



FORMULATION, OPTIMIZATION, AND ANTIMICROBIAL EVALUATION OF BENZOCAINE AND NIGELLA SATIVA SEED OIL LOADED TOPICAL NANOEMULGEL FOR ENHANCED WOUND HEALING AND LOCAL ANESTHESIA

***Mr. Ajay Kumar, Mr. Yogesh Kumar, Ms. Shalu Gautam, Ms. Charu Shishodia,
Mr. Shubham Pratap Singh**

Saraswathi College of Pharmacy, NH-09 Anwarpur, Pilkhuwa, Dist- Hapur Uttar Pradesh, India.

Article Received on 16 May 2026,
Article Revised on 06 June 2026,
Article Published on 10 June 2026,

<https://doi.org/10.5281/zenodo.20641473>

*Corresponding Author

Mr. Ajay Kumar

Saraswathi College of Pharmacy,
NH-09 Anwarpur, Pilkhuwa, Dist-
Hapur Uttar Pradesh, India.



How to cite this Article: *Mr. Ajay Kumar, Mr. Yogesh Kumar, Ms. Shalu Gautam, Ms. Charu Shishodia, Mr. Shubham Pratap Singh. (2026). Formulation, Optimization, And Antimicrobial Evaluation Of Benzocaine And Nigella Sativa Seed Oil Loaded Topical Nanoemulgel For Enhanced Wound Healing And Local Anesthesia. World Journal of Pharmacy and Pharmaceutical Sciences, 15(6), 1514-1533.

This work is licensed under Creative Commons Attribution 4.0 International license.

ABSTRACT

Background: Many synthetic and herbal APIs have been patented as nanosized emulsified gels for a range of medicinal purposes in the last few years. Unfortunately, due to their restricted therapeutic utility, almost half of these medications are classified as either class II or class IV in the biopharmaceutical categorization system (BCS) or are neither of them still in development. Benzocaine which is a BCS Class II drug is utilized in this study. Nigella sativa a well-known plant having various medicinal properties is also utilized. **Aim:** This study aimed to determine the efficacy of Benzocaine and Nigella Sativa seed oil against Gram-negative and Gram-positive microorganisms, such as Escherichia coli and Staphylococcus Aureus, as well as to observe the zone of inhibition and create a nanoemulgel to produce the combined impact of synergy. **Methods:** A formulation comprising nanosized emulsion globules created using low-speed

homogenization procedures is known as nano emulsion-based gel, or nanoemulgel, and is further transformed into nanoemulgel by including an appropriate gelling agent. It has been proven to be a viable option for topical medication delivery with globular size ranging between 5 to 500 nm. FTIR was used to study any possible incompatibilities with the drug and other excipients used in the nanoemulgel formulation. Other parameters i.e. viscosity,

pH, spreadability, etc are also evaluated to confirm the safety and efficacy of the formulation.

Result: The particle size of the optimized nanoemulsion was found to be 10.6 nm and the zeta potential was around -10.2 mV. An antimicrobial evaluation was conducted, and the competitive synergistic effect of nanoemulgel was observed. **Conclusion:** It was determined that benzocaine and Nigella Sativa seed extract separately exhibited just a negligible amount of antibacterial action against both Gram Positive and Gram Negative bacteria. The nanoemulgel demonstrated a competent combined effect after being blended.

KEYWORDS: Benzocaine, Nigella Sativa, Nanoemulsion, Nanoemulgel, Staphylococcus Aureus, Escherichia Coli.

Abbreviations

BCS- Biopharmaceutical Classification System

FTIR- Fourier Transform Infrared Spectroscopy

API- Active Pharmaceutical Ingredient

UV-Spectroscopy- Ultra Violet Spectroscopy

°C - Degree Celsius

cP- Centipoise

%- Percentage

Hrs- Hours

INTRODUCTION

Topical drug delivery system: The skin is the principal route for topical drug delivery systems, and it is also one of the easiest organs in the human body to access for topical administration.^[1] The accessibility, large surface area, deep exposure to the lymphatic and circulatory networks, and non-invasive nature of topical delivery of drugs have made it a desirable approach for a long time. Topical systems have the advantages of appropriateness, improved patient compliance, and the removal of the hepatic first-pass effect.^[2] Topical drug delivery systems have several benefits, including the capacity to administer medication more precisely and effectively to the intended site and to maximize bioavailability by reducing first-pass hepatic metabolism.^[3]

Function of Skin

- i. Provides a protective barrier against mechanical, thermal and physical injury and hazardous substances.
- ii. Prevents loss of moisture and regulates temperature.
- iii. Reduces harmful effects of UV radiation.
- iv. Serves as a sensory organ.
- v. Generation of vitamin D.^[4]

Skin serves as a barrier in the topical drug delivery system, it is largely demonstrated that the nanocarriers result in superior penetration through skin barrier. Particle size under 100nm is in the appropriate size range for deep skin penetration.^[5]

Nigella sativa (Black cumin): The seeds of *Nigella sativa* Lin. Plant belonging to Ranunculaceae family has been used to promote health and fight disease for centuries especially in the Middle East and Southeast Asia. The plant is widely grown across the world including in India, Pakistan, Turkey, Syria and Saudi Arabia. It is known as Black seed, Kalonji, Kalojeera, Nutmeg flower, Habatul-Bakarah, Roman coriander, etc.^[6] As an oriental spice, *Nigella sativa* has long been used as a natural medicine for the treatment of many acute as well as chronic conditions. This plant species has grown in favour for its diverse range of medicinal application due to its seeds which are abundant in phytoconstituents.^[7] *Nigella sativa* contains ingredients are fixed oil, essential oil, alkaloids, protein, saponin. Most of pharmacological effect due to quinine constituent of which thymoquinone is mostly abundant. Thymoquinone possesses anticonvulsant activity, antioxidant, anti-inflammatory,^[8] anti-cancer, anti-bacterial, antifungal activity,^[9] antiparasitic activity, hepatoprotective, neuroprotective effect, etc.^[10]

