

**FORMULATION AND EVALUATION OF LIPOSOMAL GEL (HYDROQUINONE & TAZAROTENE) FOR THE TREATMENT OF ACNE****Shubham Kushwaha<sup>1\*</sup>, Deepak Katiyar<sup>1\*</sup>, Prashant Katiyar, Satya Narayan Mishra<sup>1</sup>,  
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**Abstract**

The aim of the present study was to formulate and evaluate the liposomal gel containing tazarotene and hydroquinone in the treatment of acne. Tazarotene combined with gel hydroquinone to maximize the effect of ophthalmic preparation. The optimization batches were prepared by lipid film hydration method with different concentration of lecithin and cholesterol with different varying stirring speed (100 and 200 rpm). All the prepared formulation were characterized for vesicle morphology, particle size and entrapment efficiency by transmission electron microscopy (TEM). The optimized batch of tazarotene liposome TL6 was further incorporated into gel containing hydroquinone. Three different formulations (LF1, LF2, LF3) were prepared using different composition of carbopol (0.5, 1.0 and 2%) The optimized batch of tazarotene and hydroquinone incorporated gel (1%) was characterized for pH, spreadability, viscosity (cps) and in vitro drug release. The percentage drug entrapment efficiency found higher in formulation, Vesicular size and drug entrapment efficiency of the optimized liposomes were found to be 180.4 nm and 69.10% respectively. In-vitro diffusion study demonstrated that the drug diffused from liposomal gel and conventional marketed gel was found to be 98.12% and 98.58% respectively. It can be concluded from the experimental results that the liposomal gel containing tazarotene in combination with hydroquinone has potential application in topical delivery.

**Keywords:** Tazarotene, Hydroquinone, Liposome, Gel, Topical Drug Delivery**1. INTRODUCTION**

Most favourable therapeutic outcomes necessitate appropriate drug selection. In human body, the skin is a best available space for drug delivery.<sup>1,2</sup> In human body skin covers an area of about 2m and this multi-layered.

In total skin surface consist of 1/1000 of hair follicles in every tetragon centimetre of the skin area. The most gladly reachable.<sup>3,4</sup> The aera of skin where the drug is introduced. Skin are most

## FORMULATION AND CHARACTERIZATION OF VILDAGLIPTIN LOADED TRANSFEROSOMAL GEL FOR TRANSDERMAL DELIVERY

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### **Abstract: -**

The age-old theory that imparted the status of “dead impermeable barrier devoid of biological activity” to skin had already been challenged by the development of pioneering transdermal gel. TDDS in the year 1980 by Alza Corporation of USA and subsequently introduction of many other drugs by other companies such as nitroglycerin, fentanyl, clonidine, vildagliptin etc. The extensive work in the last 25 years both by academicians and industrialists in the area of transdermal delivery have generated more than ten marketed products. But this route of administration continues to be limited owing to the scarcity of suitable drug candidates available. To focus these developments and the important criteria for the selection of drug, the objectives and plan of work have been discussed. The results indicated that, there was reduction in folding endurance values in 6 months, this change may be due loss of moisture. However, the rate of degradation of drug was not significant indicating that the drug is stable further, the integrity of the permeation enhancer was assessed by *ex vivo* permeation at regular time intervals during the accelerated stability studies. The permeation rate did not alter significantly up to 6 months. These results were found to agree well with the DSC spectral data and SEM results.

**Keywords:** TDDS, DSC, vildagliptin, SEM, etc.

### **INTRODUCTION**

The potential of utilizing intact skin as a means of drug administration has been acknowledged for several decades, as exemplified by the development of medicated patches in countries like China and Japan. This practice likely ignited curiosity in exploring the skin as a pathway for delivering drugs to produce systemic effects. The discovery of this delivery route represents a

**FORMULATION OF POLYMER GEL FOR OPHTHALMIC DRUG DELIVERY  
(NORFLOXACIN & LEVOFLOXACIN HEMIHYDRATE)****Satya Narayan Mishra<sup>1</sup>, Dr. Manoj Kumar Mishra<sup>1\*</sup>, Dr. Anurag Mishra<sup>2</sup>, Shubham  
Kushwaha<sup>1</sup>, Riya Singh<sup>1</sup>, Mayank Kumar<sup>1</sup>, Dheeraj Dubey<sup>3</sup>**<sup>1</sup>*Shambhunath Institute of Pharmacy, Jhalwa, Prayagraj (U.P.)-India- 211012*<sup>2</sup>*Department of Biochemistry, University of Allahabad, Prayagraj, (U.P.), India-211002*<sup>3</sup>*Ashoka Institute of Technology and Management, Varanasi (U.P.), India – 22100 7***\*Corresponding Author****Dr. Manoj Kumar Mishra***Department of Biochemistry, University of Allahabad,**Prayagraj, (U.P.), India-211002***Email-** bmanojmishra@gmail.com**Abstract**

The formation and development of drug is done by naturally and synthetically. It was controlled by drug molecules by pharmacology effects. due to the arrival of low molecular weight of drug molecules the drug carrier system is more difficult. By the development of gel formulation this difficulty is overcome. The eye drops medicines cover 90 to 95% of medicines. The limitation and failure should be overcome by the new technology. there is different gelling technique is used for the formulation of drugs. eye drop is very simple to manufacture, sterilized and filtered. The purpose of the study is to develop a polymer gel for the treatment of ophthalmic. In which 3 different mechanism is use for the phase transition. Different parameter is for the formulation.

