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Review Article

Ozonated Oils for Wound Healing and Role of Emulgel on the Enhanced Topical Delivery

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ABSTRACT

Wound healing, particularly in chronic and infected wounds, remains a major clinical challenge due to delayed tissue repair, poor oxygenation and rising antimicrobial resistance. Ozonated oils have emerged as promising topical agents because of their broad-spectrum antimicrobial activity, oxygen-releasing ability and capacity to stimulate tissue regeneration. However, their direct clinical use is often limited by issues such as instability, strong odour and poor spreadability. This review critically discusses the generation of medical-grade ozone, preparation and chemistry of ozonated oils and the factors influencing their stability and therapeutic performance. The mechanisms underlying the wound-healing activity of ozonated oils, including antimicrobial action, modulation of inflammation, oxygen donation, angiogenesis and fibroblast stimulation, are examined. Particular emphasis is placed on emulgel-based delivery systems as an advanced topical platform to enhance the stability, controlled release, penetration and patient acceptability of ozonated oils. Preclinical evidence supporting the wound-healing efficacy of ozonated oils and emulgels in normal, infected and diabetic wound models is also summarized. Incorporation of ozonated oils into emulgel systems represents a rational and effective strategy to overcome formulation and application-related limitations of ozonated oils. With appropriate selection of excipients and stabilization strategies, ozonated oil based emulgels have strong potential as patient-friendly, stable and therapeutically effective topical formulations for modern wound management.

Keywords: Antimicrobial therapy, Emulgel, Ozonated oil, Topical drug delivery, Wound healing.

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INTRODUCTION

Wound healing continues to be a major healthcare concern worldwide, especially when it comes to chronic and non-healing wounds. These wounds often take a long time to recover and are frequently complicated by infections, poor oxygen supply and increasing antibiotic resistance. Because of this, researchers are now focusing on advanced topical therapies that can not only prevent infection but also actively promote faster and better tissue repair (Anzolin *et al.*, 2020; Guo and DiPietro, 2010).

Among these, ozone therapy particularly in the form of ozonated oils has gained much attention in recent years. Ozone, being a strong oxidizing agent, possesses excellent antimicrobial properties against bacteria, viruses and fungi. Beyond that, it helps improve wound healing by enhancing oxygen delivery to tissues, reducing inflammation and stimulating growth factors that aid in cell regeneration and angiogenesis. When ozonated oil is applied topically, it acts as a slow-release system, gradually delivering reactive oxygen species and molecular oxygen to the wound area. This continuous oxygen supply encourages re-epithelialization, fibroblast activity, collagen formation and overall faster wound closure, while also reducing the risk of infection. Studies have shown that ozonated oils can significantly improve granulation tissue formation, reduce pain and itching and enhance wound healing even in patients with diabetes or poor circulation. Their ability to control infection and modulate inflammatory responses makes them particularly useful in chronic wound conditions (Anzolin *et al.*, 2020; Deng *et al.*, 2022).

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However, one limitation of ozonated oils is their oily nature and limited stability, which can affect their ease of application and shelf life. To overcome these drawbacks, researchers have explored emulgel formulations a combination of emulsion and gel systems. Emulgels are non-greasy, easily spreadable and enhance both the stability and controlled release of active ingredients. Incorporating ozonated oil into an emulgel not only improves patient acceptability but also provides a sustained release of active components, maintaining prolonged antimicrobial and healing effects at the site of injury (Khan *et al.*, 2025).

Therefore, the topical delivery of ozonated oils through emulgel systems offers a novel and effective approach for enhanced wound care. This combined system takes advantage of ozone's therapeutic benefits along with the favourable properties of emulgels, providing an ideal platform for treating both acute and chronic wounds (Khan *et al.*, 2025).

Search Strategy

A detailed literature search was carried out to collect relevant information related to ozonated oils, their wound-healing activity and the role of emulgel systems in topical drug delivery. The search was started in 2024-25 and various electronic databases including PubMed, Scopus, Web of Science and ScienceDirect were used for the search. Keywords such as ozonated oil, ozone therapy, wound healing, topical ozone, emulgel, topical drug delivery, antimicrobial activity and skin regeneration were used either alone or in combination. Boolean operators like AND and OR were applied to narrow down the results. Only articles published in the English language were considered. Both original research articles and review articles related to topical application of ozonated oils, emulgels and wound-healing studies were included. Studies that were not related to topical delivery or wound healing were excluded. In addition, the reference lists of selected articles were manually screened to identify other relevant studies.

Medical-Grade Ozone Generation and Safety

Medical-grade ozone is produced using specially designed ozone generators that ensure accurate control over gas concentration and flow rate. These devices are calibrated to deliver ozone within a safe and effective therapeutic range, generally between 1 to 80 µg/mL, depending on the intended application. To ensure safety during medical use, such equipment is equipped with automated controls and protective features that minimize risks associated with ozone exposure (Romary *et al.*, 2023). Important safety considerations include preventing ozone leakage into the surrounding environment, using backflow prevention systems to avoid contamination of the generator, following regular maintenance and calibration schedules, using devices that are certified and approved by relevant health authorities (Roth *et al.*, 2023).

Clinical studies have shown that ozone therapy, when administered by trained healthcare professionals using approved medical devices, has a very low incidence of side effects around 0.7 cases per 1,00,000 treatments. Most reported complications are minor and usually result from improper handling rather than from ozone itself. Therefore, maintaining proper device function, accurate dosing and traceability are key factors to ensure both therapeutic success and patient safety (Leon *et al.*, 2022).

Ozone Generation Methods

Corona Discharge Method

The corona discharge technique is the most common and reliable method used to generate ozone for both industrial and medical purposes. In this process, oxygen or air is passed through a high-voltage electric field, which splits Oxygen molecules (O_2) into individual atoms. These atoms then recombine to form Ozone (O_3). Advantages of corona discharge include ability to produce ozone at high concentrations suitability for large-scale or continuous ozone generation, high efficiency and long operational life of the generators. Because of these benefits, corona discharge is often considered the gold standard for ozone generation in medical and water treatment applications, where precision and reliability are essential (Romary *et al.*, 2023).

Ultraviolet (UV) Radiation Method

The UV radiation method simulates the natural atmospheric formation of ozone. When oxygen is exposed to UV-C light (typically 185-254 nm), the light energy breaks oxygen molecules into individual atoms, which then combine to form ozone. This method produces lower ozone concentrations compared to corona discharge and it is best suited for smaller applications, such as air purification or localized sterilization, simple, cost-effective and easy to operate, though less efficient in humid environments. Due to its limited output and sensitivity to environmental conditions, the UV method is less preferred for medical-grade ozone production, where higher precision and stability are required (Sathwik *et al.*, 2025).

Both methods (Table 1) are recognized for their ability to replicate the natural ozone cycle corona discharge resembling lightning and UV resembling solar processes in the upper atmosphere. However, for medical applications, corona discharge is preferred for consistent, high-concentration ozone production and safety (Sathwik *et al.*, 2025).

