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# Incorporating *Nigella sativa* nanoemulsion into gelatin-guar gum films for enhanced healing of wound infections

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## ABSTRACT

**Aims**: This study aims to investigate the impact of incorporating *Nigella sativa* essential oil nanoemulsion (NSNE) into gelatin (Ge) and guar gum (GG)-based films at various concentrations (0%, 2%, 4%, and 6%), to evaluate the antimicrobial properties of the resulting films against common bacterial strains associated with wound infections, as well as to assess their effects on the physical and chemical properties of the films, and to create a biomaterial with the potential to be utilized as wound dressing, possessing optimal properties capable of accelerating wound infection healing.

**Methods**: The nanoemulsion (NE) was obtained through ultrasonic irradiation. Polydispersity index (PDI), zeta potential, and particle size of NE were measured. For film preparation, gelatin (Ge) and guar gum (GG) were used, incorporating NSNE at concentrations of 0%, 2%, 4%, and 6%. Mechanical properties were evaluated using a universal testing machine, film thickness with a micrometer, and crystalline structure through X-ray diffraction (XRD) analysis. Scanning electron microscopy (SEM) was utilized for microstructure examination, and hydrophobicity was assessed by contact angle measurements. Antimicrobial activity was determined via the disk diffusion method against bacteria relevant to wound infections. Statistical analysis employed one-way ANOVA and Tukey post hoc tests with a significance level set at 5%.

**Results**: The particle size, PDI, and zeta potential of the NE were measured as 296±4.85 nm, 0.569±0.2, and -35.2±07 mV, respectively. The incorporation of NSNE into GE-GG-based films demonstrated promising antimicrobial efficacy against common wound infection bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. The films maintained mechanical integrity, with no significant alterations in tensile strength (TS) and elongation at break (EAB) (p0.05). However, higher NSNE concentrations led to decreased hydrophobicity ($p<0.05$) and structural changes, as evidenced by increased pores and cracks observed in SEM images.

**Conclusion**: This study highlights the potential of NSNE-loaded films to assist in healing wound infections, combining antimicrobial properties with a biocompatible film matrix.

**Keywords**: Biomedical engineering, wound infection, nanoemulsion, *Nigella sativa*

## INTRODUCTION

Wound care is a critical aspect of medical practice, constantly evolving with advances in medicine.[1] The process of wound healing involves the repair of the skin and underlying tissues, and it has been the subject of extensive research, including the use of medicinal plants and alternative therapies.[2] The bacteria responsible for wound infections encompass a range of pathogens, including multidrug-resistant Gram-negative bacteria such as *Pseudomonas aeruginosa (P. aeruginosa)*, *Escherichia coli (E. coli)*, and *Acinetobacter* species (spp.).[3] Additionally, *Staphylococcus aureus (S. aureus)*, *Enterobacteriaceae* family members, and *Enterococcus* spp. have been identified as common culprits in wound infections.[4,5] Furthermore, the prevalence of biofilms, where microorganisms adhere to surfaces and form a community within a self-produced matrix particularly those formed by *P. aeruginosa*, has been highlighted as a significant factor in wound infections, emphasizing the complex nature of microbial communities within infected wounds.[5,6] Wound infection is a critical issue due to its significant impact on wound healing and the overall well-being of patients. It has been noted that wound infections play a crucial role in the development of chronic wounds, leading to delays in the healing process and the current approaches to wound care are encumbered by several limitations, posing significant challenges in the treatment of chronic wounds. These limitations are multifaceted and encompass various aspects of wound care, including pathophysiological understanding, management modalities, and economic, clinical, and social impacts.

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The traditional wound dressings have several disadvantages, including the potential to cause further injury, limited antibacterial effects, and inadequate promotion of wound healing.[7] These limitations of traditional wound dressings have led to the development of novel dressings that aim to overcome these challenges.

