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Exploring the Synthesis of Nanoemulsions and Assessing Their Antimicrobial Effects

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ABSTRACT

Objective: This review investigates the synthesis of nanoemulsions utilizing prepared essential oils and evaluates their antimicrobial effects. Nanoemulsions, characterized by their small droplet size and enhanced stability, offer promising applications in various industries, including pharmaceuticals and cosmetics, due to their potent antimicrobial properties.

Methods: The synthesis process involves the preparation of essential oils through extraction methods, followed by their incorporation into nanoemulsion formulations using appropriate surfactants and homogenization techniques. The resulting nanoemulsions are then subjected to rigorous antimicrobial testing against a spectrum of microorganisms, employing standardized assays to assess their efficacy.

Results: The findings highlight the significant antimicrobial potential of these essential oil-based nanoemulsions, demonstrating their effectiveness against a variety of bacterial and fungal strains. Furthermore, the elucidation of the underlying mechanisms governing their antimicrobial activity is explored, providing valuable insights into their mode of action.

Conclusion: This study contributes to advancing the understanding of nanoemulsion synthesis using prepared essential oils and underscores their promising role as effective antimicrobial agents in diverse applications.

Keywords: Essential oils, extraction techniques, synthesis antimicrobial effects, microorganism, nanoemulsions.

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INTRODUCTION

Nanoemulsions are submicron emulsions that have gained significant attention due to their potential applications in various fields, including medicine, cosmetics, and food science. They are colloidal dispersions of two immiscible liquids stabilized by surfactant molecules.[1] The small droplet size of nanoemulsions, typically ranging from 20 to 500 nm, imparts them with unique physicochemical properties, such as increased stability and enhanced solubility of active ingredients.[2] These properties make nanoemulsions an attractive delivery system for bioactive compounds, including antimicrobial agents.

The antimicrobial properties of nanoemulsions have been extensively studied, with research demonstrating their effectiveness in delivering and enhancing the activity of antimicrobial agents. For instance, nanoemulsions incorporating essential oils, such as citral, have shown significant antimicrobial activity.[3] The formulation of nanoemulsions has been found to significantly impact their antimicrobial activities, highlighting the importance of understanding the physicochemical characteristics of these systems.[4] Furthermore, nanoemulsion gels have been developed to address the limitations of conventional nanoemulsions, such as chemical instability and skin irritation, while retaining their beneficial properties, including antimicrobial activity.[5]

In addition to their antimicrobial properties, nanoemulsions have been investigated for their potential in targeted cancer therapy and anti-inflammatory applications. Studies have demonstrated the development of stable nanoemulsion delivery systems for oral administration of anti-cancer agents, enhancing their solubility, bioavailability, and efficacy.[6]

Furthermore, nanoemulsions have been shown to improve the anti-inflammatory properties of bioactive compounds, such as nobiletin, by enhancing their delivery to target cells.[7]

The unique physicochemical characteristics of nanoemulsions, including their small droplet size and enhanced permeability, make them promising vehicles for transdermal drug delivery and wound healing applications.[8,9] Moreover, the development of novel organogel-based nanoemulsions has been explored to improve the oral bioavailability of bioactive compounds, such as curcumin, demonstrating the versatility of nanoemulsions in pharmaceutical applications.[10] Overall, nanoemulsions represent a versatile and promising platform for the delivery of antimicrobial agents and bioactive compounds, with potential applications in various fields, including medicine, cosmetics, and food science. Their unique properties, such as small droplet size, enhanced stability, and improved bioavailability, make them an attractive option for the development of advanced delivery systems.

METHODS

Synthesis Methods for Nanoemulsions

Nanoemulsions, characterized by their small droplet size and unique physicochemical properties, are synthesized using various methods, including high and low energy techniques.

High-Energy Methods

High pressure homogenization (HPH) and ultrasonication are widely used high energy methods for nanoemulsion synthesis, offering efficient means to produce stable nanoemulsions with controlled droplet size Gupta et al.[11,12]

High-pressure homogenization: In this method, a high-pressure homogenizer is utilized to force the mixture of oil, water, and surfactant through a narrow gap at high velocities, resulting in intense shear forces and droplet breakup. Ultrasonication: Ultrasonic waves are applied to the emulsion mixture, causing cavitation bubbles that collapse and generate microjets, leading to droplet fragmentation and nanoemulsion formation.

