RESEARCH
www.commprac.com
ISSN 1462 2815

GENTAMICIN SULPHATE BASED OPHTHALMIC NANOEMULGEL: FORMULATION AND EVALUATION, UNRAVELLING A PARADIGM SHIFT IN NOVEL PHARMACEUTICAL DELIVERY SYSTEMS DEVELOPMENT AND ASSESSMENT

Suraj Mandal [1]*, Dr. Prabhakar Vishvakarma [2] and Dr. Milind Sharad Pande [3]

[1] Research Scholar, Department of Pharmacy, IIMT College of Medical Sciences, IIMT University, O-Pocket, Ganganagar, Meerut, U.P., India. (*Corresponding Author)
[2] Associate Professor, Department of Pharmacy, Krishna Institute, Krishna Institute, Bijnor, U.P., India.
[3] Professor, Department of Pharmacy, IIMT College of Medical Sciences, IIMT University, O-Pocket, Ganganagar, Meerut, U.P., India.

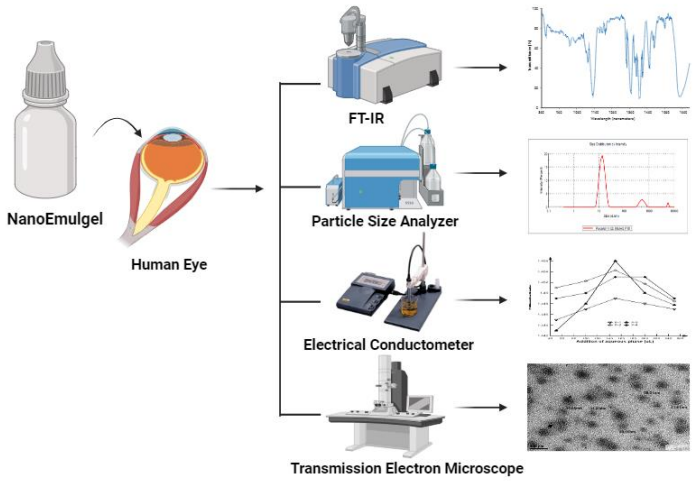
DOI: 10.5281/zenodo.10811540

Abstract

Introduction: This study presents a comprehensive exploration into the formulation and evaluation of Gentamicin Sulphate Ophthalmic Nanoemulgel, aiming to establish a novel pharmaceutical delivery system for enhanced ocular drug delivery. **Aim:** To formulate and evaluate a gentamicin sulphate based ophthalmic nanoemulgel. **Objective:** The development of this nanoemulgel involves the integration of nanotechnology with a gel-based delivery platform, combining the advantages of both systems to improve drug bioavailability and therapeutic efficacy. **Result and Discussion:** The study meticulously assesses the physicochemical characteristics of the developed nanoemulgel, including particle size, zeta potential, and drug release kinetics. Additionally, the stability and rheological properties of the formulation are investigated to ensure its suitability for ophthalmic application. The evaluation of the nanoemulgel extends to in vitro studies, providing insights into its ocular residence time, permeation, and therapeutic effectiveness. The experimental outcomes contribute valuable data on the optimization of the nanoemulgel, ensuring its efficacy, safety, and stability for potential clinical applications. **Conclusion:** This research advances the field of pharmaceutical delivery systems by presenting a detailed investigation into the development and assessment of Gentamicin Sulphate Ophthalmic Nanoemulgel. The findings underscore the potential of this novel formulation as an effective and safe means of delivering ophthalmic medications, opening avenues for further research and development in ocular drug delivery technologies.

Keywords: Gentamicin Sulphate, Ophthalmic Nanoemulgel, Physicochemical Characteristics, Ocular Drug Delivery Technologies.

Graphical Abstract



1. INTRODUCTION

An emulsion stands as a sophisticated dispersion system characterized by finely dispersed droplets within a non-agitated vehicle. Macroemulsions, denoting droplets with diameters ranging from 1 to 100 µm, are often termed conventional emulsions or colloids. These emulsion types undergo classification based on the size of the droplets. Typically unstable in water, these droplets exhibit a nearly buoyant nature, dispersing readily with one and a half parts in the medium and being readily absorbed by solid particles on the surface.

Contrastingly, microemulsions, featuring droplets between 10-100 nm, represent an isotropic liquid system distinguished by a more uniform size and physical properties. Moving further into the nanoscale, nanoemulsions boast droplet diameters between 20-200 nm, showcasing a thicker consistency compared to their micro counterparts.

The emergence of nanoemulsion gel signifies a groundbreaking development known as nanoemulsion-based hydrogelation. This involves the incorporation of an integrated nanoemulsion system into a hydrogel matrix, resulting in a formulation with superior skin penetration properties. This innovative approach enhances the therapeutic and cosmetic applications of traditional emulsions by combining the precision of nano-sized droplets with the versatility of a gel matrix.

The nanoemulsion gel, through its finely tuned composition, ensures better skin penetration, making it an optimal vehicle for the delivery of various active ingredients. This advanced formulation overcomes the limitations associated with traditional emulsions, providing enhanced stability, controlled release, and improved bioavailability. As the realms of pharmaceuticals and cosmetics continue to evolve, the integration of nanoemulsion gel stands as a testament to the continual pursuit of optimized delivery systems that cater to the intricate requirements of modern formulations.[1]

1.1 Nanoemulsion to nanoemulgel

The evolution from nanoemulsion to nanoemulgel represents a significant advancement in pharmaceutical and cosmetic formulation, introducing a hybrid structure that combines the benefits of nanotechnology with the versatile characteristics of an emulgel system. This transformation enhances the delivery, stability, and application properties of the formulation, offering a sophisticated solution to overcome challenges associated with traditional delivery systems.

Nanoemulsions, characterized by nanosized droplets of oil dispersed in water, have been widely recognized for their ability to enhance the solubility and bioavailability of hydrophobic compounds. In the pharmaceutical and cosmetic industries, nanoemulsions have played a pivotal role in improving the delivery of drugs and active ingredients, providing enhanced efficacy and targeted action. However, the inherent liquid nature of nanoemulsions may limit their application in certain scenarios, such as topical or localized drug delivery.

The transition from nanoemulsion to nanoemulgel addresses this limitation by incorporating the nanosized emulsion droplets into a gel matrix. This integration introduces a semi-solid or gel-like consistency to the formulation, combining the stability of gels with the exceptional solubilization properties of nanoemulsions. The resulting nanoemulgel inherits the advantages of both systems, offering improved stability, prolonged shelf life, and enhanced application characteristics.[2]

One of the key benefits of nanoemulgels is their ease of application. The gel matrix provides a convenient and user-friendly format, allowing for precise and controlled application, particularly in topical and dermatological formulations. The non-greasy texture further enhances the patient or user experience, making nanoemulgels an attractive choice for various therapeutic and cosmetic applications.

The formulation process involves careful selection of components such as surfactants, co-surfactants, and gelling agents to achieve optimal stability and rheological properties. The resulting nanoemulgel can be tailored to encapsulate a diverse range of active ingredients, including pharmaceutical drugs, vitamins, antioxidants, and other bioactive compounds.

In pharmaceutical applications, nanoemulgels offer advantages such as improved drug solubility, enhanced bioavailability, and controlled release, making them particularly relevant for transdermal drug delivery or localized treatments. In cosmetics, nanoemulgels provide efficient delivery of active skincare ingredients, promoting enhanced penetration and sustained effects.

The transformation from nanoemulsion to nanoemulgel showcases the dynamic nature of formulation science, where innovation meets practical application. This hybrid delivery system represents a versatile platform with potential applications in personalized medicine, targeted drug delivery, and advanced skincare formulations, contributing to the ongoing evolution of modern pharmaceutical and cosmetic technologies.[3]

1.2 Nanoemulgel:

Nanoemulgel, an advanced and multifaceted pharmaceutical and cosmetic formulation, represents a remarkable integration of nanotechnology and emulgel systems. This innovative hybrid structure combines the unique advantages of nanosized emulsion droplets with the versatility of a gel matrix, resulting in a sophisticated delivery system that overcomes several challenges encountered by traditional formulations. As a frontier in modern formulation science, nanoemulgel holds great promise for revolutionizing drug delivery and skincare applications.[4]

At its core, nanoemulgel is designed to address critical issues in the delivery of therapeutic or cosmetic agents, particularly those with limited solubility and bioavailability. The incorporation of nanosized emulsion droplets, known as nanoemulsion, serves as a pivotal strategy to enhance the absorption of hydrophobic and poorly soluble compounds. This not only contributes to improved drug solubility but also facilitates enhanced permeation through biological barriers, such as the skin or mucosal membranes.

The amalgamation of nanoemulsion into a gel matrix represents a synergy that combines the stability of gels with the exceptional solubilization and encapsulation capabilities of nanosized droplets. The resulting nanoemulgel, with its unique physicochemical properties, offers several advantages, including prolonged stability, ease of application, and the ability to deliver therapeutic or cosmetic agents in a controlled and targeted manner.[5]

In the pharmaceutical realm, nanoemulgels are gaining prominence for their potential to revolutionize drug delivery systems. The enhanced bioavailability achieved through nanoemulsion droplets addresses the challenges posed by poorly water-soluble drugs, leading to more effective and efficient therapeutic outcomes. Furthermore, the

controlled release properties of nanoemulgels contribute to sustained drug action, reducing the frequency of administration and improving patient compliance.

The cosmetic industry has also embraced the transformative potential of nanoemulgels. By encapsulating active ingredients such as vitamins, antioxidants, and anti-aging compounds within nanosized droplets, these formulations enhance skin penetration, promoting improved efficacy and desired cosmetic effects. The non-greasy and easily spreadable nature of nanoemulgels further enhances their appeal in skincare formulations.

Formulating nanoemulgels involves careful consideration of components such as surfactants, co-surfactants, and gelling agents to achieve optimal stability and rheological properties. The intricate design allows for precise control over the release kinetics of encapsulated substances, ensuring sustained and prolonged effects.[6]

As research in nanoemulgels progresses, the potential applications continue to expand, offering new avenues for personalized medicine, targeted drug delivery, and advanced skincare solutions. The convergence of nanotechnology and emulgel systems in nanoemulgels signifies a paradigm shift in formulation science, promising enhanced therapeutic outcomes and improved cosmetic benefits across diverse applications.[7]

As demonstrated in Table 1.1, emulgel is not a brand-new formulation type and is currently available on the market.

Table 1.1: Emulgel product available on the market today6-32

Sr. No.	Product Name	Manufacturer	Active Ingredients	Indications	Key Features
1.	EmulgelX Plus	ABC Pharmaceuticals	diclofenac sodium	Arthritis, Joint Pain	Fast Absorption, Long-lasting Relief, Non-Greasy Formula
2.	Dermo Gel Ultra	XYZ Cosmetics	Aloe Barbadensis Leaf Juice	Skin Conditions, Eczema, Dermatitis	Moisturizing, Hypoallergenic, Dermatologist-Tested
3.	Sports Recover Emulgel	DEF Health Solutions	Menthol 6% Camphor 4%	Muscle Soreness, Sports Injuries	Cooling Sensation, Rapid Recovery, Physical Therapist Recommended
4.	Beauty Care Revitalizing	UVW Cosmeceuticals	Zinc oxide	Anti-Aging, Wrinkle Reduction	Collagen Boost, Vitamin Enriched, Fragrance-Free
5.	Pediatric Soothe Gel	LMN Pharma	1.1% (w/v) sodium fluoride	Children's Skin Irritations, Rashes	Gentle Formula, Pediatrician-Approved, Dye-Free
6.	Eye Relief Cooling Gel	EFG Eye care Products	Cucumber Extract, Aloe barbadensis leaf juice (Aloe Vera), Glycerin, Carrageenan, Amorphophallus konjac root powder, Salicylic	Eye Fatigue, Puffiness	Refreshing, Ophthalmologist-Tested

			acid, Sorbic acid, Benzyl alcohol		
7.	Acne Control Emulgel	PQR Dermaceuticals	2% salicylic acid	Acne Treatment, Oil Control	Salicylic Acid, Non-Comedogenic, Clear Skin Complexion
8.	Sun Shield Emulgel SPF 30	UV Protection Ltd.	Glycolic Acid	Sunscreen, UV Protection	Broad Spectrum, Water-Resistant, Dermatologist-Recommended
9.	Eye Relief Emulgel	ABC Ophthalmics	Ciprofloxacin HCl	Dry Eyes, Eye Irritation	Lubricating Formula, Long-Lasting Relief, Preservative-Free
10.	Opti Gel VisionCare	XYZ Eyecare	Cucumber Extract	Redness, Itching, Allergies	Cooling Sensation, Fast Absorption, Ophthalmologist-Approved
11.	ClearSight ComfortGel	DEF Pharma	Vitamin C, Omega 3	Contact Lens Discomfort, Dry Eyes	Enhanced Hydration, Suitable for Sensitive Eyes
12.	Glauco Guard Gel Drops	LMN Ophthalmology	metoprolol, propranolol	Glaucoma Management, Intraocular Pressure Control	Sustained Release, Clinically Tested, Preservative-Free
13.	Visio Care Dry Eye Relief	UVW Vision Solutions	Hyaluronic Acid	Moderate to Severe Dry Eyes	Rapid Relief, No Blurring
14.	Blink Refresh Emulgel	PQR Optics	Polyethylene glycol	Computer Vision Syndrome, Eye Fatigue	Refreshing Gel, Protects Against Digital Eye Strain
15.	Crystal Clear Lubricating	EFG Eye Wellness	Vitamin B2, Vitamin C, Omega 3	Artificial Tears, Moisture Retention	Preservative-Free, Suitable for Nighttime Use
16.	Lumina Vision Cooling Gel	Ophthalmic Innovations	Cucumber Extract	Eye De-Puffing, Refreshment	Dermatologist-Tested, Non-Greasy
17.	Reumadep Emulgel	ErbozetaEnergi a Verde	Arnica, Ashwagandha, Myrrh, Ginger, Rosemary, Cloves, Mint.	relief to aected skin	Apply several times a day to affected areas and massage until fully absorbed
18.	Emulgel Levorag Monodose	THD LAB Farmaceutici	CM-Glucan and Glyceryl Stearate	treatment of anal fissures	emollient, lubricating and film-forming, soothing properties, protective properties
19.	Voltaren Emulgel	Novartis Consumer Health	Propylene glycol, 100 g Diclofenac diethylamine (1 g diclofena sodium) Base: An isopropanol-propylene glycol	treating osteoarthritis symptoms	Long-lasting Relief

			aqueous gel fatty emulsion.		
20.	Meloxic Emulgel	Provet	Meloxicam	Relief of pain, inflammation, and edoema in soft-tissue injuries such sprains, strains, bruising, and backache	greaseless, easily removable, easily spreadable, emollient
21.	Coolnac Gel Emulgel 1 %	Chumchon	Diclofenac Diethylammonium	Arthritis, Joint Pain	Fast Absorption, Long-lasting Relief,
22.	Benzolait AzEmulgel	Rordermal	Benzoyl peroxide and Azeloglycine	prevents the spread of bacteria and infections	easily removable, easily spreadable
23.	Miconaz-H-emulgel	Medical Union Pharmaceuticals	Miconazole nitrate, Hydrocortisone	antibacterial effect on gram – positive, bacterial, the product may also be used for mycotic affections with bacterial superinfection	easily spreadable
24.	Voveron Emulgel	Novartis Pharma	Diclofenac diethyl amine	Arthritis, Joint Pain	Long-lasting Relief, Non-Greasy Formula
25.	Diclobar emulgel	Barakat pharma	Diclofenac diethyl amine	Arthritis, Joint Pain	Fast Absorption, Non-Greasy Formula
26.	Levorag emulgel	THD Ltd	Hibiscus, liqourice and natural extracts	treatment of anal fissures	emollient, lubricating and film-forming, soothing properties, protective properties

2. DRUG PROFILE

Gentamicin, an aminoglycoside renowned for its antibacterial properties, was first isolated from Micromonospora purpurea in 1963. Widely prescribed by physicians due to its availability, affordability, and broad therapeutic range, gentamicin is particularly effective against severe gram-negative infections, including those caused by Pseudomonas aeruginosa.