Ozonated Oil Method of Preparation

Ozonated oil is prepared by reacting ozone gas with a vegetable oil that contains a good amount of unsaturated fatty acids. Oils like olive, sunflower, sesame and castor oil are commonly used because their double bonds easily interact with ozone and form the active ozonides responsible for antimicrobial and wound-healing effects (Dominguez *et al.*, 2025). The ozone oxygen mixture is

bubbled through the selected oil using a diffuser that produces fine bubbles, which helps increase the contact between the gas and the oil. As the gas passes through, ozone reacts with the double bonds in the fatty acids and gradually forms peroxides, aldehydes and stable ozonides through the Criegee reaction pathway (Slavinskienė *et al.*, 2025). The ozonation process usually continues for several hours, depending on the type of oil and the target peroxide value (1 hr, 3 hr, 6 hr and 12 hr). During this period, the reaction conditions such as temperature, flow rate and exposure time are kept controlled to avoid degradation of the active components. The progress of ozonation is monitored by checking parameters like peroxide value, iodine value, acidity and viscosity. These changes help to know how far the reaction has proceeded and ensure that the final product has the required therapeutic strength (Sathwik *et al.*, 2025). Once the desired level of ozonation is reached, the oil is removed from the ozone stream and stored in airtight, amber-coloured containers to protect it from light and maintain stability during storage. Different oils are ozonated under specific controlled conditions to achieve the required peroxide value and stability. One of the most clearly defined methods is reported for extra virgin olive oil, where ozonation was carried out at 22°C using an ozone concentration of 80 µg/mL and a gas flow rate of 4 L/min, with exposure times of 1, 3, 6 and 12 hr (Repciuc *et al.*, 2025).

Chemistry of Ozonation

Criegee Mechanism and Ozonide Formation

The ozonation of unsaturated compounds, especially vegetable oils rich in unsaturated fatty acids, mainly follows the Criegee mechanism. In this process, Ozone (O_3) reacts with the double bonds present in fatty acid chains through a series of well-defined steps (Dominguez *et al.*, 2025).

Formation of Primary Ozonide (Molozonide)

The reaction begins when ozone adds across the double bond in a 1,3-dipolar cycloaddition manner, forming an unstable cyclic compound known as the primary ozonide or molozonide shown in Figure 1 (Slavinskienė *et al.*, 2024).

Formation of Criegee Intermediate

This primary ozonide is highly unstable and quickly splits into two parts a carbonyl compound (either an aldehyde or a ketone) and a reactive zwitterionic species called the Criegee intermediate (carbonyl oxide) shown in Figure 2 (Dominguez *et al.*, 2025; Slavinskienė *et al.*, 2024).

Formation of Secondary Ozonide

The Criegee intermediate can further react with the carbonyl compound formed earlier, but in a reversed orientation, leading to a more stable secondary ozonide (Figure 3).

This reaction pathway, first proposed by Rudolf Criegee, explains how ozonides are formed during the ozonation of oils. Experimental evidence has confirmed the presence of these intermediates through trapping reactions with nucleophiles. Later, these ozonides can undergo further oxidative or reductive breakdown to yield oxygenated products such as aldehydes, ketones, alcohols and organic acids all of which contribute to the biological activity of ozonated oils (Di *et al.*, 2020).

Chemical Changes in Unsaturated Oils upon Ozonation

The ozone molecule attacks the double bonds in fatty acid chains, leading to a noticeable reduction in the levels of oleic, linoleic and linolenic acids. The cleavage of these double bonds generates a variety of oxygen-containing products, such as aldehydes (e.g., nonanal, hexanal), ketones, hydroperoxides and carboxylic acids (e.g., nonanoic acid). These compounds play a major role in the antimicrobial and healing properties of ozonated oils. Due to the formation of ozonides and other oxidation products, ozonated oils typically show an increase in both peroxide value and acidity, which are key indicators of ozonation extent. Ozonated oils become thicker and less fluid compared to the original oil because of the formation of higher molecular weight peroxidic species (Repciuc *et al.*, 2025).

For example, ozonated olive oil shows a significant reduction in its unsaturated fatty acid content (nearly 29% loss of octadec-9-enoic acid) along with a rise in aldehydic compounds. These chemical changes are directly linked to the characteristic odour, stability and enhanced biological activities particularly antimicrobial, anti-inflammatory and wound healing effects (Di *et al.*, 2020).

Comparative Chemical Properties of Ozonated Oils

Different vegetable oils respond differently to ozonation depending on their original fatty acid composition and processing conditions. Comparative studies on oils like linseed, olive, hempseed and sunflower oil have shown distinct variations in their chemical and bioactive profiles Ozonated linseed oil often shows stronger antimicrobial activity because it contains a higher proportion of polyunsaturated fatty acids that readily react with ozone oils with rich in polyunsaturated fatty acids produce higher peroxide values after ozonation. However, these high peroxide levels can sometimes reduce long-term stability, requiring careful control during storage and formulation (Slavinskienė *et al.*, 2024). The aldehydes and other volatile products formed during ozonation vary among oils and are responsible for the characteristic odour and part of the biological effects. Ozonation leads to changes in molecular weight distribution and introduces new oxygen-containing functional groups, which can be detected using spectroscopic methods such as FTIR or NMR. Because each type of oil reacts differently, the ozonation process must be optimized individually to achieve the desired therapeutic balance

ensuring strong biological activity while maintaining stability and safety for formulation use (Di *et al.*, 2020).

Factors Influencing Ozonation of Oils

The efficiency and outcome of oil ozonation depend on several factors, including the type of oil used, reaction conditions and storage parameters. These factors directly influence the chemical composition, stability and therapeutic potential of the final ozonated oil (Guerra-Blanco *et al.*, 2017).

Type and Composition of Oil

The kind of oil and its fatty acid profile play an important role in how it reacts with ozone. Oils rich in unsaturated fatty acids such as sunflower or linseed oil react more quickly with ozone, producing higher peroxide values and forming ozonides faster. On the other hand, oils with more monounsaturated fats (like olive oil) react more slowly but form more stable ozonated products. Minor components such as tocopherols (Vitamin E) and other natural antioxidants also affect the reaction, as they can slow down oxidation to some extent. Therefore, the composition of the oil determines both the speed of ozonation and the type of bioactive compounds formed, which ultimately influences the oil's antimicrobial and healing properties (Guerra-Blanco *et al.*, 2017).

Ozonation Conditions

Several experimental conditions determine how effectively the ozonation process proceeds. Increasing ozone concentration or prolonging exposure time raises the peroxide value and viscosity of the oil. However, if the exposure is too long, it can lead to over-oxidation and degradation of useful compounds. Performing ozonation at higher temperatures speeds up the reaction but can also cause thermal breakdown of ozonides and peroxides. Controlled temperatures (5-10°C) are therefore preferred to maintain product stability. The purity of the oxygen used and the flow rate through the generator influence how efficiently ozone is produced and interacts with the oil. Pure oxygen and steady flow usually yield more consistent and higher-quality ozonated products (Loureiro *et al.*, 2023).