Different extract of *Nigella sativa* as well as thymoquinone have broad spectrum antimicrobial including gram-positive and gram-negative bacteria.^[11]

Benzocaine: Ethyl 4-aminobenzoate (molecular formula $C_9H_{11}NO_2$, molecular weight 165.189 g/mol),^[12] is well known anaesthetic agent and supplemental role as antimicrobial agent.^[13] Local anaesthetics are drugs for reduction of pain and providing anaesthesia through inhibition of voltage gated channels. The action is characterized by rapid but short effect compared with duration of pain. Benzocaine is used primarily to relieve pain or irritations on the skin and mucosal surfaces. It is among the ester types of long-acting local anaesthetics, which has been used for the relief of local pain.^[14] The most familiar accessible form of

benzocaine is oral and topical. It is also known for its analgesic potency in oral for mouth cancers, dental discomforts, antibacterial, antifungal, antitumor, etc. Moreover, review on the synthesis of its analogues will lead to better ideas for future drug modifications, thereby exploring effective applications of benzocaine which is still known merely for its anaesthetic prominence.^[15]

Nanoemulsion: When a liquid is dispersed in another fluid, it forms a multiphase colloidal dispersion of two nonequilibrium structured immiscible liquids that are intended to mix with one another. Combination of surfactants and co-surfactants are used for stability. The nanoemulsion sizes between 20-200nm, giving a transparent emulsion with decreased interfacial tension between oil & water phase.^[16] This availability of large interfacial area influences the targeted drug delivery. Large volumes of oil substances are dissolved by nano emulsions, which also shields drug from the body's hydrolysis and enzyme degradation and long-term, controlled drug release is made possible. They are also referred to as Mini emulsions, ultrafine emulsions, micrometre emulsions, milky emulsions, translucent emulsions, etc. Nanoemulsions are prepared using high energy emulsification and low energy emulsification techniques.^[17]

Nanoemulgel: Nanoemulgel is an emerging drug delivery system intended to strengthen the therapeutic Profile of lipophilic drugs. Poor solubility, unpredictable absorption, and low oral bioavailability are some of the limitations of lipophilic formulations. By combining different systems nanoemulgel is formulated to deal with these limitations. Nanoemulgel is prepared by the incorporation of nanoemulsion into gel base which improves stability and enables drug delivery for both immediate and controlled release. The capacity of nanoemulgel to achieve targeted delivery, its ease of application, lack of gastrointestinal degradation and its safety profile have all contributed to its increased attention. This review focuses on the formulation components of nanoemulgel for topical drug delivery, pharmacokinetics and its ability against microorganisms.^[18]

Advantages of nanoemulgels

Avoid first pass metabolism.

Easy acceptable for the patient.

Suitably for self-medication.

Provide local drug delivery.

Easy discontinuation of medication.

Readily adapted to the skin's environment.

Proven efficacy for a controlled and sustained drug delivery system.^[19]

MATERIAL AND METHODS

Materials

The seeds of *Nigella Sativa* were purchased from a local retail shop of Satara from the Wagdole Ayurvedics. Ethanol, Benzocaine, Carbopol-934, Tween 80, Triethanolamine and all the chemicals used were of analytical grade were used throughout the study.

Equipment

Weighing balance, Mortar pestle, Beaker, Measuring Cylinder, Soxhlet apparatus, High Speed homogenizer, Sonicator, pH meter, Brookfield viscometer, etc.

Essential oil extraction of *Nigella Sativa* Seeds

The *nigella sativa* seed oil was extracted with the Soxhlet apparatus using ethanol (solvent) As shown in (figure 1). 50gm of ground *nigella sativa* seeds were weighed and placed in a thimble-shaped filter paper and placed in the glass cylinder. This cylinder is provided with a siphon tube and an inlet tube. A water condenser is attached to the cylinder at the top. This entire assembly is fitted into the neck of a round bottom flask containing 200 ml ethanol (solvent) and attached to the heating mantle. The heating mantle is set at 60°C. The solvent vapour through the inlet tube reaches the cylinder and gets condensed on passing upward into the condenser. The condensed solvent comes in contact with the crude organic substance and dissolves it as soon as the solution reaches the top end of the siphon tube. In this way, a continuous supply of solvent vapours is maintained in the cylinder, and the dissolved organic compound flows back into the flask. Finally, the heating is stopped and the solution in the flask is distilled to recover the solvent, while the organic compound is left behind.^[20]

Preparation of Nano emulsion

Utilizing the low energy emulsification technique, nanoemulsion was created. As indicated in Table 1, an oil phase comprising *nigella sativa* seed oil and the surfactant tween 80 are combined in a conical flask. After adding distilled water to the mixture dropwise, it was homogenized at 3500 rpm.^[21]

Preparation of Gel base

Weighed 3% of Carbopol is dissolved in distilled water. Add Methyl paraben and propyl paraben as preservatives in the mixture and homogenize for 2hrs at 600rpm. Slowly add

triethanolamine into the gel. After homogenization gel base was subjected for two cycles of sonication for 15 min to expel out the entrapped air bubbles from the preparation. The composition is given in (table 2).^[22]