**Keywords:** norfloxacin, levofloxacin, ophthalmic drug delivery, polymer gel

**1. INTRODUCTION**

The formation and development of drug is done by naturally and synthetically. It was controlled by drug molecules by pharmacology effects.<sup>1,2,3</sup> due to the arrival of low molecular weight of drug molecules the drug carrier system is more difficult. By the development of gel formulation this difficulty is overcome. The eye drops medicines cover 90 to 95% of medicines. The limitation and failure should be overcome by the new technology.<sup>4,5,6</sup> there are different gelling technique is used for the formulation of drugs. eye drop are very simple to manufacture, sterilized and filtered.<sup>7,8,9</sup>

The sclera covers major surface of eyeball. limbus is connect with cornea and collagen fiber. It is of different size and presentation. The inner layer of the sclera is Lamina fusca.<sup>10,11</sup> the oxygen is supply by cornea and the lacrimal fluid nutrients are by supply blood vessels. Collagen fibers made the Descemet's membrane. The single layer of flattened cells made the endothelium. It is connected by tight junctions.<sup>12,13</sup> the middle portion is pupil. This is a circular hole in between the iris by which the light is passes through.<sup>15,16</sup> the contraction and relaxation of muscle is altered by

Review

# Differential Expression of Non-Coding RNAs in Stem Cell Development and Therapeutics of Bone Disorders

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**Abstract:** Stem cells' self-renewal and multi-lineage differentiation are regulated by a complex network consisting of signaling factors, chromatin regulators, transcription factors, and non-coding RNAs (ncRNAs). Diverse role of ncRNAs in stem cell development and maintenance of bone homeostasis have been discovered recently. The ncRNAs, such as long non-coding RNAs, micro RNAs, circular RNAs, small interfering RNA, Piwi-interacting RNAs, etc., are not translated into proteins but act as essential epigenetic regulators in stem cells' self-renewal and differentiation. Different signaling pathways are monitored efficiently by the differential expression of ncRNAs, which function as regulatory elements in determining the fate of stem cells. In addition, several species of ncRNAs could serve as potential molecular biomarkers in early diagnosis of bone diseases, including osteoporosis, osteoarthritis, and bone cancers, ultimately leading to the development of new therapeutic strategies. This review aims to explore the specific roles of ncRNAs and their effective molecular mechanisms in the growth and development of stem cells, and in the regulation of osteoblast and osteoclast activities. Furthermore, we focus on and explore the association of altered ncRNA expression with stem cells and bone turnover.

**Keywords:** stem cells; lncRNA; miRNA; osteoblastogenesis; osteoclastogenesis; osteoporosis; osteoarthritis; and bone cancer

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## 1. Introduction

Stem cells can develop into various cell types in the body during early stages of life and growth. In many tissues, they serve as an internal repair system, dividing essentially to replenish other cells, throughout the life of humans and other organisms. Upon division, each new cell has the potential to either remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell. Stem cells are distinguished from other cell types by two important characteristics. First, they are unspecialized in nature, capable of renewing themselves through cell division, even after a long period of inactivity [1,2]. Previous studies suggest the potential role of non-coding (ncRNAs) in stem cell cycle regulation and developmental processes such as self-renewal, differentiation, and proliferation [3–6]. In last decades, the number of studies in ncRNAs has increased dramatically. ncRNAs are ribonucleic acid (RNA) molecules that are transcribed from DNA but not translated into protein. Sometimes ncRNAs are referred as RNA genes or functional RNA. Functionally important ncRNAs are





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## Preparation and Characterization of Topical Nano emulsion of Rifampicin and Eucalyptus Essential Oil with Study of in Vitro Anti-Microbial Activity

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### Abstract:

**Objective(s):** Eucalyptus essential oil plays a significant role in the pharmaceutical, medicine industries having antimicrobial as well as antioxidant properties. The stability of these Nano emulsions was meticulously assessed over a span of 120 days. The instability of eucalyptus oil under typical conditions can lead to a loss of its bactericidal effectiveness. To safeguard this valuable natural product, the utilization of Nano-based emulsion technology emerges as an exceptional approach.

This study's goal was to create a topical nano emulsion that could treat microbial skin infections.

**Method:** A total of five formulations were formulated, each containing varying combinations of rifampicin and eucalyptus oil. These formulations were labeled as F1, F2, F3, F4, and F5. Employing Tween 80, and water through a sonication technique, Nano emulsions for all five formulations were created.

Various physicochemical attributes, including pH, viscosity, and antimicrobial efficacy, were investigated. The stability of these nanoemulsions was meticulously assessed over a span of 120 days.

**Results:** Nanoemulsions formulated using a combination of Tween 80, eucalyptus oil and rifampicin exhibited smaller particle sizes. The stability of the developed Nano emulsions persisted over a 120-day period, as evidenced by the absence of noteworthy alterations in pH, conductivity, and droplet size. Notably, nanoemulsions displayed significant in vitro antimicrobial efficacy.

**Conclusion:** The results indicated that rifampicin and eucalyptus essential oil-based nanoemulsions could be exploited as important medication carriers in the pharmaceutical industry.