Notably, it exhibits synergistic activity when administered in combination with beta-lactam antibiotics, offering additional benefits in treating complex disorders and facilitating dose adjustment while minimizing adverse effects. Despite its historical efficacy in various therapeutic contexts, the use of gentamicin may be constrained by the risk of severe side effects, such as ototoxicity and nephrotoxicity.

Produced by the actinomycete Micromonospora purpurea, gentamicin sulphate is a water-soluble aminoglycoside antibiotic. This medication holds promise for preventing or treating a diverse range of bacterial infections, belonging to the pharmacological family of aminoglycoside antibiotics. Its mechanism of action involves inhibiting the growth of bacteria.[5]

It has the following structural formula.

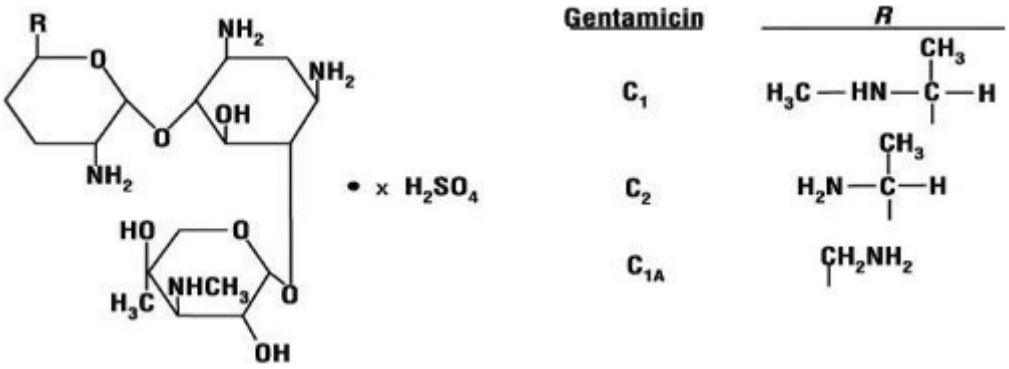


Figure 2.1: Gentamicin

3. SUMMARY

Gentamicin is an aminoglycoside used to treat a wide variety of aerobic infections in the body.

3.1. General Information:

a) **Drug Name:** Gentamicin Sulphate

b) **Class:** Aminoglycoside Antibiotic

c) **Route of Administration:** Intramuscular (IM), Intravenous (IV), Topical

d) **Common Trade Names:** Garamycin, Gentak, Gentocin, others[5,6]

4. IUPAC NAME

The International Union of Pure and Applied Chemistry (IUPAC) name for Gentamicin sulphate is (1R,2R,3R,4R,5R)-2,3,4,5-tetra-methyl-7-{[(2R,3R,4R,5S)-3,5-dihydroxy-2-[(2S,3R,4S,5S,6R)-3-amino-4,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-6-(hydroxymethyl)oxan-2-yl]oxy}-oxane-3,4,5-triol sulphate.

The structure and nomenclature are complex due to the multiple sugar and amino components in its structure. The IUPAC name provides a systematic way to represent the chemical structure of Gentamicin sulphate.[7]

5. ANTIBACTERIAL ACTIVITY

The antibacterial activity of gentamicin sulphate is well-established and widely recognized. As an aminoglycoside antibiotic, gentamicin sulphate exerts its antibacterial effects by inhibiting bacterial protein synthesis. It primarily targets and binds to the bacterial 30S ribosomal subunit, disrupting the translation process and leading to the accumulation of defective proteins within the bacterial cell.

Gentamicin sulphate demonstrates effectiveness against a broad spectrum of bacteria, including both gram-negative and some gram-positive strains. Some notable bacteria that are susceptible to gentamicin include Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter species, and certain strains of Staphylococcus aureus.

The antibiotic's ability to interfere with protein synthesis in bacteria makes it particularly valuable in treating severe infections caused by susceptible microorganisms. Gentamicin is often used in combination with other antibiotics to enhance its spectrum of activity and combat complex bacterial infections. The synergistic effects observed when gentamicin is administered with beta-lactam antibiotics contribute to its efficacy in various clinical settings.

It is crucial to note that the clinical use of gentamicin is carefully monitored due to the potential for adverse effects, particularly ototoxicity (damage to the ear) and nephrotoxicity (kidney damage). Therefore, healthcare professionals must consider the risk-benefit profile when prescribing gentamicin and monitor patients closely during its administration.[8-10]

6. MATERIALS & METHODS

To develop a drug dosage form, it is imperative to conduct preformulation studies on both the drug and its excipients. This step is crucial as it forms the basis for designing formulations with desired drug release characteristics, optimal stability, and other key attributes. The primary goal of preformulation studies is to characterize the drug sample based on specific physicochemical properties, ensuring the establishment of its identity. In addition to identity verification, these studies encompass the physicochemical characterization of solids and the evaluation of compatibility between the drug and its formulation excipients.[11, 58]

6.1 Drug Preformulation studies, characterization & Identification of Gentamycin Sulphate:

Conducting a preformulation study on a drug holds paramount significance in validating its purity. Utilizing previously described methods, organoleptic characteristics, solubility parameters, and physicochemical attributes of gentamicin sulphate were meticulously measured, and the outcomes were documented in the provided table. Molecular weight, Pka, and pharmacokinetic parameters for gentamicin sulphate were sourced from relevant literature.

6.1.1. Procurement of drug sample(s)

The sample of Gentamicin Sulphate (GS) was generously provided as a gift by Ranbaxy Laboratories Ltd., Gurgaon, India.

6.1.2. Melting Point (MP) Determination:

The drug sample's melting point was determined by using the capillary method and a melting point apparatus known as the Ambassador. The procedure included holding a capillary tube, sealing one end, and then adding dry powdered medicine to the open end. Next, the capillary tube was filled and put into the melting point apparatus. The precise temperature at which the medicine powder began to melt was noted using a thermometer. The certificate of drug sample value or the certificate of analysis for the substance was then cross-referenced with this temperature.

The observed melting point of the powdered gentamicin sulphate sample was determined to be 220-240°C. This result matches the specified melting point of the gentamicin sulphate powder, validating the measurement's accuracy and consistency.

Table 6.1: Physical Evaluation of Gentamycin Sulphate

Organoleptic characteristics	
Colour	white to buff coloured
Odour	Odourless
Texture	plane
Nature	hygroscopic powder
Solubility	
Aqueous solubility (mg/ml)	12.6 mg/mL
pKa (Strongest Acidic)	12.55
pKa (Strongest Basic)	10.12
Partition coefficient (log P o/w)	1.6
Physicochemical properties	
Melting point(ºC)	220-240 °C
Molecular weight	447.529 g/mol
Purity	99.85%
pH	6-8

The organoleptic characteristics of gentamicin sulphate closely resembled those outlined in the official monograph of gentamicin sulphate. A meticulous comparison of experimentally obtained results, including saturation solubility, melting point, and partition coefficient, with the reported values revealed no significant differences, affirming the high purity of gentamicin sulphate.

Moreover, the observed combination of high aqueous solubility and elevated lipophilicity (Log P greater than 1.5) suggested its aptness for the formulation of a nanoemulgel. With a molecular weight below 1000 daltons, gentamicin sulphate demonstrated suitability for topical administration. The comprehensive preformulation data collected strongly indicated the appropriateness of gentamicin sulphate for the formulation of a nanoemulgel.

6.1.3. Spectrophotometry

The UV spectrum of the drug sample was examined using the UV-Spectrophotometer (PerkinElmer, UV-160529). After carefully weighing a sample of 10 mg, it was placed in a 100 ml volumetric flask to be diluted with methanol. After 10 times diluting the stock solution, the UV spectrum's 200–400 nm wavelength range was studied.

The UV spectrum of the obtained sample of powdered gentamicin sulphate revealed a peak absorption at 281 nm, precisely matching the stated value in the certificate of analysis. This congruence offers further proof that the UV spectrum analysis is accurate and consistent.[18]

6.1.3.1. Analytical methodology (Spectrophotometric method)

- A precisely weighed 10mg of the drug sample was dissolved in 10 mL of methanol using an analytical balance (Shimandzu model AUX 220). This solution was transferred to an amber-colored volumetric flask, covered with black paper, marking it as the primary stock solution with a concentration of 1000 µg/ml.

- From the primary stock solution, 1ml was drawn into another volumetric container and diluted with methanol up to 10ml, resulting in a concentration of 100 µg/ml. This newly obtained solution, contained in a volumetric container wrapped in black paper, was denoted as the secondary stock solution.
- Subsequently, ten different aliquots were drawn from the secondary stock solution into separate test tubes, measuring 1, 2, 3, 4, & 5mL, and each was diluted with methanol to achieve concentrations of 1, 2, 3, 4, 5µg/mL.
- The absorbance of the resulting solutions was measured at λ max 281 nm using a UV-Vis spectrophotometer (PerkinElmer, UV-160529), with methanol as the blank. This entire process was repeated thrice for accuracy.
- A graph correlating absorption to the concentration values of the resulting solutions was plotted using MS Excel software, and statistical parameters such as correlation coefficient and regression line were determined.[119]

6.1.3.2. FTIR spectroscopy

The Fourier-transform infrared absorption spectrum of the drug was determined utilizing the KBr pellet method. Prior to the analysis, potassium bromide (KBr) powder was meticulously dried and placed in a clean mortar and pestle. For the preparation of the sample, the drug (10mg) and KBr (100mg) were ground together to reduce particle size. This step is crucial to prevent large particles from scattering the infrared beam, which could adversely affect the baseline slope of the spectrum.

Subsequently, the powdered mixture was transferred to a KBr pellet die, ensuring even distribution within the 7mm collar. The die, along with the powder, was placed into a quick handy press.

The powder-filled die underwent pressing with a hydraulic press for 1-2 minutes to form a compact pellet. Following the pressing, the die was disassembled, and the 7mm collar was removed. The collar, along with the pellet, was placed in the sample holder.

The pellet, now securely positioned in the sample holder, was subjected to Fourier-transform infrared (FTIR) instrumentation using a Shimadzu Model No. FTIR8400. The spectrum was recorded within the range of 400-4000 cm^{-1} utilizing the IR solution software. This meticulous process ensured the generation of an accurate and reliable Fourier-transform infrared absorption spectrum for the drug.

6.1.4. Differential scanning calorimetry (DSC)

The thermal analysis of the obtained sample was conducted utilizing a differential scanning calorimetry (DSC) instrument, specifically the Perkin Elmer model. In this process, a precisely weighed 20mg drug sample was transferred to a DSC pan and compacted using a hydraulic press.

Subsequently, the prepared sample pan was positioned in the DSC sample holder port, and the instrument was operated through computer-controlled software (Pyris-6 Version 4.0).

The DSC measurement was carried out with a programmed temperature increase of 10°C/min, spanning from 30°C to 400°C. This meticulous procedure allowed for the accurate determination of thermal characteristics and behavior of the drug sample under varying temperatures.[26]

6.1.5. Determination of drug solubility

The solubility of the drug was investigated to ascertain its degree of soluble in several excipients, such as oil phase, surfactant, co-surfactant, and Smix, a combination of surfactant and co-solvent. To complete the process, an excess of the medication was dissolved in a millilitre-sized Eppendorf tube that was filled with the appropriate excipient. Every Eppendorf tube with a 2-millilitre capacity also received extra extra medication, and the mixture was combined using a vortex mixer. The purpose of this was to determine whether the medicine had completely dissolved. The Eppendorf tubes were incubated at a temperature of 25 degrees Celsius with a standard variation of 5 degrees Celsius for a total of 72 hours. The goal of doing this was to achieve balance. The drug samples were centrifuged using the model number and manufacture of the apparatus for ten minutes at a speed of three thousand revolutions per minute after the allowed period of time had passed. The supernatant was carefully collected, and after that, it was filtered through a membrane filter with 0.45µm pore size. After that, it was properly diluted with methanol by following the right protocol. A UV-spectrophotometer (PerkinElmer, UV-160529) was used to measure and quantify the amount of medication that was dissolved in each component in order to ascertain the amount of medication that was dissolved in each excipient. The above formula was used in order to achieve this aim.[12]

Solubility (µg/ml) = (Absorbance± intercept)/slope × Dilution Factor

6.1.6. Mutual miscibility of excipients

Mutual miscibility, a pivotal characteristic for the development of a nanoemulsion formulation, was examined among the chosen surfactant, co-surfactant, and oil phase. This assessment involved the amalgamation of excess quantities of surfactant and co-surfactant with the oil phase, and successful miscibility was indicated by the formation of homogeneous mixtures. Here is the detailed procedure:

a) Take 1.0 mL of any surfactant (e.g., Tween-20, Tween-80, Span-20, Span-80) in an Eppendorf tube.

b) Add 1.0 mL of any co-solvent (e.g., propylene glycol, polyethylene glycol, Transcutol) to the surfactant.

c) Mix the components thoroughly using a vortex shaker.

d) Verify proper mixing by observing for a clear sign of miscibility between the surfactant and co-surfactant with the oil phase.

Repeat the process for different combinations:

Assess the mutual miscibility of oil, surfactant, and co-surfactant in various excipient combinations.

- Selected surfactants: Tween 20, Tween 80; selected oil phases: almond oil, olive oil, soybean oil, castor oil, sesame oil, oleic acid.
- Selected co-surfactants: propylene glycol (PG), polyethylene glycol 200 (PEG 200), polyethylene glycol 400 (PEG 400).

This comprehensive evaluation ensures the suitability of the selected components for the design of a nanoemulsion formulation.

6.2. Excipients selected for nanoemulsion formulation

In the development of a nanoemulsion formulation for gentamicin sulphate, careful consideration will be directed towards characterizing the physical, chemical, and biological properties of both the drug and the excipients.

6.2.1. Screening of Oil

The drug loading capacity in nanoemulsion plays a crucial role in screening the oil phase. Typically, a higher solubility of the drug sample in the oil phase is preferred. Additionally, it is essential for the drug to form a homogenous mixture with surfactants and co-surfactants to facilitate the formation of the nanoemulsion formulation.

To screen the oils, 54 mg of the drug was placed in 1.0 mL of the selected oil phase in an Eppendorf tube. The system was allowed to reach drug equilibration over a span of 4 weeks at a constant temperature. This meticulous process aims to identify the most suitable oil phase based on the drug's solubility and compatibility with surfactants and co-surfactants for the subsequent nanoemulsion formulation.[11]

6.2.2. Screening of surfactants

To formulate the nanoemulsion, the surfactant plays a pivotal role in its preparation. Non-ionic surfactants were specifically chosen for the creation of the nanoemulsion due to their exceptional capabilities in development and ease of miscibility with the selected components. The selection of non-ionic surfactants was also motivated by their lower toxicity and enhanced safety compared to cationic and anionic surfactants.

