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Fabrication, characterization, and assessment of curcumin-loaded natural gum-based nanoemulsion gels: an innovative strategy for UTI treatment

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ABSTRACT

The current effort aimed to improve the permeability, solubility, and efficacy of curcumin against uropathogens by forming a gel based on nanoemulsion. Because the mixture was composed of natural, non-irritating, and pharmaceutically approved substances, it was safe to use and did not create sensitization. Chitosan, a natural polymer used to create the gel, was combined with natural gums such gum acacia and xanthan to create the formulation. The enhanced composition of the nanoemulsion gel was completed and described with success. The results of the experiments showed that at the 12-hour mark, the produced nanoemulsions and nanoemulsion gel released up to 86.87±2.18 and 76.94±1.93% of the medication, respectively. It was demonstrated that the nanoemulsion gel was more functional than pure medication. A noteworthy antibacterial activity was also observed in the nanoemulsion gel formulation (NEGF3) when four gramme negative uropathogenic bacteria were compared to nanoemulsion (NEF2) and pure curcumin. Therefore, compared to conventional formulations, the suggested Nanoemulsion-based gel offers a better potential for topical drug administration.

Keywords: Nanogel, Nanoemulsion, Uropathogens, Curcumin, UTIs, Antibacterial

INTRODUCTION

In contrast to the milky-white colour associated with coarse dispersion, nanoemulsion, often referred to as nanometric-sized emulsions, are fine water-in-oil (w/o) and oil-in-water (o/w) dispersions of two immiscible fluids. To stabilise these 20–200 nm droplets, add the suitable emulsifiers or amphiphilic emulsifiers. As a result, mini-emulsions are another name for nanoemulsion. Unlike microemulsions (ME), nanoemulsion (NE) are stable on heterogeneous systems because of their kinetic stability. Nanoemulsion, often referred to as "potential thermodynamic stability," are distinct because of their prolonged physical consistency; as a result, they don't seem to collect or flocculate. The earliest known instances of nanoemulsion are from the early 1900s, when scientists started experimenting with colloidal systems. Although early research concentrated on macroemulsions and microemulsions, it set the foundation for the subsequent development of nanoemulsion. Significant attention was paid to nanoemulsion as a separate class of emulsions during the 1990s. Scholars began delving into their distinctive characteristics, like their minuscule droplet sizes, which generally fall between 20 and 200 nanometers. This time period saw a change in perspective towards the possible uses of nanoemulsion, especially in the food and pharmaceutical industries [1-3]. The following advantages provide an explanation of why nanoemulsion is popular in the healthcare and personal care and cosmetics sectors:

- Compared to ME, which requires a high concentration (20%) of emulsifier, nanoemulsion can be created with concentrations as low as 3–10%.
- Because of its enormous surface area and ability to effectively transport active chemicals through semipermeable membranes, nanoemulsion enhances emulsion system penetration.
- The small globule size of nanoemulsion not only prevents droplet flocculation but also bigger droplet flocculation. Because of this, the system may function independently and without division.
- The decrease in Brownian motion and gravitational forces is brought about by tiny globules or droplets in a nanoemulsion. As a result, the product does not cream or sediment while it is being preserved.
- Nanoemulsion are easy to prepare and don't take a lot of energy. It is claimed that nanoemulsion formulations increase bioavailability and the repeatability of the plasma concentration profile.
- Because nanoemulsion include both lipophilic and hydrophilic medications, they are super solvents.
- The medication is shielded from environmental factors including pH hydrolysis and oxidation when the active ingredient is contained within a nanoemulsion formulation.
- Among other dosage forms, nanoemulsion can take the form of gels, creams, foams, aerosols, and sprays. They can also be administered topically, intravenously, intrapulmonaryly, intranasally, intramuscularly, orally. Nanoemulsion are more thermo-kinetically stable and have a greater solubilization capability than micelles dispersion.

- Because it uses an oil or lipid-based drug delivery method, it helps prevent hepatic first-pass metabolism.
- Additionally, the metallic and bitter tastes of drugs that may cause unpleasant side effects like nausea and vomiting can be efficiently covered up by nanoemulsion.
- Occasionally, lamellar liquid crystalline surrounding globules can be formed from nanoemulsion, which can be a valuable substitute for unstable liposomes and vesicles [4, 5].

Even with these instruments, the conception, fabrication, assembly, and manipulation of nanoemulsion remain very trivial. This impression stems mainly from the fact that conventional ideas about the stability and emulsion formation hardly get expanded upon. This kind of collective inadequacy serves as evidence for the way things are now perceived. In order to improve poorly aqueous soluble drug candidates' bioavailability and decrease dose-related side effects, this study focuses on the nanoemulsion concept as a novel delivery system. The drugs can be used to treat a variety of diseases, including cancer, diabetes, and hypertension.