**Keywords:** Nano-emulsion, Tween 80, Eucalyptus oil, Rifampicin, Anti-microbial activity

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### Introduction

Plants based essential oils represent secondary metabolites derived from aromatic plants. These essential oils are intricate blends comprising diverse bioactive chemical constituents, including terpenes, esters, alcohols, and various aromatic compounds. [1] Because of these different complex bio-structures that work together, essential oils have a variety of biological actions, including antibacterial, antiviral, fungicidal, insecticidal, and other properties herbicidal. Because of the herbal nature and the safer alternative in comparison to pharmaceutical medications, interest in these types of plants The number of secondary metabolites based on is growing by the day. Because of its antioxidant and antibacterial properties essential oils have a lot of promise in terms of activities in medical industries. As a result, over time, an essentials oils have received a lot of attention medicinal agents [2] Rifampicin (C<sub>43</sub>H<sub>58</sub>N<sub>4</sub>O<sub>12</sub>) falls within the borderline of high molecular weight class II drugs. The dissolution rate and extent of Rifampicin are pivotal factors influencing its optimal bioavailability [3] Rifampicin (RIF), characterized by its lipophilic nature, encounters challenges in penetrating bacterial cell walls. To address this, drug delivery system for RIF has been devised, incorporating salicylic acid and benzoic acid. This formulation has demonstrated efficacy against bacterial and fungal infections. Additionally, RIF-loaded niosomes have been explored for treating acne topically. With its broad-spectrum effectiveness against Mycobacterium and gram-positive bacteria, Rifampicin proves potent even at low concentrations. However, its vulnerability to acidic pH compromises oral absorption, stability, and overall bioavailability. Consequently,



nanoemulsions have been employed for parenteral administration of RIF. [4] Droplet sizes in NE range from 10 to 500 nm and are made up of isotropic mixes of medicines, lipids, hydrophilic surfactants, and co-solvents. [5]

A nanoemulsion, as implied by its name, constitutes an emulsion with particle dimensions on the nanometer scale. At present, this technology is being harnessed to enhance the delivery of therapeutic agents. [6] The process of ultrasonic emulsification has arisen as a remarkably effective approach, conquering the challenge of high viscosity by utilizing intense shear forces generated by high-energy ultrasound. This technique is employed to diminish the size of droplets. In laboratory settings, powerful ultrasound has proven successful in creating transparent nanoemulsions. These nanoemulsions, characterized by their reduced particle size, contribute to prolonged drug retention and enhanced bioavailability. Consequently, this size reduction plays a pivotal role in preventing drug loss. [7]

Due to its limited solubility in water, the effective permeation of the bacterial cell walls by conventional topical formulations for rifampicin presents challenges. In prior investigations, we developed two innovative drug delivery systems aimed at facilitating the targeted topical administration of rifampicin for acne treatment [8] Rifampicin displays broad-spectrum antibiotic properties, exhibiting effectiveness against *Mycobacterium tuberculosis* and *M. laprae*. Additionally, rifampicin holds a modest degree of solubility in water and is moderately soluble in other solvents. [9] When tested against the bacterial strains responsible for acne, namely *Propionibacterium acnes* and *Staphylococcus epidermidis*, the antibiotic used for acne treatment exhibited sensitivity. Among these species, both rifampicin and tetracycline displayed the most potent antibacterial effects. [9] Eucalyptus oil is part of a vast array of essential oils, each with its own unique properties. Thanks to the inclusion of 1-8 cineole (eucalyptol), eucalyptus oil (EO) exhibits a range of functions, such as antimicrobial, insecticidal, and expectorant activities. Nevertheless, the high volatility of 1-8 cineole (eucalyptol) under regular circumstances renders it unstable and prone to oxidative degradation upon exposure to air. This vulnerability can lead to a decline in the oil's overall integrity and its antimicrobial efficacy. [10] Hence, the utilization of nanoemulsion technology emerges as a promising solution to encapsulate, enhance water solubility, and safeguard this bioactive element. It can serve as the oil-soluble constituent within oil-in-water (O/W) nanoemulsions. Through this approach, the concentration of photoactive components like eucalyptol can be amplified in regions abundant with microorganisms. Consequently, this research endeavors to create nanoemulsions containing eucalyptus oil and explore their antimicrobial potential. These findings will contribute novel insights into the applications of eucalyptus essential oil-based nanoemulsions across diverse domains such as food, cosmetics, and in pharmacy drug delivery methods. [11]

## Materials And Method

### Materials

Rifampicin was kindly donated by OTTO CHEMEI PVT LTD (Zaveri Mumbai), Tween 80 and oleic acid were obtained from Thomas Baker 4/86 Bharat mahal Marine Drive Mumbai, Eucalyptus oil were purchased from Hi Media (India) All emulsions were made using ultrapure water that was acquired using a Cicada Biowater System and had a resistivity of no more than 18.2 M cm<sup>-1</sup>. The only other chemicals were analytical reagent-grade substances.

### Methods

#### Formulation of Rifampicin Nanoemulsion

Nano emulsions of Eucalyptus based essential oil and rifampicin were prepared by method given by Moradi and Barati [10] Rifampicin Nanoemulsion was formulated alongside excipients comprising oleic acid, Tween 80, and distilled water. Initially, rifampicin was quantified and introduced into a 20 ml screw-cap vial containing oleic acid. In a separate 100 ml glass bottle, Tween 80 was measured and added. Likewise, distilled water was poured into another 100 ml glass bottle. All the three containers were then placed within an ultrasonic water bath and temperature was maintained to 70°C. This process was sustained until rifampicin had fully dissolved, yielding a clear solution. Once equilibrium was reached at the common temperature of 70°C, the rifampicin-infused oleic acid was incorporated into the glass bottle containing Tween 80. Sequentially, gradual addition of distilled water to this amalgamation ensued, accompanied by intermittent shaking through a vortex mechanism. This procedure persisted until a turbid nanoemulsion emerged. Subsequently, the nanoemulsion was allowed to cool at ambient temperature and kept between 4-10 °C.