Additionally, the phase inversion temperature (PIT) method has been employed for nanoemulsion preparation, demonstrating its effectiveness in producing nanoemulsions with specific characteristics.[13]

Low-Energy Methods

Phase Inversion Temperature (PIT) method: This technique exploits the phase transition of surfactants in water-oil systems at specific temperatures, inducing spontaneous emulsification and nanoemulsion formation.

Spontaneous emulsification: Also known as the phase inversion composition (PIC) method, this approach relies on the gradual addition of water to an oil-surfactant mixture under constant stirring, leading to spontaneous emulsion formation.

Solvent displacement method: In this method, a water-soluble solvent (e.g., ethanol) containing the surfactant is mixed with the oil phase, followed by the addition of water, resulting in nanoemulsion formation due to solvent displacement.

Furthermore, the ultrasonic method has been utilized for the synthesis of nanoemulsions, offering a low energy approach to achieve homogenous nanoemulsions.[14,15] The inverse nanoemulsion technique has also been employed for the synthesis of nanoemulsions, providing a unique route for the production of nanoemulsions with specific properties.[16] Moreover, the Ouzo effect has been utilized as a simple nanoemulsion synthesis method, demonstrating its potential for the production of nanoemulsions with tailored properties.[17]

In contrast, low energy methods, such as the emulsion phase inversion (EPI) method, have been explored for the production of nanoemulsions, offering a versatile approach to generate nanoemulsions with controlled droplet size and stability.[18] Additionally, the use of self-assembly processes and water-in-oil nanoemulsion cross-linking techniques has been investigated for nanogel synthesis, providing alternative routes for the fabrication of nanoemulsions with specific applications.[19] Furthermore, pH regulation based on fatty acid/amine complexes has been proposed as a new low-energy method for nanoemulsion formation, offering a novel approach to synthesize nanoemulsions with tailored properties.[20] The low energy nanoemulsions have also been utilized as templates for the formulation of hydrophobic drugs, demonstrating their potential in drug delivery applications.

Overall, the synthesis of nanoemulsions encompasses a diverse range of methods, including high and low energy techniques, each offering unique advantages for the production of nanoemulsions with tailored properties and applications.

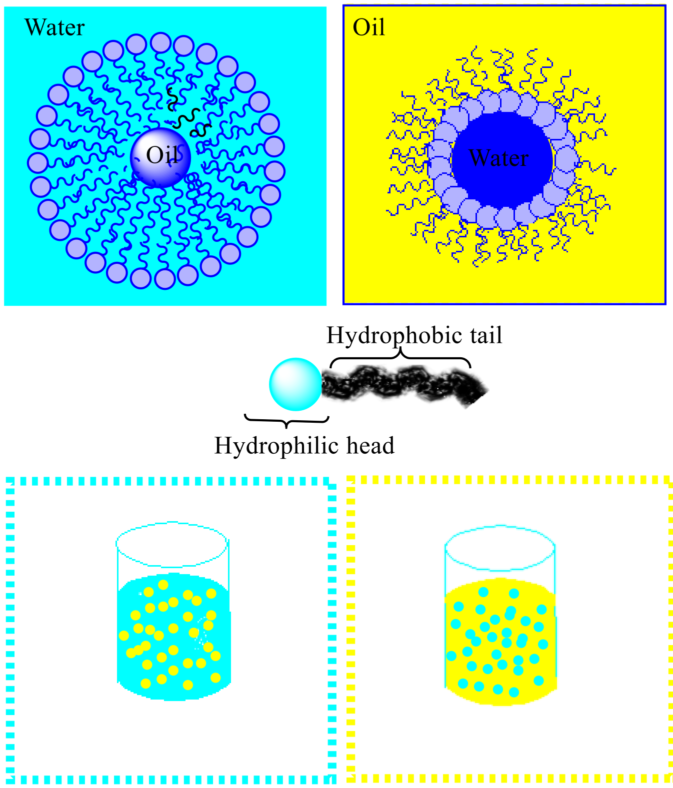

Figure 1. Nanoemulsion formulation demonstration

Membrane Emulsification:
Membrane-based emulsification techniques involve the use of membranes with defined pore sizes to control droplet size and distribution. Techniques such as microfluidic membrane emulsification and membrane dispersion enable precise control over nanoemulsion synthesis.