Tween 20 and Tween 80 were specifically chosen as they exhibit maximum solubilization capacity for the drug and demonstrate high miscibility with the oil phase. These surfactants, being non-ionic in nature, are well-suited for the intended nanoemulsion formulation, contributing to its stability and efficacy.[12]

6.2.3. Screening of co-surfactants

In the process of creating nanoemulsion formulations, the co-surfactant plays a crucial role due to its high miscibility with both oil and surfactant. The reason for this is because both of these chemicals are present. It does this by helping to reduce the interfacial tension between the oil and the combination of surfactants. The co-surfactant used for this particular experiment was chosen based on its potential to significantly improve the drug's solubilization and demonstrate its mutual miscibility. Depending on the extent to which they dissolved the drug, lipidic excipients were put through rigorous testing in order to create the nanoemulsion for gentamicin sulphate. This was done to ensure that the formulation goals and the lipidic excipients were compatible with each other.[13]

6.3. Aqueous Dispersibility of pre-concentrate mix

The performance of the preconcentrate mixture, comprising Smix along with the oil phase and water, is a crucial aspect in the development of a dilutable nanoemulsion system. The individual components, namely oil, surfactant, and co-surfactant, play pivotal roles in formulating the nanoemulsion and understanding its dilution behavior in the presence of aqueous media. The aqueous dispersibility of the nanoemulsion was assessed through a visual examination involving the gradual addition of the aqueous phase to the preconcentrate mix. The dilatation of the preconcentrate mixture with the aqueous phase could lead to either a clear system or a turbid dispersion,

signifying the formation of nanoemulsion or coarse emulsion, respectively. Following visual inspection, the quantity of water needed to maintain transparency in the preconcentrate mix was determined.[14]

The procedure comprised the following steps:

a) Transfer 5.0 mL each of Tween 20 and polyethylene glycol to a 15 mL capacity Falcon tube, gradually mix well for 20 minutes, and label it as Smix (1:1), containing surfactant and co-surfactant fractions in equal proportions.

b) Take 100 µL of oleic acid in a 2 mL Eppendorf tube as the oil phase, add 900 µL of Smix (prepared in step 1), and mix using a vortex shaker.

c) Add a small quantity of water to the Eppendorf tube containing the oil phase and Smix.

d) Initially, add 100 µL of water and shake using a vortex until a clear solution is observed.

e) Repeatedly add subsequent 100 µL increments of water until the clarity of the dispersion is lost.

f) Repeat the above procedure by taking different combinations of surfactants, co-solvents, and oils as indicated in the tables, representing different trials.

6.4. Construction of Ternary Phase Diagram

To comprehensively investigate the phase behavior of the chosen ternary components (Smix, Oil, and aqueous phase), a series of ternary phase diagrams were meticulously constructed using the aqueous dilution technique. Various Smix ratios (1:1, 1:1.5, 1.5:1, 1:2, and 2:1) were considered to offer a comprehensive perspective.

To prepare a phase diagram at a Smix ratio of 1:1, diverse weight combinations of oil (10, 20, 30, and 40 mg) and Smix (60, 70, 80, 90, 100...160 mg) were individually placed in 10 mL beakers. Employing the specified instrument model, each beaker's contents underwent sonication. Subsequently, the resulting mixture was titrated against distilled water, maintained at 25°C, using a 25 mL burette. The titration proceeded until the mixture lost its clarity, signaling the endpoint titration. The percentage (in w/w) of each consumed component (oil, Smix, and aqueous phase) during the titration was meticulously calculated for each combination. The acquired data were then utilized to construct a ternary phase diagram with the aid of specialized software. In this diagram, shaded regions delineated coarse-emulsion areas, while non-shaded regions indicated the nanoemulsion zone.

This method facilitated a comprehensive exploration of the ternary system's phase behavior, providing insights into the conditions conducive to nanoemulsion formation.[15]

6.5. Formulation of Gentamycin Sulphate Nanoemulsions

Utilizing the data from the ternary phase diagram, several formulations of gentamycin sulphate nanoemulsion were developed. The selection of oil, surfactant, and co-surfactant fractions was primarily based on the non-shaded regions of the diagram. The proportions of these components, chosen from the non-shaded regions, were combined as a preconcentrate mix to formulate the gentamycin sulphate nanoemulsion.[16]

6.5.1. Procedure for preparation of nanoemulsion

The oil phase was blended with the Smix at a specific ratio and then subjected to the aqueous phase. The drug-loaded discontinuous phase received the aqueous phase in a dropwise manner with continuous stirring and sonication.[17]

6.6. Physical characterization of Nanoemulsion Formulation

6.6.1 Droplet size distribution

The determination of droplet size for different formulations was conducted using the Malvern Zeta Sizer. In this methodology, 5 mL of the sample underwent filtration through a nylon membrane filter, followed by a 10-minute sonication period. Following this preparation, 1-2 mL of the sample was extracted and transferred into the cuvette of the Zeta Sizer, where it underwent a scanning process lasting 10 minutes, and the corresponding data was meticulously recorded. The recorded data were then presented in the form of a percentage, illustrating droplet intensity or volume versus the droplet size within the nanoemulsion system.[18-19]

6.6.2. Measurement of Electrical Conductivity of Formulation

It was discovered that the electrical conductivity of the composition was measured using a digital conductometer metre of type 611 E. The cell constant of the apparatus was determined by using a normal KCl solution at 25 degrees Celsius. This step was done in an official capacity to start the procedure. After this calibration was finished, one millilitre of the formulation was put to a beaker with a ten-millilitre capacity and a platinum electrode was inserted inside. To get a constant and dependable result, the conductivity value shown on the conductometer was meticulously recorded until it was reached. The next step was gradually diluting each mixture with the aqueous phase. This was the subsequent phase. To do this, 50 microliters of distilled water were added to the beaker progressively, in increments of 50 microliters. The material was fully mixed in line with the previously stated process, and the dispersion system's conductance was then assessed.[20]

6.6.3. Determination of Refractive Index

For each preconcentrate formulation (1 mL), equal aliquots (100 µL each) were dispensed into five separate 10 mL capacity beakers. Distilled water (50, 100, 150, 200, and 250 µL) was added to each beaker and thoroughly mixed for a duration of half an hour. The ABBE Refractometer was positioned appropriately to receive ample light. Methanol was used to carefully clean the refractometer's stage index, and the mirror assembly was positioned to provide enough light over the eyepieces. A single drop from the system was positioned over the refractometer's stage assembly, for which the refractive index required to be ascertained. After that, the assembly closure was turned on to distribute the liquid evenly throughout its prisms. The knobs were then adjusted such that the cross-mark was clearly visible, and the matching value was appropriately noted. To get the drug-loaded formulation's refractive index, the same process was repeated.[21-22]

6.6.4. pH determination

Consequently, the pH of the formulation was pre-adjusted to be within the range of 6.5 to 7.2. The pH measurements of the formulations were conducted using a digital pH metre, namely a Symtronic model SE 962 P. The pH metre was used to begin measuring the solution's pH once the calibration process using standard buffer

solutions was complete. One crucial factor that is important in reducing irritation to the nasal mucosa is the formulations' pH. The formulations' concentration is another important factor.[23]

6.6.5. Zeta potential determination:

Double-distilled water was used to dilute 0.1 mL of the formulation to 10 mL in order to conduct the zeta potential study. The zeta potential was then determined using the Zeecom-2000 zeta potential measurement device (Zeecom-2000, Microtec Co. Ltd., Chiba, Japan). Equipped with a 4.0 mW He-Ne red laser (633 nm), the apparatus identified potential within the range of -120 to 120 V. Additionally, a zeta potential analysis was conducted utilising the same measuring apparatus, but with the nanoemulsion formulation (0.1 mL) diluted 100 times with double-distilled water. At 25°C, each measurement was made. In general, zeta potentials of +30 mV or −30 mV were considered high. The improved formulations exhibited negative values of zeta potential, indicating a negatively charged system and ensuring stability. Importantly, a wide pattern that illustrated the connection between a decline in negative surface charge values and a decrease in emulsion particle size was seen when comparing the zeta potential and particle size data.[24]

6.6.6. Transmission electron microscopic:

The morphology of the dispersed phase was ascertained by means of research using transmission electron microscopy (TEM). A 300-mesh copper grid was covered with a drop of diluted nanoemulsion, which was let to stand for a minute. Phosphotungstic acid (PTA) was then added to the grid and allowed to sit for 10 seconds while it was upside down. Excess PTA was carefully absorbed onto filter paper. The grid was analysed using the Morgagni 268D (FEI Company, USA), which operates at 60–80 kV and has a 1550X magnification.

6.7. Preparation of nanoemulgel

In accordance with the methodology described by Bruschi et al. (2007), a combination gel consisting of Carbopol 934P (CP 934P) and Poloxamer 407 (P407) was developed. At first, a mechanical stirrer was used to dissolve CP934P in distilled water at concentrations of 0.25 percent, 0.50 percent, and 1 percent by weight. After the dissolving was finished, P407 was added to this gel at concentrations of 15%, 20%, and 25% by weight. The amalgamation was then kept at a temperature of 4 degrees Celsius for a period of twelve hours in order to guarantee full wetting using the cold technique. After that, the formulations were swirled to ensure that the two components were well combined inside the formulation. In the subsequent step, the optimised nanoemulsion that was loaded with the medication was introduced to the sol system in a drop-by-drop manner while magnetic stirring was continuously performed. First, the mixture was neutralised with TEA, and then it was kept at 4 degrees Celsius for twenty-four hours.[25]

6.7.1. Drug content

To find out how much medication was in each formulation overall, it was required to weigh and inspect the various formulations. These weighed samples were dissolved in methanol, and the liquid was agitated using a vortex mixer. Subsequently, the solutions were filtered using Whatman filter paper, and the concentrations of the solutions were determined using the developed HPLC procedure.

6.7.2. pH determination

A digital pH metre, produced by Hicon Enterprises in New Delhi, India, was utilised to ascertain the pH levels of drug-loaded NEGs and thermoreversible syringeable periodontal medications. The purpose of this procedure was to ascertain the pH value of the substances. Following the dissolution of 0.5 grammes of sol in fifty millilitres of distilled water, the resulting solution was maintained at a temperature of five degrees Celsius until complete solubilization occurred.[25]

6.7.3. Sol-gel transition and syringeability

The test tube inverting approach was used to estimate the periodontal sol's sol-gel transition temperature. Five millilitres of sol were poured into test tubes, which were then covered with aluminium foil and submerged in an electric water bath with a thermostat set to 4°C. The water bath's temperature was raised by 0.5°C increments, and the sol was given a minute to acclimatise to each new setting. When the meniscus stopped moving when tilted at a 90° angle, it was thought to have gelled. It was noted what temperature gelled. Five millilitres of the sol were taken in a test tube and put in a water bath that was kept at 37°C to measure the gelling time. Three separate measurements were made.[26]

A plastic syringe with a 22-gauge needle (1 mL) was used to conduct syringeability tests on nanoemulgels. The syringe was filled with a predetermined quantity of the nanoemulgel formulation (in sol form) that was kept at 4°C. The injector portion of the syringe was then pressed with a mild, consistent force. Throughout the experiment, air bubbles were prevented. A qualitative evaluation of syringeability was conducted.[27]

6.7.4. *In vitro* drug release kinetics of NEG

Dialysis bag approach was used for in vitro drug release research. To be more precise, a dialysis bag (molecular weight cutoff 6000-8000) containing 400 mg of periodontal sol with 2% medication was soaked for 48 hours in simulated gingival crevicular fluid (SGCF), a phosphate buffer with a pH of 7.4 to mimic the pH in periodontal disorders. After that, the dialysis bag was put in a vessel with 100 mL of SGCF, which was kept at 37°C and agitated at 100 rpm. At predefined intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, and 24 hours), samples of the dissolving fluid were taken, and the established HPLC technique was used to analyse the drug(s) concentration.

The acquired data from these release investigations were fitted to the general release equation (Eq-1) utilising logarithmic transformations and least squares regression analysis to look into the kinetics of drug release.

$$Mt / M\infty = ktn \quad (1)$$

The drug's release at time t is denoted by Mt, and at time ∞ by M∞; the kinetic constant is k, and the diffusion coefficient is n. The goodness of fit (R) criterion was used to determine which model was the best.[28]

6.7.5. Rheological measurements

At 80 revolutions per minute, rheological analysis was performed on the optimised gel formulation (0.5 gm) utilising an R/S CPS Plus Rheometer (Version:9.00, Serial number: #303169, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) and a Spindle C 50-1 (diameter = 50mm). 25±0.5°C was the temperature that was maintained. Rheo3000 was the utilised software. A controlled stress rate investigation

was conducted in two stages (step 1: 1.528-80 rpm, step 2: 80-1.528 rpm) in order to obtain vital data regarding the flow characteristics in response to variations in shear strain and spindle speed (rpm). There was a 180-second delay before the operation. the flow characteristics of the optimised gel formulation sample using a variety of models, including the Newtonian model (Eq-2), the Power Law model (Eq-3), the Bingham model (Eq-4), the Herschel-Bulkley model (Eq-5), and the Casson model (Eq-6).[29]

$$\tau = \eta \gamma \quad (2)$$

$$\tau = K\, yn \quad (3)$$

$$\tau = \tau o + \eta p\, \gamma \quad (4)$$

$$\tau = \tau o + K\, yn \quad (5)$$

$$\tau\ 0.5 = \tau o 0.5 + K\, y0.5 \quad (6)$$

Where, τ - shear stress, γ - shear rate, η - viscosity, ηp - plastic viscosity, τo - yield value, K - consistency index and n - flow behaviour index.[30]

6.7.6. HPLC analysis of Drugs

In this study, we explored the drug concentrations in the in-vitro release and drug content of Gentamycin Sulphate. Additionally, we investigated the combination of Carbopol 934P (CP 934P) and Polaxamer 407 (P407) loaded Nanoemulgels (NEGs). Utilising a technique known as High-Performance Liquid Chromatography (HPLC) allowed for the successful completion of these tasks. The separation of the particles was accomplished by the use of chromatography; specifically, a Capacel Pak C18 Type MG column measuring 250mm x 4.6mm and possessing a particle size of 5 µm was employed. For the purpose of carrying out isocratic elution, a mobile phase was used that was composed of acetonitrile and a buffer that contained 0.02M potassium dihydrogen orthophosphate at a pH of 3.0. The ratio of the mobile phase to the buffer was 40:60. Throughout the whole of the experiment, a flow rate of one millilitre per minute was maintained, and the wavelength at which the detection was performed was measured to be 254 nanometers. Through the use of this technology, it became far less difficult to accurately estimate the quantities of the pharmaceuticals that were included into the formulations that were being researched.[31]

7. RESULTS AND DISCUSSION

7.1. Drug Preformulation studies, characterization & Identification of Gentamycin Sulphate:

7.1.1. Drug Preformulation studies

The organoleptic qualities, melting point, solubility, partition coefficient, and spectroscopic properties of drug samples (GS) were assessed using the following techniques. The results of the previously stated identification tests are shown in Table 7.1. The stated standard values obtained from many monographs—are also given for comparison's purposes.[32]

Table 7.1: Observations for GS sample

Sr. No.	Identification parameters	Observed Value	Reported / Standard Value (Reference)	Remarks
1	Physical Appearance	White, crystalline, odourless, tasteless powder with an irritant dust.	Same as observed value	Complies
2	Melting Point	237°C	Within the range of 220-240 °C (BP 2010: 846)	Complies
3	Solubility	Insoluble in water, soluble in ether, ethanol, octanol, acetone, methanol and ethyl acetate.	Same as observed value (BP 2010: 846)	Complies
4	FTIR spectrum	Principal peaks at Wavenumbers 3200-2500, 2977, 2730, 2880, 1697, 1654, 1596, 1444, 1369, 860-690	Same as observed value (Analytical profile of drug substance by Florey)	Complies
5	UV scan, λmax	In methanol = 202,	Same as observed value(Analytical profile of drug substance by Florey)	Complies

The organoleptic characteristics of gentamycin sulphate were similar to that described in official monograph of gentamycin sulphate. There was no significant difference between experimentally obtained results of saturation solubility, melting point and partition coefficient in comparison with the reported values confirmed the purity of gentamycin sulphate.