Storage Conditions

Storing ozonated oils at cooler temperatures helps preserve ozonides and peroxides. High temperatures accelerate their breakdown and reduce antimicrobial effects. Direct exposure to light or air promotes oxidation and rancidity, leading to a loss of therapeutic activity. For packaging, air-tight and dark containers help protect ozonated oils from degradation and extends their shelf life (Lima and Ramos, 2024).

Impact of Peroxide Value and Viscosity

The peroxide value is an important measure of how much oxidation has occurred in an ozonated oil. It often correlates with antimicrobial strength, but very high peroxide values can indicate over-oxidation, which might reduce safety or cause irritation. As ozonation proceeds, viscosity also increases because of polymerization and formation of heavier ozonide molecules. While a thicker consistency can help with longer skin contact, too much viscosity can make the product harder to spread (Repciuc *et al.*, 2025).

Mechanism Of Action of Ozonated Oil in Wound Healing

The wound-healing effects of ozonated oil are linked to several biological mechanisms working together. These include antimicrobial action, modulation of inflammation, improved oxygen delivery and stimulation of tissue regeneration (Ugazio *et al.*, 2020).

Antimicrobial Effects

Ozonated oil has strong broad-spectrum antimicrobial activity against bacteria, fungi and viruses commonly found in wounds. The ozonides and peroxides formed during ozonation produce mild oxidative stress that damages microbial cell walls and membranes, leading to cell death. This action is effective even against drug-resistant and biofilm-forming microorganisms, which are major causes of delayed wound healing. By breaking down biofilms and reducing microbial load, ozonated oil helps maintain a clean wound bed and supports faster recovery, especially in chronic wounds where infection control is critical (Silva *et al.*, 2021; Prtani *et al.*, 2024).

Immunomodulatory and Anti-Inflammatory Actions

Ozonated oil also plays a role in regulating the immune and inflammatory responses at the wound site. It enhances the activity of white blood cells and stimulates the release of cytokines and growth factors that attract cells involved in tissue repair, such as fibroblasts and keratinocytes. The mild oxidative stress generated by ozonated oil activates the body's antioxidant defence mechanisms, which helps balance inflammation. This prevents prolonged inflammatory phases and promotes a smoother transition to the proliferative phase of healing. As a result, swelling, redness and pain in the wound area are reduced, creating a favourable environment for tissue regeneration (Kim *et al.*, 2024).

Oxygen Donation and Angiogenesis

Another important function of ozonated oil is its ability to deliver molecular oxygen directly to oxygen-deprived (hypoxic) wound sites. The improved oxygen supply enhances cellular respiration and ATP production, which are essential for fibroblast activity, collagen synthesis and overall tissue repair. Ozonated oil also stimulates the release of angiogenic factors, encouraging the growth of new blood vessels. This neovascularization supplies oxygen and nutrients to regenerating tissues, accelerating

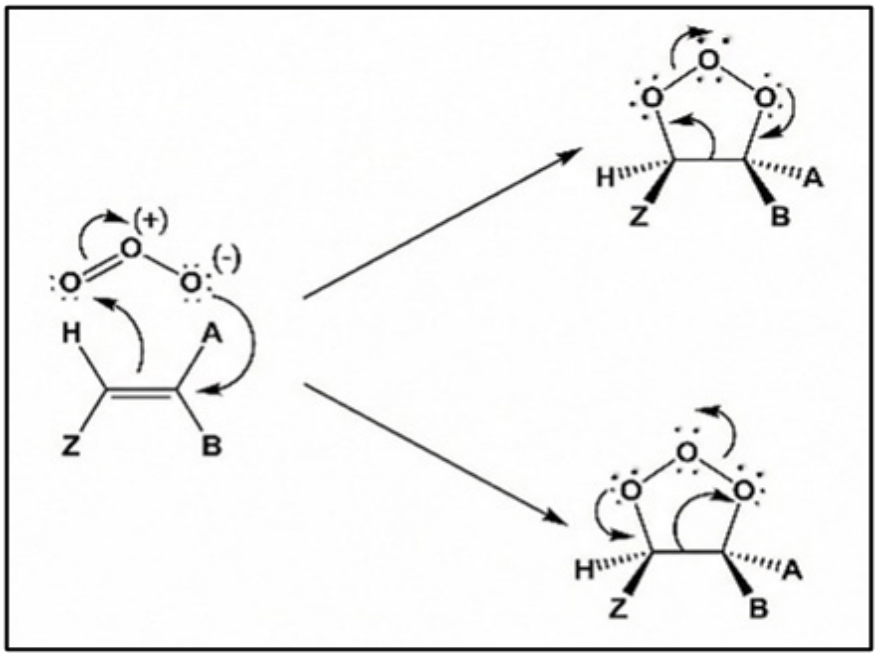

Figure 1: Formation of primary ozonide.

granulation tissue formation and wound closure (Kim *et al.*, 2009; Lu *et al.*, 2023).

Enhancement of Fibroblast Migration and Tissue Regeneration

Animal studies have shown that topical application of ozonated oil promotes fibroblast migration and proliferation in the wound bed. Fibroblasts are key cells responsible for producing collagen and extracellular matrix, which form the foundation for new tissue growth and re-epithelialization. This leads to stronger and more organized tissue repair, resulting in improved wound strength and faster healing (Xiao *et al.*, 2017; Borges *et al.*, 2017).

Types of Oils Used for Ozonation

Different vegetable oils can be used to prepare ozonated oils and their chemical composition greatly influences the reactivity, stability and therapeutic properties of the final product.

Olive Oil

Olive oil contains mainly monounsaturated fatty acids, particularly oleic acid, giving it moderate reactivity with ozone. It is widely used due to its excellent skin compatibility, natural antioxidants (like tocopherols) and soothing properties. Ozonated olive oil shows strong antimicrobial and wound-healing effects and is preferred for sensitive or inflamed skin (Travagli *et al.*, 2010; Tualzik *et al.*, 2021).

Sunflower Oil

Sunflower oil is rich in polyunsaturated fatty acids, mainly linoleic acid, which makes it highly reactive to ozone. The resulting ozonated sunflower oil exhibits powerful antimicrobial activity, though it often has higher peroxide values, requiring careful control during ozonation. It is commonly used in topical formulations where rapid disinfection and tissue regeneration are desired (Cho *et al.*, 2023).

Sesame Oil

Sesame oil has a balanced ratio of oleic and linoleic acids along with natural antioxidants such as lignans. These antioxidants help stabilize the peroxidic compounds formed during ozonation, leading to better shelf life and strong therapeutic effects. It is gaining popularity as a base oil due to its stability and biological activity (Pai *et al.*, 2014).

Castor Oil

Castor oil contains ricinoleic acid, a unique fatty acid that influences its reactivity during ozonation. It ozonated form has been found useful in treating autoimmune and inflammatory skin disorders, such as bullous pemphigoid. Ozonation enhances its antimicrobial and wound healing properties, making it suitable for chronic and hard-to-heal wounds (Li *et al.*, 2025).