### Preparation of Rifampicin and Eucalyptus oil Nanoemulsion

The technique outlined [10] was employed to create nanoemulsions of Eucalyptus essential oil, following the procedure summarized in Table 1. In summary, for the formulation of essential oil (EO)- based nanoemulsions, Tween 80 were dissolved in distilled water at room temperature. The resulting mixture was subjected to vortexed for 15 minutes to achieve a uniform solution. Subsequently, Eucalyptus essential oil was slowly introduced into the aforementioned mixture and agitated using a magnetic stirrer for 15 minutes. The mixture was then allowed to continue stirring with the magnetic stirrer for an additional 10 minutes.

In a separate experimental setup Rifampicin Nanoemulsion was added to a solutions containing Tween 80 (2% v/v), and distilled water was augmented with The resultant mixture underwent stirring using a magnetic stirrer for a duration of 10 minutes, the mixture was stirred using a magnetic stirrer. Likewise, the essential oil was gradually introduced into the mixture over 15 minutes while maintaining continuous stirring. Subsequently, the mixture was subjected to an additional 10 minutes of stirring using the magnetic stirrer. The specific composition of the nanoemulsion created in this investigation is outlined in Table 1.

**Table 1: Components of nano Emulsion**

NAME SAMPLE	OF	TYPE OF ESSENTIAL OIL	ESSENTIAL OIL (% V/V)	TWEEN 80 (% V/V)	RIFAMPICIN (% V/V)
F1		Eucalyptus globulus	2	2	0.25
F2		Eucalyptus globulus	4	4	0.5
F3		Eucalyptus globulus	6	6	0.75
F4		Eucalyptus globulus	8	8	1
F5		Eucalyptus globulus	10	10	1.25

**Table: 2 Organoleptic Properties of Rifampicin**

Properties	Specification	Observation
Colour	Reddish or Brown colour	Dark Red colour
Nature	Amorphous or crystalline powder	Crystalline powder
Odour	NA	Odourless

### SIZE AND SIZE DISTRIBUTION

By using the Malvern Zetasizer Nano ZS 90 (produced by Malvern Instruments Ltd.) at a temperature of  $25\pm 0.1^\circ\text{C}$ , photon correlation spectroscopy was used to measure the average droplet size (Z-average) and the distribution of sizes (PDI) for the nanoemulsions. Measurements were made in a quartz cell with a 90-degree detection angle. Prior to measuring particle sizes, the samples were properly diluted (10-fold) with distilled water to lessen the effects of multiple scattering. The results that were given were calculated from the average of at least three measurements. [12]

### ZETA POTENTIAL

The zeta potential of the nanoemulsions was evaluated through the photon correlation spectroscopy, by the use of Malvern Zetasizer ver 8.2 ZS from Malvern Instruments. This measurement took place at a temperature of  $25\pm 1^\circ\text{C}$  using a zeta potential cell that was set at a detection angle of 90 degrees. Each NE sample, totaling 700 ml, was introduced into the folded capillary cell that incorporated a gold electrode. A set of three measurements were performed and the results were subsequently averaged. [13]

### PH

The pH of the prepared products was assessed using a Digital pH meter (Model 361, Systronics). The pH meter's electrode was cleaned with distilled water and dried with a tissue paper. [14] Subsequently, the electrode and temperature probe were submerged into a beaker containing the emulsion formulations. The pH readings of the samples were recorded from the pH meter This experiment was conducted in triplicate, and average values were presented in Table 3.



## VISCOSITY

Viscosity measurement is the procedure used to assess a fluid's resistance to flow. It is a fundamental property that characterizes the internal friction of a moving fluid. Viscosity is typically quantified using units such as Poise, Centipoises, or Pascal-seconds.

In this study, the viscosity of the formulation nanoemulsions was evaluated with the assistance of a Brookfield Viscometer, specifically the DV-E model with the spindle S-64. [15] The testing was conducted in triplicate, and the resulting mean values for each prepared formulation were tabulated in Table 3

## FTIR MEASUREMENTS

The Rifampicin and Eucalyptus oil Nano emulsion underwent FTIR analysis using the potassium bromide technique on a Nicolet 6700 FT-IR Spectrometer manufactured by Thermo Fisher Scientific. The analysis encompassed a scanning range spanning from 4,000 to 500  $\text{cm}^{-1}$ . [16] The primary objectives of the FTIR analysis were to investigate the interactions between drug and the oil nano emulsions.