Ge-based hydrogels have been characterized and investigated for their potential as wound dressings, showing high water uptake capacity and cell-interactive properties, which are essential for wound healing.[8,9] Besides, GG has shown potential for wound dressing applications due to its ability to form hydrogen bonding with water molecules, which is essential for maintaining a moist environment conducive to wound healing.[10]

Studies have shown that essential oils (EOs), such as those from St. John's Wort and Salvia cacaliifolia Benth., promote fibroblast migration and tissue repair, indicating their role in the wound healing process.[11,12] Furthermore, EOs have been reported to inhibit tissue remodeling-related proteins, suggesting promising wound healing properties.[13] The EO and seeds of *Nigella sativa (N. sativa)* have exhibited antimicrobial activity, making them potential candidates for combating bacterial and fungal infections.[14,15] Thymoquinone, an active compound in *N. sativa*, has been identified as having antimicrobial effects against both Gram-negative and Gram-positive bacteria, further supporting its potential for antimicrobial applications.[16]

NE form of EOs that have much smaller droplet sizes than coarse emulsions offer several advantages over coarse emulsions in microbiological applications. Their small droplet sizes provide improved emulsion homogeneity, stability against oxidation, coalescence, and creaming, enhanced solubility, and controlled release of volatile organic compounds.[17] The small droplet size of NEs expands their application options and presents a greater surface area, providing advantages over conventional macroemulsions.[18] Overall, the use of NEs in microbiological applications offers enhanced stability, improved bioavailability, and increased functionality of active compounds, making them a promising system for drug delivery and other microbiological applications.

This study introduces a novel approach by incorporating NSNE into GE/GG-based films, thereby aiming to enhance their antimicrobial efficacy for wound infection treatment. Additionally, the study evaluates the impact of NSNE incorporation on the physical and chemical properties of the films, contributing to the development of advanced wound dressing biomaterials with optimized healing properties.

## METHODS

This study does not require an ethics committee approval. approval. All procedures were carried out in accordance with the ethical rules and the principles.

### Preparation of *Nigella sativa* oil Nanoemulsion and Characterization

A two-step methodology was employed to generate NSNE. In the initial phase, the oil phase was formulated by blending 5% (v/v) *N. sativa* oil, 3% (v/v) ethanol, and 3% (v/v) Tween 80, followed by an incubation period at 85 °C for 1 hour. Subsequently, distilled water was added dropwise into the solution while consistently stirring at 25°C, during the second phase. The resulting blend, featuring a final concentration of 5% oil (v/v), underwent homogenization at 13,000 rpm for 5 minutes using a Wiggenhauser homogenizer (D-130, Germany). To transform the coarse emulsion into NE, the prepared mixture underwent ultrasonic irradiation in a Bandelin Electronic RK 255H ultrasonic bath (Germany) at 20°C, with a power of 160 W and a frequency of 35 kHz, for a duration of 15 minutes. The resultant NE was then preserved in a dark bottle at +4°C.

The PDI, zeta potential, and particle size values of NSNE were measured by the Malvern Zetasizer Nano ZSP instrument (Malvern Instruments Ltd., Malvern, UK). The analyses were run in triplicate.

### Preparation of *Nigella sativa* oil Nanoemulsion-loaded Films

In the initial phase, 2% (w/v) Ge was hydrated in distilled water at room temperature for 30 minutes and subsequently heated to 60°C using a magnetic stirrer. After cooling to room temperature, GG was introduced to the Ge solution at a concentration of 50% (w/w, Ge/GG) and stirred at 45°C for 3 hours. Glycerol, serving as a plasticizer, was then added (20% of the weight of Ge) and stirred for an additional 15 minutes. Following this, NSNE was incorporated into the solution at concentrations of 0%, 2%, 4%, and 6% (v/v), resulting in films named Ge/GG, Ge/GG-NSNE 2%, Ge/GG-NSNE 4%, and Ge/GG-NSNE 6%, respectively.

To eliminate bubbles, the film solutions underwent stirring at a reduced speed using a magnetic stirrer for an additional 30 minutes. Approximately 20 g of each film solution was poured into petri dishes (with a diameter of 9 cm) and allowed to dry at room temperature for 24 hours. After drying, the films were detached from the petri dishes and transferred to a desiccator with a controlled environment of 25°C and a relative humidity of 50±3%, saturated with magnesium nitrate, for a duration of 48 hours.