The production of nanoemulsion for various drug delivery modalities offers a multitude of notable benefits and a broad spectrum of advantages, substantially improving the efficacy and versatility of pharmaceutical formulations. Comparing nanoemulsion formulations to nanoemulsion-based formulations, transdermal gel formulations demonstrate a notable increase in drug bioavailability. With a wide interfacial area for drug dissolution and enhanced solubility for drugs with low water solubility, nanoemulsion are identifiable by their tiny droplets. This advantage leads to higher drug-loading capacities, which facilitate the delivery of a wider range of therapeutic medicines. In the case of transdermal gels, nanoemulsion can improve drug penetration through the skin, resulting in a faster onset of action and more effective therapeutic outcomes [1-3]. Furthermore, the exceptional stability of nanoemulsion prevents medication deterioration and increases the shelf life of medications. This feature is very crucial when talking about transdermal gels because the formulation's endurance over time is what determines how consistently medications are administered.

The benefits of the latter are highlighted by the distinctions between transdermal gels with and without nanoemulsion. Gels derived from nanoemulsion have superior skin penetration, a greater capacity to load drugs, and a reduced potential for skin irritation. They enable more accurate control over the kinetics of drug release, leading to increased therapeutic efficacy and patient compliance. Furthermore, there is considerable flexibility in the administration of nanoemulsion via oral, intravenous, or topical routes. They can satisfy different patient demands and are helpful in a range of medicine administration applications because of their versatility [6-8],

Furthermore, nanoemulsion serve as an efficient carrier that can improve a drug's solubility and bioavailability after it has been applied topically. Additionally, the encapsulation of curcumin in the nanoemulsion formulation enhances drug penetration into the afflicted area, resulting in a potent antibacterial activity with minimal side effects to the system. This study designed and investigated a nanoemulsion-based gel for curcumin administration in an effort to enhance the drug's permeability, solubility, and efficacy against uropathogens.

MATERIAL AND METHODS

Drugs and chemicals

Curcumin was received as gift sample from Alpha remedies, Ambala, India. We purchased xanthan gum from Merck in India. We bought chitosan from Sigma Aldrich in Mumbai, India. The remaining chemicals and reagents were exclusively acquired and arranged from reliable and verified suppliers. Every chemical and reagent used was of analytical grade.

Preparation of nanoemulsion formulation (NEF)

The method described above was used to get the nanoemulsion ready [9] with slight modifications. To make nanoemulsion, high-speed homogenizers and stirrers were employed. Ten grammes of eucalyptus oil and twenty-five grammes of xanthan gum were combined to generate the oil phase (A) of the nanoemulsion. To the oil phase was introduced the medication. 39.9 g of distilled water and 25 g of tween-80 (Polysorbate-80) were combined to create the aqueous phase (B). Prior to the preparation of the nanoemulsion, both phases (A+B) were kept at 45 oC ± 5 for thirty minutes on a water bath (Labco, Model 345, India). After both phases were heated, the oil phase was progressively added to the aqueous and rapidly agitated for seven minutes at 3500 rpm. After the mixture was gradually cooled, the stirrer's speed was reduced to 1400 rpm for a whole 12-minute period. The stirrer's speed was increased again to 3500 rpm for 12 minutes, or until a uniform emulsion was created, in order to achieve uniform mixing. At least an hour was allowed for the emulsion to cool. This emulsion was passed through a high speed/high shear homogenizer at 14000 rpm for a duration of 12 minutes. In order to optimise, many formulations with different component counts were made and saved for early stabilities. Table 1 following is a list of the prepared formulas.

Table 1. Table of formula composition for nanoemulsion with varying component concentrations

Code for the formulations	Eucalyptus oil w/w	Xanthan gum (w/w)	Tween-80	Drug (w/w)	Distilled water Q. S to make 100 g
NEF-1 (Blank)	17 g	22 g	17 g	-	44 g
NEF-2	22 g	17 g	16 g	100 mg	44.9 g
NEF-3	19 g	20 g	14 g	100 mg	46.9 g
NEF-4	22 g	17 g	12 g	100 mg	51.1 g

Evaluation of nanoemulsions

Before being mixed with gel, a variety of nanoemulsion formulations were examined in person. The phase separation, consistency, and colour shifts of the various formulations were recorded for this reason. For a duration of 28 days, the samples of every formulation were stored at 8 ^{o} C, 25 ^{o}C, 40 ^{o} C, and 40 ^{o} C/75% relative humidity (RH).

Preparation of chitosan gel

Chitosan was dissolved in distilled water to create the chitosan gel. Three grammes of chitosan were properly weighed, and then it was dissolved in 100 millilitres of distilled water with 2% acetic acid (Zhou et al., 2016). A high-speed mixer set to run at 5000 rpm for 10 minutes was

used to create the gel. Before the generated gel was put to the emulsion, it was kept in storage for a whole night.

Preparation of nanoemulsion gel (NEG)

After choosing a stable nanoemulsion, it was combined 1:1 with chitosan gel and constantly stirred gently for at least 25 minutes. The result was a homogeneous, semisolid nano-emulsion gel composition. Triethanolamine (TEA) was used to reduce the pH of the formulation.