Furthermore, high aqueous solubility and high lipophilicity (Log P greater than 1.5) indicated suitability for preparation of nanoemulgel. Molecular weight of gentamycin sulphate is less than 1000 daltons which indicated its suitability for topical administration. The preformulation data obtained indicated suitability of gentamycin sulphate for preparation of nanoemulgel.

7.1.2. Spectrophotometry

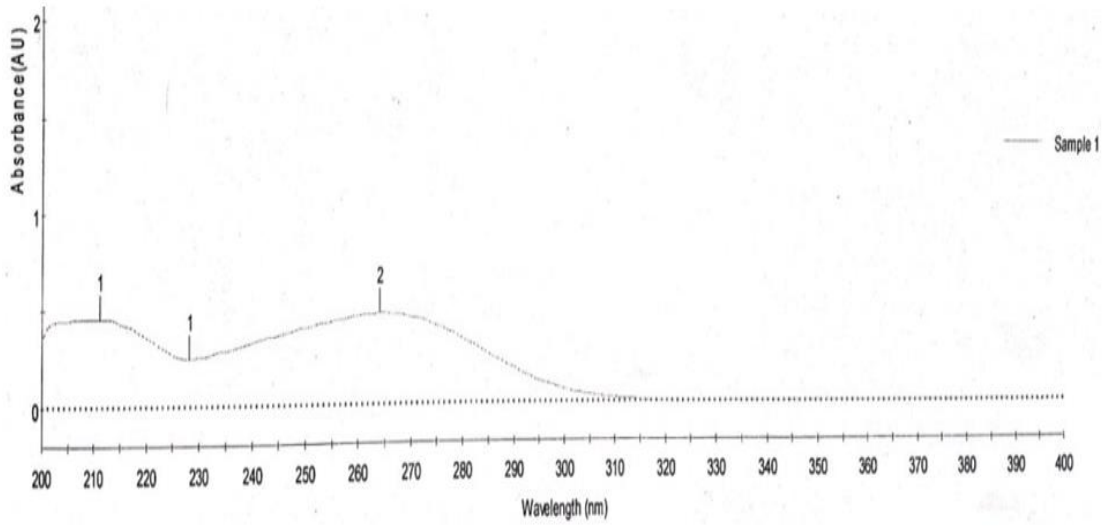


Figure 7.1: Representation of UV spectrum of *Gentamycin Sulphate* powder in methanol (sample)

7.1.2.1. Analytical methodology (Spectrophotometric method)

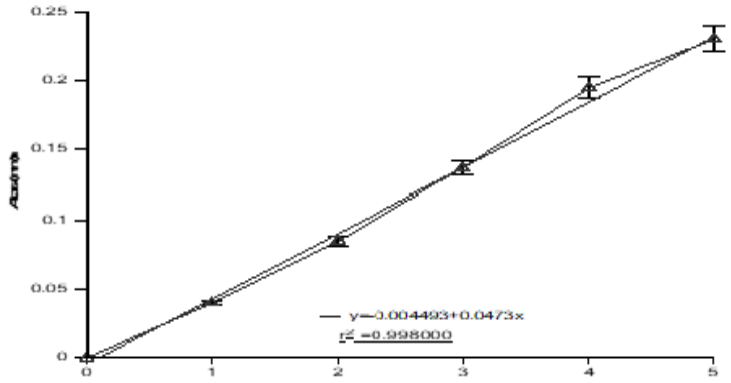


Figure 7.2: Standard curve of Gentamycin Sulphate powder in methanol

Table 7.2: Calibration curve of Gentamycin Sulphate powder in methanol

Sr. No.	Concentration (µg/ml)	Absorbance (nm)
1	1.00	0.0220 ± 0.005
2	2.00	0.0885 ± 0.007
3	3.00	0.1574 ± 0.009
4	4.00	0.1995 ± 0.011
5	5.00	0.2425 ± 0.013

Table 7.3: Analytical parameters calculated from Spectrophotometry of Gentamycin Sulphate powder

Sr. No.	Solvents	Equation of line	λ_{max} (nm)	r^2 value
1.	Ethanol	Y=0.044x	281	0.966
2.	Iso-propyl alcohol (IPA)	Y=0.051x	281	0.978
3.	10% Ethanol in water	Y=0.052x	281	0.989

7.1.2.2. FTIR spectroscopy

FTIR spectrum of drug sample and its reference spectrum were given in the figure (7.3 & 7.4 respectively) measured in the range of 4000 to 400 cm^{-1} through KBr pellet method. Interpretation of FTIR spectra of sample showed that the vibration frequencies observed at 3500, 1600, and 2500 cm^{-1} could be due to functional groups viz. –OH, $-NH_2$, and benzene ring in its chemical structure and found to be almost identical to the reference spectrum of drug Tugarova A.V. et al. [101].

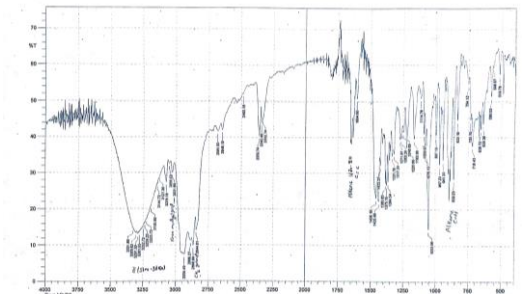


Figure 7.3: FTIR spectrum of procured drug sample (Gentamycin Sulphate)

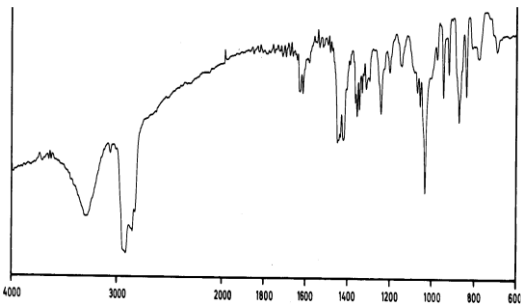


Figure 7.4: FTIR spectrum of Gentamycin Sulphate (reference)

Different physical mixture of Gentamycin Sulphate and excipient ratio (1:1) was taken and FTIR was recorded.

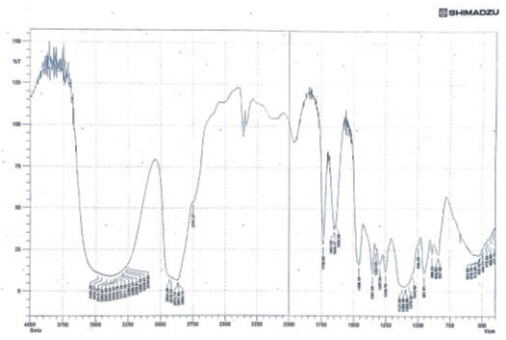


Figure 7.5: FTIR spectrum of S_{mix} Tween-20/PEG-400 (1:1)

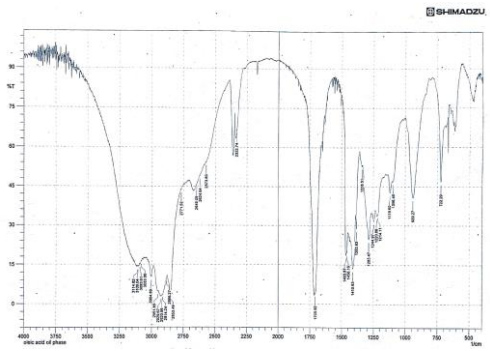


Figure 7.6: FTIR spectrum of oleic acid (oil phase)

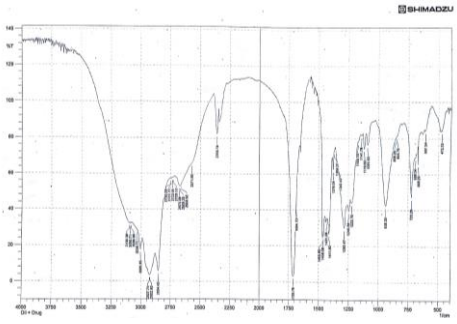


Figure 7.7: FTIR spectrum of mixture containing oleic acid and Gentamycin Sulphate

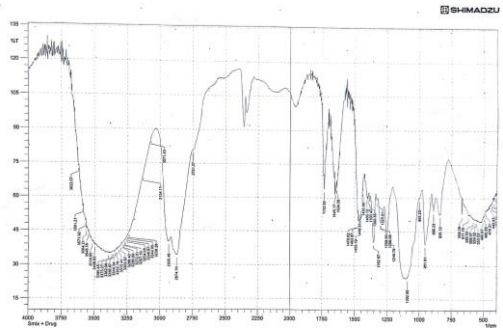


Figure 7.8: FTIR spectrum of mixture containing S_{mix} and drug

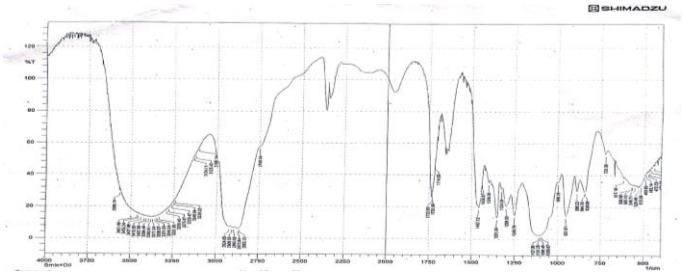


Figure 7.9: FTIR spectrum of mixture containing S_{mix} and oleic acid

7.1.3. Differential scanning calorimetry (DSC)

Differential scanning colorimeter (DSC) characterization of sample was carried out using crippling pan method in the scanning range of 40 to 400°C. Result of DSC analysis is given in the figure 7.10. It indicates from the results that two thermal events were observed; one melting endothermic appeared at 98° C with ΔH_t value 78.147 J/g while second endothermic event observed at 98°C.

Appurtenance of sharp peak at C indicates purity of sample while second exothermic event observed at 220°C revealed that drug sample possesses different polymorph or impurities with high melting range may be present.

Above results are confirmed by melting point determination carried out using capillary tube method, indicated its melting point at 237°C. On the basis of above results, it can be said that the procured sample was Gentamycin Sulphate..

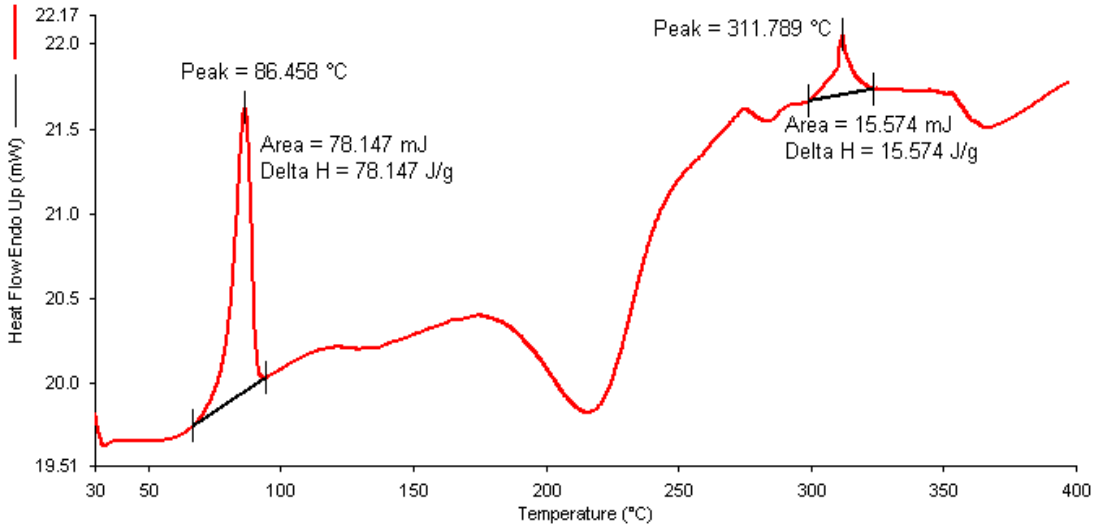


Figure 7.10: DSC thermo gram of Gentamycin Sulphate

7.1.4. Determination of drug solubility

Drug solubility in the various excipients/ solvents, its miscibility with liquid excipients like drug solubility in oil phase, surfactant and co-surfactant is most essential tool to develop a nanoemulsion formulation.

Solubility of drug in different excipients to be required in the formulation development stage is given in table 7.4. Highest solubility of drug was found to be 52 mg/mL, 48 mg/mL and 43 mg/mL in PEG 400, Tween 20, and oleic acid respectively.

Besides the drug solubility in different nanoemulsion excipients, it is essential to investigate the mutual miscibility of excipient-excipient like surfactant, co-surfactant and oil phase components to be used in the subsequent development stage of nanoemulsion formulation. Mutual miscibility is shown in table 7.5.

Table 7.4: Solubility of *Gentamycin Sulphate* powder in oils, surfactant and co-surfactants

Oil		Surfactant		Co-surfactant	
Almond oil	26mg/mL	Tween 80	40mg/mL	Propylene Glycol (PG)	30mg/mL
Sesame oil	30mg/mL	Tween 20	50mg/mL	Polyethylene Glycol 200	34mg/mL
Olive oil	34mg/mL	Span 80	32mg/mL	Polyethylene Glycol 400	50mg/mL
Castor oil	32mg/mL	Span 20	32mg/mL	Transutol IP	38mg/mL
Soybean oil	44mg/mL	Solutol HS-15	32mg/mL	Propylene Glycol (PG)	30mg/mL
Oleic acid	54mg/mL	Tween 20	48 mg/mL	Polyethylene Glycol 400	45 mg/mL

7.1.5. Mutual miscibility of excipients

Table 7.5: Mutual miscibility of *Gentamycin Sulphate* powder in various oil, surfactants and co-surfactants

	With Transutol IP						With PG-400						With PEG						Without solvent					
	A	S	O	C	S	O	A	S	O	C	S	O	A	S	O	C	S	O	A	S	O	C	S	O
T-80	M	M	S	S	M	M	I	M	S	M	S	S	I	M	S	S	M	M	S	S	S	S	S	M
T-20	M	M	M	S	M	I	M	M	M	M	S	M	M	S	I	M	M	M	M	M	M	M	M	M
S-80	M	S	I	S	M	M	M	S	M	M	S	M	M	M	M	S	S	M	I	I	S	I	I	M
S-20	M	S	S	S	S	M	M	S	I	S	M	M	I	M	S	S	M	M	I	I	S	I	S	M
SL15	M	I	I	I	S	M	S	S	I	S	S	I	I	I	I	S	I	S	S	S	S	S	S	S

A-Almond oil, S- Sesame oil, O- Olive oil, C- Castor oil, S- Soybean oil, O- Oleic acid, T-80- Tween-80, T-20- Tween-20, S-80- Span-80, S-20- Span-20, SL-15- Solutol HS-15, PG- Propylene Glycol, PEG- Polyethylene Glycol, I- Immiscible, S- Slightly miscible, M- Miscible

7.2. Excipients selected for nanoemulsion formulation

Oil & S_{mix} both are selected as the as per the solubility and miscibility report, shown in the table 7.4 & 7.5.