Each synthesis method offers distinct advantages and challenges, and the choice depends on factors such as desired droplet size, stability requirements, scalability, and compatibility with specific formulations. Optimization of synthesis parameters is crucial to achieve nanoemulsions with desired characteristics for various applications in pharmaceuticals, cosmetics, food, and biomedical fields.

Characterization Techniques for Nanoemulsions
Nanoemulsions, owing to their unique properties and potential applications, require thorough characterization to assess their physical and chemical attributes. Various techniques have been employed for the comprehensive characterization of nanoemulsions, including microscopy imaging, cryogenic transmission electron microscopy (cryo-TEM), zeta potential analysis, and dynamic light scattering (DLS) . Microscopy imaging techniques have been utilized to assess the microstructure of nanoemulsions, providing insights into their droplet size and distribution. Various techniques are employed to characterize nanoemulsions, including:

Particle Size Analysis

Dynamic Light Scattering (DLS): DLS measures the intensity fluctuations of scattered light by particles in solution, providing information on the hydrodynamic diameter and size distribution of nanoemulsion droplets.

Laser Diffraction: This technique measures the angular distribution of light scattered by particles, allowing for the determination of droplet size distribution in nanoemulsions.

Zeta Potential Measurement:

Electrophoretic Light Scattering (ELS): ELS determines the zeta potential, which reflects the surface charge and stability of nanoemulsion droplets. It provides insights into the electrostatic repulsion between particles, influencing colloidal stability.[21]

Microscopy Techniques

Transmission Electron Microscopy (TEM): TEM offers high-resolution imaging of nanoemulsion morphology, allowing visualization of individual droplets and assessment of size, shape, and uniformity.

Scanning Electron Microscopy (SEM): SEM provides detailed surface morphology information, aiding in the characterization of nanoemulsion structure and stability.

Rheological Analysis
Rheometry: Rheological measurements assess the flow behavior and viscoelastic properties of nanoemulsions under different shear conditions, offering insights into their stability and suitability for various applications.

Stability Assessment

Centrifugation: Centrifugation tests evaluate the sedimentation stability of nanoemulsions by subjecting them to centrifugal forces, allowing observation of phase separation and creaming.

Turbidity Measurement: Turbidity analysis measures the optical density of nanoemulsions over time, providing information on stability against coalescence and aggregation.

Chemical Analysis

Fourier Transform Infrared Spectroscopy (FTIR): FTIR identifies functional groups and chemical bonds in nanoemulsion components, elucidating molecular interactions and composition.

Nuclear Magnetic Resonance (NMR): NMR spectroscopy enables the identification and quantification of molecular species in nanoemulsions, aiding in understanding their formulation and stability.

These characterization techniques collectively provide a comprehensive understanding of nanoemulsion properties, aiding in formulation optimization, quality control, and assessing their suitability for specific applications in pharmaceuticals, cosmetics, food, and biotechnology industries.

Zeta potential analysis and DLS have been employed to determine the surface charge and particle size distribution of nanoemulsions, respectively, contributing to the understanding of their stability and colloidal behavior.

In addition to imaging and particle size analysis, other techniques such as atomic force microscopy (AFM), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Fourier Transform Infrared Spectroscopy (FT-IR), Thermo-Gravimetric Analysis (TGA), and Contact Angle Measurement (CAM) have been utilized for the comprehensive characterization of nanoemulsions AFM has been established as an important technique for interface characterization, offering unique advantages over traditional imaging and surface force-determining approaches. XRD, SEM, TEM, FT-IR, TGA, and CAM have been employed to assess the structural, thermal, and surface properties of nanoemulsions, providing valuable insights into their composition, morphology, and stability. These analyses provide insights into the chemical composition, thermal stability, and surface charge of the nanoemulsions, which can influence their antimicrobial behavior.

Furthermore, techniques such as laser diffraction, multiple light scattering, and second harmonic scattering (SHS) have been utilized to analyze the physical properties, stability, and orientational order of interfacial water molecules in nanoemulsions, contributing to a comprehensive understanding of their behavior and interactions. Laser diffraction and multiple light scattering techniques have been employed to analyze the droplet size and stability of nanoemulsions, while SHS has been utilized to characterize the orientational order of interfacial water molecules, providing insights into their surface properties.