Characterizations

Thermodynamic stability and Heat cooling cycle

In order to test the optimised formulations under stressful conditions, they were both subjected to thermodynamic stabilities for 28 days in compliance with ICH (International Conference on Harmonisation) guidelines. With a few changes, this test was carried out utilising the previously published study as a guide [10]. The prepared nanoemulsion (NE) and the chitosan-based nanoemulsion gel (NEG) formulations were kept at 43 oC in an incubator for the first 28 days. After then, both formulations were allowed to come to room temperature. This test was designed to look for outward indications of turbidity, creaming, and cracking.

Freeze thaw cycle and Centrifugation

The NE and NEG underwent a 28-day freeze-thaw cycle, during which time they were maintained at a temperature of 3–4 ºC in a deep freezer. After being removed from the freezer, the two formulations (NE and NEG) were allowed to reach room temperature. After undergoing intense therapy, the formulations were inspected to determine whether they had reverted to their initial state. Both mixes were centrifuged using a high-speed centrifuge (Remi, India). After being kept in an Eppendorf, the two sample formulations were centrifuged for 15 minutes at 5000 and 11000 rpm. It was investigated if the formulations could split into an aqueous and an oily phase.

The pH of NE (NEF2) and NEG (NEGF3)

All of the freshly created NE and NEG formulations were evaluated after 12 hours, 24 hours, 7 days, and 14 days following a pH study [10].

Droplet size, surface charge and PDI

This test was conducted to determine the droplet size, surface charge, and polydispersity index of the nanoemulsion formulation. Helium-neon laser and zeta sizer (Nano ZS 90, Malvern Instruments, UK) were utilised for this objective based on the previously described methods. For three minutes, the mixture of one millilitre of nanoemulsion and ten millilitres of deionized water was thoroughly mixed. Following this procedure three times, the outcomes were averaged [11].

Drug content

Drug content analysis was carried out utilising the previously outlined methods with a few minor modifications [10]. To do this, one gramme (10 μg/g) of weighed nanoemulsion gel was combined with nine millilitres (w/v) of ethanol. Following that, this combination was centrifuged for two to six minutes at 5000 rpm using a Remi centrifuge (India). It was filtered via a nylon filter membrane with pores the size of 0.45 micrometres. In a UV spectrophotometer, three analyses of the sample filtrate (Shimadzu, Japan) were conducted, and the average findings were computed.

Viscosity of NEG and Morphological studies

The viscosities of the NE (NEF2) and NEG (NEGF3) formulations were measured on days 0, 1, 2, 7, 14, and 28 at 8 ºC, 25 ºC, and 40 ºC using a viscometer [12]. Spline #4 is mostly used to assess the viscosity of semisolid dosage forms, including nanoemulsion [13]. Beakers were used to hold the 50 g formulations of NE (NEF2) and NEG (NEGF3). The viscometer's spindle was positioned in the middle and slowly dipped into the beakers without coming into contact with the bottom. Values for both formulations were recorded while the spindle's rotation speed was set at 7 rpm for a duration of six minutes. Scanning electron microscopy was used to examine the morphology and apparent shape of the chitosan-based nanoemulsion gel (Quanta FEG, UK). Using double-sided sticky tape, the metal stubs were covered with NEG (blank) and drug-loaded samples. The stubs loaded with formulation were kept in a vacuum chamber for drying. Before inspection, the samples were coated for six minutes with an 8–11 nm thick layer of gold using a sputter coater. The stubs were subjected to an accelerating voltage of 12.5 KV and a magnification of 14,000X for the SEM investigation. The samples were shot using the previously mentioned techniques in the allocated areas [10, 13].

FTIR study and Spreadibility of NEG

FTIR spectroscopy was used to examine the drug crystals, chitosan powder, nanoemulsion, and nanoemulsion gel (Perkin, Elmer, United Kingdom). The objective of the study was to determine the degree of compatibility between the polymer and the formulation's component parts and to confirm that functional groups and wave numbers were present in the formulations. Using the knob, a sample of each mixture and component was applied to the diamond crystal, where it was crushed. The spectrum of each sample was recorded in triplicate in the 400–4000 cm–1 wave number range (Khan et al., 2020). With a few minor modifications, the "Drag & Slip" apparatus was used to evaluate the Spreadibility of the NEG, as previously reported [11]. All that's on this apparatus is a wooden block with a pulley fastened to one end. It consists of two glass slides of the same size, one of which is fixed to the block and the other of which is movable. Placed between the top mobile slide and the stationery slide, the sample whose Spreadibility needs to be assessed is given a measured weight. The improved nanoemulsion gel's Spreadibility was tested by sandwiching an upper slide between a fixed (Stationery) slide containing 2.0 g of NEG. A 50 g weight was placed on top of the glass slide. The time it took for the upper slide to move 8 cm was noted. The following formulas were used to calculate the test NEG's Spreadibility.