7.3. Aqueous Dispersibility of pre-concentrate mix

Table 7.6: Trial A composition prepared using S_{mix} with almond oil

Trial	Smix (mg)		Smix Ratio	Almond oil (mg)	Water (mg)	Visually Examination
	Tween 80	PEG 400				
A 1	100	100	1:1	50	70	Turbid
A 2	400	100	4:1	100	70	Turbid
A 3	100	200	1:2	100	56	Turbid
A 4	100	300	1:3	100	84	Turbid
A 5	300	200	3:2	100	98	Turbid

Table 7.7: Trial B composition prepared using S_{mix} (1:1) with almond oil

Trial	Almond oil (mg)	Smix (mg)	Water (mg)	Visual Examination
B 1	80	400	560	Turbid
B 2	80	400	560	Turbid
B 3	80	400	490	Turbid
B 4	80	400	420	Turbid
B5	80	400	420	Turbid

(Smix= Tween 20 / PEG 400, 1:1)

Table 7.8: Trial C composition prepared using S_{mix} (1:1) with olive oil

Trial	Olive oil (mg)	Smix (mg)	Water (mg)	Visual Examination
C 1	80	400	560	Clear
C 2	80	400	574	Clear
C 3	80	400	588	Clear
C 4	80	400	602	Turbid
C 5	80	400	630	Turbid

(Smix= Tween 20 / PEG 400, 1:1)

Table 7.9: Trial D composition prepared using S_{mix} (1:1) with castor oil

Trial	Castor oil (mg)	Smix (mg)	Water (mg)	Visual Examination
D 1	80	400	560	Turbid
D 2	80	400	574	Turbid
D 3	80	400	490	Turbid
D 4	80	400	420	Turbid
D 5	80	400	420	Turbid

(Smix= Tween 20 / PEG 400, 1:1)

Table 7.10: Trial E composition prepared using S_{mix} (1:1) with soybean oil

Trial	Soybean oil (mg)	Smix (mg)	Water (mg)	Visual Examination
E 1	80	400	560	Clear
E 2	80	400	574	Turbid
E 3	80	400	588	Turbid
E 4	80	400	602	Turbid
E 5	80	400	630	Turbid

(Smix= Tween 20 / PEG 400, 1:1)

Table 7.11: Trial F composition prepared using S_{mix} (1:1) with sesame oil

Trial	Sesame oil (mg)	Smix (mg)	Water (mg)	Visual Examination
F 1	80	400	560	Clear
F 2	80	400	574	Turbid
F 3	80	400	588	Turbid
F 4	80	400	602	Turbid
F 5	80	400	630	Turbid

(Smix= Tween 20 / PEG 400, 1:1)

Table 7.12: Trial G composition prepared using S_{mix} (1:1) with oleic acid

Trial	Oleic oil (mg)	Smix (mg)	Water (mg)	Visual Examination
G 1	80	400	560	Clear
G 2	80	400	574	Turbid
G 3	80	400	588	Turbid
G 4	80	400	602	Turbid
G 5	80	400	630	Turbid

(Smix= Tween 80 / PG, 1:1)

Table 7.13: Trial H composition prepared using S_{mix} (1:1) with oleic acid

Trial	Oleic oil (mg)	Smix (mg)	Water (mg)	Visual Examination
H 1	80	400	560	Clear
H 2	80	400	574	Clear
H 3	80	400	588	Turbid
H 4	80	400	602	Turbid
H 5	80	400	630	Turbid

(Smix= Tween 80 / PEG400, 1:1)

Table 7.14: Trial I composition prepared using S_{mix} (1:1) with oleic acid

Trial	Oleic oil (mg)	Smix (mg)	Water (mg)	Visual Examination
I 1	80	400	560	Clear
I 2	80	400	574	Clear
I 3	80	400	588	Clear
I 4	80	400	602	Clear
I 5	80	400	630	Turbid

(Smix= Tween 20 / PEG 400, 1:1)

Table 7.15: Trial J composition prepared using S_{mix} (1.5:1) with oleic acid

Trial	Oleic oil (mg)	Smix (mg)	Water (mg)	Visual Examination
J 1	40	360	500	Clear
J 2	40	360	530	Clear
J 3	40	360	560	Clear
J 4	40	360	580	Clear
J 5	40	360	620	Clear

(Smix= Tween 20 / PEG 400, 1.5:1)

Table 7.16: Trial K composition prepared using S_{mix} (1:1.5) with oleic acid

Trial	Oleic oil (mg)	Smix (mg)	Water (mg)	Visual Examination
K 1	80	360	500	Clear
K 2	80	360	530	Clear
K 3	80	360	560	Clear
K 4	80	360	580	Clear
K 5	80	360	620	Clear

(Smix= Tween 20 / PEG 400, 1:1.5)

Table 7.17: Trial L composition prepared using S_{mix} (2:1) with oleic acid

Trial	Oleic oil (mg)	Smix (mg)	Water (mg)	Visual Examination
L 1	80	360	500	Clear
L 2	80	360	530	Clear
L 3	80	360	560	Clear
L 4	80	360	590	Clear
L 5	80	360	650	Clear

(Smix= Tween 20 / PEG 400, 2:1)

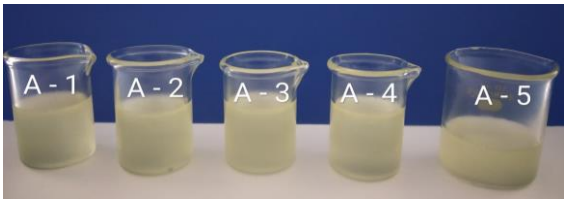


Figure 7.11: Preconcentrate mix consisted of Tween 80 / PEG 400 and almond oil upon aqueous dispersion

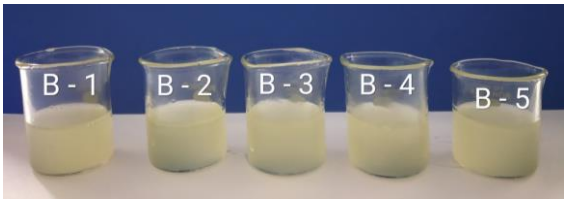


Figure 7.12: Preconcentrate mix consisted of S_{mix} and almond oil upon aqueous dispersion

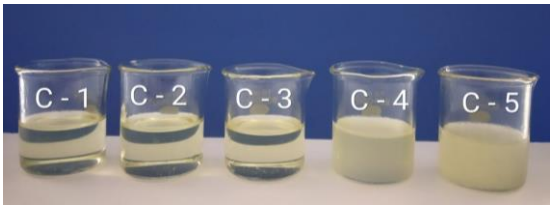


Figure 7.13: Preconcentrate mix consisted of S_{mix} and olive oil upon aqueous dispersion

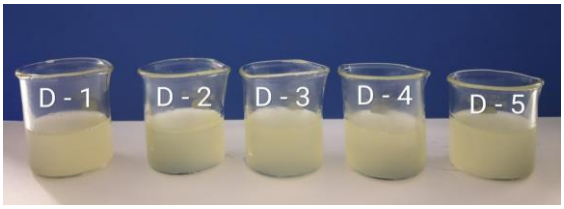


Figure 7.14: Preconcentrate mix consisted of S_{mix} and castor oil upon aqueous dispersion

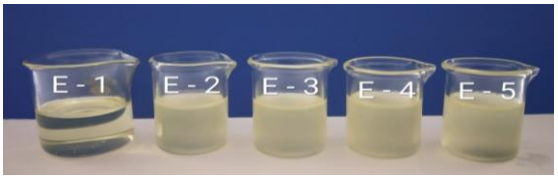


Figure 7.15: Preconcentrate mix consisted of S_{mix} and soybean oil upon aqueous dispersion

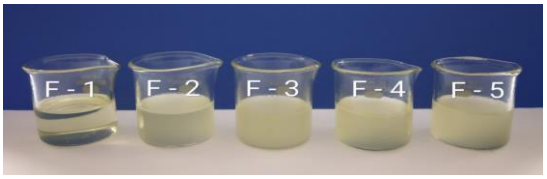


Figure 7.16: Preconcentrate mix consisted of S_{mix} and sesame oil upon aqueous dispersion

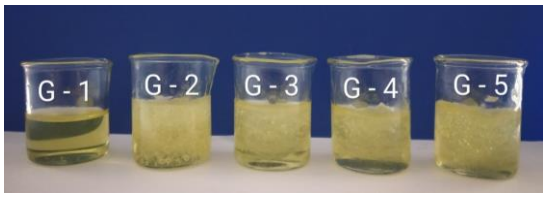


Figure 7.17: Preconcentrate mix consisted of S_{mix} and oleic acid upon aqueous dispersion

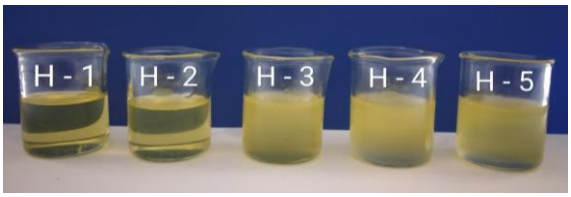


Figure 7.18: Preconcentrate mix consisted of S_{mix} and oleic oil upon aqueous dispersion

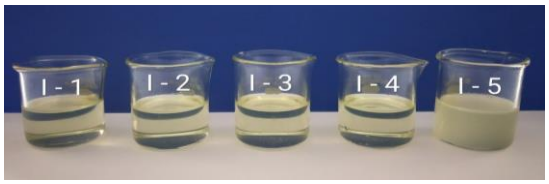


Figure 7.19: Preconcentrate mix consisted of S_{mix} and oleic oil upon aqueous dilutions

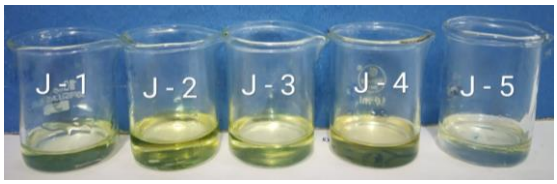


Figure 7.20: Preconcentrate mix consisted of S_{mix} and oleic oil upon aqueous dispersion

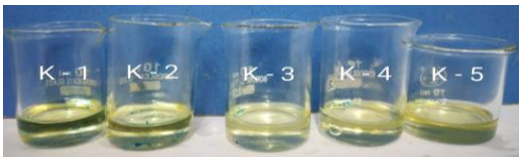


Figure 7.21: Preconcentrate mix consisted of S_{mix} and oleic oil upon aqueous dispersion

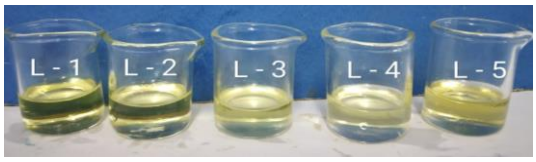


Figure 7.22: Preconcentrate mix consisted of S_{mix} and oleic oil upon aqueous dispersion

Table 7.18: Summary of different trial batches

Trial	Smix components	Smix ratio	Oil type	Water (mg)	Visual Exam
A	Tween 80/ PEG 400	1:1	Almond oil	Infinite	Turbid
B	Tween 20/ PEG 400	1:1	Almond oil	Infinite	Turbid
C	Tween 20/ PEG 400	1:1	Olive oil	Infinite	Clear
D	Tween 20/ PEG 400	1:1	Castor oil	Infinite	Turbid
E	Tween 20/ PEG 400	1:1	Soybean oil	Infinite	Turbid
F	Tween 20/ PEG 400	1:1	Sesame oil	Infinite	Turbid
G	Tween 80/ PG	1:1	Oleic acid	Infinite	Turbid
H	Tween 80/ PEG 400	1:1	Oleic acid	Infinite	Turbid
I	Tween 20/ PEG 400	1:1	Oleic acid	Infinite	Clear
J	Tween 20/PEG 400	1.5:1	Oleic acid	Infinite	Clear
K	Tween 20/PEG 400	1:1.5	Oleic acid	Infinite	Clear
L	Tween 20/PEG 400	2:1	Oleic acid	Infinite	Clear

Systems containing Span 80 or Span 20 yielded turbid systems

Various pre-concentrate mix consisted of surfactant, co-surfactant and oil phase upon dilution with fixed quantity of water were subject to visually examination (aqueous dilution test). Appearance of clear dispersion upon aqueous dilution indicated that formation of nanoemulsion resulted due to solubilization of oil phase in surfactant co-solvent system. However, loss of clarity in the system indicates formation of coarse dispersion (coarse-emulsion) in spite to nanoemulsion formation.

7.4. Construction of Ternary Phase Diagram

Ternary phase diagram is the mapping tool of various ternary components mixed at specific proportions which command the formation of micellar, reverse micellar, nanoemulsion, bicontinous systems and coarse-emulsion.

Such plots are very helpful in the preformulation studies on nanoemulsion in the identification of possible dispersion system were formed when a particular proportion of S_{mix}, oil and water phase taken

Table 7.19: Ternary phase diagram drawn at (1:1) Smix ratio, oleic acid and water

Sr. No	Oil (mg)	Smix (mg)	Water (mg)	Oil %	Smix %	Water %	Visual examination
1	20	100	480	3.33	16.67	80.00	Clear
2	30	70	185	10.53	24.56	64.91	Clear
3	40	60	65	24.24	36.36	39.40	Clear
4	20	80	65	12.12	48.48	39.40	Clear
5	20	160	97	7.22	57.76	35.02	Clear
6	10	90	55	6.45	58.06	35.49	Clear
7	20	120	60	10.00	60.00	30.00	Clear
8	20	140	70	8.70	60.87	30.43	Clear

Table 7.20: Ternary phase diagram drawn at (1:1.5) Smix ratio, oleic acid and water

Sr. No.	Oil (mg)	Smix (mg)	Water (mg)	Oil %	Smix %	Water %	Visual examination
1	20	140	30	10.53	73.68	15.79	Clear
2	20	160	45	8.89	71.11	20.00	Clear
3	20	120	35	11.43	68.57	20.00	Clear
4	10	90	35	7.41	66.67	25.92	Clear
5	20	80	45	13.79	55.17	31.04	Clear
6	40	60	25	32.00	48.00	20.00	Clear
7	30	70	65	18.18	42.42	39.40	Clear
8	20	100	225	5.8	28.99	65.21	Clear

Table 7.21: Ternary phase diagram drawn at (1.5:1) Smix ratio, oleic acid and water

Sr.No.	Oil (mg)	Smix (mg)	Water (mg)	Oil %	Smix %	Water %	Visual examination
1	20	140	107	7.49	52.43	40.08	Clear
2	10	90	80	5.56	50.00	44.44	Clear
3	20	160	180	5.56	44.44	50.00	Clear
4	20	120	172	6.41	38.46	55.13	Clear
5	20	80	120	9.09	36.36	54.55	Clear
6	40	60	120	18.18	27.27	54.55	Clear
7	30	70	235	8.96	20.90	70.14	Clear
8	20	100	360	4.17	20.83	75.00	Clear

Table 7.22: Ternary phase diagram drawn at (1:2) Smix ratio, oleic acid and water

Sr. No.	Oil (mg)	Smix (mg)	Water (mg)	Oil %	Smix %	Water %	Visual examination
1	10	90	25	8	72.00	20.00	Clear
2	20	140	40	10	70.00	20.00	Clear
3	20	160	60	8.33	66.67	25.00	Clear
4	20	80	65	12.12	48.48	39.40	Clear
5	40	60	45	27.59	41.38	31.03	Clear
6	20	40	60	16.67	38.89	44.44	Clear
7	20	100	180	6.67	33.33	60.00	Clear
8	20	120	560	2.86	17.14	80.00	Clear

Table 7.23: Ternary phase diagram drawn at (2:1) Smix ratio, oleic acid and water

Sr. No.	Oil (mg)	Smix (mg)	Water (mg)	Oil %	Smix %	Water %	Visual examination
1	20	160	45	8.89	71.11	20.00	Clear
2	10	90	35	7.41	66.67	25.92	Clear
3	20	140	70	8.7	60.87	30.43	Clear
4	20	80	35	14.81	59.36	25.83	Clear
5	30	70	45	20.69	48.28	31.03	Clear
6	40	60	25	32.00	48.00	20.00	Clear
7	20	100	120	8.33	41.67	50.00	Clear
8	20	120	330	4.26	25.53	70.21	Clear