Overall, the characterization of nanoemulsions involves a diverse range of techniques, each offering unique insights into their physical, chemical, and structural properties, contributing to a comprehensive understanding of their behavior and potential applications.

The synthesis and characterization of nanoemulsions play a pivotal role in elucidating their physical and chemical properties, which in turn dictate their suitability for various applications. Overall, the results of nanoemulsion synthesis and characterization provide valuable insights into their formulation stability, physical properties, and potential applications. These findings form the basis for further research into the antimicrobial effectiveness and biomedical importance of synthesized nanoemulsions, which will be discussed in subsequent chapters.

Antimicrobial Assays and Testing Protocols

The assessment of antimicrobial activity in nanoemulsions requires robust assays and standardized protocols to ensure accurate and reliable results. Several methodologies are employed to evaluate the effectiveness of nanoemulsions against various microbial strains. Key antimicrobial assays and testing protocols include:

Agar Diffusion Assay (Disk Diffusion Method):

The disc diffusion method, a widely used assay, has been employed to examine the antibacterial ability of nanoemulsions against specific pathogens, such as Escherichia coli Doan et al.[22] This method involves the diffusion of the nanoemulsion onto an agar plate inoculated with the target microorganism, followed by the measurement of the zone of inhibition, providing insights into the antimicrobial activity of the nanoemulsion. This assay involves impregnating paper disks with nanoemulsions and placing them onto agar plates inoculated with microbial cultures. The zone of inhibition around the disk indicates antimicrobial activity, with larger zones correlating to higher efficacy.[23,24]

Minimum Inhibitory Concentration (MIC) Determination:

In addition to the disc diffusion method, the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) has been utilized to quantify the lowest concentration of the nanoemulsion required to inhibit microbial growth and induce microbial death, respectively.[25] These assays provide valuable information on the potency of the nanoemulsion against specific microorganisms, contributing to the understanding of their antimicrobial efficacy. MIC testing determines the lowest concentration of nanoemulsion required to inhibit microbial growth. Dilutions of nanoemulsions are prepared in growth media and inoculated with microbial strains. MIC values are determined based on the absence of visible growth.

Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) Assays

MBC and MFC assays determine the lowest concentration of nanoemulsion required to kill microbial cells. Following MIC determination, aliquots from wells showing no visible growth are subcultured onto agar plates to assess viability.[26-28]

Time-Kill Kinetics:

Time-kill kinetics evaluates the rate and extent of microbial killing by nanoemulsions over time. Microbial suspensions are exposed to sub-inhibitory concentrations of nanoemulsions, and viable cell counts are determined at specified time intervals.

Biofilm Inhibition and Disruption Assays:

These assays assess the ability of nanoemulsions to prevent biofilm formation or disrupt preformed biofilms. Nanoemulsions are incubated with biofilm-producing microbial strains, followed by quantification of biofilm biomass or visualization using microscopy.[29-34]

The use of nanoemulsions as antimicrobial agents has also been explored in the medical field, where they have demonstrated notable antimicrobial activity against biofilm organisms, such as Streptococcus mutans and Acinetobacter baumannii.[35,36] Additionally, nanoemulsions have been reported to possess strong antibacterial and antioxidant activities, making them potential candidates for applications in wound healing and burn injury treatments.[35,37]

Nanoemulsions have gained significant attention due to their potential antimicrobial activity. These emulsions, composed of nanometer-sized droplets stabilized with surfactants, have been shown to exhibit a broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria.[38] The antimicrobial activity of nanoemulsions is attributed to their ability to encapsulate bioactive compounds, leading to better fusion with bacterial cell walls and subsequent disruption of the bacterial cells.[39] Furthermore, the increase in surfactant concentration in nanoemulsions has been found to enhance their antimicrobial activity compared to those with lower surfactant concentration or bulk essential oils.[40] Additionally, the encapsulation of essential oils in nanoemulsions has been reported to significantly enhance their antimicrobial activity, making them promising tools for insect pest and pathogen management.[41]

Evaluation of antimicrobial activity is critical to assessing the effectiveness of nanoemulsions as potential anti-microbial agents. Overall, the results show the significant antimicrobial potential of synthetic nanoemulsions, leading to their development as an effective antibiotic for applications in health care, food protection and environmental improvement. Further explanation of the mechanisms of action and optimization of nanoemulsion formulations will enhance their effectiveness and extend their benefits in the fight against microbial infections.