$S = M \times L \div T$

In this case, S stands for Spreadibility.

"M" indicates the weight on the upper glass slide.

"L" displays the length of the glass slides, while "T" indicates how long they travel.

***In vitro* drug release**

The in vitro drug release analysis was conducted in accordance with the previous study's conclusions [14]. This was accomplished by using a Franz diffusion cell (IPS Technologies, India) with donor and receptor compartment capacities of 6 ml and 3 ml, respectively. The temperature was maintained at 37 oC ± 1 oC and the stirring speed was 300 rpm prior to the addition of the NE and NEG samples. Because in vitro release experiments use artificial

membranes, a tuffryn membrane (Sortorius, Germany) was clamped between the receptor and donor compartments. Each of the two cell receptor compartments on the donor compartment had two grammes of each formulation sample. The receptor compartments were then filled with pH 5. 5 sodium acetate buffers. The sample (2 ml) was taken out of the receptor compartment using a spinal syringe at pre-arranged times: 0 h, 1 h, 2 h, 4 h, 8 h, and 12 h. New buffer was added in order to preserve the sink state and sodium acetate buffer level in the receptor compartment. After the sample was collected, the drug's release behaviour was determined by UV-spectrophotometer analysis (at 421).

Drug release kinetics

Subsequently, the medication's release behaviour was ascertained by fitting the drug release data into many mathematical models [10].

***In vitro* Antibacterial activities against uropathogenic bacterial strains**

Biological screening was done to ascertain the formulations' potential as antimicrobials against uropathogenic bacterial strains.

Microorganisms

These microbiological strains were used to assess the antibacterial qualities of *Escherichia coli* and *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* and *Escherichia coli*, two Gram-negative bacteria, were employed in the antibacterial activity screening. All strains were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC). The bacteria were grown on nutrient agar slants. The cultures were stored at 4 °C before usage, and they were subcultured on a regular basis.

Table 2. Specifics and growing conditions for the investigated microorganisms.

Standard Antibiotic	**Solvent used**	**MTCC No.**	**Micro-organisms**	**Strain**	**Incubation time**	**Temp**
Ciprofloxacin	Dimethyl sulfoxide (DMSO)	424	*Pseudomonas aeruginosa*	gram -ve	24h	37˚C
Ciprofloxacin	Dimethyl sulfoxide (DMSO)	1687	*Escherichia coli*	gram -ve	24h	30˚C

Screening for antimicrobial activity (antibacterial)

The agar-well diffusion method was used to measure the antimicrobial (or antibacterial) activity [15-17]. Test samples were reconstituted in dimethylsulphoxide (DMSO) to produce different concentrations of the sample (50, 100, 200, 250, and 500 µg/ml). By carefully mixing 0.5 ml of the 24-hour fresh cultures with 25 ml of sterile melted agar that had been cooled to about 30-37 °C, the test microorganisms were added to the medium (bacterial cultures were adjusted to #0.5 McFarland turbidity standards to determine antibacterial activity). To do this, sterilised Petri plates were used. When the material hardened, four wells with a diameter of 6 mm were made with a sterile borer. Each well received 100 µl of the test sample or solvent blanks. The antimicrobial assay plates were incubated at 30-37 °C for 24 hours. The positive

antibacterial controls employed were the standard antibiotics. The diameter of the zones of inhibition surrounding each well, including the well diameter, served as an indicator of the antibacterial activity. The mean diameter of the inhibitory zone was measured after each experiment was conducted three times [15-17]**.**

Statistical analysis

A One Way ANOVA and a student's T-test were applied to the data using GraphPad Prism. Once each variable was averaged three times, the data was shown as mean ± SD.

RESULTS & DISCUSSION

Characterizations of formulations (NE and NEG)

Physical appraisal and thermodynamic stability

Three formulations were kept at different temperatures (8 ºC, 25 ºC, 40 ºC, and 40 ºC + 75 relative humidity (RH)) for a period of 28 days. The formulations included blank formulations, chitosan-based nano-emulsion gel, and eucalyptus oil nanoemulsion. Physical assessments were conducted on these compositions periodically to evaluate their phase separation, consistency, liquefaction, colour change, and cracking. The freshly prepared formulations had a yellowish colour and a smooth, elegant appearance. After centrifugation at 5000 and 11000 rpm, no phase separation was observed. When the initial formulations were evaluated, their pH was found to be 5. 5. The pH of human skin was measured as a reference [18]. The pH of the formulations was measured using the student t test at various intervals following 12 hours, 24 hours, 7 days, 14 days, 1 month, 2 months, and 3 months. Each formulation's pH did not differ noticeably, or $p > 0.05$. In order to avoid skin irritations, the pH of drugs applied topically must be between 5 and 6 [18, 19]. Over time, the pH value significantly dropped, which could be due to water diffusing out of its phase or the oil in the formulations creating acidic chemicals [19, 20]. This pH fluctuation was, nevertheless, within the typical range for human skin. According to the characteristics listed above, each formulation was thermodynamically stable.

