no substantial variation in medication solubility among these surfactants and cosurfactants, screening surfactants and cosurfactants on the basis of solubility is challenging.

Melting point Determination:

TABLE4: MELTING POINT OF FRAXIDIN FOUND AS FOLLOWS

Reported melting point	163°C
Observed melting point	164°C

UV spectral Analysis:

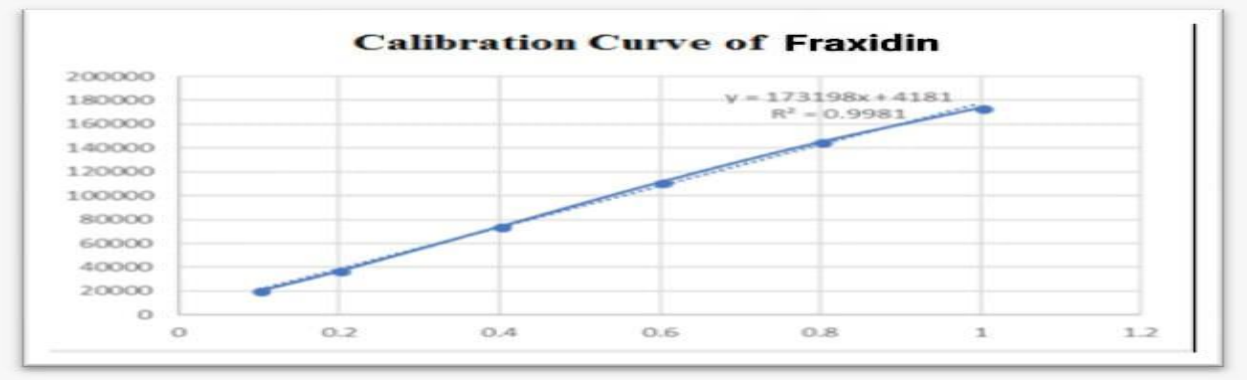


FIG.2: FRAXIDIN CALIBRATION PLOT IN METHANOL (UV)

Inference: The λ_{max} of Fraxidin in methanol was 293 nm and was discovered to be the same as the reference spectrum. A Shimadzu Double Beam Spectrophotometer was used to scan the prepared sample between 290 and 310 nm (UV 1601). The solution has absorption maxima at 293 nm. With the regression equation y=0.0465x+0.1356 and R^2 =0.9996.

High Performance Liquid Chromatography Method:

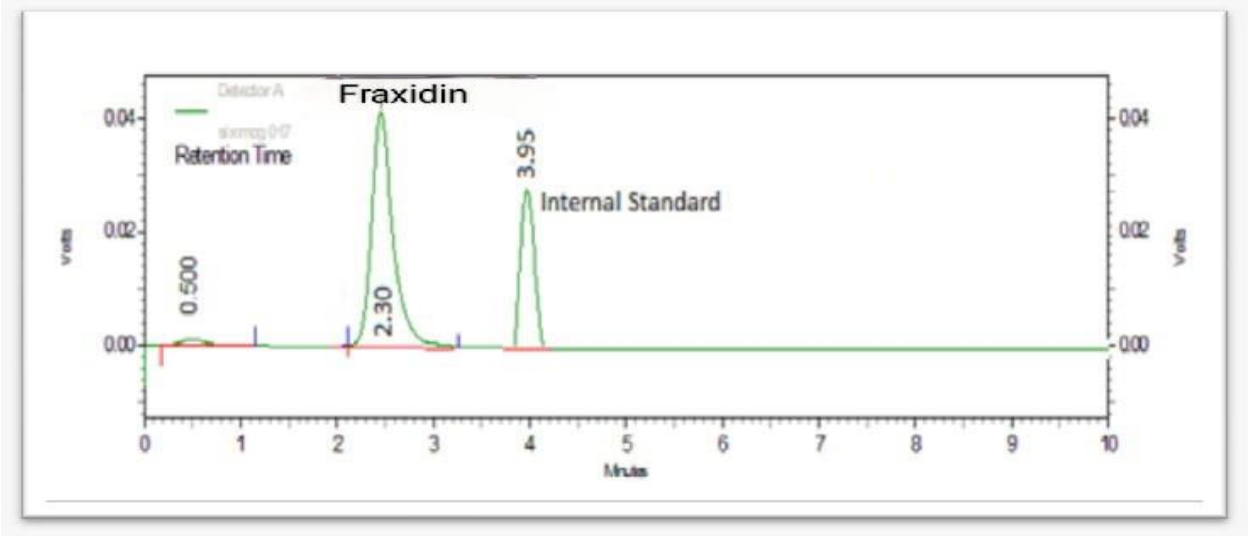


FIG.3: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF FRAXIDIN NANO EMULSION GEL

5: FRAXIDIN CALIBRATION CURVE AT PH 7.4 IN PHOSPHATE

S.no.	Conc. (µg/ml)		Peak Area		Mean	SD	%CV=x100
1	2	65039.33	57198.45	61034.7	61091.43	3206.86	5.24
2	4	128979	114391.93	120571.7	121313.87	5985.10	4.92
3	6	172127.3	161582.72	169862.4	167857.47	4538.24	2.70
4	8	229093.3	238774.48	225691.8	231186.19	5548.61	2.39
5	10	284425.7	295966.6	289661.7	290017.66	4724.30	1.62
6	12	34218.2	363158.4	346554.7	352643.76	7471.92	2.11
7	14	417974.7	430351.3	414791.9	421038.96	6717.09	1.59
8	16	462416	477541.5	459871.7	466611.06	7805.86	1.67
9	18	519788	544734.8	517881.7	527468.16	12241.13	2.31
10	20	583773.7	599926.1	581482.4	588394.06	8213.85	1.39