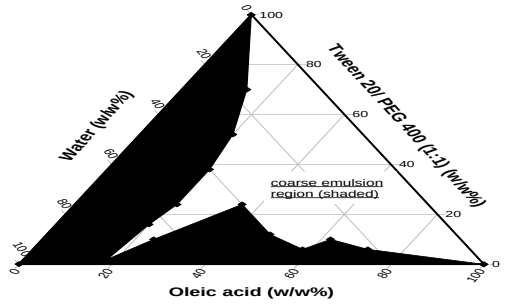


Figure 7.23: Ternary phase drawn at (1:1) S_{mix} ratio, oleic acid and water

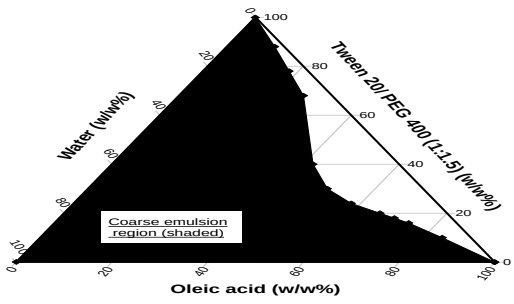


Figure 7.24: Ternary phase drawn at (1:1.5) S_{mix} ratio, oleic acid and water

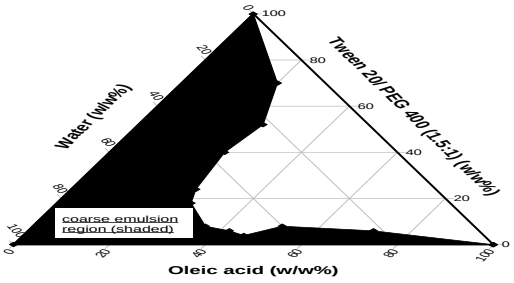


Figure 7.25: Ternary phase drawn at (1.5:1) S_{mix} ratio, oleic acid and water

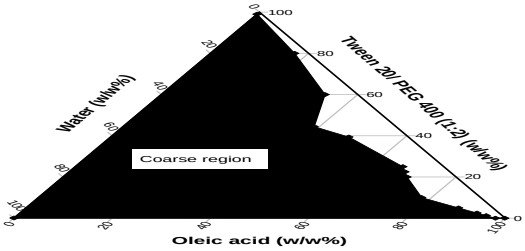


Figure 7.26: Ternary phase drawn at (1:2) S_{mix} ratio, oleic acid and water

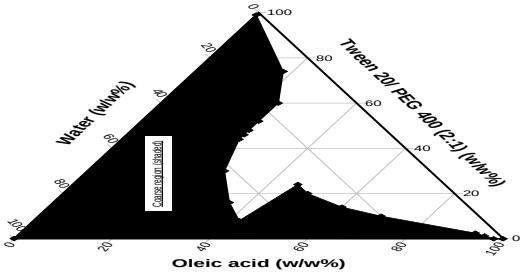


Figure 7.27: Ternary phase drawn at (2:1) S_{mix} ratio, oleic acid and water

7.5. Formulation of Gentamycin Sulphate Nanoemulsions

A 3-factor 3-level Box-Behnken statistical design, utilizing Expert Software (Version 8.0.7.1, Stat-Ease Inc, Minneapolis, MN), was employed to investigate quadratic response surfaces and formulate second-order polynomial equations. This approach

aimed to optimize the nanoemulsion system by systematically exploring the effects of multiple factors and their interactions.

7.5.1. Procedure for preparation of nanoemulsion

Table 7.24: Design and composition of the nanoemulsion formulations showing various components

Formulation code	Oil (mg)	Smix (mg)	Fixed amount of drug (mg)	Total weight* (mg)
Smix = Tween20/PEG 400 (1:1); oil phase (Oleic acid)				
F1 (20:80)	26.8	107.2	1	135
F2 (25:75)	33.7	100.3	1	135
F3 (30:70)	40.5	93.5	1	135
F4 (10:90)	13.4	120.6	1	135
F5 (15:85)	20.2	113.8	1	135
F6 (12:88)	16.2	117.8	1	135
Smix = Tween20/PEG 400 (1.5:1); oil phase (Oleic acid)				
F7 (20:80)	26.8	107.2	1	135
F8 (10:90)	13.4	120.6	1	135
F9 (15:85)	20.2	113.8	1	135
F10 (12:88)	16.2	117.8	1	135
F11 (25:75)	33.7	100.3	1	135
F12 (30:70)	40.5	93.5	1	135
Smix = Tween20/PEG 400 (2:1); oil phase (Oleic acid)				
F13 (20:80)	26.8	107.2	1	135
F14 (25:75)	33.7	100.3	1	135
F15 (30:70)	40.5	93.5	1	135
F16 (10:90)	13.4	120.6	1	135
F17 (15:85)	20.2	113.8	1	135

* Fixed amount of water (14mg) was added to each formulation

7.6. Physical characterization of Nanoemulsion Formulation

7.6.1 Droplet size distribution

Table 7.25: Droplet size and PDI of the formulation (Zeta sizer)

Formulations	Droplet size (nm)	PDI
F1	13.77	0.197
F2	15.9	0.125
F3	14.8	0.128
F4	14.6	0.16
F5	13.8	0.1255
F6	13.15	0.1147
F7	12.5	0.1039
F8	11.85	0.0931
F9	11.2	0.0823
F10	10.55	0.0715
F11	9.9	0.0607
F12	9.25	0.0499
F13	14.7	0.123
F14	7.95	0.0283
F15	7.3	0.0175
F16	6.65	0.0067
F17	15.7	0.125

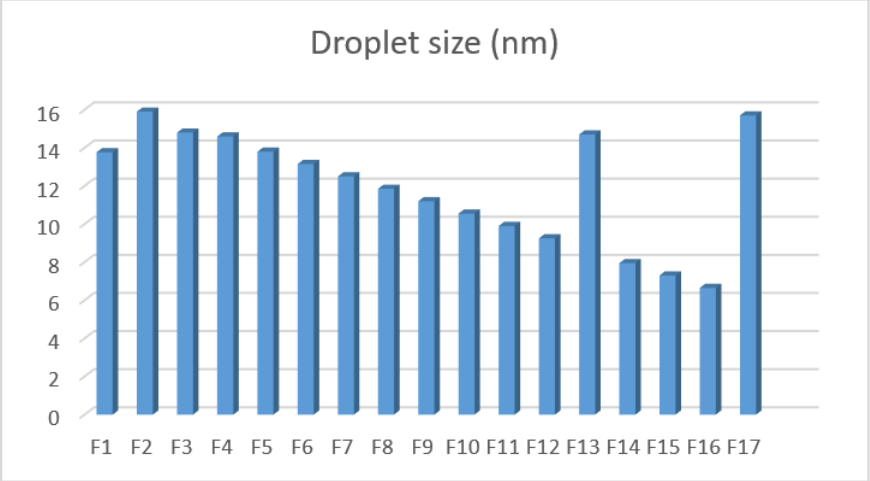


Figure 7.27: Droplet size chart

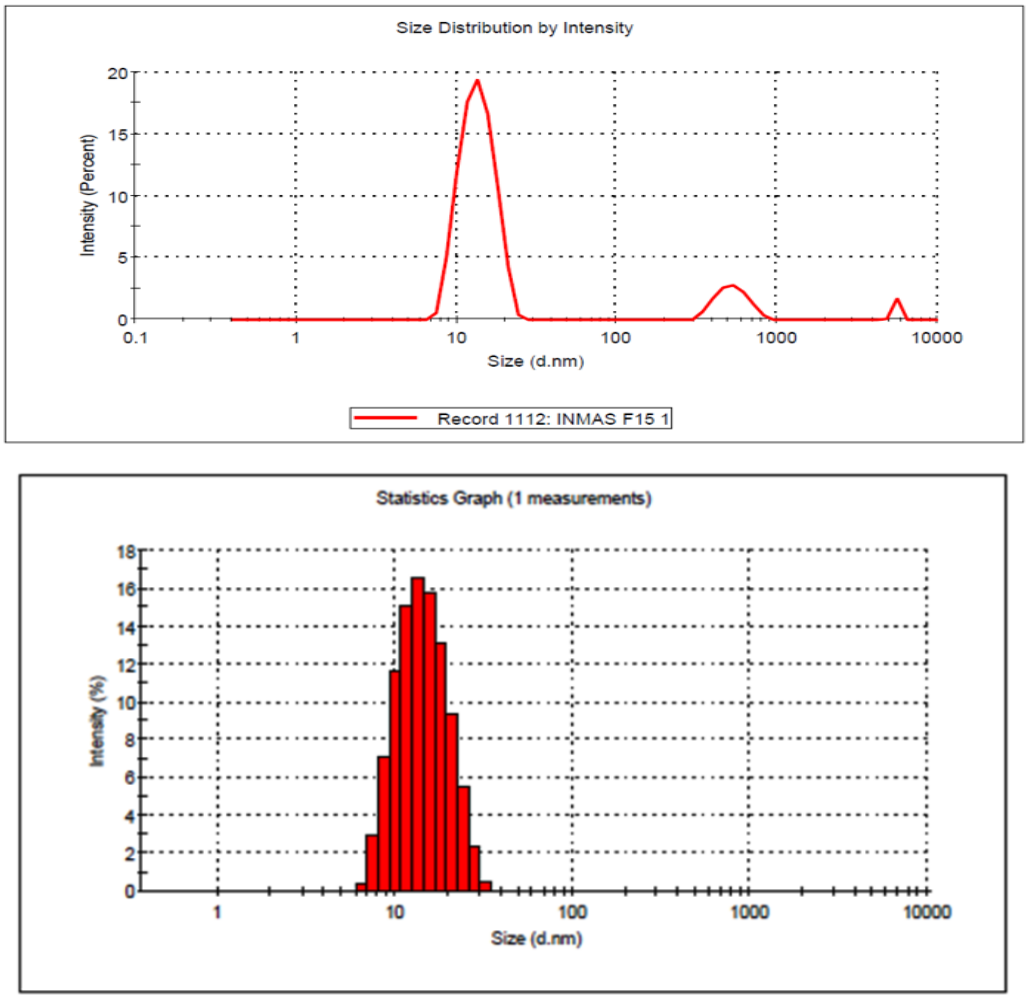


Figure 7.29: Particle size distribution of optimized nanoemulsion formulation

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -26.1	Peak 1:	-26.1	100.0	7.09
Zeta Deviation (mV): 7.09	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0817	Peak 3:	0.00	0.0	0.00

Result quality Good

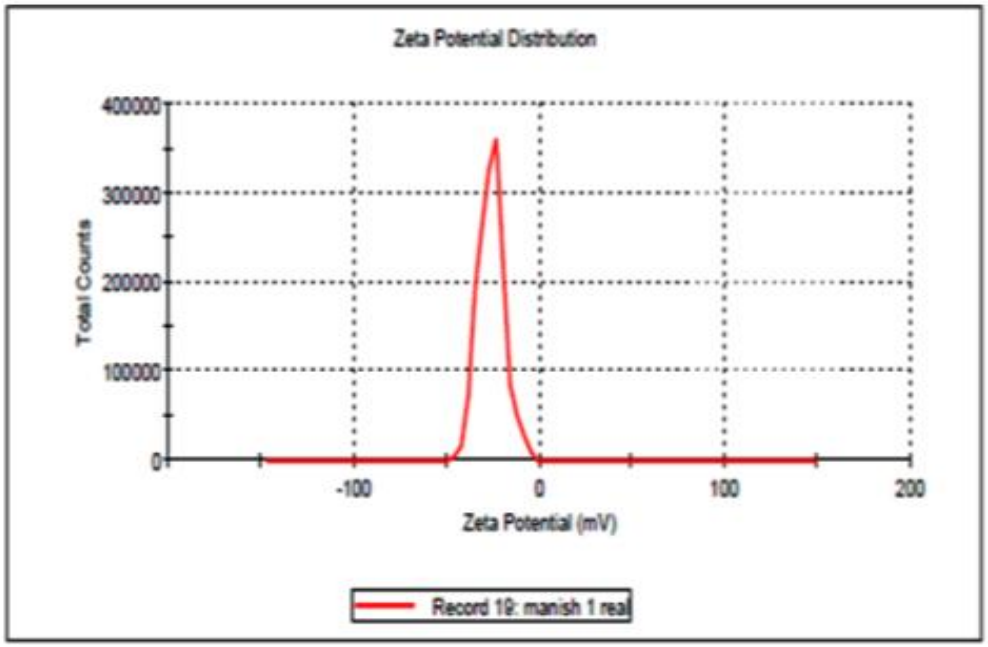


Figure 7.30: Zeta potential of optimized nanoemulsion formulation

7.6.2. Measurement of Electrical Conductivity of Formulation

Table 7.26: Variations in the electrical conductivity values of the formulations incorporating of aqueous phase dilutions

Formulations	Addition of water (µL)				
	50	100	150	200	250
F1	1.6	3.0	3.5	2.2	1.8
F2	0.6	0.9	1.9	1.5	1
F3	0.6	1.5	2.8	2.3	0.9
F4	0.5	0.6	1.7	1.4	0.8
F5	3.0	2.2	1.5	0.6	3.0
F6	0.9	1.5	2.8	1.7	2.2
F7	1.5	2.3	2.3	1.4	1.5
F8	0.6	1.4	0.9	0.8	0.6
F9	1.2	1.5	1.3	0.8	1.5
F10	1.2	1.5	1.1	0.6	1.5
F11	1.2	1.5	0.9	0.5	1.5
F12	1.2	1.4	0.7	0.3	1.5
F13	1.3	1.4	0.5	0.2	1.5
F14	1.3	1.4	2.3	2.3	1.5
F15	1.3	1.4	0.9	0.9	1.5
F16	1.3	1.3	1.3	1.3	1.5
F17	1.3	1.3	1.1	1.1	1.5

ISSN 1462 2815

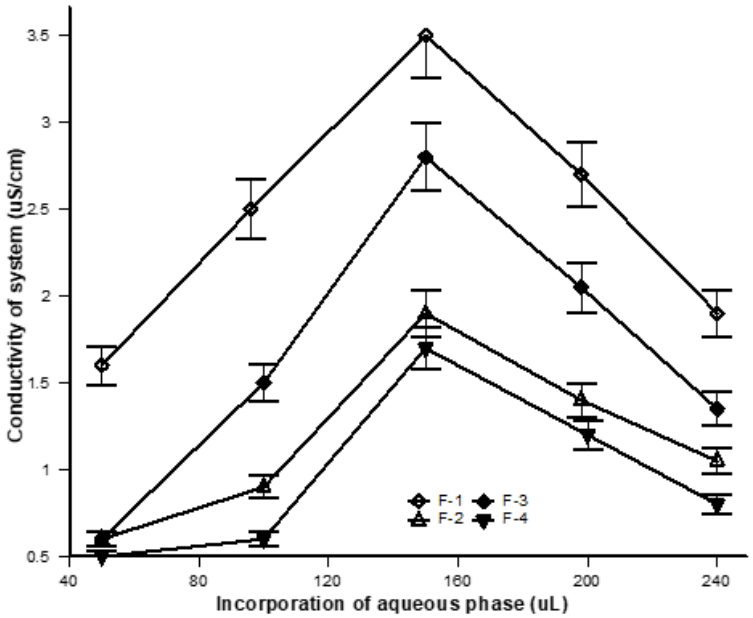


Figure 7.31: Electrical conductivity of F1, F2, F3, and F4, at different aqueous phase dilution