### Characterization of Films

**Mechanical properties of films:** The thickness of the films was determined using a micrometer (Loyka, 5203, Ankara, Turkiye) with measurements taken at five random locations on films and subsequent calculation of mean values. The TS and EAB values of the films were determined employing a universal testing machine (Testform/AS1, Ankara, Turkiye). Film samples were prepared in strips measuring 6×1 cm and then subjected to testing. The initial grip separation was set at 40 mm/min, and the crosshead speed was 50 mm/min. Each film was tested three times.

**X-ray diffraction analysis:** To examine the crystalline structure of the films, XRD patterns were acquired utilizing a Bruker AXS D8 Advance X-ray diffractometer (Madison, WI, USA) operating at 42 kV, 30 mA, and 1.540 A° with CuKa radiation. The spectra were recorded over a range of 2θ angles from 5 to 60°C at room temperature.

**Microstructure of films:** The surface morphology of the film samples was investigated using a SEM (Carl Zeiss Gemini 300, Germany) after gold coating. The samples were examined under low vacuum conditions at a voltage of 15 kV.

**Hydrophobicity of films:** The contact angles were assessed employing a Theta Attension optical tensiometer (Biolin Scientific, Gothenburg, Sweden). Approximately 5 µL of ultrapure water was delicately dispensed onto the film surface using a micro-syringe. Measurements were taken on both sides of the water droplet at room temperature, and the results were reported as the average of three determinations.

### Antimicrobial Activity

To investigate the antimicrobial properties of the produced films, the disk diffusion method was used, selecting certain bacteria responsible for wound infections. The pathogens were cultured in Mueller Hinton Agar at 37 °C for 18 hours. Subsequently, they were transferred into sterile saline, and the bacterial suspension's turbidity was adjusted ~$1.5x10^6$–$10^7$ CFU/mL. Sterile swabs were utilized to evenly spread the suspension onto Mueller-Hinton agar plates. Films with a diameter of 12 mm were sterilized using ultraviolet irradiation for 3 minutes. Following sterilization, the films were positioned on the plates and then incubated for 24 hours at 37°C. The diameter of the inhibition zones (mm) was measured as the diameter of the film+the zone, after three repeated measurements, and recorded as the mean±standard deviation (SD).

### Statistical Analysis

To assess variations in mean values, a one-way analysis of variance (ANOVA) followed by Tukey post hoc tests was conducted using SPSS software (version 22, Chicago, IL), with a predetermined significance level set at 5%.

## RESULTS

### Mechanical Properties of Films

Thickness and mechanical properties (TS and EAB) of films are shown Table 1. When compared to the control film (Ge/GG) the thickness of films incorporated with 2% NSNE remained unchanged, while an increase in thickness was observed in films incorporated with 4% NSNE and 6% NSNE. ($p<0.05$). The increase in film thickness can be attributed to the rising oil content in the film. The films incorporated with NSNE at different concentrations retained their mechanical integrity, exhibiting no notable changes in TS and EAB values ($p>0.05$).

**Table 1. Thickness and mechanical properties of films**

| Sample | Thickness (mm) | TS (MPa) | EAB (%) |
|---|---|---|---|
| Ge/GG | 0.256±0.05[a] | 3.0011.146[a] | 78.192±12.286[a] |
| Ge/GG-NSNE 2% | 0.258±0.11[a] | 3.2940.619[a] | 65.788±5.333[a] |
| Ge/GG-NSNE 4% | 0.262±0.03[b] | 3.1030.800[a] | 73.036±6.918[a] |
| Ge/GG-NSNE 6% | 0.265±0.06[b] | 2.9100.264[a] | 70.918±3.665[a] |

Data are mean±SD. Different letters in the same column indicate significant differences ($p<0.05$)

### X-Ray Diffraction Analysis

The main peaks of Ge in XRD graphs are typically observed at 2θ of 7° and 20°, representing the triple helix structure and single left-handed helix chain of Ge. The main peaks of GG in XRD graphs are typically observed at 2θ of 15°, 17°, 18°, and 23°, indicating the characteristic crystalline structure of GG. As seen in Figure 1 the sharpness of the 2θ of 17° peaks was observed to diminish in films incorporated with NSNE at concentrations of 4% and 6%. The XRD graph illustrates the typical semi-crystalline structure of polymers, and the addition of EOs has further reduced sharp peaks.