Linseed Oil (Flaxseed Oil)

Linseed oil is highly unsaturated and rich in alpha-linolenic acid, making it one of the most reactive oils for ozone treatment. Ozonated linseed oil exhibits strong anti-inflammatory and antimicrobial activity, but the process must be carefully controlled to prevent over-oxidation. It is often used in advanced wound care formulations designed for faster tissue regeneration and repair (Pagano *et al.*, 2021).

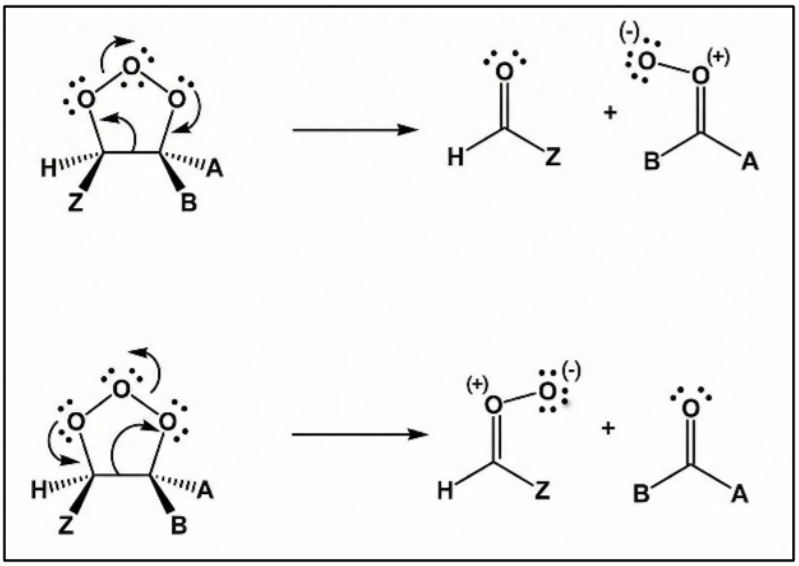

Figure 2: Formation of Criegee intermediate.

Physico-Chemical Properties, Stability and Pharmaceutical Handling of Ozonated Oils

Post-Ozonation Viscosity and Peroxide Value

Viscosity

The viscosity of vegetable oils such as sunflower or olive oil increases markedly after ozonation due to the formation of ozonides and peroxidic compounds derived from oxidative cleavage of unsaturated fatty acids. The degree of viscosity elevation directly correlates with ozone exposure time and the extent of double-bond saturation. Increased viscosity enhances retention time of the formulation on the wound surface, providing prolonged contact and sustained antimicrobial effect. However, excessive viscosity can affect spreadability and patient compliance. Therefore, optimization of ozonation time and formulation excipients is essential to achieve a balance between ease of application and therapeutic efficiency (Slavinskienė *et al.*, 2024; Guerra-Blanco *et al.*, 2017).

Peroxide Value (PV)

The Peroxide Value (PV) serves as a primary indicator of oxidative products such as peroxides and hydroperoxides generated during ozonation. PV typically rises proportionally with ozone dosage, representing increased oxidative load and antimicrobial activity. However, excessive oxidation may lead to unstable intermediates that exhibit cytotoxic effects or reduced shelf stability. Proper control of ozonation parameters ensures therapeutic peroxide levels while minimizing adverse oxidative degradation. PV stability is temperature and light-sensitive, elevated temperatures or UV exposure accelerate peroxide decomposition, diminishing biological activity (Reociuc *et al.*, 2025; Guerra-Blanco *et al.*, 2017).

Stability Considerations

Ozonated sunflower oil and similar preparations exhibit gradual peroxide degradation during storage, especially at ambient or higher temperatures. Incorporation of stabilizers such as natural or synthetic antioxidants (e.g., tocopherols, BHT) can retard degradation and preserve the desired physico-chemical profile. Continuous monitoring of PV and viscosity over time provides a reliable indication of formulation stability and product shelf life (Cirlini *et al.*, 2012).

Stability and Storage of Ozonized Vegetable Oils

Degradation Challenges

Ozonized oils are inherently unstable due to the reactive nature of ozonides and peroxides formed during ozonation. Environmental factors temperature, light exposure and oxygen contact trigger decomposition, leading to loss of antimicrobial activity and therapeutic potency. Physical indicators of degradation include increased viscosity, rancid odour and colour change, corresponding to peroxide decomposition and secondary oxidation. Monitoring of Peroxide Value (PV) and Acid Value (AV) provides essential data to quantify degradation and assess product viability (Lima and Ramos, 2024).

Packaging Recommendations

To minimize oxidative degradation, ozonized oils should be stored in airtight, light-resistant containers, such as amber glass or UV-blocking multilayer plastics. Minimal headspace is recommended to limit oxygen exposure and retard auto-oxidation. Storage below 25°C, preferably under refrigeration, significantly extends shelf life and preserves peroxidic activity (Table 2). Packaging systems must ensure an effective oxygen barrier while maintaining product sterility and integrity (Satwik *et al.*, 2025).

Table 1: Ozone Generation Methods.

Method	Principle	Ozone Output	Advantages	Limitations	Reference
Corona Discharge	High-voltage electric field splits O_2	High (1-16% w/w)	Scalable, efficient	Needs dry input gas, more complex	(Romary *et al.*, 2023)
UV Radiation	UV-C light splits O_2	Low (0.1-0.001% w/w)	Simple, cost-effective	Lower output, short-lived ozone	(Sathwik *et al.*, 2025)

Table 2: Storage conditions and stability.

Storage Condition	Approximate Stability	Reference
Room temperature (Dark, Airtight)	1-2 months	(Satwik *et al.*, 2025)
Refrigerator (2-8°C)	6-12 months	(Cirlini *et al.*, 2012)
Freezer (-20°C)	1-2 years	(Lima and Ramos, 2024)

Quality Control in Pharmaceutical Applications

Pharmaceutical-grade ozonated oils require standardized ozonation processes to ensure consistent peroxide concentration and therapeutic reproducibility. Routine quality control parameters include Peroxide Value (PV) and Acid Value (AV) determination (Repciuc *et al.*, 2025).

8.Ozonated Water: Antimicrobial Activity And Clinical Applications

Ozonated water is an aqueous solution containing Dissolved Ozone (O_3), which generates Reactive Oxygen Species (ROS) such as ozone molecules, singlet oxygen and hydroxyl radicals. These species exhibit potent antimicrobial effects through oxidative degradation of microbial cell walls, membrane lipids, proteins and nucleic acids. The resulting oxidative stress leads to rapid inactivation of a broad range of microorganisms, including bacteria, fungi and viruses. Clinically, ozonated water is primarily utilized for wound irrigation and surface disinfection. Its strong oxidizing capacity effectively reduces microbial load and disrupts established biofilms, a key factor in the management of chronic and infected wounds. Additionally, ozonated water is non-toxic, non-irritant and leaves no chemical residues, making it a safe and convenient adjunct before application of topical dressings or medications (Romary *et al.*, 2023).