7.6.3. Determination of Refractive Index

Table 7.27: Refractive index of formulations with addition of aqueous phase dilution

Formulations	Addition of water (µL)				
	50	100	150	200	250
F1	1.445	1.447	1.449	1.448	1.447
F2	1.451	1.451	1.453	1.453	1.451
F3	1.443	1.447	1.456	1.45	1.449
F4	1.449	1.451	1.453	1.453	1.456
F5	1.447	1.448	1.449	1.449	1.453
F6	1.451	1.45	1.447	1.453	1.449
F7	1.449	1.451	1.451	1.456	1.447
F8	1.447	1.449	1.447	1.453	1.451
F9	1.451	1.453	1.451	1.449	1.447
F10	1.448	1.456	1.447	1.447	1.451
F11	1.45	1.453	1.451	1.451	1.448
F12	1.453	1.449	1.447	1.448	1.45
F13	1.456	1.447	1.451	1.45	1.451
F14	1.453	1.451	1.448	1.447	1.449
F15	1.447	1.447	1.45	1.451	1.447
F16	1.451	1.451	1.453	1.448	1.451
F17	1.448	1.448	1.456	1.45	1.448

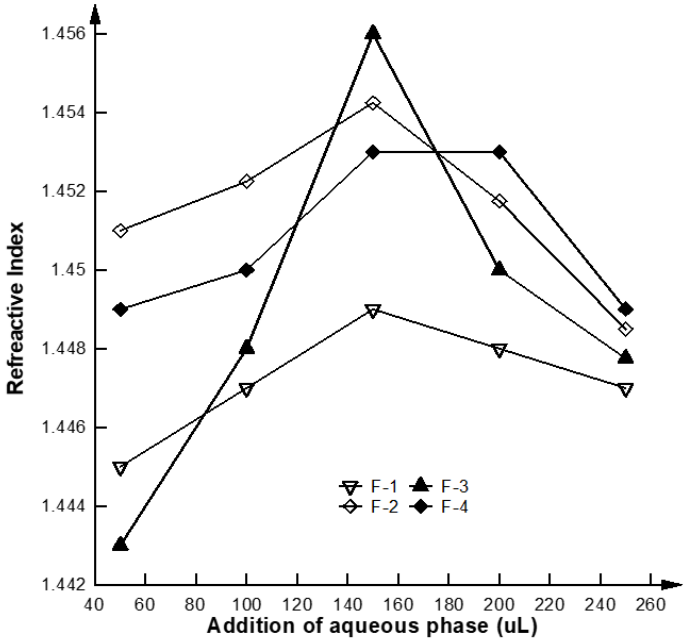


Figure 7.32: Change in refractive index of formulations with addition of aqueous phase dilutions

7.6.4. Transmission electron microscopic:

Through the use of transmission electron microscopy (TEM) research, further information on the shape and distribution of the particles that comprise the nanoemulsion systems was collected.

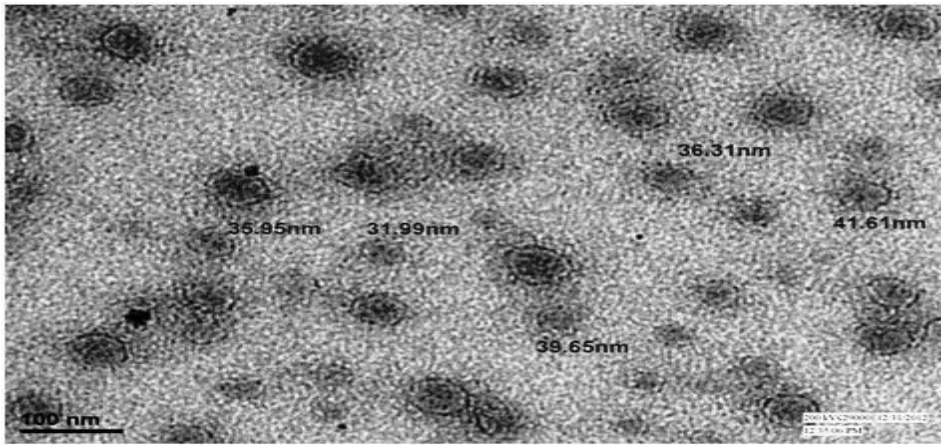


Figure 7.33: TEM photomicrographs of optimized nanoemulsion

7.7. Preparation of nanoemulgel

Carbopol 934P (CP 934P) and Polaxamer 407 (P407) were the two components that were blended together to create the combination gel. This was accomplished by first dissolving CP934P in distilled water at concentrations of 0.25, 0.50%, and 1% by weight. This was accomplished with the use of a mechanical stirrer. P407 was added to this gel at doses of 15, 20, and 25% by weight after it had been completely dissolved. The combination was then maintained at a temperature of four degrees Celsius for a period of twelve hours in order to ensure that the cold approach was used to ensure full soaking.

Following that, the formulations were blended in order to guarantee that the two components mixed together in an effective manner. After that, a nanoemulsion that had been optimised and loaded with the medication was added to the sol system in a drop-by-drop fashion while magnetic stirring was taking place continuously. Last but not least, the nanoemulsion was neutralised with TEA and then kept at a temperature of 4 degrees Celsius for a period of twenty-four hours.[59-77]

Note: Taking those formulations having lower particle size will only be considered.

Table 7.27: Composition of all the nanoemulgels of GS for Ophthalmic delivery

Optimoized formula / Composition	A1*	A2*	A3*	A4*	A5*	A6*	A7*	A8*	A9*
CP 974P (%)	0.3	0.5	1	0.3	0.5	1	0.3	0.5	1
Polaxamer	15	20	25	15	20	25	15	20	25
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben	0	0	0	0	0	0	0	0	0
Purified water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
*Oil phase containing 4 % w/w of GS in NE and all the formulation containing NE: gel (1:1) ratio									

7.7.1. Drug content and pH determination

A wide range of consistency could be obtained using the GS loaded nanoemulgel formulations, which had different concentrations of P407 and C934P polymers. As per the observational results, the drug content of every formulation, from F1 to F9, was found to be almost same and fell between 97.82 ± 0.88% and 100.23 ± 0.54%. The pH values of all the nanoemulgels were found to be practically neutral, ranging from 6.56±0.004 to 6.74±0.006, as shown in Table 7.28. This range demonstrated that the nanoemulgels could administer periodontal drugs without irritating the gums.

7.7.2. Sol-gel transition, mucoadhesive strength and syringeability studies

The sol-gel transition was found to occur between 20.91 ± 0.31C (lowest in A9) and 37.21 ± 0.34C (highest in A1). The gelling time varied between 26.33 ± 0.34 and 39.42 ± 0.63 seconds. The syringeability test was not passed by the formulations containing 1% CP934P (A3, A6, and A9), and their gelation temperature was below 25ºC due to the stiff network of the polymer. Formations A5 and A8 were selected for further study since A1, A2, A4, and A7 were discovered to have less syringe-ability features. (Table 7.28).

Table 7.28: Pharmaceutical characteristics properties of all NEG formulations like pH determination, gelling temperature, gelling time, % drug content and syringe ability

Optimoized formula /Composition	pH Determination	Gelling Temperature (°C)	Gelling Time (sec)	% Drug content	Syringe ability
A1*	6.71±0.021	37.21 ± 0.34	39.42 ± 0.63	99.52 ± 0.92	Passed
A2*	6.64±0.031	33.27 ± 0.53	32.21 ± 0.24	98.82 ± 1.11	Passed
A3*	6.64±0.01	20.21 ± 0.31	26.33 ± 0.34	99.56 ± 0.87	Failed
A4*	6.74±0.021	37.11 ± 0.21	39.42 ± 0.63	99.52 ± 0.92	Passed
A5*	6.63±0.021	33.77 ± 0.53	32.21 ± 0.28	98.82 ± 1.11	Passed
A6*	6.64±0.12	20.21 ± 0.35	26.37 ± 0.34	99.56 ± 0.87	Failed
A7*	6.73±0.022	37.21 ± 0.32	39.42 ± 0.63	99.52 ± 0.99	Passed
A8*	6.64±0.031	29.87 ± 0.50	32.21 ± 0.29	98.82 ± 1.16	Passed
A9*	6.55±0.33	20.91 ± 0.31	26.33 ± 0.34	99.56 ± 0.88	Failed

8. CONCLUSION

In conclusion, this study represents a significant stride in the realm of pharmaceutical sciences, specifically in the development and assessment of Gentamicin Sulphate Ophthalmic Nanoemulgel as a novel and promising pharmaceutical delivery system.

The integration of nanotechnology with a gel-based matrix offers a multifaceted approach to enhancing ocular drug delivery, addressing challenges associated with bioavailability and therapeutic efficacy.

Through meticulous formulation and characterization, the study achieved a nanoemulgel with favourable physicochemical properties, including a controlled particle size, appropriate zeta potential, and sustained drug release kinetics.

The stability and rheological assessments confirmed the robustness and suitability of the formulation for ophthalmic applications, a crucial aspect for ensuring patient compliance and effective therapeutic outcomes.

This study's findings collectively underscore the potential of Gentamicin Sulphate Ophthalmic Nanoemulgel as an advanced and viable alternative for ocular drug delivery.

The success of this comprehensive investigation paves the way for future advancements in nanoemulgel formulations, fostering innovation in pharmaceutical sciences and contributing to the enhancement of therapeutic strategies for ocular diseases.

As research in this domain progresses, the outcomes of this study stand as a foundational contribution to the evolving landscape of novel pharmaceutical delivery systems.

Conflict of Interest:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgement:

I would like to express my sincere gratitude to my guide, Dr. Prabhakar Vishvakarma, for his invaluable guidance, continuous support, and expertise throughout the course of this research. His encouragement, insightful feedback, and dedication have been instrumental in shaping the progress and success of this work.

I also extend my heartfelt thanks to the Department of Pharmacy at IIMT College of Medical Sciences, IIMT University, Meerut for providing the necessary resources and a conducive research environment. The academic excellence and collaborative spirit of the department have significantly contributed to the quality and depth of this research endeavour.

I am thankful for the opportunity to be a part of such an esteemed institution, and I appreciate the encouragement and cooperation extended by all faculty members and fellow researchers.

Funding: None.

Ethics approval and consent to participate: Not applicable.

Human and animal rights: Not applicable.

Consent for publication: Not applicable.

Code availability: Not applicable.

List of Abbreviations:

Abbreviation	Full Form
µm	Micron meter
nm	Nanometre
mg	Milligram
g	Gram
HLB	Hydrophilic lipophilic balance
%	Percentage
SC	Secretory compartment
DSC	Differential scanning calorimetry
C_{max}	Maximum concentration
AUC	Area under curve
°C	Degree Centigrade
L	litter
ng/ml	Nanogram per millilitre
T_{max}	Transport maximum
g/ml	Gram per millilitre
h	Hours
N_{mix}	surfactant and co-surfactant

References

1) Lieberman, H.A., M.M. Rieger, and G.S. Banker, Pharmaceutical Dosage Forms Disperse Systems Emulsion and Microemulsions, 2014(2), 335-369.

2) Chellapa P, Mohamed AT, Keleb EI, Elmahgoubi A, Eid AM, Issa YS, Elmarzugi NA. Nanoemulsion and nanoemulgel as a topical formulation. IOSR J Pharm. 2015 Oct;5(10):43-7.

3) Marti-Mestres G, Nielloud F. Emulsions in health care applications—an overview. Journal of dispersion science and technology. 2002 Jan 1;23(1-3):419-39.

4) Singh RP, Parpani S, Narke R, Chavan R. Emulgel: A recent approach for topical drug delivery system. Asian Journal of Pharmaceutical Research and Development. 2014 Mar 1:112-23.

5) Jain A, Deveda P, Vyas N, Chauhan J, Khambete H, Jain S. Development of antifungal emulsion based gel for topical fungal infection (s). IJPRD. 2011;2(12):18-22.

6) Verma S, Singh AK, Mukharjee A. Formulation and evaluation of ketoconazole nanoemulgel. World journal of pharmacy and pharmaceutical sciences. 2016;5(2):899-911.

7) Joshi B, Singh G, Rana AC, Saini S, Singla V. Emulgel: a comprehensive review on the recent advances in topical drug delivery. Int Res J Pharm. 2011;2(11):66-70.

8) Panwar N. Upadhyay, M Bairagi, S Gujar, G Darwhekar Emulgel. A Review. Asian Journal of Pharmacy and Life Science 2011; 1(3): 333-43.

9) Bandyopadhyay S, Katare OP, Singh B. Optimized self-nanoemulsifying systems of ezetimibe with enhanced bioavailability potential using long chain and medium chain triglycerides. Colloids Surf B Biointerfaces 2012; 100: 50-61. [http://dx.doi.org/10.1016/j.colsurfb.2012.05.019] [PMID: 22766282]

10) Singh Y, Meher JG, Raval K, *et al.* Nanoemulsion: Concepts, development and applications in drug delivery. J Control Release 2017; 252: 28-49. [http://dx.doi.org/10.1016/j.jconrel.2017.03.008] [PMID: 28279798]

11) Calder PC. Hot topics in parenteral nutrition. Rationale for using new lipid emulsions in parenteral nutrition and a review of the trials performed in adults. Proc Nutr Soc 2009; 68(3): 252-60. [http://dx.doi.org/10.1017/S0029665109001268] [PMID: 19426581]

12) Manuel-y-Keenoy B, Nonneman L, De Bosscher H, *et al.* Effects of intravenous supplementation with alpha-tocopherol in patients receiving total parenteral nutrition containing medium- and longchain triglycerides. Eur J Clin Nutr 2002; 56(2): 121-8. [http://dx.doi.org/10.1038/sj.ejcn.1601294] [PMID: 11857045]

13) Hippalgaonkar K, Majumdar S, Kansara V. Injectable lipid emulsions- advancements, opportunities and challenges. AAPS PharmSciTech 2010; 11(4): 1526-40. [http://dx.doi.org/10.1208/s12249-010-9526-5] [PMID: 20976577]

14) Choudhury H, Gorain B, Karmakar S, *et al.* Improvement of cellular uptake, *in vitro* antitumor activity and sustained release profile with increased bioavailability from a nanoemulsion platform. Int J Pharm 2014; 460(1-2): 131-43. [http://dx.doi.org/10.1016/j.ijpharm.2013.10.055] [PMID: 24239580]

15) Derle D, Sagar B, Sagar P. Microemulsion as a vehicle for transdermal permeation of nimesulide. Indian J Pharm Sci 2006; 68(5): 622-5. [http://dx.doi.org/10.4103/0250-474X.29630]

16) Kasturi R. Pawar & R. Jayachandra Babu. Lipid Materials for Topical and Transdermal Delivery of Nanoemulsions, Critical Reviews ™ in Therapeutic Drug Carrier Systems 2014; 31(5): 429–458.

17) Nastiti CMRR, Ponto T, Abd E, Grice JE, Benson HAE, Roberts MS. Topical Nano and Microemulsions for Skin Delivery. Pharmaceutics 2017; 9(4)9040037 [http://dx.doi.org/10.3390/pharmaceutics9040037] [PMID: 28934172]

18) Siang Yin Lee, *et al.* Lipid-Based Delivery System for Topical Phenytoin. In: Journal of Applied Pharmaceutical Science Vol. November 2016; 6: p. (11)014-020.