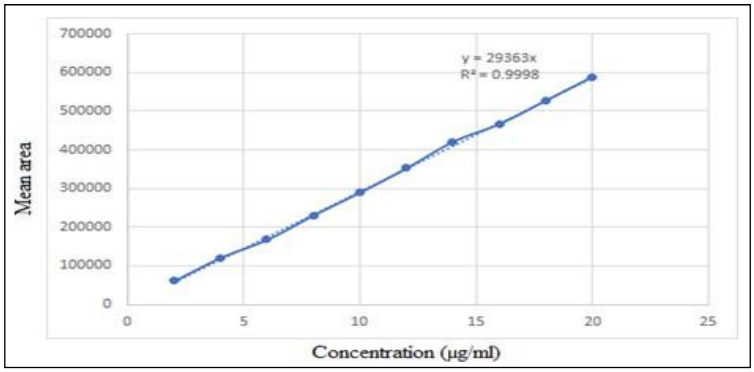
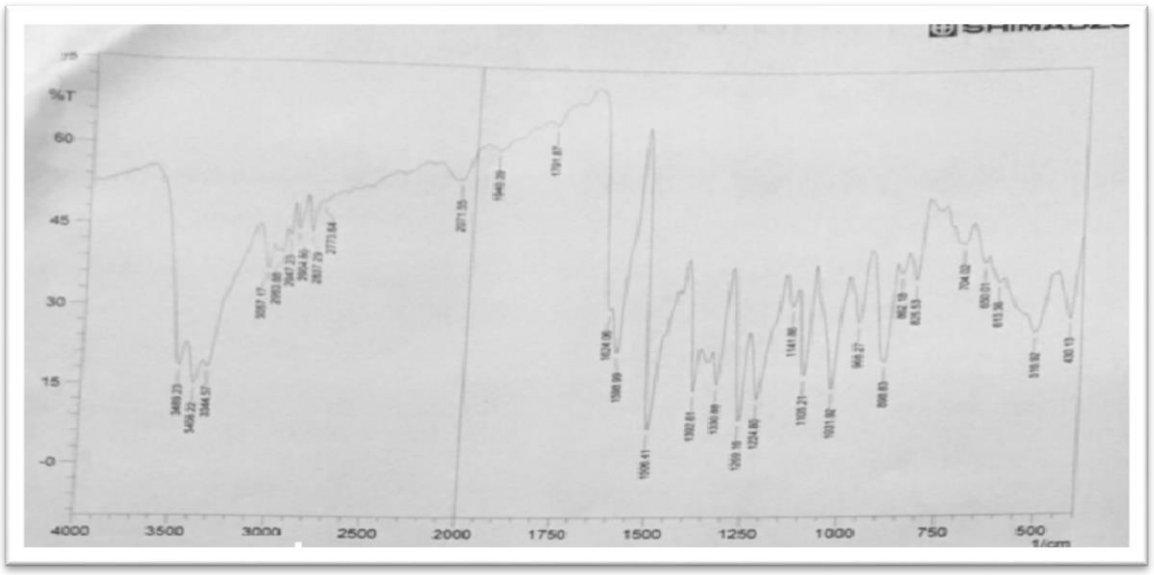


FIG.4: Fraxidin Calibration Using the Rp-Hplc Technique

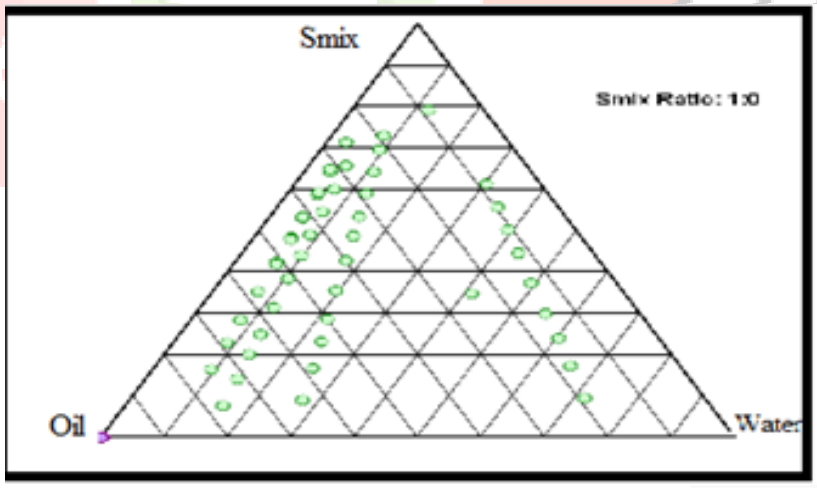
Fourier Transformations Infrared Absorption Spectrum (FTIR):