Cytotoxicity Testing:

Cytotoxicity assays evaluate the impact of nanoemulsions on mammalian cell viability. Cell lines are exposed to varying concentrations of nanoemulsions, and cell viability is assessed using assays such as MTT or alamarBlue.[42,43]

Furthermore, the evaluation of cytotoxic effects on various cell lines, such as CT26 colon cancer cells and umbilical vein endothelial cells (HUVEC), has been conducted to assess the impact of nanoemulsions on both cancerous and normal cells, providing insights into their potential cytotoxicity and biocompatibility.[13] This testing protocol contributes to the comprehensive assessment of the safety profile of nanoemulsions for potential biomedical applications.

Quality Control and Standardization:

Adherence to established guidelines and standards, such as those outlined by the Clinical and Laboratory Standards Institute (CLSI), ensures consistency and comparability of antimicrobial assay results.

By employing these antimicrobial assays and testing protocols, researchers can comprehensively evaluate the efficacy, mechanisms of action, and safety profiles of nanoemulsions, facilitating their development as potent antimicrobial agents for diverse applications in healthcare, agriculture, and food preservation.

Future Directions and Applications in Biomedical and Food Industries

Nanoemulsions offer promising prospects for future applications in both biomedical and food industries. In the biomedical realm, these nanostructures could revolutionize antimicrobial therapy by facilitating targeted drug delivery to infection sites while minimizing systemic side effects. Additionally, nanoemulsions may find utility in wound healing, where their antimicrobial properties could be harnessed to prevent infection and promote tissue regeneration. Exploring their potential in eradicating biofilms associated with chronic wounds or medical implants represents another avenue for research. Furthermore, investigating synergistic combinations of nanoemulsions with existing antimicrobial agents could enhance therapeutic efficacy and mitigate resistance.

In the food industry, nanoemulsions hold considerable potential for improving food preservation, safety, and flavor enhancement. They could be integrated into food packaging materials to inhibit microbial growth and extend shelf life, thereby reducing food spoilage and waste. Moreover, nanoemulsions containing natural antimicrobial agents, such as essential oils or plant extracts, could serve as alternatives to synthetic preservatives, aligning with consumer preferences for clean-label products. Nanoemulsions also offer a means of encapsulating and delivering hydrophobic flavor compounds, enhancing flavor stability and sensory attributes in food products. Additionally, nanoemulsions with antimicrobial properties could be employed as sanitizing agents for food contact surfaces or as wash solutions for fresh produce, bolstering food safety measures.[44-46]

Despite the promising prospects, several challenges must be addressed before widespread adoption of nanoemulsions in biomedical and food applications. Safety assessments and regulatory approvals are paramount to ensure the safe use of nanoemulsions in consumer products and medical devices. Achieving long-term stability and scalability of nanoemulsion formulations is critical for industrial production and commercialization. Educating consumers about the benefits and safety of nanoemulsion-based products is essential to foster acceptance and market penetration. Additionally, assessing the environmental impact of nanoemulsions and their byproducts is crucial to mitigate potential ecological risks. Overall, nanoemulsions hold significant promise for addressing current challenges and advancing various applications in biomedical and food industries. Continued research efforts, collaborative initiatives, and regulatory oversight will be instrumental in realizing the full potential of nanoemulsion technology and translating it into impactful solutions for global health and food security. The future directions and applications of nanoemulsions in the biomedical and food industries hold significant promise for addressing various challenges and advancing innovative solutions. Nanoemulsions, with their unique properties and versatile applications, are poised to revolutionize several sectors, including food preservation, drug delivery, wound healing, and tissue engineering.

In the biomedical field, nanoemulsions offer potential applications in drug delivery systems, where their ability to encapsulate and deliver bioactive compounds to specific targets holds great promise for improving therapeutic outcomes. Furthermore, the use of nanoemulsions in wound healing and burn injury treatments has shown significant potential, with their strong antibacterial and antioxidant activities making them valuable for medical applications. Additionally, the antimicrobial properties of nanoemulsions make them suitable for addressing microbial threats in biomedical settings, such as in the development of antimicrobial coatings for medical devices and implants.[47-53]

In conclusion, the future applications of nanoemulsions in the biomedical and food industries hold great promise for addressing various challenges and advancing innovative solutions. The unique properties of nanoemulsions, coupled with their potential for sustainable and eco-friendly applications, position them as valuable tools for revolutionizing drug delivery, food preservation, and functional food development.