19) Dhawan B, Aggarwal G, Harikumar S. Enhanced transdermal permeability of piroxicam through novel nanoemulgel formulation. nt J Pharm Investig 2014; 4(2): 65-76. [http://dx.doi.org/10.4103/2230-973X.133053] [PMID: 25006551]

20) Bajerski L, Michels LR, Colomé LM, *et al.* The use of Brazilian vegetable oils in nanoemulsions: an update on preparation and biological applications. Braz J Pharm Sci 2016; 52(3): 347-63. [http://dx.doi.org/10.1590/s1984-82502016000300001]

21) Deapsari , *et al.* Penetration of Ubiquinone (Q10) Nanoemulsion Using Olive Oil Through Rat Skin. International Journal of Pharmaceutical and Clinical Research 2017; 9(2): 169-72.

22) C. R. SCHOLFIELD. Composition of Soybean Lecithin. Reprinted from the jozmzal of the American Oil Chemists'. Society 1981; 58(10): 889-92.

23) van Hoogevest P, Wendel A. The use of natural and synthetic phospholipids as pharmaceutical excipients. Eur J Lipid Sci Technol 2014; 116(9): 1088-107. [http://dx.doi.org/10.1002/ejlt.201400219] [PMID: 25400504]

24) Kato A, Ishibashi Y, Miyake Y. Effect of egg yolk lecithin on transdermal delivery of bunazosin hydrochloride. J Pharm Pharmacol 1987; 39(5): 399-400. [http://dx.doi.org/10.1111/j.2042-7158.1987.tb03407.x] [PMID: 2886592]

25) Hoeller S, Sperger A, Valenta C. Lecithin based nanoemulsions: A comparative study of the influence of non-ionic surfactants and the cationic phytosphingosine on physicochemical behaviour and skin permeation. Int J Pharm 2009; 370(1-2): 181-6. [http://dx.doi.org/10.1016/j.ijpharm.2008.11.014] [PMID: 19073240]

26) Wu H, Ramachandran C, Weiner ND, Roessler BJ. Topical transport of hydrophilic compounds using water-in-oil nanoemulsions. Int J Pharm 2001; 220(1-2): 63-75. [http://dx.doi.org/10.1016/S0378-5173(01)00671-8] [PMID: 11376968]

27) Barry BW. Lipid-protein-partitioning theory of skin penetration enhancement. J Control Release 1991; 15(3): 237-48. [http://dx.doi.org/10.1016/0168-3659(91)90115-T]

28) Lopes LB, VanDeWall H, Li HT, *et al.* Topical delivery of lycopene using microemulsions: enhanced skin penetration and tissue antioxidant activity. J Pharm Sci 2010; 99(3): 1346-57. [http://dx.doi.org/10.1002/jps.21929] [PMID: 19798758]

29) Khani S, Keyhanfar F, Amani A. Design and evaluation of oral nanoemulsion drug delivery system of mebudipine. Drug Deliv 2016; 23(6): 2035-43. [http://dx.doi.org/10.3109/10717544.2015.1088597] [PMID: 26406153]

30) UrmilaSri Syamala. Development & Optimization Of Allyl Amine Antifungal Nanoemulgel Using 23 Factorial Design: For The Treatment Of Tinea Pedis. European Scientific Journal December 2013. SPECIAL/ edition vol.4 ISSN: 1857 – 7881 (Print) e – ISSN 1857- 7431.

31) Pund S, Pawar S, Gangurde S, Divate D. Transcutaneous delivery of leflunomide nanoemulgel: Mechanistic investigation into physicomechanical characteristics, *in vitro* anti-psoriatic and antimelanoma activity. Int J Pharm 2015; 487(1-2): 148-56. [http://dx.doi.org/10.1016/j.ijpharm.2015.04.015] [PMID: 25869452]

32) Sengupta P, Chatterjee B. Potential and future scope of nanoemulgel formulation for topical delivery of lipophilic drugs. International journal of pharmaceutics. 2017 Jun 30;526(1-2):353-65.

33) Setya Sonal. Appraisal of Transdermal Water-in-Oil Nanoemulgel of Selegiline HCl for the Effective Management of Parkinson's Disease: Pharmacodynamic, Pharmacokinetic, and Biochemical Investigations. AAPS PharmSciTech February 2018; 19(2)

34) Hauss DJ. Oral lipid-based formulations. Adv Drug Deliv Rev 2007; 59(7): 667-76. [http://dx.doi.org/10.1016/j.addr.2007.05.006] [PMID: 17618704]

35) Mortazavi S, Aboofazeli R. An Investigation into the Effect of Various Penetration Enhancers on Percutaneous Absorption of Piroxicam. Iran J Pharm Res 2003; 2: 135-40.

36) Inayat Bashir Pathan. C Mallikarjuna Setty. Chemical Penetration Enhancers for Transdermal Drug Delivery Systems. Trop J Pharm Res 2009; 8(2): 173-9.

37) Srivastava M, Kohli K, Ali M. Formulation development of novel in situ nanoemulgel (NEG) of ketoprofen for the treatment of periodontitis. Drug Deliv 2016; 23(1): 154-66. [http://dx.doi.org/10.3109/10717544.2014.907842] [PMID: 24786482]

38) Ernoviya Ernoviya *et al.* Optimization and evaluation of topical ketoconazole nanoemulsion. Asian J Pharm Clin Res 2018; 11(5): 143-6. [http://dx.doi.org/10.22159/ajpcr.2018.v11i5.23540]

39) Solè I, Maestro A, Gonzalez C, Solans C, Gutiérrez JM. Optimization of nano-emulsion preparation by low-energy methods in an ionic surfactant system. Langmuir 2006; 22(20): 8326-32. [http://dx.doi.org/10.1021/la0613676] [PMID: 16981744]

40) Mason TG, *et al.* Extreme Emulsification: Formation and Structure of Nanoemulsions. J Phys Condens Matter 2006; 9(1): 193-9. [http://dx.doi.org/10.5488/CMP.9.1.193]

41) Graves S, Meleson K, Wilking J, Lin MY, Mason TG. Structure of concentrated nanoemulsions. J Chem Phys 2005; 122(13)134703 [http://dx.doi.org/10.1063/1.1874952] [PMID: 15847485]

42) Peng Jie, Dong Wu-jun, Li Ling, *et al.* Effect of high-pressure homogenization preparation on mean globule size and large diameter tail of oil-in-water injectable emulsions journal of food and drug analysis 2015; 23: 828 -835.

43) Kotta S, Khan AW, Ansari SH, Sharma RK, Ali J. Formulation of nanoemulsion: a comparison between phase inversion composition method and high-pressure homogenization method. Drug Deliv 2015; 22(4): 455-66. [http://dx.doi.org/10.3109/10717544.2013.866992] [PMID: 24329559]]

44) Rehman A, Iqbal M, Khan BA, Khan MK, Huwaimel B, Alshehri S, Alamri AH, Alzhrani RM, Bukhary DM, Safhi AY, Hosny KM. Fabrication, in vitro, and in vivo assessment of eucalyptol-loaded nanoemulgel as a novel paradigm for wound healing. Pharmaceutics. 2022 Sep 19;14(9):1971.

45) Rehman A, Iqbal M, Khan BA, Khan MK, Huwaimel B, Alshehri S, Alamri AH, Alzhrani RM, Bukhary DM, Safhi AY, Hosny KM. Fabrication, in vitro, and in vivo assessment of eucalyptol-loaded nanoemulgel as a novel paradigm for wound healing. Pharmaceutics. 2022 Sep 19;14(9):1971.

46) Yeo E, Chieng CJ, Choudhury H, Pandey M, Gorain B. Tocotrienols-rich naringenin nanoemulgel for the management of diabetic wound: Fabrication, characterization and comparative in vitro evaluations. Current Research in Pharmacology and Drug Discovery. 2021 Jan 1;2:100019.

47) Morteza-Semnani K, Saeedi M, Akbari J, Eghbali M, Babaei A, Hashemi SM, Nokhodchi A. Development of a novel nanoemulgel formulation containing cumin essential oil as skin permeation enhancer. Drug delivery and translational research. 2021 Jul 17:1-1.

48) Algahtani MS, Ahmad MZ, Shaikh IA, Abdel-Wahab BA, Nourein IH, Ahmad J. Thymoquinone loaded topical nanoemulgel for wound healing: formulation design and in-vivo evaluation. Molecules. 2021 Jun 24;26(13):3863.

49) Soliman WE, Shehata TM, Mohamed ME, Younis NS, Elsewedy HS. Enhancement of curcumin anti-inflammatory effect via formulation into myrrh oil-based nanoemulgel. Polymers. 2021 Feb 14;13(4):577.

50) Abdallah MH, Lila AS, Unissa R, Elsewedy HS, Elghamry HA, Soliman MS. Preparation, characterization and evaluation of anti-inflammatory and anti-nociceptive effects of brucine-loaded nanoemulgel. Colloids and Surfaces B: Biointerfaces. 2021 Sep 1;205:111868.

51) Ojha B, Jain VK, Gupta S, Talegaonkar S, Jain K. Nanoemulgel: a promising novel formulation for treatment of skin ailments. Polymer Bulletin. 2021 May 27:1-25.

52) Mehanna MM, Mneimneh AT, Abed El Jalil K. Levofloxacin-loaded naturally occurring monoterpene-based nanoemulgel: a feasible efficient system to circumvent MRSA ocular infections. Drug Development and Industrial Pharmacy. 2020 Nov 1;46(11):1787-99.

53) Vartak R, Menon S, Patki M, Billack B, Patel K. Ebselen nanoemulgel for the treatment of topical fungal infection. European Journal of Pharmaceutical Sciences. 2020 May 30;148:105323.

54) Aithal GC, Narayan R, Nayak UY. Nanoemulgel: A promising phase in drug delivery. Current pharmaceutical design. 2020 Jan 1;26(2):279-91.

55) Abdel-Rashid RS, Helal DA, Omar MM, El Sisi AM. Nanogel loaded with surfactant based nanovesicles for enhanced ocular delivery of acetazolamide. International journal of nanomedicine. 2019 Apr 29:2973-83.

56) Morsy MA, Abdel-Latif RG, Nair AB, Venugopala KN, Ahmed AF, Elsewedy HS, Shehata TM. Preparation and evaluation of atorvastatin-loaded nanoemulgel on wound-healing efficacy. Pharmaceutics. 2019 Nov 13;11(11):609.

57) Mao Y, Chen X, Xu B, Shen Y, Ye Z, Chaurasiya B, Liu L, Li Y, Xing X, Chen D. Eprinomectin nanoemulgel for transdermal delivery against endoparasites and ectoparasites: preparation, in vitro and in vivo evaluation. Drug delivery. 2019 Jan 1;26(1):1104-14.

58) Rambharose S, Kalhapure RS, Govender T. Nanoemulgel using a bicephalous heterolipid as a novel approach to enhance transdermal permeation of tenofovir. Colloids and Surfaces B: Biointerfaces. 2017 Jun 1;154:221-7.

59) Mandal S, Vishvakarma P. Nanoemulgel: A Smarter Topical Lipidic Emulsion-based Nanocarrier. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s481-s498.

60) Mandal S, Jaiswal DV, Shiva K. A review on marketed Carica papaya leaf extract (CPLE) supplements for the treatment of dengue fever with thrombocytopenia and its drawback. International Journal of Pharmaceutical Research. 2020 Jul;12(3).

61) Mandal S, Bhumika K, Kumar M, Hak J, Vishvakarma P, Sharma UK. A Novel Approach on Micro Sponges Drug Delivery System: Method of Preparations, Application, and its Future Prospective. Indian J of Pharmaceutical Education and Research. 2024;58(1):45-63.

62) Pal N, Mandal S, Shiva K, Kumar B. Pharmacognostical, Phytochemical and Pharmacological Evaluation of Mallotus philippensis. Journal of Drug Delivery and Therapeutics. 2022 Sep 20;12(5):175-81.

63) Singh A, Mandal S. Ajwain (Trachyspermum ammi Linn): A review on Tremendous Herbal Plant with Various Pharmacological Activity. International Journal of Recent Advances in Multidisciplinary Topics. 2021 Jun 9;2(6):36-8.

64) Mandal S, Jaiswal V, Sagar MK, Kumar S. Formulation and evaluation of carica papaya nanoemulsion for treatment of dengue and thrombocytopenia. Plant Arch. 2021;21:1345-54.

65) Mandal S, Shiva K, Kumar KP, Goel S, Patel RK, Sharma S, Chaudhary R, Bhati A, Pal N, Dixit AK. Ocular drug delivery system (ODDS): Exploration the challenges and approaches to improve ODDS. Journal of Pharmaceutical and Biological Sciences. 2021 Jul 1;9(2):88-94.

66) Shiva K, Mandal S, Kumar S. Formulation and evaluation of topical antifungal gel of fluconazole using aloe vera gel. Int J Sci Res Develop. 2021;1:187-93.

67) Ali S, Farooqui NA, Ahmad S, Salman M, Mandal S. Catharanthus roseus (sadabahar): a brief study on medicinal plant having different pharmacological activities. Plant Archives. 2021;21(2):556-9.

68) Mandal S, Vishvakarma P, Verma M, Alam MS, Agrawal A, Mishra A. Solanum Nigrum Linn: An Analysis Of The Medicinal Properties Of The Plant. Journal of Pharmaceutical Negative Results. 2023 Jan 1:1595-600.

69) Vishvakarma P, Mandal S, Pandey J, Bhatt AK, Banerjee VB, Gupta JK. An Analysis Of The Most Recent Trends In Flavoring Herbal Medicines In Today's Market. Journal of Pharmaceutical Negative Results. 2022 Dec 31:9189-98.

70) Mandal S, Vishvakarma P, Mandal S. Future Aspects And Applications Of Nanoemulgel Formulation For Topical Lipophilic Drug Delivery. European Journal of Molecular & Clinical Medicine.;10(01):2023.

71) Chawla A, Mandal S, Vishvakarma P, Nile NP, Lokhande VN, Kakad VK, Chawla A. Ultra-Performance Liquid Chromatography (Uplc).

72) Mandal S, Raju D, Namdeo P, Patel A, Bhatt AK, Gupta JK, Haneef M, Vishvakarma P, Sharma UK. Development, characterization, and evaluation of rosa alba l extract-loaded phytosomes.

73) Mandal S, Goel S, Saxena M, Gupta P, Kumari J, Kumar P, Kumar M, Kumar R, Shiva K. Screening of catharanthus roseus stem extract for anti-ulcer potential in wistar rat.

74) Shiva K, Kaushik A, Irshad M, Sharma G, Mandal S. Evaluation and preparation: herbal gel containing thuja occidentalis and curcuma longa extracts.

75) Vishvakarma P, Kumari R, Vanmathi SM, Korni RD, Bhattacharya V, Jesudasan RE, Mandal S. Oral Delivery of Peptide and Protein Therapeutics: Challenges And Strategies. Journal of Experimental Zoology India. 2023 Jul 1;26(2).

76) Mishra, N., Alagusundaram, M., Sinha, A., Jain, A. V., Kenia, H., Mandal, S., & Sharma, M. (2024). Analytical Method, Development and Validation for Evaluating Repaglinide Efficacy in Type Ii Diabetes Mellitus Management: a Pharmaceutical Perspective. *Community Practitioner*, *21*(2), 29–37. https://doi.org/10.5281/zenodo.10642768

77) Singh, M., Aparna, T. N., Vasanthi, S., Mandal, S., Nemade, L. S., Bali, S., & Kar, N. R. (2024). Enhancement and Evaluation of Soursop (Annona Muricata L.) Leaf Extract in Nanoemulgel: a Comprehensive Study Investigating Its Optimized Formulation and Anti-Acne Potential Against Propionibacterium Acnes, Staphylococcus Aureus, and Staphylococcus Epidermidis Bacteria. *Community Practitioner*, *21*(1), 102–115. https://doi.org/10.5281/zenodo.10570746