### 6. Scanning Electron Microscopy (SEM)

SEM images were captured and analyzed for the formulation, which had been optimized, to examine both droplet size distribution and surface morphology (as depicted in Figure 2). The droplet sizes observed ranged from 10 to 200 nm, aligning well with the droplet size measurements obtained through Zetasizer analysis. These droplets exhibited a spherical shape and were evenly distributed within the nanometer range. [17]

### Thermal Stress, Heating/Cooling Cycles, And Centrifugation

Stability analysis were carried out 24 hours after preparation through a series of tests:

(i) Centrifugation test: Samples of 5 ml each were subjected to three sequential rotational speed (1000, 2000, and 3500 rpm) using a Fanem 206R centrifuge. [18] Each speed was maintained for 15 minutes. Only the formulations that exhibited stability after the centrifugation test was proceeded to the subsequent cycles of heating and cooling and thermal stress.

(ii) Thermal stress test: Vials holding 5 ml of the sample were heated in 5 °C increments from 40°C - 85 °C. The samples were kept for 30 minutes at each temperature. An electronic bath (Nova an NT281) with a thermostat was used to alter the temperature.

(iii) Heating/cooling cycles: These cycles were run to evaluate sample stability in high-temperature environments. The samples were initially heated to 45°C for 24 hours inside an air stove (Fanem 002-CB). They were then kept in refrigerator (Electrolux RDE-37) at a temperature of 4°C for an additional 24 hours, completing one cycle. A total of six cycles (equivalent to 12 days) were performed.

Throughout these stability tests, various physicochemical characteristics were monitored, including zeta potential, polydispersity index, density, refractive index, viscosity values, pH, electrical conductivity, and other parameters.

## MACROSCOPIC ANALYSIS

All formulations underwent macroscopic analyses, 24 hours after preparation to look for any signs of macroscopic instability. This involved testing the samples both before and after centrifugation, thermal stress, and heating/cooling cycles to look for phenomena like creaming, sedimentation, flocculation, or coalescence.

## ANTI-BACTERIAL ACTIVITY AGAINST THE CLINICAL PATHOGEN:

Bacterium *S. aureus* MTCC 3160 was obtained from Microbial Culture Collection Bank, Microbiology Department of Sam Higinbottom University of Agriculture Technology and Sciences.

The Gram-positive Bacterium *S. aureus* MTCC 3160 was used as the test organism for the in vitro evaluation of the antibacterial activity of Eucalyptus essential oil and Rifampicin nanoemulsions [19] using the agar disc diffusion method. The Luria Broth-filled petri dishes were equally dotted with a bacterial suspension swab. 6 mm-diameter sterile paper discs were placed on the agar surface after being dipped in NE. The nanoemulsions were put on culture plates, and they were then cultured for 24 hours at 37°C. To quantify the antibacterial activity, the zone of inhibition was determined.

## Results And Discussion

### PH AND VISCOSITY

The mentioned PH value (table 3) for all the formulation was  $4.14 \pm 4.25$  which is considered suitable for topical application [20]





Five different formulations were created for optimization, and their viscosities were measured. Among the various data collected, it was observed that formulation F2 exhibited lower viscosity and superior flow properties compared to the other formulations. Reducing viscosity is evidently linked to the improvement of flow characteristics. Flow properties are inherent to nanoemulsions, which are classified by their high-flow characteristics.

**Table 3- Physicochemical properties of nanoemulsions**

S.NO	FORMULATION	PH	VISCOSITY
<u>1</u>	<u>F1</u>	<u>4.14</u>	<u>875</u>
<u>2</u>	F2	4.69	350
<u>3</u>	F3	4.76	1750
<u>4</u>	F4	4.37	1275
<u>5</u>	F5	4.25	600

### MACROSCOPIC ANALYSIS

Analysis were made on the basis of observation after keeping the samples for 24 to 48 hours the result was found to be excellent as there was no creaming sedimentation or breaking and cracking of the nanoemulsion prepared [21]. The study was concluded on the basis that after keeping samples under observation the all the 5 formulations showed no signs of creaming and sedimentation but out of all only F2 formulation was found to be more stable which led to the stability of a perfect Nano emulsion.



**Figure 1- Visual Appearance and storage of nanoemulsion**

### PARTICLE SIZE AND ZETA POTENTIAL

#### Droplet size analysis

Figure 2 provides an overview of the droplet size distribution observed in the nanoemulsions. Achieving a suitable Hydrophilic-Lipophilic Balance (HLB) value was a main factor in ensuring the stability of the prepared nanoemulsions. In our current investigation, the droplet sizes ranged from  $10.96 \pm 93.75$  nm. This size range, characterized by a tight distribution, was achieved through the high-pressure homogenization method, resulting in nanoemulsions with small droplets meeting the defined criteria of being in range within 200.

Different concentrations of NE by weight yielded corresponding droplet sizes of nm, respectively. It's worth noting that a nanoemulsion formed using a blend of surfactant, oil and drug in different was found to yield the lowest droplet size for a nanoemulsion. The formation of nanoemulsions with the lowest droplet size and a narrow distribution can be achieved by combining surfactants with noticeably different HLB values. surfactant with a low HLB, which dissolves in oil phase, and water may be responsible for this event.



The interaction between the oxyethylene group of Tween 80 and water molecules in the presence of hydroxyl ions at the interface between the water and oil phases may also be a contributing cause to this phenomena. The nanoemulsion droplets' measured zeta potentials ranged from - 40.5 to - 25.5 mv (F4). Previous studies have shown that the comparatively large hydrophilic (polyoxyethylene) head groups in Tween surfactants of non-ionic emulsifiers, which exert significant repulsive forces based on steric hindrance, can successfully inhibit droplet aggregation.