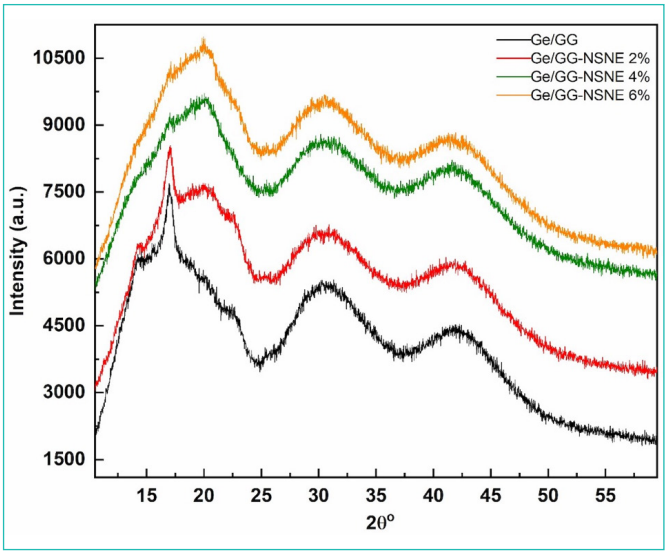


**Figure 1.** XRD diagram of film without NSNE and films loaded with 2% (w/w) NSNE, 4% (w/w) NSNE and 6% (w/w) NSNE

### Microstructure of Films

The incorporation of NSNE has been shown to alter the microstructural characteristics of polymer films. As seen in Figure 2, the number of pores and the cracks in the films has increased proportionally with the increasing concentration of NSNE.

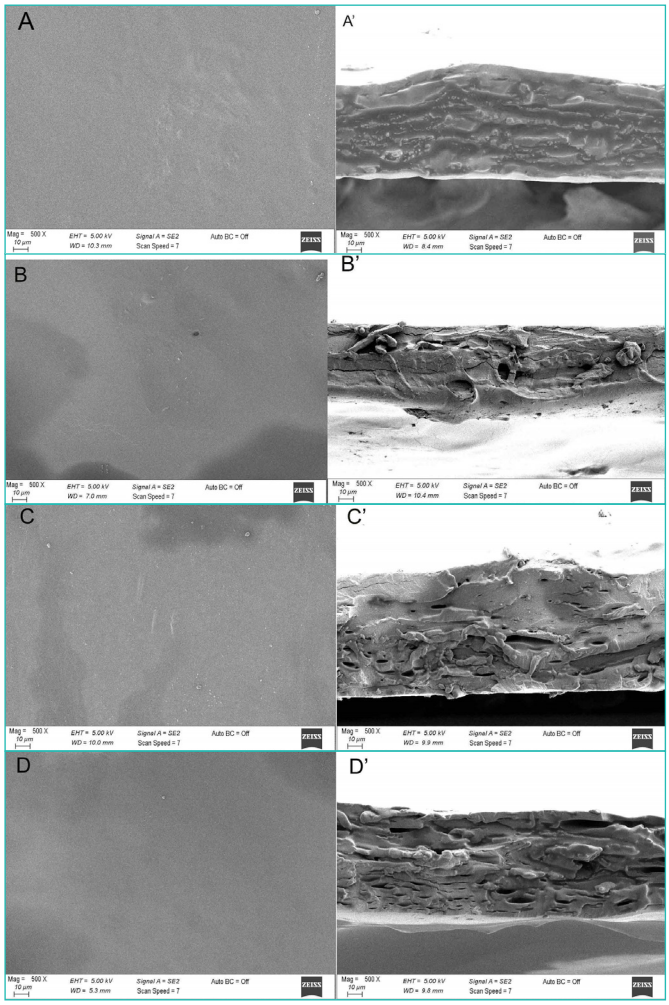


**Figure 2.** SEM images of surface (A, B, C, and D) and cross-section (A', B', C', and D') of the control film and films containing 2%, 4% and 6% of NSNE, respectively