Preparation of Nanoemulgel Formulation

The nanoemulgel is made using the spontaneous emulsification technique. A high speed homogenizer is used to combine the optimised nano emulsion with the benzocaine medication and homogenise it with the prepared gel base for 30 minutes at 3500 rpm. Refer (table 3) for the formulation table and (Figure 2).^[23]

EVALUATION PARAMETERS

PREFORMULATION STUDY

Oil extract

Colour: Yellowish Brown

Boiling point: 230°C

Solubility study: Solubility of extract was checked by dissolving 2 ml of extract in ethanol, Tween 80 surfactant. Extract was freely soluble in ethanol, easily soluble in surfactant.

Calibration curve: 10 ml extract was diluted in 100 ml of ethanol Solution (A). 10 ml Sol (A) was again diluted in 100 ml ethanol sol (B). Further dilutions 0.2, 0.4, 0.6, 0.8, 1ml in 10 ml were done and $\lambda_{\max}$ is obtained at 271nm.^[24]

Chemical tests for Extract

Test for Alkaloids: Take 2ml Filtrate and saturated solution of picric acid to it. If yellow colour precipitate is formed alkaloids present in the extract.

Test for Fixed oil: Few drops of filtrate were taken on the filter paper. If stain appeared on filter paper fixed oil was present in the extract.

Test for Volatile oil

- (i) Few drops of filtrate are taken on filter paper. If permanent stain was observed on the filter paper volatile oil is present in the extract.
- (ii) Take 2 ml of Filtrate and add few drops of Sudan III dye to it. If red colour obtained volatile oil is present.

Test for Tannins: Take 5% solution of FeCl and add 2ml filtrate if it gives green or yellow colour tannins are present in the extract.

Test for Flavonoids: Add NaOH solution in 2ml filtrate if yellow colour is obtained, flavonoids are present.^[25]

Benzocaine

Colour: White crystalline powder.

Odour: It is Odourless.

Melting point: Benzocaine M. P. was determined at 90°C.

Solubility: 1mg Benzocaine was added in 10 ml water, ethanol, chloroform and dilute acid. Benzocaine is sparingly soluble in water, very soluble in ethanol, chloroform and more soluble in dilute acids.^[26]

Calibration curve: 10 mg benzocaine was diluted in 100 ml of ethanol and named Solution (A). 10 ml Solution (A) was again diluted in 100 ml ethanol and solution (B) was obtained. Further dilutions 0.2, 0.4, 0.6, 0.8, 1ml in 10 ml were done and $\lambda_{\max}$ is obtained at 532nm.^[27]

FTIR spectroscopy

FTIR Characterization of Benzocaine was carried out to detect the functional groups present in the drug.^[28]

Compatibility study (FTIR)

FTIR characterization of the sample containing drug (Benzocaine), surfactant and polymer. FTIR analysis can be carried out for the assessment of drug excipient interaction, polymerization, crosslinking as well as drug loading in the formulation. The test helps in determining any potential incompatibility in between the drug and other excipients used in the nanoemulgel formulation.^[28]

EVALUATION OF NANOEMULSION

Physical appearance- Different physical characteristics of the formulations were carried out such as colour, odour, homogeneity, etc.

Measurement of pH- The apparent pH of nanoemulsion is determined by using digital pH meter. Calibration of pH meter was carried out in buffer 4 and buffer 7 solutions. One gram

of nanoemulsion is dissolved in 100 ml distilled water and stored for two hours. The measurement of pH is carried out three times and an average mean value is calculated.

Viscosity study- The measurement of viscosity of the prepared nanoemulsion was done with a Brookfield Viscometer. The nanoemulsion was rotated at 50 rotations per minute. At each speed, the corresponding dial reading was noted. The viscosity of the nanoemulsion was obtained by multiplication of the control reading with factor given in the Brookfield Viscometer catalogues.

Dilutability test- The rationale of dilution test is that continuous phase can be added in larger proportion into a nanoemulsion without causing any problem in its stability. Thus O/W nano emulsions are dilutable with water, but W/O nano emulsions are not.^[29]

Particle size measurement - Particle size of nanoemulsion was measured by scattering light intensity for all the batches to determine whether the prepared batches remained nanosized.^[30]

Zeta potential measurement- Zeta potential is an important parameter in determining the stability of an emulsion and other colloidal dispersion. It was performed for the study of physical stability study of the formulations. It is a method for measuring surface charge of particles when it is placed in a liquid. It was carried out at 25°C temperature. Electrostatic repulsion between negatively charged droplets can result in a well-separated and coalescence-free nanoemulsion formulation.^[30]

EVALUATION OF GEL

Measurement of pH- The pH of nanoemulsion formulation is determined by using digital pH meter. Calibration of pH meter was carried out in buffer 4 and buffer 7 solutions. One gram of nanoemulsion is dissolved in 100 ml distilled water and stored for two hours. The measurement of pH is carried out three times and average mean values are calculated.

Viscosity study- The Brookfield Viscometer was utilised to measure the viscosity of the prepared nanoemulsion. The nanoemulsion was rotated at 50 rotations per minute. At each speed, the corresponding dial reading was noted. The viscosity of the nanoemulsion was obtained by multiplication of the dial reading with factor given in the Brookfield Viscometer catalogues.^[31]

EVALUATION OF NANOEMULGEL

Physical Appearance

Colour: The colour of nanoemulgel was found to be transparent yellowish brown in colour.