Moreover, smaller-sized nanoemulsions provide a large surface area. Consequently, a high concentration of tween 80 ( lipophilic surfactant) was necessary in the surfactant blend to ensure comprehensive surface coverage of nanoemulsion droplets, ultimately stabilizing the nanoemulsion [22].

A study ( Enayatifard et al) states that using varying proportions of Span 80, Tween 60, and Tween 20 as surfactants. Their findings demonstrated that the smallest particle size and the most favorable Polydispersity Index (PDI) were achieved when Tween 80 was employed as the surfactant. It is important to note that the choice of emulsifier for nanoemulsion preparation, characterized by its distinct Hydrophilic-Lipophilic Balance (HLB) value, can exert an influence on the composition, structure, and the electric charge of the interfacial layer enveloping the oil droplets.[23]

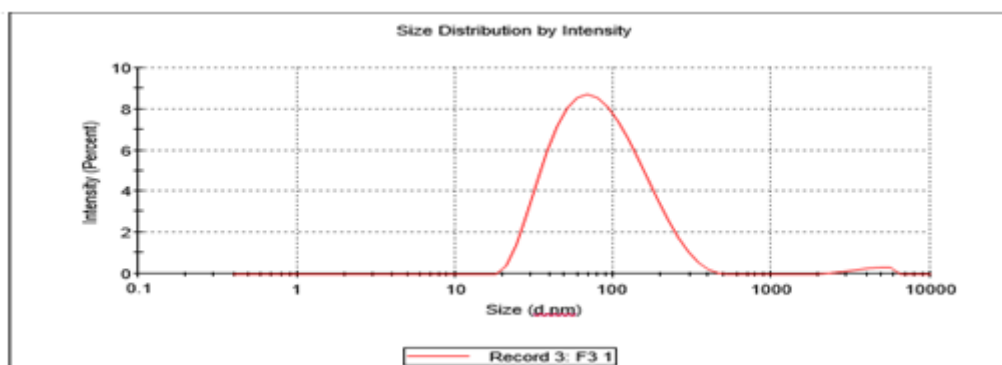


Figure 2 (a) Zeta Potential Distribution

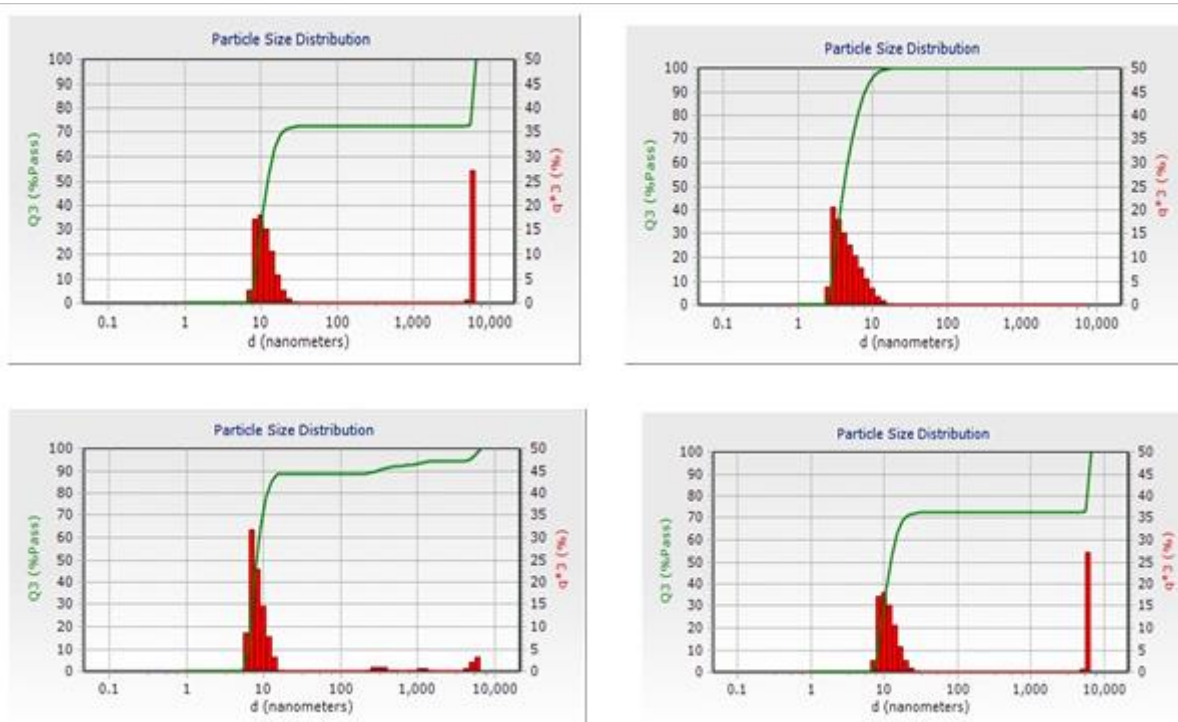


Figure 2 (b) Size Distribution by volume



### SCANNING ELECTRON MICROSCOPY (SEM)

Direct measurement of the size distribution and form of nanomaterials is possible with SEM.[24]. In the case of the prepared nano-emulsions, its average size was determined to fall within the range of 10-200 nanometers, and its shape observed is mentioned in Figure 3.