Droplet size, polydispersity (PDI) and surface charge

Droplet size is important when it comes to formulations used for transdermal or topical drug delivery [21]. The droplet size and size distribution have an impact on a number of intriguing properties, including medication release, drug penetration, and bio distribution [21]. The Malvern equipment from the UK was used to measure the droplet size, polydispersity, and zeta potential (Zetasizer) using dynamic light scattering (DLS). The droplet size, PDI, and surface charge of the drug-loaded, blank, and chitosan-based nanoemulsion gels, respectively, are shown in Table 3 and are designated as NEF1, NEF2, and NEGF3. In comparison to the blank nanoemulsion, which has a droplet size of 54.65±0.99 nm, the drug-loaded and chitosan-based nanoemulsion gels have higher droplet sizes. Drug loading has an impact on the droplet size in the formulation [22]. After the drug was loaded, the formulation's (NEF2) droplet size grew to 69.62±1.01 nm; in contrast, the mean droplet size of the chitosan-based nanoemulsion gel was 82.52±1.02 nm. The addition of chitosan as a gelling agent did not significantly increase the mean droplet size as some other authors have previously reported [23]. They came to the conclusion that adding polymers to the nanoemulsion system produced a significant amount of cross linking, increasing the formulations' viscosity and resulting in the formation of larger droplet sizes.

The negative charge on the surfaces of the drug-loaded and blank nanoemulsion is -17.8 mV and -20.6 mV, respectively. The formulations become more stable with the help of zeta potential, or the negative surface charge [24, 25]. The positive surface charge of 25.4 mV of the nanoemulsion gel can be explained by the addition of chitosan, a highly positive charged polymer, to the formulation, which imparts a positive charge to the mixture [25]. According to studies, formulations with positive or negative surface charge values exhibit greater stability because to the increased electrostatic repulsion between emulsion droplets, which prevents droplet coalescence [26, 27].

The formulas' PDI indicates the homogeneity and uniformity of the size distribution or droplet size. The higher the PDI, the less regular the droplet size and size dispersion are in the formulation [28]. If the PDI of a formulation is less than 0.45, it is considered homogeneous distributed [29]. The current investigation shows that the NEF1, NEF2, and NEGF3 formulations' droplet sizes were consistent, as seen by their corresponding PDI values of 0.139, 0.127, and 0. 346.

Table 3. PDI, droplet size, and zeta potential of the NEF1, NEF2, and NEGF3 formulations

Prepared Formulations	**Size of droplets (nm)**	**Zeta potential (Mv)**	**PDI Ration**
NEF1 - Blank	54.65±0.99	-20.6	0.139
NEF2 - Nanoemulsion	69.62±1.01	-17.8	0.127
NEGF3 – Nanoemulsion gel	82.52±1.02	25.4	0.346

FTIR study

Figure 1 displays the FTIR spectra of curcumin, pure chitosan, a gel containing chitosan and eucalyptus oil, and a nano-emulsion based on eucalyptus oil. FTIR spectroscopy investigation corroborated the novel drug's high concentration of phenols, carboxylic acids (COOH), alkanes, amines (NH2), and aliphatic amine (C–N) compounds [30]. The active ingredient in eucalyptus oil, 1,8-cineole, is responsible for its therapeutic properties. FTIR analysis was used to confirm the active ingredient in the created nanoemulsion compositions. The distinctive but less intense bands of 1,8-cineole in the FTIR spectrum are caused by the C-O-C symmetrical (1094.21 cm-1) and C-H3 symmetrical deformation modes, which are situated at 1470.12 cm-1 [30].

The spectra of the eucalyptus oil nanoemulsion showed two distinct peaks: the OH/NH stretching vibrations are represented at 3437.90 cm−1, while the methylene group is situated at 2930.88 cm−1. Larger bands were seen at 2988.12 cm-1, which corresponded to the N–H2 group, and at 1090.12 cm-1, which resulted from the C–O–C stretching. Chitosan's principal peaks were discovered at 3395.87 cm-1, primarily due to OH and NH stretching vibrations.

As seen in Figure 1, the FTIR spectrum was utilised to distinguish between the functional groups found in chitosan-loaded eucalyptus oil nano-emulsion and pure chitosan. The stretching vibrations of the O-H and N-H groups were indicated by the major peaks of chitosan, which were approximately 3375.90 cm−1 and 2880.64 cm-1. The peaks at 1641 cm−1 were found to originate from the secondary amide C=O bond of the remaining acetamido groups. The high signal at 1090.80 cm−1 is a result of O-H deformation of carboxyl groups [31].