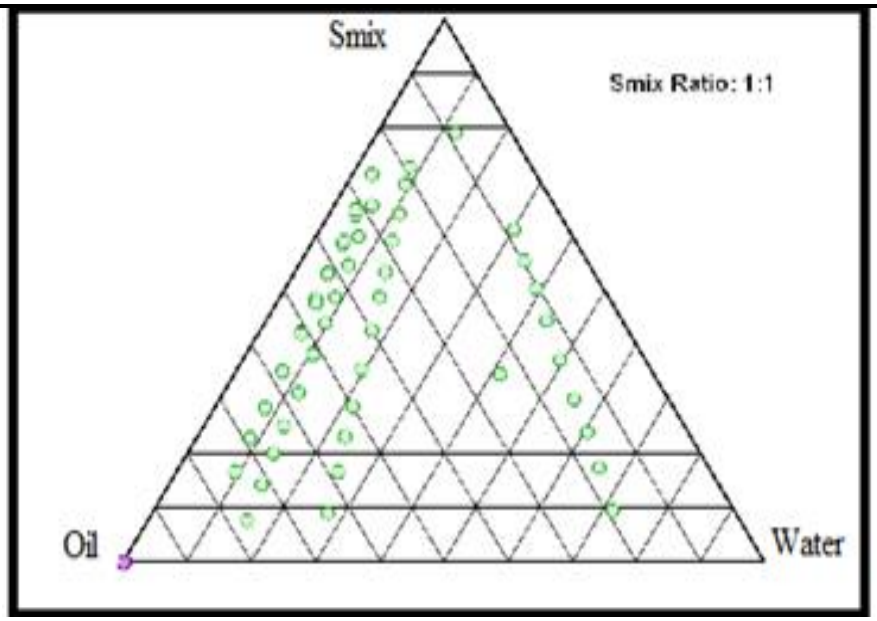
Fraxidin Ftir Displays the Drug's Distinctive Band and Stretching

Fourier transformations infrared absorption Spectrum absorption spectrum of Fraxidin was acquired utilizing the KBr pellet method and exhibited the characteristics (IR) peak sat approximately one or more of the positions about 3862,3745,3680,3650,3620,3454,3390,3358,2875, 2362,2017,1867,1741,1546,1396,1205, 1101, 1054, 1017 and 758.

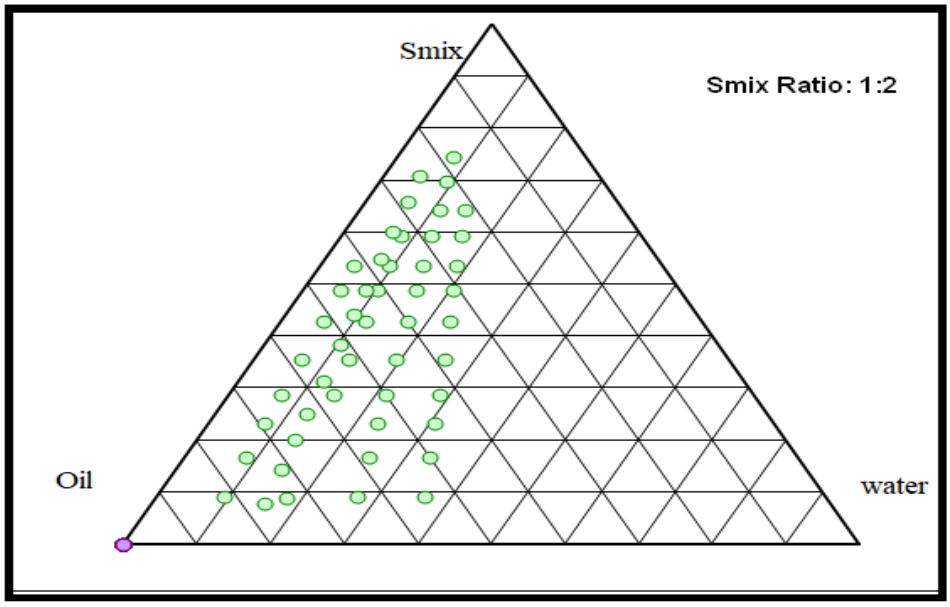
Development of Nano emulsion by using Phase Tertiary Method:



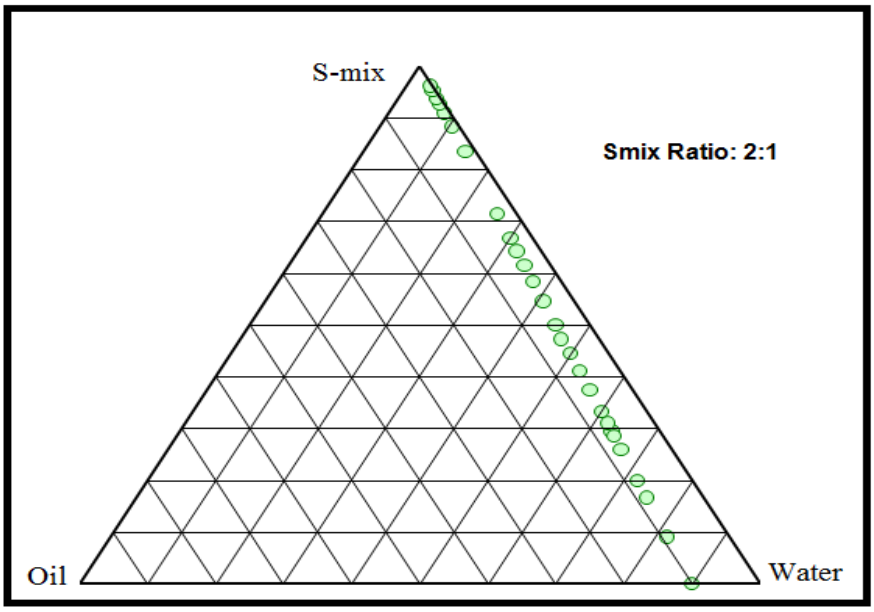
Pseudo ternary phase diagram of the o/w Nano-emulsion area for the surfactant/cosurfactant ratio of 1:0 (Tween 20/glycerol).