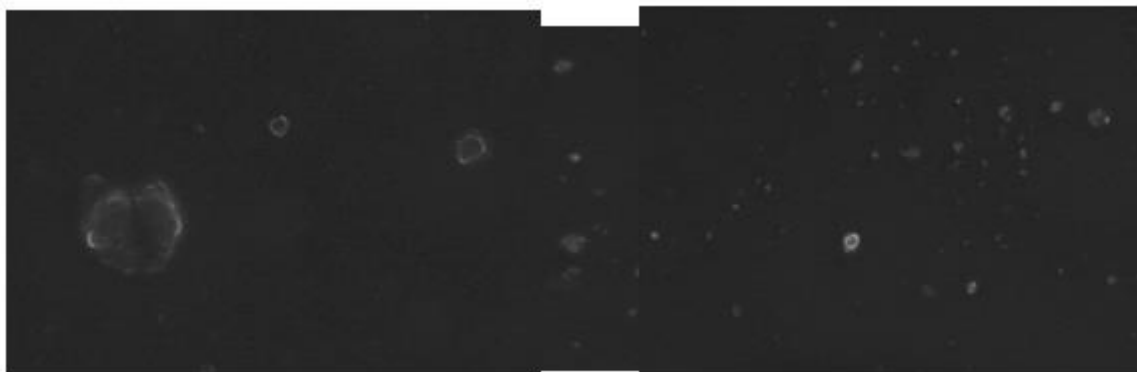


Figure 3: SEM Image of prepared Nano - Emulsions

### FTIR MEASUREMENTS

The compatibility between the crude medication NE, oil, and the synthesized product was evaluated using FTIR spectra. Examining the FTIR spectra of the resultant conjugate as well as the raw drug NE and EO[25]. In the FTIR study, all unique peaks corresponding to the crude drug's NE, EO, and conjugate were found. The results show that there were no chemical interactions or changes during the nano-conjugate's formulation.

The C-H stretch at 1958.8 cm<sup>-1</sup>, C=O stretch at 1621.16 cm<sup>-1</sup>, C-O stretch in the range of 1500–2000 cm<sup>-1</sup>, O-H aromatic stretch at 1299.04 cm<sup>-1</sup>, C-H aromatic stretch in the range of 1000–1500 cm<sup>-1</sup>, and C=C aromatic stretch at 1148.59 cm<sup>-1</sup> were among the characteristic peaks that could be seen in the FTIR spectra of crude drug NE.

The C-H stretch at 1958.79 cm<sup>-1</sup>, the C=O stretch at 1621.23 cm<sup>-1</sup>, the C-O stretch in the 1500–2000 cm<sup>-1</sup> range, the O-H aromatic stretch at 1301.35 cm<sup>-1</sup>, the C-H aromatic stretch in the 1000–1500 cm<sup>-1</sup> range, and the C=C aromatic stretch at 1146.53 cm<sup>-1</sup> were all distinguishable peaks in FTIR spectrum of the essential oil of eucalyptus.

Furthermore, the C-H stretch at 2923.32 cm<sup>-1</sup>, the C=O stretch at 1637.68 cm<sup>-1</sup>, the C-O stretch in the 1500–2000 cm<sup>-1</sup> range, the C-H aromatic stretch in the 1000–1500 cm<sup>-1</sup> region, and the O-H aromatic stretch at 1252.53 cm<sup>-1</sup> were all peaks in the FTIR spectra of the Rifampicin and essential oil conjugate.

Overall, the outcomes of the FTIR study supported the that the oil and medication did not interact chemically during the creation of the nano-conjugate resulting the compatibilness of drug and oil with each other.

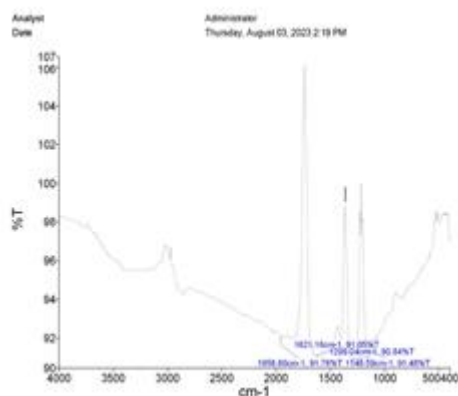


Fig 5 (a) FTIR spectra of crude rifampicin NE

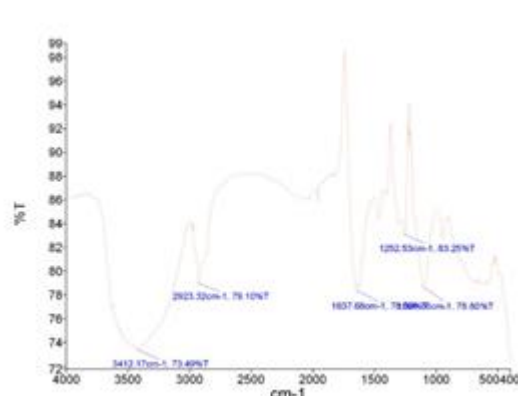


Fig 5 (b) FTIR spectra of Eucalyptus oil

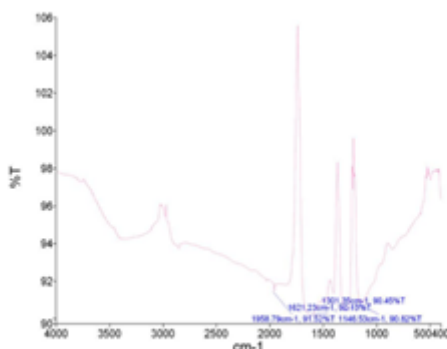


Fig 5 (c) FTIR spectra of Rifampicin NE, Eucalyptus oil based conjugate

#### THERMAL STRESS, HEATING/COOLING CYCLES, AND CENTRIFUGATION

The study of centrifugation and multiple heating/cooling cycles shows there were no indications of instability. In centrifugation test cycles were runned for all formulations for 15 minutes of time interval and all the formulations was found stable out which F2 formulation came out to be in the best stable form showing no indication of creaming and sedimentation. Furthermore, for all the testing like thermal stress and heating/cooling cycles the samples were found stable with no sedimentation and creaming in nanoemulsions