Odour: Characteristic odour was observed.

Consistency: It was checked by applying on the skin.

Spreadability: The spreadability was evaluated by applying it to the skin.

Homogeneity: Through visual inspection.

Greasiness: Checked by applying on the skin.

Skin irritancy: It was checked by applying on the skin.

Phase separation: It was checked by visual inspection.^[32]

Measurement of pH- The apparent pH of formulation was measured by a digital pH meter in triplicate. Calibration of apparatus was carried out using buffer 4 and buffer 7 solutions. One gram of nanoemulsion is dissolved in 100 ml distilled water and stored for two hours.

Viscosity study- The measurement of viscosity of the prepared nanoemulsion was done using Brookfield Viscometer. The nanoemulsion was rotated at 50 rotations per minute. At each speed, the corresponding dial reading was noted. The viscosity of the nanoemulsion was obtained by multiplication of the dial reading with factor given in the Brookfield Viscometer catalogues.

Extrudability- Nanoemulgel was filled in an aluminium collapsible tube and by using hardness tester. 5gm of amount of formulation extruded from the tube was checked. This process was repeated three times.^[33]

$$\text{Extrudability} = \frac{\text{Applied weight to extrude nanoemulgel from tube}}{\text{Area}}$$

ANTIMICROBIAL ACTIVITY

The antimicrobial assay is carried out for both qualitative and quantitative estimation of antimicrobial agents using the microorganisms. This assay was carried out by using the agar well diffusion method. In the agar well diffusion technique, initially, the agar plates were prepared using a sterile nutrient agar medium. 4.6 mg Nutrient Agar was dissolved in 120ml distilled water. It was boiled, cooled and kept in the autoclave for 15 mins. The agar plates were incubated for 48 hrs. Then the cavities of 6 mm in diameter are prepared on the agar plates via glass bend rod. The test formulations (benzocaine, extract and nanoemulgel) were

then added into each cavity separately. Finally, all the plates are incubated for 24 h-48 h at 37°C and the diameter of the zone of inhibition in mm is measured.^[34]

RESULTS

PREFORMULATION STUDY

Oil extract

Colour: Observed as yellowish brown.

Boiling point: 230°C B.P. was estimated.

Solubility study: It is soluble in ethanol, Tween 80 surfactant.

Calibration curve: The $\lambda_{\max}$ is obtained at 271nm. The absorbance for different concentrations 0.2, 0.6, 0.8, 1 and 1.2 µg/ml was carried out and the absorbance was 0.121, 0.135, 0.169, 0.198, 0.225 & 0.268. The coefficient of determination (R^2) of the nigella sativa oil extract was 0.9802 which falls under the standard range. Refer figure 3.

Chemical tests for Extract

The chemical components contained in the nigella sativa seed oil extract were found using a variety of chemicals and reagents. Chemical constituents such as alkaloid, fixed oil, volatile oil, tannins, and flavonoids were confirmed in the extract as shown in table 4.

Benzocaine

Melting point: M. P. was observed at 90°C.

Solubility: Benzocaine is sparingly soluble in water, very soluble in ethanol, chloroform and more soluble in dilute acids.

Calibration curve: $\lambda_{\max}$ was found to be 532 nm. Absorbance noted at different concentrations 0.2, 0.4, 0.6, 0.8 and 1 µg/ml was 0.168, 0.225, 0.387, 0.432, 0.520. The reported coefficient of determination (R^2) of benzocaine was 0.9715 which falls under the standard range. Refer (figure 4).

Benzocaine (Fourier Transform Infrared) FTIR spectroscopy

Following was the graph (figure 5) obtained from IR Spectroscopy and the functional groups present were detected. Refer table given below for the functional groups present in benzocaine. As per the IR analysis of benzocaine the obtained IR data is checked against the standard absorption frequencies to detect the functional groups present in benzocaine. Aromatic C-H, Aromatic C-C Double bond, C-O, CH₃, Alcohol, Ether, Ester, Carboxylic

acid, C-H Alkane and Anhydride are the functional groups present in benzocaine. Refer (table 5).

Compatibility study (FTIR)

FTIR analysis is conducted in order to assist any drug-excipient interaction, polymerization, crosslinking as well as drug loading in the formulation. Non-ionic surfactant was selected since they are least affected by pH and are generally regarded safe and biocompatible. The test proved no potential incompatibility in between the drug and other excipients used in the nanoemulgel formulation. All the characteristic peak of drug such as Hydrogen-bonded, C-H Alkane, CH₃, etc. appeared at same point in the formulation. Refer (Figure 6).

EVALUATION OF NANOEMULSION

Physical appearance: The nanoemulsion appeared to be transparent yellowish in colour. No visible turbidity or phase separation observed in the formulation.

Dilutability test: The emulsion was soluble in water; hence, we can conclude that the formulated nanoemulsion is a o/w type of emulsion.

Particle size: The particle size of the formulations was determined using scattering light intensity at scattering angle 90 with 25°C temperature of the holder. Dispersion medium viscosity was 0.895 mPa·s and the count rate were 212 kCPs. Batches F1-F5 had particle size in the range of 10.6-50.1 nm. Refer (figure 7).

Zeta potential study: Zeta potential of the nanoemulsion was carried out at 0.188 mS/cm conductivity. Temperature of the holder was 25°C with dispersion medium viscosity 0.896 mPa.s. The electrode voltage was 3.4 V. Zeta potential of batches F1-F5 was -10.2 to -30.0 mV. (Refer table 6 for the readings obtained for the stability studies).