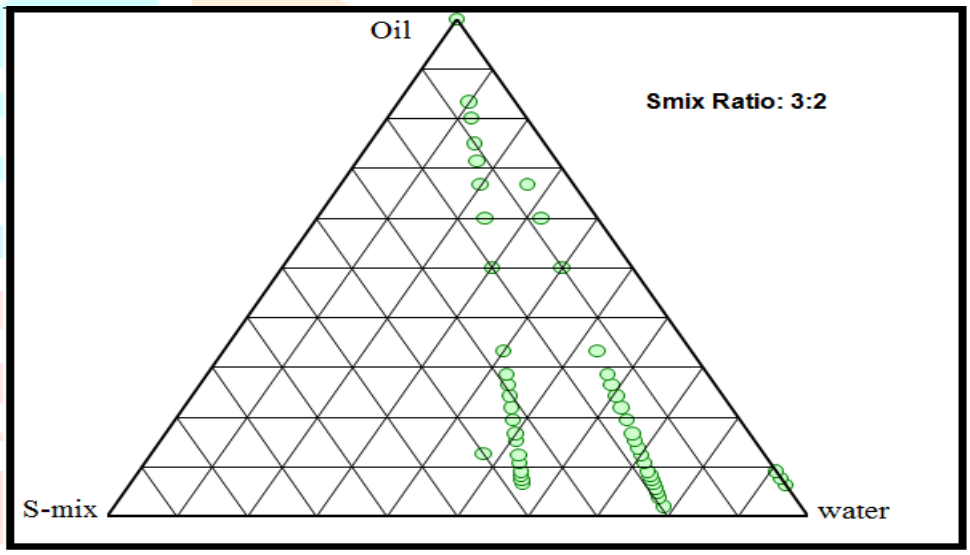
Pseudo ternary phase diagram of the o/w Nano-emulsion area for the surfactant/cosurfactant ratio of 1:1 (Tween 20/glycerol).



Pseudo-ternary phase diagram of the o/w Nano-emulsion area for the surfactant/co-surfactant ratio of 1:2 (Tween 20/glycerol).



Pseudo-ternary phase diagram of the o/w Nano-emulsion area for the surfactant/co-surfactant ratio of 2:1 (Tween 20/glycerol).



Pseudo-ternary phase diagram of the o/w Nano-emulsion area for the surfactant/co-surfactant ratio of 3:2 (Tween 20/glycerol).

Thermodynamic Stability of Nano emulsion: All of the formulations had good physical stability, as demonstrated by stress tests that included centrifugation, freeze-thaw cycles, and heating- cooling cycles.

Fraxidin was discovered to be stable after three months, with recovery rates of >97% for all the chosen formulations. Over the course of three months, there was no discernible change in the formulations' refractive indices' mean values (data not shown).

Inlight of this, it can be said that the Nano emulsion formulations were both physically and chemically stable.

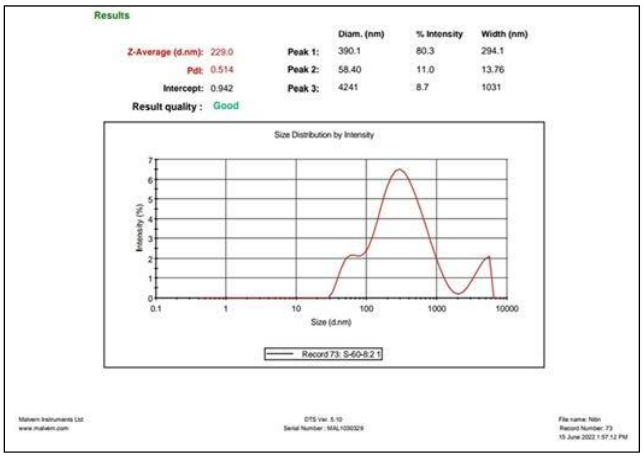
Characterization of Nanoemulsion:

Viscosity: The viscosity of the resulting nanoemulsion is measured by concentric cylinder Brookfield viscometer having a viscosity of 82 centipoise.

pH: pH was measured with a pH meter, and 5.95 was discovered.

Refractive Index: Determined using Abbe's refractometer at 25ºC and was found to be 1.463.

Particle size: Utilizing a quasi-dynamic light scattering device, the optimum nanoemulsion particle size was calculated and has been given in **Fig. 6** and **7.**



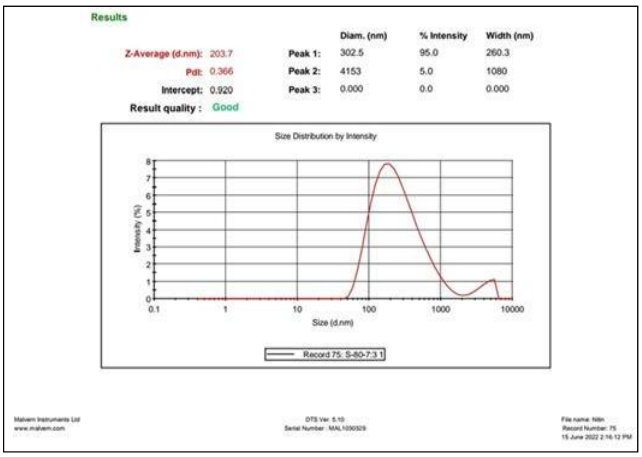


FIG.6: ANALYSIS OF PARTICLE SIZE (NA) **FIG.7: ANALYSIS OF PARTICLE SIZE(NB)**

Zeta Potential:

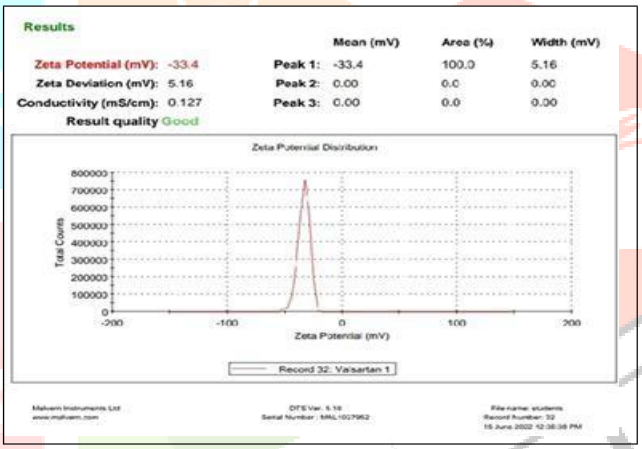


FIG.8: ANALYSISOFZETAPOTENTIAL

***In-vitro* Release Profile:**

TABLE6: FRAXIDIN DRUG RELEASE *IN-VITRO* AT PH 6.4 IN PHOSPHATE BUFFER

Time(hr.)	Mean arean=3 (±S. D)	The cumulative amount of drug release	Cumulative percentage of drug release
0	**0**	**0**	**0**
1	228130	644	3.22
2	387538	1094	5.47
3	685454	1935	9.67
4	900478	2542	12.71
5	1365595	3855	19.27
6	1637297	4622	23.11
7	1956821	5524	27.62
8	2251195	6355	31.77
10	2841713	8022	40.11
12	3384763	9555	47.77
24	4967153	14022	70.11

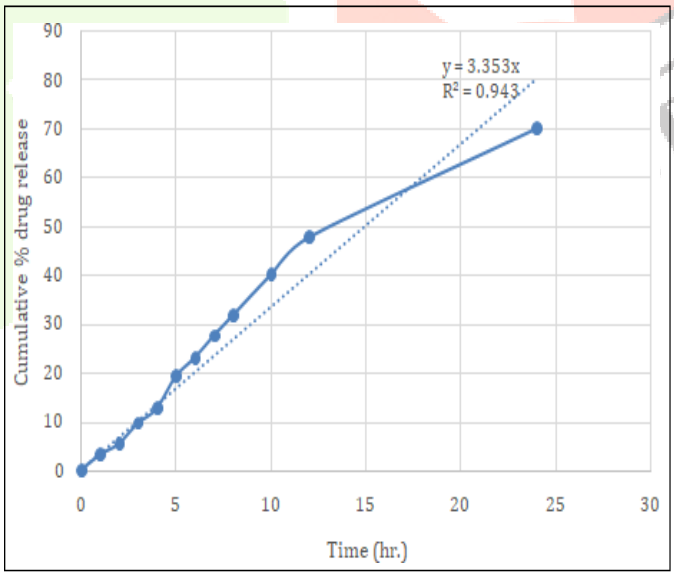
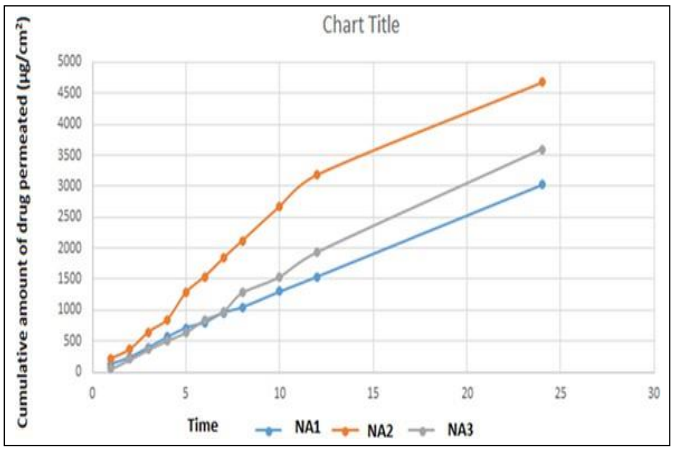


FIG.9: FRAXIDIN NANOEMULSION *IN-VITRO* RELEASE

***Invitro* Skin Permeation Studies:** In order to evaluate the effect of Nanoemulsion vehicles on Skin permeation, various Nanoemulsion formulas were selected from the phase diagrams.