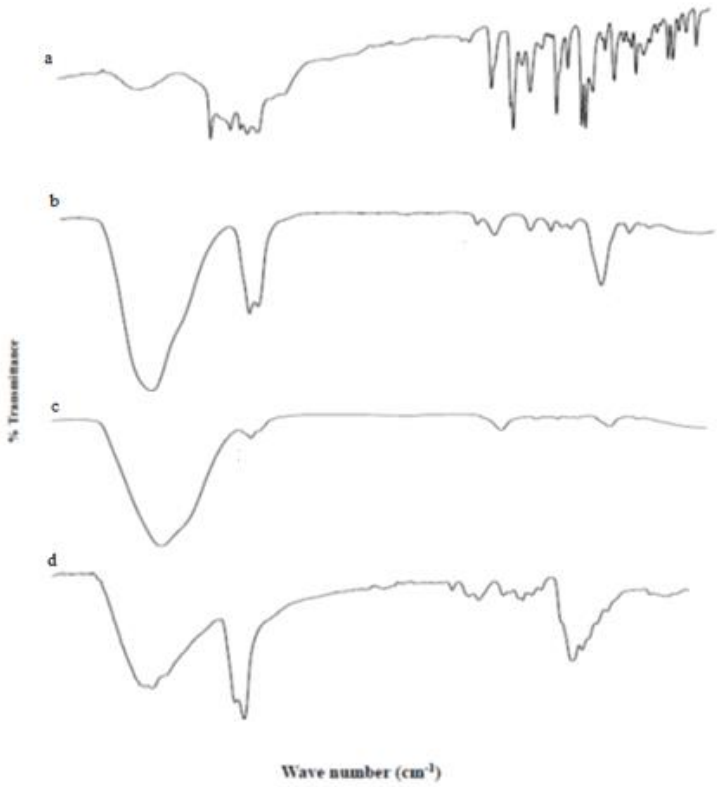

Figure 1. FTIR spectra of a. Drug, b. NE with Eucalyptus oil, c. Chitosan and d. NEG

Drug content analysis

The percentage of drug content in any pharmaceutical composition confirms that the medications are distributed uniformly. The percentage of drug in each formulation is shown in Table 4. The drug content of the NEF2 formulation was found to be 92.91±1.12%, whereas the NEGF3 formulation had 90.84±1.09%, according to the spectrophotometric analysis. Additionally, it demonstrated that the procedures followed in the current effort to create the nanoemulsion and nanoemulsion gel were appropriate for that goal.

Table 4. Shows the percentage of drug in the formulations for nanoemulsion and nanoemulsion gel.

Formulation code	**Drug Required (µg)**	**Drug Obtained (µg)**	**% Drug content**
NEF1	-	-	-
NEF2	100	92.91±1.12	92.91±1.12
NEGF3	100	90.84±1.09	90.84±1.09

Viscosity of formulations

When applied topically or transdermally, viscosity has a significant impact on drug delivery; it affects drug release, stability, Spreadibility, and application ease. The kind of gelling agent employed, the emulsifiers (surfactants and co-surfactants), the oils and co-solvents used in the formulations, and other elements can also have an impact on the viscosity of a formulation. The viscosities of the NEF2 and NEGF3 formulations, which were measured at various times and temperatures, are listed in Tables 5. The chitosan-based formulation (NEGF3) appears to have higher viscosities than the NEF2 formulation. The explanation might be the polymer

chitosan, which was included in the NEGF3 formulation as a gelling agent. The viscosities of the formulations at various temperatures were also tested in a similar way. It was found that higher temperatures led to lower viscosities and vice versa [14]. A study claims that the migration of water molecules from the dispersed aqueous phase to the continuous aqueous phase is what causes the inverse association between viscosities and temperature variations. They continued by saying that the fracturing of many globules due to osmotic pressure is another factor causing the inverse association between viscosities and temperature [10, 12-14].

Table 5. The viscosities of the formulations (NEF2 and NEGF3) were shown as centipoise at different times and temperatures.

NEF2			
Time	**Viscosities (Mean ± SD)**		
	8 ^{0}C	**25 ^{0}C**	**40 ^{0}C**
Day 0	6957±12.71	6957±11.19	6957±11.79
Day 7	6948±13.78	6928±13.89	5898±10.27
Day 28	6945±12.76	5989±12.89	5652±9.98
NEGF3			
	Viscosities (Mean ± SD)		
Time	**8 ^{0}C**	**25 ^{0}C**	**40 ^{0}C**
Day 0	13270±11.79	13279±12.49	13389±12.09
Day 7	13256±13.93	13076±11.98	11038±14.95
Day 28	13223±13.99	12986±11.88	11882±12.89

Morphological studies by SEM

Scanning electron microscopy (SEM) was used to examine the morphologies and forms of the drug-loaded nanoemulsion (NEF2), chitosan-based nanoemulsion gel (NEGF3), and blank nanoemulsion (NEF1), as illustrated in Figure 2. The blank nano-emulsion formulation (NEF1) looked like a mesh in the SEM image. However, connected holes with a random distribution of diameters are seen in the SEM images of the drug-loaded nanoemulsion (NEF2) and nanoemulsion gel (NEGF3). The formulations' porosity is advantageous since it offers lots of room for drug loading. In a similar vein, the formulations' drug mobility and release rate have been improved.