Measurement of pH and Viscosity study

Following (Table 6) are the obtained pH and viscosity for all the prepared batches for the nanoemulsion formulation. All the formulations comply to the standard range.

EVALUATION OF GEL BASE

From table 7 we can conclude the gel base had pH 6.01 and Viscosity 1603.1 which complies to the standard reading as mentioned in the IP.

EVALUATION OF NANOEMULGEL

Physical Appearance

Colour: The colour of nanoemulgel was found to be transparent yellowish brown in colour.

Odour: Characteristic odour.

Consistency: It was checked by applying on the skin.

Spreadability: Easily spreadable.

Homogeneity: Homogenous.

Greasiness: No greasiness.

Skin irritancy: No sign of irritancy on the skin.

Phase separation: It was checked by visual inspection.

Measurement of pH and Viscosity

Measurement of pH was carried out using pH meter. F4 has pH 5.99 while F1, F2, F3, F5 have pH 6-6.7 which complies the standard range. pH of F1, F2, F4 has increased while F3 decreased and F5 remained unchanged. Utilizing Brookfield viscometer, the formulation's viscosity was assessed. Viscosity of all F1 to F5 batches increased but F1 and F4 were more viscous. Refer (Table 8).

Extrudability

As mentioned in (Table 9) among all the batches F1 & F4 showed excellent extrudability.

ANTIMICROBIAL ACTIVITY

The zone of inhibition observed for both the microorganisms were adequate to conclude the extract and benzocaine drug had antimicrobial activity against the microorganisms. While the nanoemulgel also had sufficiently good antimicrobial activity. As shown in (table 10 & figure 8).

Tables

Table 1: Compositions of nigella sativa loaded Nanoemulsion.

Formulation no.	Oil(%w/v)	Surfactant (%w/v)	Distilled water(%w/v)
F1	3	17.01	9.99
F2	3.99	16.02	9.99
F3	6.21	18.81	4.98
F4	7.59	17.4	5.01
F5	9	13.89	7.11

Table 2: formulation table for gel base.

Formulation no.	Carbopol 934 (%w/v)	Triethanolamine (%w/v)	Methyl paraben (%w/v)	Propyl paraben (%w/v)	Distilled water
F1	0.9	0.06	0.06	0.06	q.s.

Table 3: Composition of benzocaine and nanoemulsion loaded nanoemulgel using gel base.

Formulation no.	Nanoemulsion(gm)	Benzocaine(gm)	Gel base(gm)
F1	15	-	Up to 15
F2	15	220	Up to 15
F3	10	220	Up to 15
F4	10	220	Up to 15
F5	-	220	Up to 15

Table 4: chemical constituents detected in nigella sativa seed oil.

Test	Chemical Test	Observation	Result
Test for Alkaloids	Filtrate + saturated solution of picric acid	Yellow colour precipitate	Alkaloids present
Test for Fixed oil	Drop of filtrate on the filter paper	Stain on filter paper	Fixed oil present
Test for Volatile oil	1.Drop of filtrate on filter paper 2.Filtrate +Sudan III dye	Permanent stain on filter paper Red colour obtained	Volatile oil present Volatile oil present
Test for Tannins	5% solution of FeCl ₃ + filtrate	Green or yellow colour obtained	Tannins present
Test for Flavonoids	NaOH solution + filtrate	Yellow colour obtained	Flavonoids present
Test for Glycosides	Dilute H ₂ SO ₄ +filtrate+5% NaOH solution+ add Fehling's (A+B) solution	Red colour produced	Glycosides present

Table 5: Functional groups present in Benzocaine and API and the frequency range.

Standard Absorption Frequency Range (cm ⁻¹)	Observed Absorption Frequency Range (cm ⁻¹)	Functional Group Present
3500-3200	3415	Hydrogen-bonded
3300-3000	3331.11, 3213.90	Aromatic C-H
3000-2850	2972.40, 2896.41	C-H Alkane
1600, 1580, 1500, 1450	1676.88, 1587.40, 1503.84, 1439.69	Aromatic C-C Double Bond
1380-1370	1364.78	CH ₃

1300-1000	1268. 20	Alcohol, Ether, Ester, Carboxylic acid, Anhydride
1150-1060	1109.42, 1013.60	C-O

Table 6: Stability study(particle size and zeta potential), pH and viscosity observations noted for the nanoemulsion.

Sr. No.	Test	Observations				
		F1	F2	F3	F4	F5
1	Particle size	10.6 nm	35.5 nm	50.1 nm	15.3 nm	22.7 nm
2	Zeta Potential	-10.2mV	-25.4mV	-12.5mV	30.0mV	-12.3mV
3	pH	6.3	5.92	6.7	5.76	6.4
4	Viscosity	986cps	564cps	876.7cps	924.1cps	769.9cps

Table 7: pH and viscosity evaluation of the gel base.

Formulation no.	Measurement of pH	Viscosity Study (cps)
F1	6.01	1603.1

Table 8: Evaluation of pH and viscosity of nanoemulgel.

Formulation no.	Measurement of pH	Viscosity(cps)
F1	6.7	14235.2
F2	6	9935.6
F3	6.5	7593.5
F4	5.99	11512.8
F5	6.4	8622.1

Table 9: Extrudability study of nanoemulgel to determine amount of pressure required to extrude the formulation from the container.