However, its therapeutic potential is limited by the short half-life of ozone in aqueous media, typically lasting only seconds to a few minutes. This rapid decomposition results in transient antimicrobial activity, requiring on-site generation and immediate use, which restricts its practicality in routine wound care. Although both ozonated oil and ozonated water exploit the antimicrobial and healing potential of ozone-derived ROS, their clinical applications differ significantly due to contrasting stability and physicochemical properties. Overall, as shown in Table 3, ozonated oils are preferred for long-term wound management owing to their superior stability, prolonged bioactivity and compatibility with modern topical delivery systems such as emulgels. In contrast, ozonated water remains valuable as a pre-treatment step for wound cleansing and microbial reduction before the application of ozonated oil-based formulations (Roth *et al.*, 2023).

Concept and Discussion of Emulgel as A Topical Drug Delivery System

Emulgels are modern topical drug delivery systems that combine the properties of both emulsions and gels as shown in Figure 4, offering several benefits over conventional formulations. In simple terms, an emulgel is formed by incorporating an emulsion (oil-in-water or water-in-oil) into a gel base, resulting in a semisolid preparation that is smooth, stable and patient-friendly (Chandel and Sharma, 2021). The gel matrix provides desirable qualities like a non-greasy feel, smooth spreadability and easy washability, which make the formulation more acceptable for patients. At the same time, the emulsion phase plays an important role in dissolving and stabilizing both hydrophilic and lipophilic drugs, something that is often difficult to achieve with traditional dosage forms. This dual nature allows emulgels to overcome the drawbacks of older topical systems such as ointments (which are greasy and sticky) and creams (which may not provide adequate solubilization or stability) (Milutinov *et al.*, 2023). Because of this, emulgels have become a popular choice for delivering a wide range of drugs including anti-inflammatory, antimicrobial, antifungal and wound-healing agents. In addition, emulgels can offer controlled or sustained drug release, improve the duration of therapeutic action and reduce the frequency of application. Their structure can also be easily adjusted by modifying the type or concentration of gelling agents and emulsifiers, allowing formulators to achieve the desired viscosity, texture and release profile for specific clinical needs (Jadhav *et al.*, 2024).

Advantages of Emulgels Over Conventional Topical Systems

Emulgels are smooth, non-sticky and cosmetically pleasant compared to greasy ointments or heavy creams. Their light texture spreads easily on the skin, making them more comfortable and improving patient adherence, especially for long-term treatments. The gel structure helps stabilize emulsion droplets and prevents phase separation. This leads to a longer shelf life and consistent

drug distribution throughout the formulation. Emulgels can carry both water-soluble and oil-soluble drugs in a single system, making them suitable for a wide range of therapeutic agents something most conventional formulations cannot achieve. The gel network can be tailored to control the release of the drug, providing prolonged action, reducing frequent application and enhancing overall therapeutic outcomes (Chaudhari and Vispute, 2021).

Emulgels form a thin, semi-occlusive layer that keeps the wound area moist, creating an ideal environment for faster healing and protecting it from external contamination. Being water-washable, emulgels can be applied and removed easily without leaving residue, which makes them convenient and hygienic for daily use. The fine droplet size of the emulsion phase enhances drug penetration through the skin layers, leading to better absorption and improved bioavailability compared to ordinary gels or creams (Vanpariya *et al.*, 2021).

Role of Emulgels in Enhancing the Topical Delivery of Ozonated Oils

Improved Spreadability and Patient Acceptability

Ozonated oils tend to be highly viscous due to the formation of ozonides and polymeric peroxides, which makes them difficult to spread uniformly on the skin or wound surface. When the oil is incorporated into an emulgel, its heavy, sticky texture transforms into a smooth semisolid that is much easier to apply. The aqueous gel phase reduces the greasiness and allows the formulation to glide across the wound bed with minimal friction. This becomes especially important for chronic wounds, where patients undergo repeated dressing changes and need a formulation that does not cause discomfort. Emulgels also improve patient compliance because they feel lighter, absorb better and leave less residue on the skin compared to pure ozonated oil (Charyulu *et al.*, 2021).

Stabilization of Reactive Oxygen Species (ROS)

The therapeutic activity of ozonated oil mainly depends on its reactive species such as ozonides, organic peroxides and aldehydes. However, these compounds break down quickly when exposed to heat, light, air, or metals. Entrapping ozonated oil within an emulgel system protects these unstable components by encapsulating them inside microscopic emulsion droplets. The surrounding gel matrix acts as an additional barrier that prevents direct exposure to oxygen and environmental triggers. This slows the rate of oxidative degradation and helps maintain a consistent peroxide value for longer periods. As a result, the emulgel keeps the biological activity intact during storage and ensures that the patient receives a stable and effective product (Papagari and Vihetha, 2021).

Controlled and Sustained Release of Active Compounds

Pure ozonated oil releases its reactive oxygen species immediately, which may result in a short duration of action. Emulgels overcome this problem by creating a dual-phase system where the oil droplets are embedded inside the gel. This structure allows the peroxides and ozonides to diffuse gradually from the oil phase into the gel and then onto the wound site. The slow

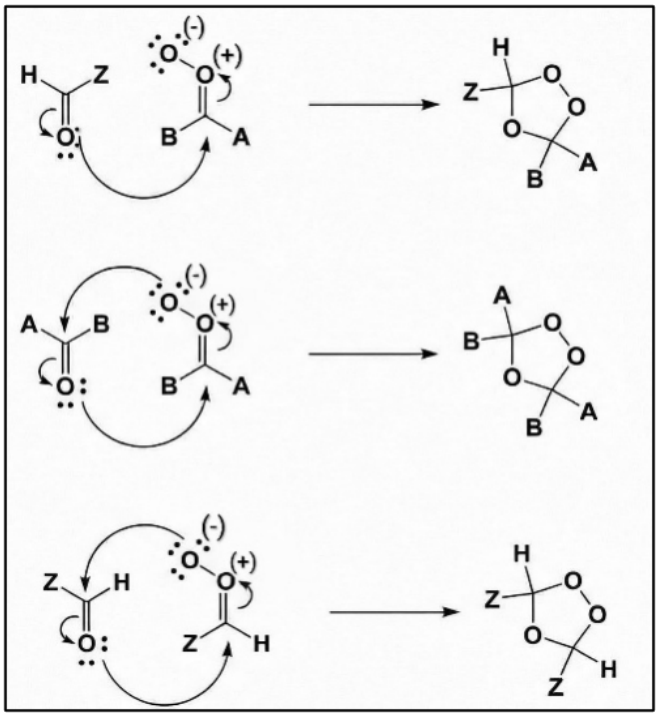

Figure 3: Formation of secondary ozonide.