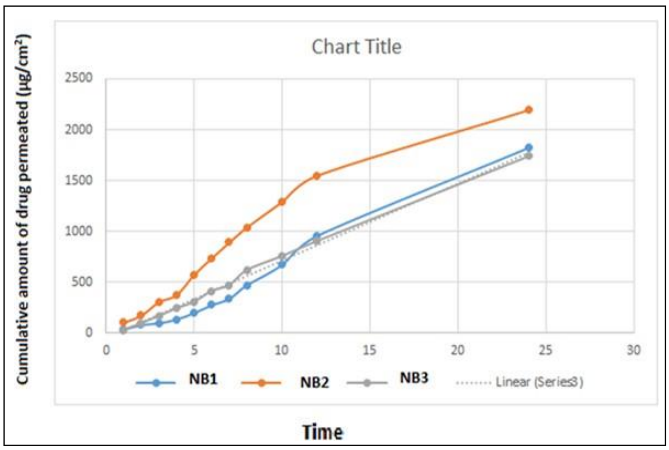


FIG. 10: FRAXIDIN NANOEMULSION WITH A 1:1

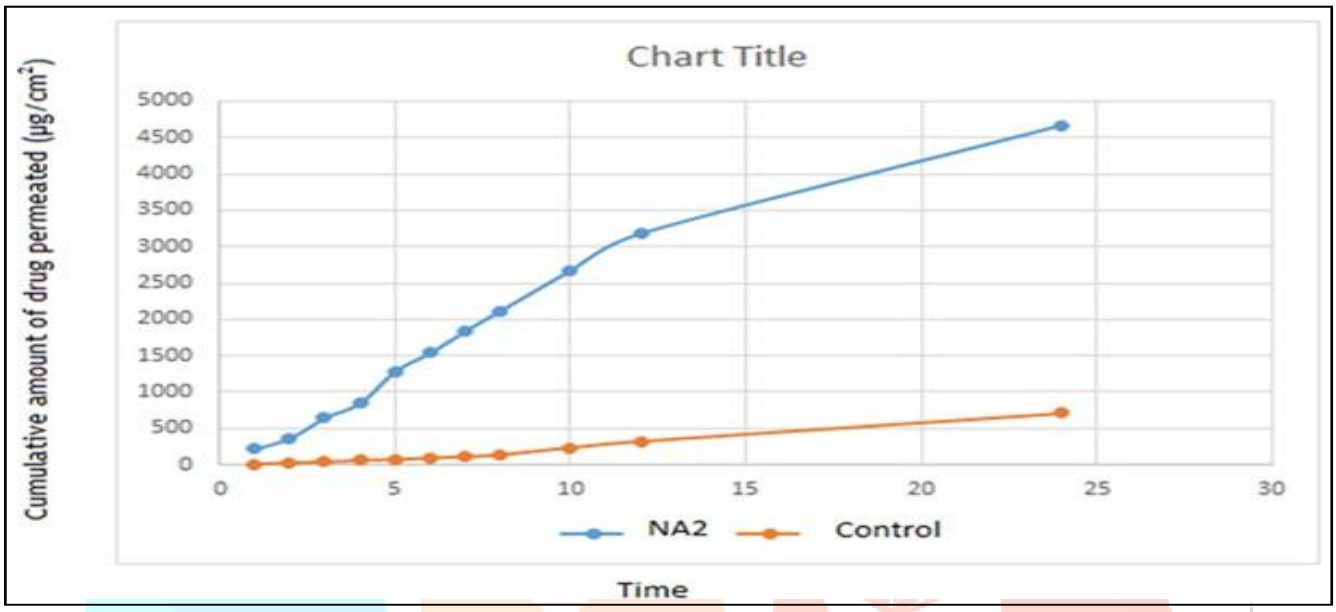
FIG. 11: FRAXIDIN NANOEMULSION WITH AN S/COS RATIO *IN-VITRO* SKIN PERMEABILITY 1:2S/COS RATIO *IN-VITRO* SKIN PERMEABILITY

***Ex-vivo* Permeation Study:**

TABLE7: *IN-VITRO* SKIN PERMEATION STUDIES

Formulation	Flux(µg/cm^2/h)	Permeability coefficient	Enhancement ratio
NA1	125.4	0.627	4

NA2	204.41	1.022	6.52
NA3	157	0.7859	5
NB1	82.055	0.410	2.61
NB2	97.10	0.485	3.09
NB3	75.94	0.3797	2.42
Control	31.353	0.156	-



***IN-VITRO* SKIN PERMEATION OF FRAXIDIN FROM NANOEMULSION**

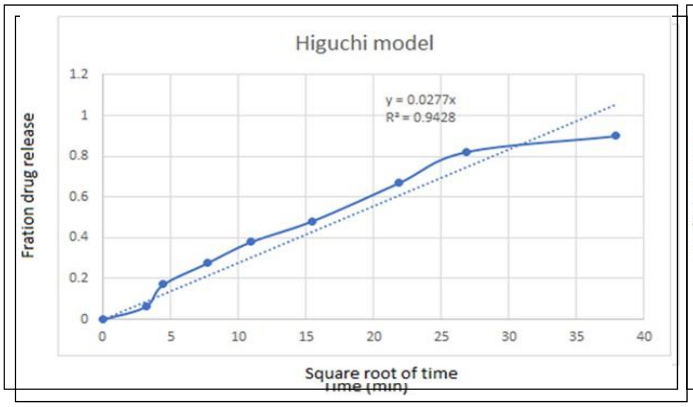
***In-vitro* Release Kinetics Study:**

TABLE 8: KINETICS ANALYSIS OF FRAXIDIN LOADED NANOEMULSION

Time(min)	Square root of time	Log time	%drug release	Fraction drug release	Log% drug release	% drug remaining	Log%drug remaining
0	0	0	0	0	0	100	2
10	3.162	1	6.3	0.063	0.799	93.7	1.9717
20	4.472	1.301	17.1	0.171	1.232	82.9	1.9185
60	7.745	1.778	27.7	0.277	1.442	72.3	1.8591
120	10.954	2.079	38.1	0.381	1.580	61.9	1.7916
240	15.491	2.380	48.3	0.483	1.683	51.7	1.7134
480	21.908	2.681	67.1	0.671	1.826	32.9	1.5171
720	26.832	2.857	82.2	0.822	1.914	17.8	1.2504
1440	37.947	3.158	90.1	0.901	1.954	9.9	0.9956