Sr. No.	Formulation	Extrudability (g)
1.	F1	1.32
2.	F2	0.97
3.	F3	1.03
4.	F4	1.35
5.	F5	1.20

Table 10: Observations of Zone of inhibition obtained for the extract, benzocaine drug and nanoemulgel formulation using Staphylococcus aureus and Escherichiacoli.

Microorganisms	Zone of inhibition (mm)									
	S. Aureus					E. Coli				
	F1	F2	F3	F4	F5	F1	F2	F3	F4	F5
Extract	8	7.2	10	10.3	9.5	11	8.9	13	9	11.3
Benzocaine	10.2	6.7	12	7	10	9	9	10	11.4	8.7
Nanoemulgel	20	12	19	15.6	18.5	18	15	21	17.3	20.2

Figures

Figure 1: Extraction of oil from *nigella sativa* seeds using soxhlet apparatus.

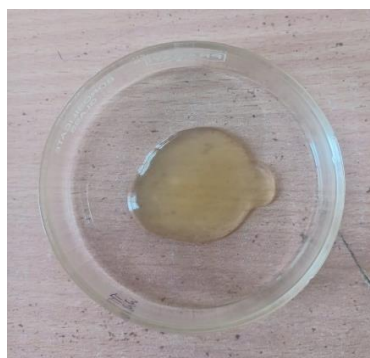


Figure 2: Nanoemulgel.

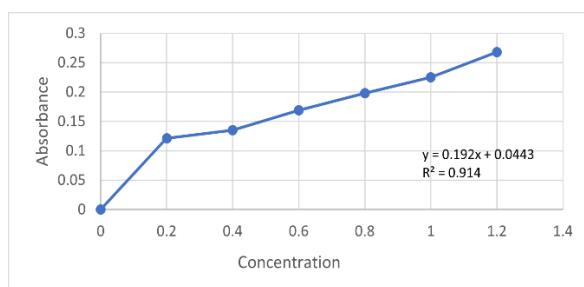


Figure 3: Calibration curve obtained at λ_{max} 271nm.

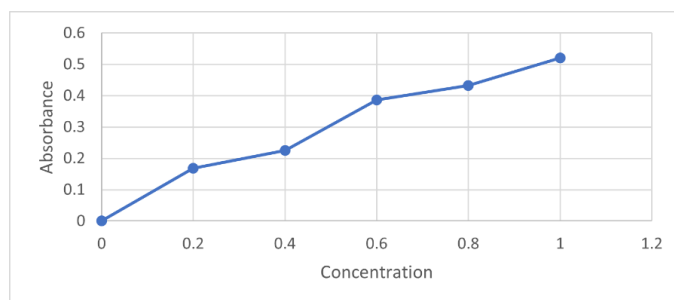


Figure 4: Calibration curve of benzocaine at λ_{max} 532nm.

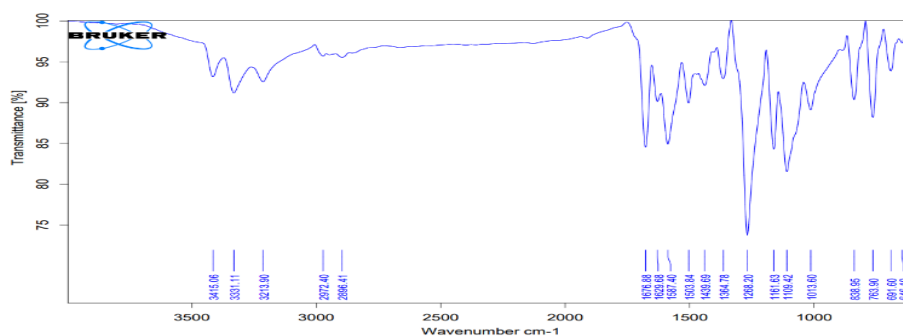


Figure 5: FTIR characterization of benzocaine.

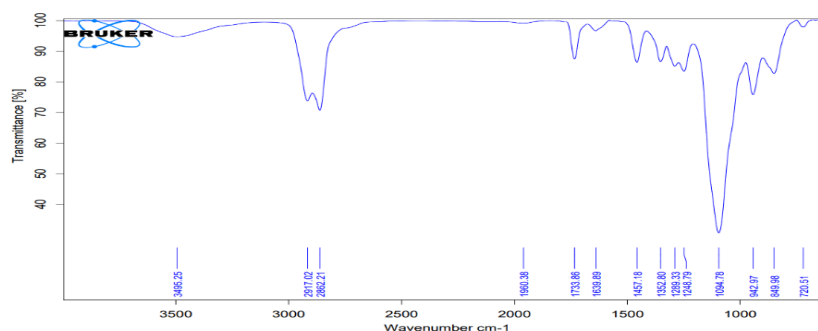


Figure 6: Compatibility study of drug and other excipients using FTIR to check any possible interaction.