#### ANTI BACTERIAL PROPERTIES OF EUCALYPTUS OIL AND RIFAMPICIN NANO EMULSIONS

To assess its antiseptic activity, Nano emulsion form were tested against the gram-positive bacterium *S. Aureus* [26] The antimicrobial effectiveness of eucalyptus oil and rifampicin exhibited a significant enhancement when converted into a nanoemulsion. notably the nanoemulsion comprised of Tween 80 and Eucalyptus essential oil. exhibited robust inhibition of bacterial growth [27].

In this study we prepared five different nanoemulsions using varying combinations of rifampicin and eucalyptus oil These formulations were denoted as F1, F2, F3, F4& F5 to assess their antibacterial properties, we measured the size of the inhibition zones, as depicted in Figure 7. Only F2 and F4 nanoemulsions demonstrated positive outcomes against bacterial strain The inhibition zones achieved by F2 against *S. aureus* exceeded 1.62mm. Conversely, F4 and F3 displayed better results On the other hand, F1, and F5 nanoemulsions did not exhibit significant activity against the bacterial strain.

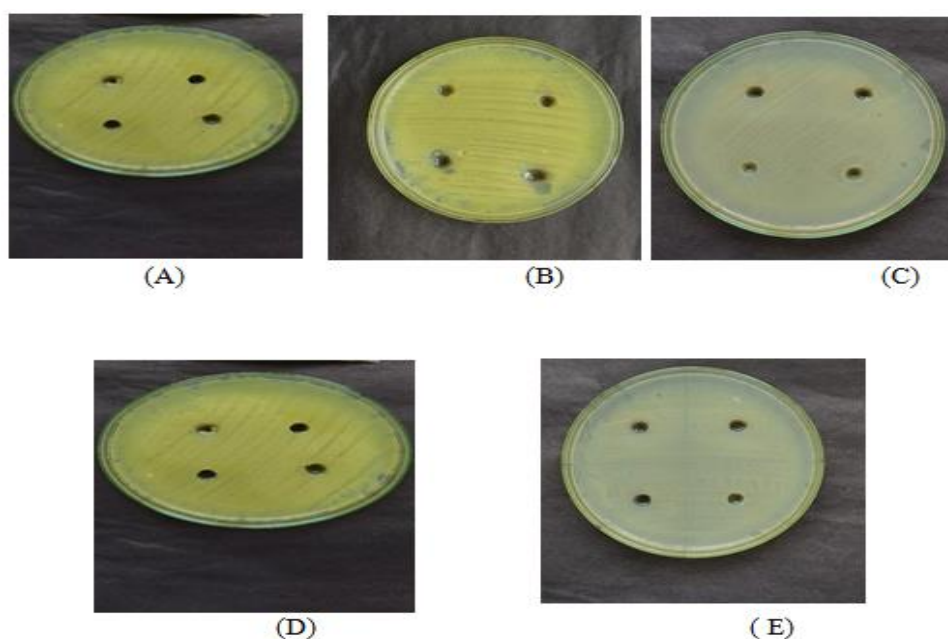
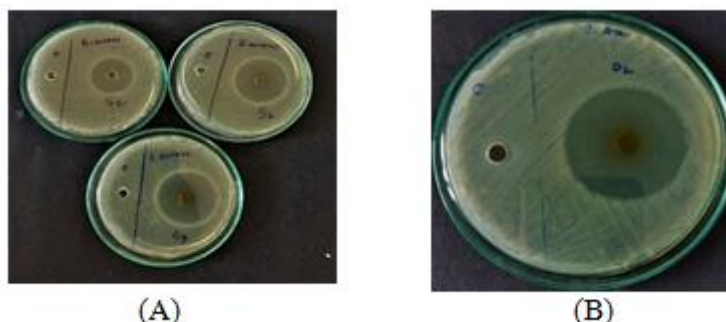


Fig 6 : Preparation of bacterium cultutres on petri plates with prepared nanoemulsions for observation





**Figure7: Anti-bacterial activity of induced nano emulsion against S.aureus by agar diffusion method**

## CONCLUSION

The nano emulsion composed of rifampicin infused with nanoemulsified eucalyptus oil was effectively created. Subsequent investigations were made into the physicochemical characteristics of this formulation to elucidate the interplay between nanoemulsion and bacterial activity. Notably, augmenting the concentration of drug and oil within nanoemulsion Furthermore, it was discerned that the NE exhibited superior antibacterial efficacy against the tested clinical pathogen, *S. aureus*. These findings substantiate the potential incorporation of the rifampicin and EO nano emulsion in wound management applications within the pharmaceutical sector.

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Consent for publication

The consent of all the authors has taken to publish the research in this journal.

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