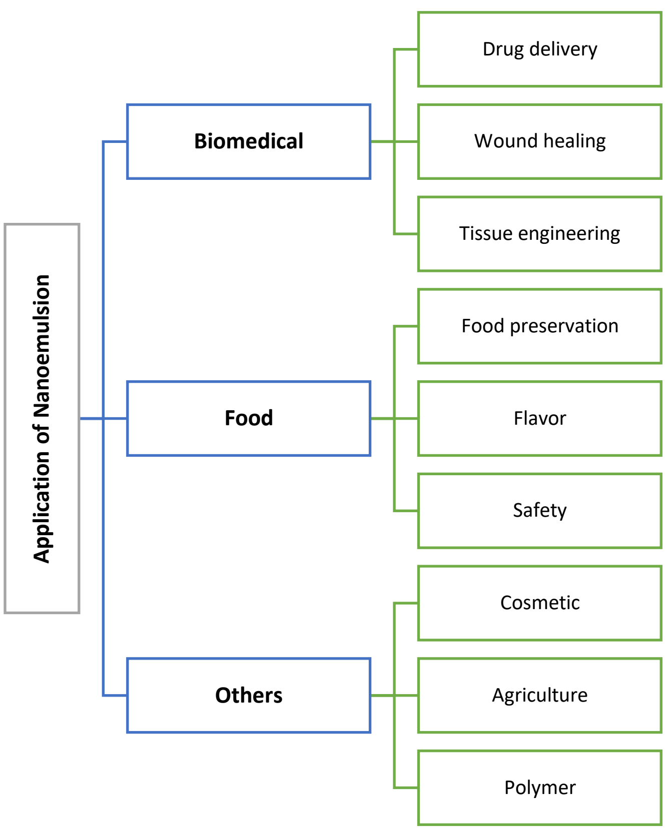

Figure 2. Applications fields of nanoemulsions

DISCUSSION

Conclusion: Harnessing Nanoemulsions for Antimicrobial Solutions

In conclusion, nanoemulsions represent a promising platform for the development of effective antimicrobial solutions with diverse applications in healthcare, food preservation, and environmental hygiene. Through meticulous synthesis methods and comprehensive characterization techniques, nanoemulsions can be tailored to exhibit desired properties such as small droplet size, enhanced stability, and potent antimicrobial activity. The results of our study have shown that the significant antimicrobial potential of synthesized nanoemulsions, demonstrating their efficacy against a wide range of pathogenic microorganisms, including bacteria, fungi, and biofilm-forming strains.

Moving forward, harnessing nanoemulsions for antimicrobial solutions requires concerted efforts in research, development, and application. Future directions may include optimizing synthesis methods to enhance efficiency and scalability, elucidating mechanisms of antimicrobial action to inform rational design of nanoemulsion formulations, and evaluating safety profiles and efficacy in vivo and clinical settings. Additionally, exploring synergistic combinations with existing antimicrobial agents and addressing challenges related to regulatory compliance, consumer acceptance, and environmental impact will be critical for advancing nanoemulsion-based antimicrobial therapies.

Overall, the potential of nanoemulsions in combating microbial infections and addressing antimicrobial resistance is immense. By leveraging their unique physicochemical properties and antimicrobial efficacy, nanoemulsions have the capacity to revolutionize antimicrobial therapy and contribute to the global effort to combat infectious diseases. As we continue to harness the power of nanoemulsions, collaboration between academia, industry, and regulatory agencies will be essential to realize their full potential and translate them into impactful solutions for public health and food safety challenges.

Ethics Committee Approval: Ethical approval and informed consent are not required in our study as no research was conducted on human or animal specimens.

Peer-review: Externally peer-reviewed.

Author Contributions: H.N. and K.K. contributed equally. Conceptualization, literature search, writing-original draft preparation, reviewing and editing, visualization was carried out by both authors. All authors have read and agree to the published version of the manuscript.

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