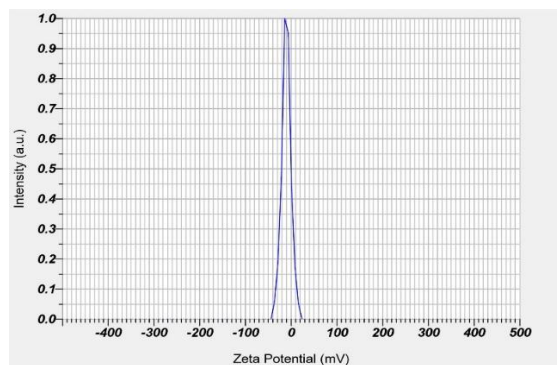
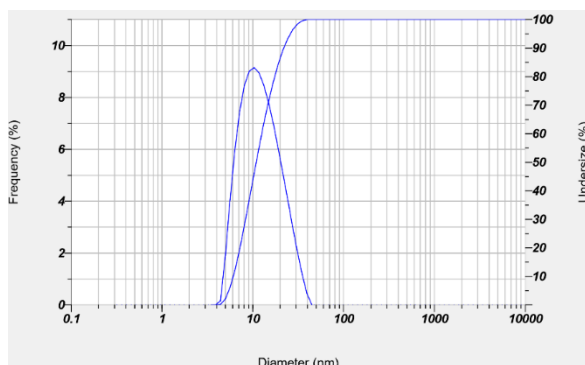


Figure 7: Particle size and zeta potential of nanoemulsion.



Figure 8: Zone of inhibition obtained for the extract, benzocaine and Nanoemulgel.

DISCUSSION

The purpose of this study was to develop and characterise nigella sativa seed oil and benzocaine-containing nanoemulgel (NEGs) for antibacterial activity optimisation. The extracted oil from nigella sativa seeds using Soxhlet apparatus had various phytochemicals constituents present in it. FTIR confirmed compatibility of the drug and other excipients used in the nanoemulgel formulation. Proper selection of the surfactant enhanced the nano emulsification process and stability of the formulations. Studies on phase behaviour for optimization of nanoemulsion properties are important when low energy homogenization method is used, because the phases involved during emulsification are determinant in order to obtain nanoemulsion of small particle size. The particle size of the F1 batch was found to be 10.6 nm and the zeta potential was around -10.2 mV. Gel base was prepared using Carbopol 934 and its pH and viscosity was evaluated which was 6.01 and 1603.1cps respectively. Gel base and optimised nanoemulsion were combined to formulate nanoemulgel. Furthermore, every relevant parameter was assessed. Due to the addition of the gel base the low viscosity problem of the nanoemulsion was overcome and stiff nanoemulgel was achieved and there was no significant change in the pH of all formulation batches.

CONCLUSION

The results showed that the batches F1, F4, and F5 had the best viscosity, pH, and extrudability, while the batches F2, F3, and F5 also performed well. The antibacterial activity of alone benzocaine and Nigella Sativa seed extract against Gramme Positive and Gramme Negative microorganisms was found to be weak. A competent combination effect was observed in the nanoemulgel. Nanoemulsion and nanoemulgel F1 batches outperformed the other batches in terms of different evaluation factors. The results from the remaining batches were also satisfactory and met the minimum standards.

ACKNOWLEDGMENT

The Gourishankar Institute of Pharmaceutical Education and Research Limb, Satara, along with its principal Dr. A. S. Kulkarni, vice-principal Dr. Y. A. Gurav and teachers are all greatly appreciated by the authors for their invaluable support throughout the work. For assistance with the FTIR, zeta potential, and particle size analyses, we are grateful to the principal of Annasaheb Dange College of Pharmacy. Thanks to WagdoleAurvedics for providing the Nigella Sativa seeds.

DECLARATION OF INTEREST: The authors report no conflicts of interest.

REFERENCES

1. Bhowmik D. Recent advances in novel topical drug delivery system. *The Pharma Innovation*, 2012 Nov 1; 1(9).
2. Donthi MR, Munnangi SR, Krishna KV, Saha RN, Singhvi G, Dubey SK. Nanoemulgel: A Novel Nano Carrier as a Tool for Topical Drug Delivery. *Pharmaceutics*, 2023 Jan 3; 15(1): 164.
3. Azeez AR, Alkotaji M. Nanoemulgel As A Recent Drug Delivery System. *Military Medical Science Letters/Vojenské Zdravotnické Listy*, 2022 Apr 1; 91(2).
4. Yousef H, Alhajj M, Sharma S. *Anatomy, skin (integument), epidermis*, 2017.
5. Salvioni L, Morelli L, Ochoa E, Labra M, Fiandra L, Palugan L, Prosperi D, Colombo M. The emerging role of nanotechnology in skincare. *Advances in colloid and interface science*, 2021 Jul 1; 293: 102437.
6. Srinivasan K. Cumin (*Cuminum cyminum*) and black cumin (*Nigella sativa*) seeds: traditional uses, chemical constituents, and nutraceutical effects. *Food quality and safety*, 2018 Mar; 2(1): 1-6.
7. Alrashidi M, Derawi D, Salimon J, Yusoff MF. The effects of different extraction solvents on the yield and antioxidant properties of *Nigella sativa* oil from Saudi Arabia. *Journal of Taibah University for Science*, 2022 Dec 31; 16(1): 330-6.
8. Bader AA. Black cumin (*Nigella sativa*) to treat bronchial asthma, ischemia, and cardiovascular diseases. In *Biochemistry, Nutrition, and Therapeutics of Black Cumin Seed*, 2023 Jan 1; (231-236). Academic Press.
9. Yimer EM, Tuem KB, Karim A, Ur-Rehman N, Anwar F. *Nigella sativa* L.(black cumin): a promising natural remedy for wide range of illnesses. *Evidence-Based Complementary and Alternative Medicine*, 2019 Oct; 2019.
10. Forouzanfar F, Bazzaz BS, Hosseinzadeh H. Black cumin (*Nigella sativa*) and its constituent (thymoquinone): a review on antimicrobial effects. *Iranian journal of basic medical sciences*, 2014 Dec; 17(12): 929.
11. D VR, R VD, K Prof DP, P.A Dr PS. Formulation & Evaluation of Mucoadhesive Benzocaine Gel. *European Journal of Biomedical and Pharmaceutical Sciences*, 2019; 6(13): 318–319.
12. Mouwakeh A, Kincses A, Nové M, Mosolygó T, Mohácsi-Farkas C, Kiskó G, Spengler G. *Nigella sativa* essential oil and its bioactive compounds as resistance modifiers against *Staphylococcus aureus*. *Phytotherapy Research*, 2019 Apr; 33(4): 1010-8.