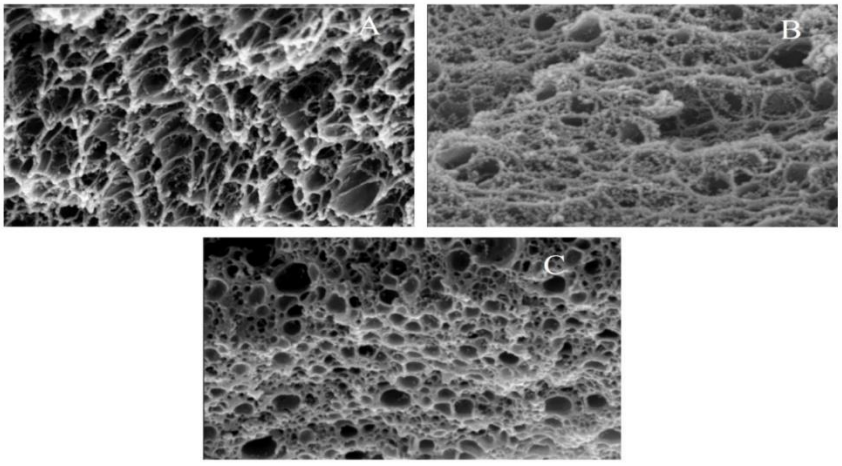

Figure 2. SEM photomicrographs of the formulations A. Blank formulation (NEF1), B. Nanoemulsion formulation (NEF2) and C. Nanoemulsion gel formulation (NEGF3)

Spreadibility

Spreadibility is the measure of a medicinal composition's skin dispersion when applied topically. Spreadibility is a crucial factor in topical preparations that impacts their medicinal efficacy. There must be a small shear for a formulation to come out of a container with the maximum amount of Spreadibility. There are several factors that affect the Spreadibility coefficient of topical application, including temperature extremes. It could be as a result of the formulation's increased viscosity at low temperatures, which decreased Spreadibility. In a similar vein, topical formulations' significant Spreadibility was caused by a drop in viscosity at high temperatures. Spreadibility and viscosity have an inverse relationship [10, 14]. The average Spreadibility values for NEF1, NEF2, and NEGF3 are shown in Table 6. The information is displayed as mean ± SD. Formulations kept at a high temperature showed greater Spreadibility than those kept at a low temperature. Moreover, formulations NEF1 and NEF2 spread more readily than formulation NEGF3 because they lacked a gelling ingredient. While NEF1 and NEF2 revealed no difference ($p > 0.05$), NEGF3 differed from them statistically significantly ($p < 0.05$).

Table 6. The spreadability for the formulations (NEF1, NEF2, and NEGF3) at the different temperatures under study is shown as an average value.

	Spreadibility		
Formulation codes	**8 ^{0}C**	**25 ^{0}C**	**40 ^{0}C**
NEF1	17.72±1.11	21.75±1.14	27.43±1.21
NEF2	17.89±1.12	21.77±1.15	27.35±1.20
NEGF3	13.30±1.09	15.73±1.12	17.28±1.09

***In vitro* drug release**

The therapeutic efficacy of a medicine is determined by its release from its pharmaceutical dosage form. A topical formulation's ability to release the medicine depends on a number of factors, including viscosity, emulsifying agents, gelling agents (polymers), and Spreadibility. Table 7 and Figure 3 illustrates the drug release from the nanoemulsion gel (NEGF3) and nanoemulsion (NEF2) formulations. At various time intervals, the percent drug release of the NEF2 formulation was determined to be 0%, 55.74±2.11, 65.89±2.02, 74.09±2.04, 81.73±2.25, 84.27±2.19, and 86.5±2.12%. Furthermore, the following drug release values were observed at different time intervals from the NEGF3 formulation: 0%, 52.34±1.98, 59.75±1.95, 70.65±1.99, 75.28±1.90, 78.89±1.96, and 76.83±1.89%. There is a significant difference ($p < 0.05$) in the amount of medication released between the NEF2 and NEGF3 formulations. The NEGF3 formulation released a somewhat lesser quantity of medicine in comparison to NEF2. The decrease in drug release from NEGF3 may be related to the presence of a gelling agent, which lengthens the diffusion routes for the drug to pass across the membrane and increases the intricacy of the gel network [10, 14].

Table 7. Drug release data in vitro expressed as a percentage for the formulations (NEF2 and NEGF3).