### Hydrophobicity of Films

Contact angles of films are given in Table 2. While it is expected that the nature of the oil would increase hydrophobicity, it was observed that the hydrophobicity remained unchanged in films incorporated with 2% and 4% NSNE and decreased in the film incorporated with 6% NSNE ($p<0.05$).

**Table 2.** Contact angle of films

| Sample | Contact Angel (°) | Droplet photographs |
|---|---|---|
| Ge/GG | 108.27±12.28[a] | |
| Ge/GG-NSNE 2% | 36.47±1.68[a] | |
| Ge/GG-NSNE 4% | 41.61±1.30[a] | |
| Ge/GG-NSNE 6% | 25.78±1.25[b] | |

Data are mean±SD. Different letters in the same column indicate significant differences ($p<0.05$)

### Antimicrobial Activity of Films

The antimicrobial effects of the films against *E. coli*, *P. aeruginosa*, *Enterococcus faecalis (E. faecalis)*, *S. aureus*, and *Klebsiella pneumoniae (K. pneumoniae)* were determined. As seen in Table 3 it was observed that the effectiveness against the other bacteria, except for *S. aureus*, was not dependent on the incorporated oil ratio. While the effectiveness in films other than the control film showed a slight increase with the dose, this increase was not statistically significant ($p>0.05$). For *S. aureus*, films with 2% and 4% added oil were found to be statistically similar, but those with 6% incorporated oil exhibited a significantly higher efficacy ($p<0.05$).

## DISCUSSION

NEs are a category of emulsions characterized by their transparency or translucency, with droplet sizes falling within the range of 20 to 500 nm.[19] These droplets are smaller than those found in conventional emulsions, contributing to the kinetic stability, and improved functional

**Table 3.** Inhibition zones of films

| Strains | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | Ge/GG | Ge/GG-NSNE 2% | Ge/GG-NSNE 4% | Ge/GG-NSNE 6% |
| *E. coli* | 12.0[b] | 14.36±0.37[a] | 14.66±0.57[a] | 14.78±0.54[a] |
| *P. aeruginosa* | 12.0[c] | 16,51±0.44[b] | 16.08±0.80[b] | 16.06±1.10[b] |
| *E. faecalis* | 12.0[d] | 17.23±0.25[c] | 17.11±0.59[c] | 17.16±0.14[c] |
| *S. aureus* | 12.0[f] | 13.0[e] | 13.0[e] | 15.23±0.25[d] |
| *K. pneumoniae* | 12.0g | 15.58±0.14[f] | 15.50±0.43[f] | 15.92±0.14[f] |

Data are mean±SD. Different letters in the same row indicate significant differences ($p<0.05$)

performance of NEs.[20] Studies have shown that the droplet size of NEs can be influenced by various factors such as the type of oil used and the emulsification process.[21,22] The NE exhibited a mean particle size of 296 nm, a PDI of 0.569, and a zeta potential of -35.2 mV, indicating a moderately sized and well-stabilized colloidal system with a relatively uniform size distribution, thus holding promise for various applications in wound dressing.