13. Stamova S, Koleva Y, Georgieva S, Ermenlieva N, Georgieva E. Probable microbial evaluation of the benzocaine as an antimicrobial agent, 2020; 545.
14. Üstündağ Okur N, Çağlar EŞ, Arpa MD, Karasulu HY. Preparation and evaluation of novel microemulsion-based hydrogels for dermal delivery of benzocaine. *Pharmaceutical development and technology*, 2017 May 19; 22(4): 500-10.
15. Khair-ul-Bariyah S, Arshad M, Ali M, Din MI, Sharif A, Ahmed E. Benzocaine: review on a drug with unfold potential. *Mini Reviews in Medicinal Chemistry*, 2020 Jan 1; 20(1): 3-11.
16. Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: an advanced mode of drug delivery system. *3 Biotech.*, 2015 Apr; 5: 123-7.
17. Khanna V, Singh S, Baghel DS, Kaur B, Nayar A, Ghimire R. Nanoemulsions—Present And Future Perspective—An Overview. *Journal of Emerging technologies and Innovative research*, 2018; 5(12): 354-370.
18. Sandeep DS. Development, characterization, and in vitro evaluation of aceclofenac emulgel. *Asian Journal of Pharmaceutics (AJP)*, 2020 Jul 22; 14(03).
19. Sharma RB, Kumar G, Thakur H, Tomar S. Nanoemulgel: A Novel Approach for Topical Delivery System: Updated Review. *International Journal of Drug Development and Research*, 2023; 15(1): 988
20. Redfern J, Kinninmonth M, Burdass D, Verran J. Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. *Journal of microbiology & biology education*, 2014 May; 15(1): 45-6.
21. Franklyne JS, Gopinath PM, Mukherjee A, Chandrasekaran N. Nanoemulsions: The rising star of antiviral therapeutics and nanodelivery system—Current status and prospects. *Current Opinion in Colloid & Interface Science*, 2021 Aug 1; 54: 101458.
22. Tamboli S, Ambekar AW. Formulation, Development and Evaluation of Herbal Antifungal Nanoemulgel containing Neem seed oil and Aloe-vera gel. *International Journal of All Research Education and Scientific Methods*, 2021; 9(8).
23. Rao SV, Vani GS, Kiranmai C, Padmalatha K. Effectiveness Of Topical Nanoemulgel In Promoting Wound Healing. *Journal of Population Therapeutics and Clinical Pharmacology*, 2023 Mar 14; 30(3): 904-13.
24. Sharma D, Kosankar KV, Lanjewar AM. Extraction and Chemical Tests on *Nigella sativa* L. Collected from Vidarbha Region of India.

25. Cheuk D, Svård M, Rasmuson ÅC. Thermodynamics of the enantiotropic pharmaceutical compound benzocaine and solubility in pure organic solvents. *Journal of Pharmaceutical Sciences*, 2020 Nov 1; 109(11): 3370-7.
26. Mohammeda DH, Sarsamb LA. Spectrophotometric determination of benzocaine by azodye formation reaction. *J Univ Anbar Pure Sci.*, 2011; 5(1).
27. Solomons TG, Fryhle CB. *Organic chemistry*. John Wiley & Sons, 2008.
28. Che Marzuki NH, Wahab RA, Abdul Hamid M. An overview of nanoemulsion: Concepts of development and cosmeceutical applications. *Biotechnology & biotechnological equipment*, 2019 Jan 1; 33(1): 779-97.
29. Donthi MR, Munnangi SR, Krishna KV, Saha RN, Singhvi G, Dubey SK. Nanoemulgel: A Novel Nano Carrier as a Tool for Topical Drug Delivery. *Pharmaceutics*, 2023 Jan 3; 15(1): 164.
30. Bolzinger MA, Briançon S, Pelletier J, Fessi H, Chevalier Y. Percutaneous release of caffeine from microemulsion, emulsion and gel dosage forms. *European Journal of Pharmaceutics and Biopharmaceutics*, 2008 Feb 1; 68(2): 446-51.
31. Arianto A, Lie DY, Bangun HB. Preparation and evaluation of nanoemulgels containing a combination of grape seed oil and anisotriazine as sunscreen. *Open Access Macedonian Journal of Medical Sciences*, 2020 Oct 4; 8(B): 994-9.
32. Malay NJ, Chandresh PP, Bhupendra GP. Nanoemulgel innovative approach for topical gel based formulation. *Nanoemulgel Innov approach Top gel based Formul*, 2018 Feb 15.
33. Malavi S, Kumbhar P, Manjappa A, Disouza J, Dwivedi J. Emulgel for improved topical delivery of Tretinoin: Formulation design and characterization. In *Annales Pharmaceutiques Françaises*, 2022 Mar 1; 80(2): 157-168. Elsevier Masson.
34. Balouiri M, Sadiki M, Ibensouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, 2016 Apr 1; 6(2): 71-9.