FIG.: ZERO ORDER RELEASE KINETICS OPTIMIZED NA2 FORMULATION

FIG.: FIRST ORDER KINETICS OF OPTIMIZED OF NA2 FORMULATION



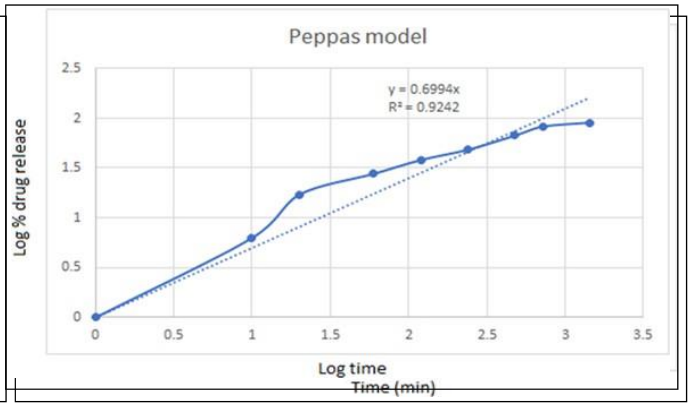
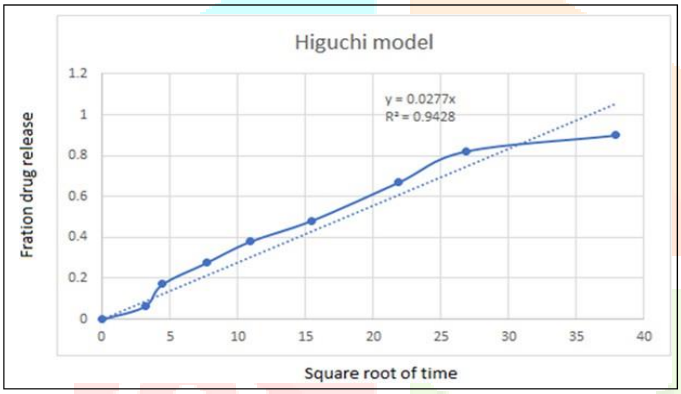


FIG. : HIGUCHI'S PLOT OF OPTIMIZED NA2 FORMULATION

FIG.: KORSEMEYERPEPPAS PLOT OF OPTIMIZED NA2 FORMULATION



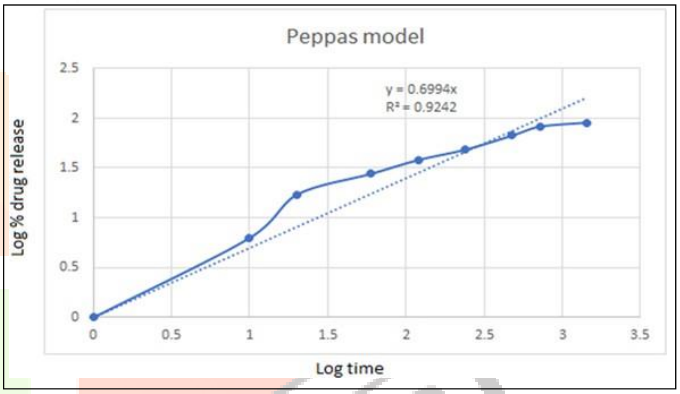


TABLE 9: MODEL FITTING OF THE DRUG RELEASE PROFILE OF FORMULATION NA2

Release model	R^2
Zero order model	0.4699
First order model	-1.754
Higuchi's square root of time plot	0.9428
Korsemeyerpeppas plot	0.9242

Interpretation of Release Model (*In-vitro*): The formulation as stated uses first order release kinetics, and the drug is released by diffusion (first order release model, R^2= -1.754; Higuchi model, R^2=0.9428).

***In-vitro* Kinetics Analyses of Nanoemulsion:** The rate constants for zero -order and first-order were computed along with standard deviation (SD)and Coefficient of variation (Vc)after each time interval during *in-vitro* skin permeation studies. The calculations were based on the cumulative percentage of drug that penetrated the skin.

Zero order rate constant, K_o

K_o=X/t= (Percentage drug permeated)/ (Time (h)

First order rate Constant, K_1

K_1=2.303/T log (Co/C)

Where, Co = Initial drug concentration. C = Amount of drug remaining at time t.

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Zero order rate constant, K_o

K_o=X/t= (Percentage drug permeated)/ (Time (h)

First order rate Constant, K_1

K_1=2.303/T log (Co/C)

Where, Co = Initial drug concentration. C = Amount of drug remaining at time t.