NEs may disrupt the structural integrity of the films, leading to a reduction in TS.[23] However, in this study, the incorporation of NSNE did not cause any change in TS and EAB values ($p>0.05$). The mechanical properties of films with the addition of oil NE may remain unaltered due to the specific properties of the oil and its interaction with the film matrix. For instance, similar to present study, castor oil has been found not to plasticize the film matrix and may produce in situ blend compatibilizers due to the presence of certain functional groups.[24,25] Similarly, the incorporation of oils such as palm oil and essential oils has been observed to increase the elasticity and thickness of film packages without significantly affecting the TS.[26]

The addition of oils into Ge-based films has been found to have several effects on the properties of the resulting films. Incorporating oils into polymer films can indeed alter the sharp XRD peaks and reduce the crystallinity of the films. Similar to present study, the addition of limonene to polylactic acid resulted in a decrease in the degree of crystallinity due to enhanced polymer chain mobility and the plasticization effect.[27] Similarly, the incorporation of essential oils into carrageenan-based films was found to influence the mechanical properties and decrease the crystallinity of the films.[28]

The formation of pores in the film matrix can be attributed to various factors, including the reduction in intermolecular forces between the polymer chains due to the incorporation of NEs.[29] Furthermore, the incorporation of lipid compounds into hydrocolloid-based films has been reported to decrease water vapor permeability, potentially leading to the formation of pores in the film matrix.[30] The addition of EOs to film formulations has been reported to weaken the film by decreasing cohesion forces within the structure, potentially leading to the formation of pores.[31] These factors collectively contribute to the formation of pores in the film matrix following the incorporation of oil NEs.

Hydrophobicity is an important feature in film samples intended for wound dressing applications. The hydrophobic nature of the film can prevent the ingress of water and microorganisms, while maintaining a moist environment at the wound interface, which is conducive to wound healing. Additionally, hydrophobic films can effectively act as a barrier to microorganisms and remove excess exudates from the wound surface, promoting an optimal environment for wound healing.[32] The decrease in hydrophobicity when oil NE is incorporated into the film matrix can be attributed to several factors. The addition of NEs can lead to an increase in the molecular spaces between the protein, resulting in decreased hydrophobicity.[33] Furthermore, the reduction in intermolecular forces between the polymer chains due to the incorporation of NEs can contribute to the decrease in hydrophobicity. These factors collectively contribute to the observed decrease in hydrophobicity when oil NE is incorporated into the film matrix. Consistent with the present study, films developed by Acharya[34] and Mutlu[35] addition NEs to the film matrix decreases surface hydrophobicity.

The antibacterial effect of *N. sativa* has been extensively studied. Thymoquinone, an active principle of *N. sativa*, has been found to possess strong antibacterial properties against Gram-positive bacteria such as *S. aureus* and *Streptococcus* mutants.[36] Thymoquinone has been shown to have potency in preventing bacterial biofilm formation.[37] Topical gel formulations prepared using ethylacetate extract of *N. sativa* have demonstrated antibacterial properties against acne-causing microorganisms, indicating its potential as an alternative remedy for dermal acne.[38] These findings highlight the potential of *N. sativa* and its active components in combating bacterial infections and supporting skin health.

These findings collectively suggest that the developed films, particularly those supplemented with 2% and 4% NSNE, hold promise as wound dressing materials for wound healing applications. Further research could explore optimization strategies to fine-tune the material properties, considering the observed changes in hydrophobicity and microstructure. Additionally, in vivo studies would be crucial to validate the efficacy and safety of these films in practical wound care scenarios. Overall, this study contributes valuable insights into the development of functionalized films for potential biomedical applications.

## CONCLUSION

The incorporation of NSNE into Ge/GG-based films demonstrated minimal impact on mechanical properties, underscoring their potential for wound healing applications. Notably, the films maintained their structural integrity, with no significant alterations in TS and EAB values, while exhibiting enhanced antimicrobial efficacy. These findings emphasize the clinical relevance of the developed films, especially those enriched with 2% and 4% NSNE, as promising wound dressings with both structural resilience and heightened antimicrobial properties for effective biomedical applications.

## ETHICAL DECLARATIONS

### Ethics Committee Approval

This study does not require an ethics committee approval due to its design.

### Informed Consent

This study does not require an informed consent.

### Referee Evaluation Process

Externally peer reviewed.

### Conflict of Interest Statement

The authors have no conflicts of interest to declare.

### Financial Disclosure

The authors declared that this study has received no financial support.

### Author Contributions

All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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