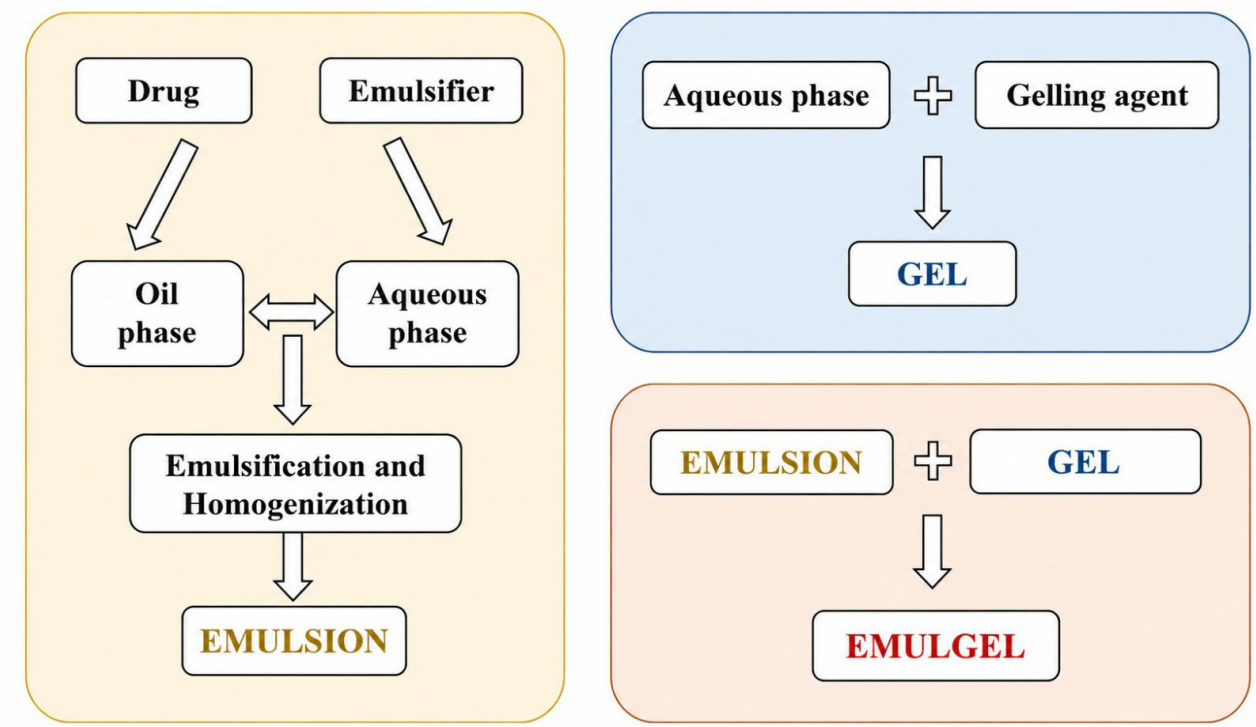

Figure 4: Preparation process of emulgel.

and controlled release ensures a prolonged antimicrobial effect, steady oxygen donation and continuous stimulation of fibroblast activity. This sustained release pattern is beneficial in chronic or infected wounds, where ongoing oxidative support helps in breaking down biofilms, reducing microbial load and promoting tissue regeneration (Kaif *et al.*, 2024).

Formation of a Moist, Protective Wound Environment

Emulgels form a thin, uniform film on the skin that provides a balanced moist environment neither too wet nor too dry. Moist wound healing is known to promote faster epithelial cell migration, increase angiogenesis and prevent scab formation. The gel base also allows limited gaseous exchange, which helps maintain oxygen availability at the wound site. The presence of ozonated oil further increases local oxygenation due to the slow breakdown of ozonides into peroxides and molecular oxygen. In addition, the protective film shields the wound from dust, microbes and external irritants, reducing the chances of reinfection and minimizing trauma during dressing removal (Ahmed *et al.*, 2022).

Reduction of Odour and Irritation

Ozonated oils produce a strong, sharp odour due to aldehydes and short-chain oxidation products formed during ozonation. This odour can be unpleasant and often leads to poor patient compliance. Incorporating the oil into an emulgel significantly reduces the smell because the gel network helps trap these volatile compounds. Moreover, the gel phase acts as a mild diluent, lowering the immediate concentration of oxidative species coming in contact with the skin. This reduces the chances of irritation, stinging, or burning sensations, especially in sensitive or inflamed wounds. The patient-friendly profile of emulgels makes them more suitable for long-term or repeated application (Neacşu *et al.*, 2025).

Enhanced Penetration and Bioavailability

The penetration of ozonated oil alone can be limited because of its thick consistency and hydrophobic nature. Emulgels allow better penetration by combining the solubilizing ability of emulsions with the hydrating effect of gels. Hydrated skin has higher permeability, which facilitates the entry of peroxides and oxygen-releasing species into the upper layers of the epidermis. Emulsion droplets also improve the distribution of the oil across the skin surface, increasing contact area and enhancing absorption. Better penetration ensures that the reactive oxygen species reach microbial colonies and deeper tissue layers where they can stimulate healing, collagen deposition and local circulation (Pappu *et al.*, 2022).

Better Retention on the Wound Surface

Liquid ozonated oil can easily run off, get absorbed into the dressing, or be wiped away unintentionally. Emulgels adhere more firmly to the wound bed due to their semisolid nature. Once applied, they remain in place even with slight movements, sweating, or minor washing. This stronger bio-adhesion increases the residence time of ozonated oil on the wound, ensuring that the active compounds remain in contact with tissues for an extended

period. Prolonged retention supports sustained antimicrobial action, improved oxygenation and longer interaction with fibroblasts and keratinocytes ultimately speeding up the overall healing process (Varghese *et al.*, 2024).

Formulation Challenges of Ozonated Oil Emulgels

chemical instability and peroxide degradation

Ozonated oils contain reactive oxygen species such as peroxides and ozonides, which are highly unstable. These compounds degrade easily under heat, light, or oxygen exposure, leading to reduced antimicrobial and wound-healing effects. Maintaining peroxide stability is one of the major formulation challenges (Cirlini *et al.*, 2012).

Compatibility Issues

Because ozonated oils are strong oxidizing agents, they can react with other formulation components like polymers, surfactants, or preservatives. Such reactions may alter the rheological properties or cause instability. Hence, selecting compatible excipients through careful pre-formulation studies is crucial (Cirlini *et al.*, 2012).

Odour and Sensory Problems

Ozonated oils often have a sharp, unpleasant odour due to the presence of aldehydic compounds. This can affect user compliance. Formulators must find effective odour-masking techniques without altering the chemical stability of the product (Lima and Ramos, 2024).

Control of Oxidative Degradation

Incorporating antioxidants such as vitamin E or natural polyphenols can help slow peroxide degradation. However, care must be taken so that these antioxidants do not interfere with the therapeutic activity of the ozonated oil. Advanced techniques like nano-encapsulation can also help stabilize the reactive species and control their release (Lima and Ramos, 2024).

Rheological and Cosmetic Challenges

Ozonated oils are often viscous, which can affect the consistency of the final emulgel. The polymer type and concentration must be carefully adjusted to achieve the right balance between spreadability and structural integrity (Sabalingam and Siriwaedhene, 2022).

Storage and Shelf-Life Stability

Ozonated oil-based emulgels are highly sensitive to light and temperature. They must be packaged in airtight, light-protected containers and stored under cool conditions. Maintaining peroxide content and overall product stability throughout shelf life remains a key concern (Sabalingam and Siriwaedhene, 2022).