	Formulations	
Time (Hr)	**NEF2**	**NEGF3**
0	0	0

1	55.74±2.11	52.34±1.98
2	65.89±2.02	59.75±1.95
4	74.09±2.04	70.65±1.99
6	81.73±2.25	75.28±1.90
8	84.27±2.19	78.89±1.96
12	86.87±2.18	76.94±1.93

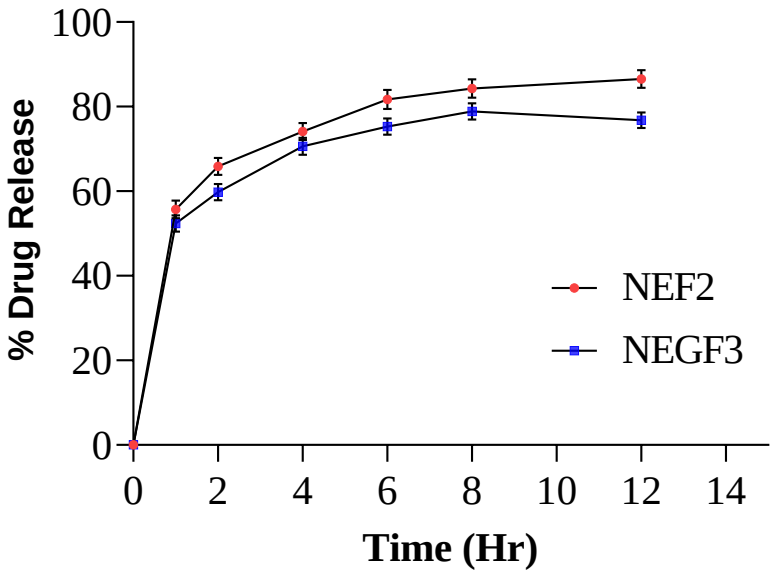

Figure 3. Drug release data in vitro expressed as a percentage for the formulations (NEF2 and NEGF3).

In vitro Antibacterial activities against uropathogenic bacterial strains

The zone of inhibition (ZOI) was used to assess the antibacterial activity of the formulation (NEGF3) against a range of bacterial species, including *Pseudomonas aeruginosa* and *Escherichia coli*. The formulation (NEGF3) demonstrated noteworthy antibacterial activity against all tested uropathogenic strains, according to the data, with larger ZOI values corresponding to higher concentrations. The ZOI for Pseudomonas aeruginosa rose gradually as the formulation concentration increased, from 13.62±1.04 mm at 50 µg/mL to 23.77±1.05 mm at 500 µg/mL. These findings demonstrated the improved antibacterial activity of the formulation (NEGF3) against *Pseudomonas aeruginosa*, as they were significantly greater than those of the pure medication (15.63±1.03 mm). ZOI values for *Escherichia coli* also showed a similar tendency of rising with increased formulation concentrations (NEGF3). For Escherichia coli, the ZOI values were 25.58±1.09 mm at the maximum dose examined (500 µg/mL). By contrast, the ZOI values for *Escherichia coli* were 16.95±1.04 mm when the pure medication was used. These results imply that the formulation (NEGF3) has strong antibacterial activity against a wide range of gram-negative uropathogenic bacteria, including pathogens that are clinically significant like Escherichia coli and *Pseudomonas aeruginosa*. The observed rise in ZOI with increasing doses highlights the formulation's (NEGF3) potential as powerful antibacterial agents for the treatment of bacterial infections in urinary tract infections (UTIs) and suggests a dose-dependent response. Overall, the study's findings demonstrate the

formulation's (NEGF3) improved and promising antibacterial activity when compared to the pure drug and call for more research to clarify its pharmacokinetic characteristics, mechanism of action, and potential for clinical application in the treatment of bacterial infections that are resistant to multiple drugs.

Table 8. ZOI data demonstrates the antibacterial effectiveness of nanoformulation (NEGF3) against uropathogens.

Conc. (µg/mL)	**Zone of inhibition (mm)**	
	Pseudomonas aeruginosa	*Escherichia coli*
50	13.62±1.04	11.38±1.05
100	15.66±1.01	15.59±1.04
200	18.72±1.02	18.28±1.10
250	21.63±1.08	21.83±1.12
500	23.77±1.05	25.58±1.09
DMSO	0	0
Pure Drug (Curcumin)	15.63±1.03	16.95±1.04

DMSO = Dimethyl sulfoxide (Control), Curcumin = (200µg/mL)

CONCLUSION

The improved nanoemulsion gel composition was successfully finished and characterised. The produced nanoemulsion and nanoemulsion gel released up to 86.87±2.18 and 76.94±1.93% of the drug at the 12-hour mark, respectively, according to the trials. The mixture was non-sensitizing and safe to use because it was made of natural, non-irritating, and pharmaceutically authorised ingredients. Natural gums and polymers were employed to create the formulation. The nanoemulsion gel was shown to have higher functionality when compared to pure medicine. When four gramme negative uropathogenic bacteria were compared to nanoemulsion (NEF2) and pure curcumin, a significant antibacterial activity was also shown in the case of nanoemulsion gel formulation (NEGF3). Thus, it can be said that the suggested Nanoemulsion-based gel offers a greater potential for topical drug administration than conventional formulations.

DECLARATION OF INTEREST

None

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Nil

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