TABLE10: KINETICPROFILEOFIN-*VITRO*SKINPERMEATIONDATAOFNANOEMULSION

Time(h)	Cumulative % drug permeated	% of drug remaining	Zero order rate constant (K_o)	First order rate constant (K_1) (h^{-1})
1	3.22	96.78	3.22	0.032
2	5.47	94.53	2.73	0.027
3	9.67	90.33	3.223	0.033
4	12.71	87.29	3.177	0.0338
5	19.27	80.73	3.854	0.0426
6	23.11	76.89	3.851	0.0433
7	27.62	72.38	3.945	0.0460
8	31.77	68.23	3.971	0.049
10	40.11	59.89	4.011	0.0511
12	47.77	52.23	3.98	0.054
24	70.11	29.89	2.92	0.050
	Average		3.5347	0.04198
	SD		0.48325	0.009116

Cv was calculated using the following formulation:

Cv =(SD×100)/Average Thus, Cv (K_o) = 13.671

Cv (K_1) =21.7

TABLE11: KINETIC PROFILE OF *IN-VITRO* SKIN PERMEATION DATA OF CONTROL

Time(h)	Cumulative %drug permeated	% of drug remaining	Zero order rate constant (K_o)	First order rate constant(K_1) (h^{-1})
1	.076	99.24	0.076	0.00755
2	2.6	97.4	1.3	0.02625
3	3.16	96.84	1.053	0.03208
4	3.8	96.2	0.95	0.03873
5	3.84	96.16	0.768	0.03912
6	4.16	95.84	0.693	0.04249
7	4.36	95.64	0.622	0.04449
8	4.52	95.48	0.565	0.04622
10	4.72	95.28	0.472	0.04831
12	5.04	94.96	0.42	0.05163
24	5.44	94.56	0.226	0.05591
	Average		0.649545	0.039343
	SD		0.359517	0.013509

Cv=(SD×100)/Average Thus, Cv (K_o) = 13.671

Cv(K_1) =21.7

Franz diffusion cells containing rat skin were used to conduct *in-vitro* skin permeation tests on the control and the nano emulsions. According to the findings of the investigation, formulation NA2 produced good results since the total quantity of medicine penetrated. To determine penetration

kinetics, the data from the nano emulsion's skin permeation trials was further assessed. The permeation investigations yielded the rate constant, coefficient of variation (Cv), and deviation from the mean (SD) for zero and first order rate kinetics for each time period.

In the instance of nanoemulsion, lower Cv was recorded for zero order kinetics and higher Cv was seen for first order kinetics.

Skin Irritation Test Data:

kinetics, the data from the nano emulsion's skin permeation trials was further assessed. The permeation investigations yielded the rate constant, coefficient of variation (Cv), and deviation from the mean (SD) for zero and first order rate kinetics for each time period.

In the instance of nanoemulsion, lower Cv was recorded for zero order kinetics and higher Cv was seen for first order kinetics.

TABLE12: SKIN IRRITATION SCORES OF FORMULATIONS NA2

Scores for skin irritation caused by the formulations are measured using two types of scores: A for-erythema formation and B for edema formation.

	Intact skin				Abraded skin			
Rats	**24 hrs.**		**72 hrs.**		**24 hrs.**		**72 hrs.**	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
1	0	0	1	0	0	1	0	1
2	0	1	0	1	0	1	1	0
3	1	1	0	1	0	2	1	1

The ultimate scores for skin irritation of the formulations (where*indicates the total of A and B from the section, andrepresents the average of all skin reading stake at 24and72 hours).**

Rats	**Intact skin**		**Abraded skin**		**Total**	**Average**
	24 hrs.	**72hrs.**	**24 hrs.**	**72hrs.**	**(i)+(ii)**	
	(i)		**(ii)**			
1	1	1	1	1	1,1.75,1	
2	2	1	2	2		
3	0	1	1	2		
					Combinative. =1.25	

The skin irritability test was carried out to verify the formulation's safety. The formulation used is considered safe for human skin and does not cause irritation as long as the skin irritancy score is between 0 and 2. The average score for skin irritancy of the formulation was determined to be 1.25. This number shows that every excipient employed in the formulation was suitable for the topical administration of drugs.

CONCLUSION:

Preformulation tests were carried out to establish the drug's solubility and partition coefficient in order to determine its appropriateness for use in a topical system. The drug's solubility in different oils was also investigated in order to choose the best oil phase for nanoemulsion formulations. Fraxidin HPLC was performed using Acetonitrile: Potassium Dihydrogen Phosphate Buffer with a flow rate of 1.5 ml/min. Particle size of the optimized nanoemulsion was found to be 229 nm. Zeta potential was found to be33. 4mV.In the Optimize nanoemulsion drug delivery system, the derived kinetic profile demonstrates the existence of two distinct release processes. *In-vitro* Fraxidin release was rapid at first, then continued steadily. Fraxidin's release from the nanoemulsion surface may be responsible for the initial medication to be released quickly. A release

order was established using a kinetic analysis of the *in-vitro* release profile of the Optimize nanoemulsion. For fraxidin loaded nanoemulsion, the Higuchi model was best fitted. Fraxidin nanoemulsion (NA2) was reported to have a maximal skin permeation flux of 204.41 µg/cm^2 /hr. and that of control is 31.353 µg/cm^2/hr. The enhancement ratio of the optimize formulation was found to be 6.52 as compare to control. The optimize nanoemulsion was found to be non-irritating and safe for human skin with average skin irritancy score of 1.25. The develop nanoemulsion drug delivery system will be proved to be a potential carrier for delivery of antimicrobial drug fraxidin through transdermal route with increased solubility with increased skin permeation flux and permeability coefficient, with increase enhancement ratio and better efficacy.

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CONFLICTS OF INTEREST: There is no any conflict of interest among the authors.

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