Strategies to Overcome Formulation Challenges of Ozonated Oil Emulgels

Ozonated oil based emulgels face several formulation challenges due to the inherent instability and high reactivity of ozone-derived peroxidic species. To address these limitations, various formulation strategies have been explored as shown in Table 4.

Preclinical Evidence of Ozonated Oils and Emulgels in Wound Healing

A study evaluated ozonated sesame oil in a full-thickness excision wound model using Sprague-Dawley rats Animals were divided into four groups and treated topically once daily with plain

Table 3: Comparison between Ozonated oil and Ozonated water.

Parameter	Ozonated oil	Ozonated water	References
Stability and Duration of Action	Contains stable ozonides and peroxides that release ROS gradually, providing sustained antimicrobial and wound-healing activity for several hours to days.	Exhibits an extremely short half-life; ozone decomposes rapidly, resulting in brief antimicrobial activity and requiring immediate use after generation.	(Romary *et al.*, 2023)
Physical Characteristics	Forms a viscous, semi-occlusive film on the wound, maintaining a moist healing environment and acting as a protective barrier.	Volatile and non-viscous; evaporates quickly without forming a protective layer.	
Ease of Use and Compliance	Can be formulated into creams, ointments, or emulgels, allowing controlled delivery, ease of application and good patient acceptability.	Must be freshly prepared and used immediately, limiting practicality in outpatient or home-care settings.	(Leon *et al.*, 2022)
Clinical Efficacy	Demonstrates enhanced healing in burns, ulcers and chronic wounds due to its sustained activity, stimulation of angiogenesis and fibroblast proliferation.	Primarily used as an adjunctive cleanser or initial debridement agent, not as a standalone therapeutic.	(Roth *et al.*, 2023)

Table 4: Strategies to Overcome Formulation Challenges of Ozonated Oil Emulgels.

Strategies	Advantages	References
Controlled Release Systems	Advanced delivery systems, such as dual-controlled release platforms, help regulate the release of reactive ozone species. This approach protects peroxides from rapid degradation, prolonging antimicrobial activity at the wound site.	(Zerillo *et al.*, 2022)
Stabilizing Polymers and Surfactants	Selecting polymers and emulsifiers that are chemically inert toward peroxidic species minimizes instability. Synthetic polymers with antioxidant properties or inert behavior are preferred to maintain formulation integrity.	(Varghese *et al.*, 2024)
Incorporation of Antioxidants	Natural antioxidants such as vitamin E, polyphenols, or flavonoids can neutralize free radicals and prevent peroxide breakdown without compromising the biological activity of ozonated oil.	(Mirgorodshaya *et al.*, 2022)
Odour Masking Agents	Essential oils or fragrances not only reduce the pungent odour of ozonated oils but may also add complementary antimicrobial and anti-inflammatory benefits.	(Zerillo *et al.*, 2022)
Nanocarriers and Liposomal Encapsulation	Encapsulating ozonated oils in nanocarriers or liposomes protects unstable compounds, enhances skin penetration and allows controlled release. This reduces potential irritation and improves therapeutic efficacy.	(Zerillo *et al.*, 2022)
Rheology Optimization	Adjusting polymer concentration and the degree of crosslinking ensures appropriate viscosity and spreadability, improving patient comfort and adherence.	(Chandel and Sharma, 2021)
Packaging and Storage	Using airtight, UV-resistant containers and storing emulgels at low temperatures helps maintain the stability of ozonides and peroxides over extended periods.	(Lima and Ramos, 2024)

sesame oil (vehicle), framycetin (standard) or ozonated sesame oil with peroxide values of 500 and 700 mEq/1000 g (low and high dose). By day 11, wounds treated with the higher-peroxide ozonated oil showed markedly smaller residual wound area compared with vehicle controls, indicating faster wound contraction. Biomechanical testing revealed significantly higher tensile strength of the healed skin in both ozonated oil groups versus vehicle, suggesting better collagen remodelling and stronger scar tissue. Biochemical analysis demonstrated increased hydroxyproline content, reflecting higher collagen deposition and elevated Superoxide Dismutase (SOD) activity at the wound site, indicating an improved endogenous antioxidant response. Histopathology further confirmed more mature granulation tissue, better re-epithelialization and denser collagen fibres in the ozonated oil groups compared to plain oil (Pai *et al.*, 2014).

Another study shows the effect of ozonated olive oil on acute full-thickness skin wounds in guinea pigs. Circular wounds were created on the dorsal skin and animals were assigned to three groups: no treatment (control), pure olive oil and ozonated olive oil. Topical application of ozonated oil resulted in significantly smaller wound size and residual wound area on days 5 and 7 compared to the olive oil group, indicating more rapid wound repair. Histological staining (H&E and Masson's trichrome) revealed a higher density of fibroblasts and more intense collagen staining in the ozonated oil group on day 7, suggesting enhanced granulation tissue formation and extracellular matrix deposition. Immunohistochemistry demonstrated up-regulation of key growth factors involved in repair Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor-β (TGF-β) and Vascular Endothelial Growth Factor (VEGF) in the ozonated oil group, whereas Fibroblast Growth Factor (FGF) expression remained unchanged (Kim *et al.*, 2009).

To clarify cellular mechanisms, the effect of ozone oil on fibroblast behaviour is investigated using both a murine skin injury model and *in vitro* fibroblast cultures. *In vivo*, topical ozone oil significantly reduced wound area and accelerated closure compared with control treatment. Fibroblasts isolated from granulation tissue of ozone-treated wounds showed increased expression of collagen I, α-Smooth Muscle Actin (α-SMA) and TGF-β1 at mRNA and protein levels, which are markers associated with myofibroblast differentiation and matrix production. *In vitro*, ozone oil enhanced fibroblast migration in scratch assays and promoted an Epithelial Mesenchymal Transition (EMT) like phenotype, characterised by up-regulation of fibronectin, vimentin, N-cadherin, MMP-2 and MMP-9 and down-regulation of E-cadherin. These changes were accompanied by activation (phosphorylation) of the PI3K/Akt/mTOR pathway; pharmacological inhibition of PI3K reversed the pro-migratory effect of ozone oil. Additionally, inflammatory mediators were reduced, indicating an anti-inflammatory effect at the fibroblast level. This study suggests that ozonated oil can directly stimulate fibroblast migration and matrix remodelling

via PI3K/Akt/mTOR-dependent signalling, thereby accelerating the proliferative phase of wound healing (Xiao *et al*., 2017).

In a diabetic mouse model where, wound healing is typically delayed, daily application of ozonated camellia oil significantly accelerated closure of full-thickness skin wounds compared with untreated and vehicle groups. The wounds in the ozonated oil group showed much faster reduction in wound area, with nearly complete closure by day 8. Histological examination revealed increased keratinocyte proliferation along the wound edge, improved re-epithelialization and a narrower epidermal gap. Molecular analysis showed that ozonated oil activated key growth-factor pathways involved in repair, particularly IGF-1 receptor, EGFR and VEGF signalling. These pathways further triggered downstream PI3K/Akt and ERK activation, which enhanced keratinocyte migration and supported faster wound resurfacing. *In vitro* studies on epidermal keratinocytes showed similar effects, with ozonated medium increasing both migration and proliferation. Together, these findings indicate that ozonated oils can overcome the delayed healing seen in diabetic conditions by strengthening growth-factor-dependent re-epithelialization (Lu *et al*., 2023).

In an animal model of diabetic ulcers complicated by Methicillin-Resistant *Staphylococcus aureus* (MRSA), topical treatment with ozonated vegetable oil showed clear benefits over non-ozonated oil. The ozonated oil group demonstrated better wound appearance, more organized granulation tissue and more advanced re-epithelialization on histology. Bacterial cultures from the wound area showed a reduction in microbial load, indicating that the oxidative components of ozonated oil helped suppress MRSA growth. Although the decrease in bacterial count was modest, the overall improvement in wound structure and tissue regeneration was significant compared to the non-ozonated control. These results suggest that ozonated oils offer a dual advantage in infected diabetic wounds by supporting tissue repair while also exerting antimicrobial effects that limit bacterial persistence (Silva *et al*., 2021).

Preclinical work consistently shows that converting active agents into an emulgel base can markedly improve their wound-healing performance. In a burn wound model, a 5% *Ocimum basilicum* emulgel showed acceptable rheology and spreadability, released more than 80% of the drug within a few hours *in vitro* and produced wound contraction in rabbits comparable to silver sulfadiazine cream. Histology after 16 days showed almost restored epidermis with dense collagen and minimal inflammation, confirming true tissue repair rather than just surface closure. Similar findings are reported with other botanicals. An emulgel loaded with essential oil from *Withania somnifera* accelerated excision wound healing in rodents, with a markedly smaller residual wound area by day 20 compared with untreated controls, along with evidence of antioxidant and anti-inflammatory support to the healing bed. A polyherbal emulgel combining comfrey, yarrow and myrrh was shown in human fibroblast scratch assays to significantly enhance cell migration and wound closure *in vitro*, suggesting that emulgel vehicles can deliver phytoconstituents in a way that directly stimulates fibroblast activity (Khan *et al*., 2025).

Animal studies with polyherbal emulgels further support these effects. Formulations containing multiple plant extracts and essential oils improved contraction in excision and burn wounds, increased re-epithelialization and promoted fibroblast proliferation and collagen deposition compared with gels or crude extracts alone, indicating that the emulgel structure itself contributes to better retention and penetration at the wound site. Protein-rich *Channa striata* extract formulated as an emulgel also accelerated incision wound healing in rats, reducing wound length and increasing neutrophil/macrophage influx early, followed by higher fibroblast counts and collagen density on days 3 and 7. In a chemical-burn model, *Senna podocarpa* emulgel (7.5%) produced well-organized epidermis, abundant fibroblasts and thick collagen bundles on histology, confirming structural regeneration rather than incomplete scar tissue (Isaac *et al*., 2022).

FUTURE PERSPECTIVES

The field of topical drug delivery is progressing rapidly and emulgel formulations are emerging as versatile platforms with expanding therapeutic potential. Advances in formulation science are opening new possibilities through the integration of nanocarrier-based delivery systems, including nanoemulgels, liposomes and nanofibrous structures. These technologies offer several advantages enhanced drug bioavailability, improved skin penetration and the ability to provide controlled or sustained release of active compounds making them particularly useful for sensitive or highly reactive ingredients.

Nanoemulgels, which combine the benefits of nanoemulsions and gels, have shown improved stability, reduced skin irritation and better patient acceptance. Liposomal encapsulation further strengthens formulation performance by protecting delicate actives such as ozonated oils, leading to improved antimicrobial action and enhanced tissue repair. In parallel, nanofibrous dressings produced via electrospinning are gaining attention for their structurally optimized design, offering superior wound protection, targeted drug delivery and the potential for personalized treatment strategies.

Addressing the formulation challenges associated with labile or unstable compounds remains a key focus for future research. Techniques such as the incorporation of antioxidants, micro or nanoencapsulation and the use of optimized polymer matrices are being explored to maintain molecular stability, extend shelf life and enable scalable production. These innovations, combined with the inherent advantages of emulgels favourable physicochemical properties, easy application and strong patient compliance support their growing potential for clinical translation and commercial development.

The therapeutic applications of emulgels are also broadening beyond traditional wound healing. Current investigations highlight their usefulness in treating complex dermatological problems such as chronic ulcers, biofilm-associated infections and inflammatory skin disorders. Their utility is also extending into cosmetic and regenerative medicine. Incorporating bioactive compounds like ozonated oils into multifunctional emulgel systems has shown promising antibiofilm effects and notable enhancement in tissue regeneration, positioning these formulations as promising candidates for precision dermatology.

CONCLUSION

Ozonated oils have shown strong potential in wound care because of their antimicrobial, oxygen-releasing and tissue-repairing abilities. However, their direct use is often limited by issues like instability, strong odour and difficulty in application. Incorporating ozonated oils into an emulgel helps overcome these problems by improving stability, spreadability and patient comfort while allowing a more controlled release of active components. With proper selection of polymers, emulsifiers and stabilizers, emulgels can deliver consistent therapeutic effects and remain physically stable during storage. Although some formulation challenges still exist, new approaches such as antioxidants, nanocarriers and improved packaging continue to strengthen their performance. Overall, ozonated oil based emulgels appear to be a promising and practical option for modern wound-healing applications, with good potential for further development in clinical use.

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ABBREVIATIONS

ROS: Reactive oxygen species; **UV:** Ultraviolet; **ATP:** Adenosine triphosphate; **SOD:** Superoxide dismutase; **PDGF:** Platelet-derived growth factor; **TGF-β:** Transforming growth factor-β; **VEGF:** Vascular endothelial growth factor; **FGF:** Fibroblast growth factor; **α-SMA:** α-smooth muscle actin; **mRNA:** Messenger RNA; **EMT:** Epithelial-mesenchymal transition; **MMP:** Matrix metalloproteinase; **PI3K:** Phosphoinositide 3-kinase; **Akt:** Protein kinase B; **mTOR:** Mammalian target of rapamycin; **ERK:** Extracellular signal-regulated kinase; **MRSA:** Methicillin-resistant Staphylococcus aureus; **PV:** Peroxide value; **AV:** Acid value; **FTIR:** Fourier-transform infrared spectroscopy; **NMR:** Nuclear magnetic resonance; **BHT:** Butylated hydroxytoluene.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

S.M. and H.N.S. conceptualized and designed the review. S.M., M.M.Y., S.A.P., and V.M. performed the literature search and data curation. S.M. and M.M.Y. prepared the original draft of the manuscript. F.S.D. and K.T.K. contributed to the analysis of emulgel formulations and topical delivery aspects. A.G.R. provided inputs on regulatory and translational considerations. S.T.B. contributed to the discussion on wound-healing mechanisms and clinical relevance. H.N.S. supervised the work and critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

CONSENT FOR PUBLICATION

All authors have read the content of the manuscript and agreed on its submission for publication.

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