

## Research Article

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## Advances in the use of Herbal Drugs via the use of Nanotechnology for Impaired Wound Healing in Diabetic Patients with Anti Inflammatory Activity

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### ABSTRACT

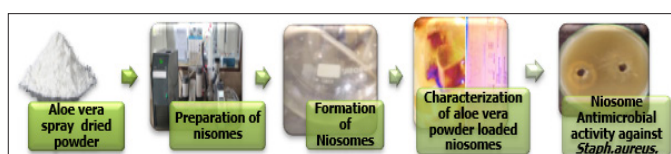
Diabetes mellitus (DM) is a chronic condition characterized by elevated blood glucose levels, leading to nerve and blood vessel damage, organ failure, and impaired wound healing. Wound healing in diabetic patients is further complicated by reduced cytokine responses and delayed healing processes, which can lead to severe complications such as sepsis and gangrene if left untreated.

In this study, we explored the potential of acemannan, an herbal extract from aloe gel incorporated into niosomes, for wound healing. Acemannan exhibits antihyperglycemic, antiseptic, and anti-inflammatory properties with discrete delivery mechanisms and minimal side effects.

The aim of this study was to develop herbal-loaded niosomes for wound healing. Niosomes, self-assemblies of nonionic surfactants with or without cholesterol, offer versatile delivery systems for both hydrophilic and hydrophobic substances. The formulation consisted of Span 60, cholesterol, chloroform, methanol, buffer, and spray-dried aloe vera powder, prepared using the Thin Hydration method. The resulting herbal niosomes were evaluated for entrapment efficiency, particle size, and zeta potential. A batch with a 2:2 ratio of Span 60 and cholesterol exhibited favorable characteristics with a particle size of 144.1 nm and a zeta potential of -17.1 mV.

In conclusion, the developed herbal-loaded niosome formulation shows promise for wound healing, offering a potentially safer and more effective alternative to traditional antibiotic treatments for diabetic wounds.

### Graphical Abstract



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### Background

Diabetes is a chronic medical condition characterized by high levels of glucose in the blood. It occurs when the body either does not produce enough insulin or cannot use the insulin it produces effectively. Insulin is a hormone produced by the pancreas that helps glucose from food enter cells to be used for energy [1].

### There are Several Types of Diabetes

#### Type 1 Diabetes

This type of diabetes occurs when the immune system mistakenly attacks and destroys insulin-producing cells in the pancreas. People with type 1 diabetes require insulin injections or an insulin pump to survive. All often develops in childhood or adolescence, but it can occur at any age [2].

#### Type 2 Diabetes

This is the most common type of diabetes; it typically occurs in

adults but is increasingly common in children and adolescents. In type 2 diabetes, the body becomes resistant to insulin or does not produce enough insulin to maintain normal glucose levels. It is often associated with lifestyle factors such as obesity, lack of physical activity, and poor diet [2].

### **Gestational Diabetes**

This type of diabetes occurs during pregnancy when the body cannot produce enough insulin to meet the increased needs of pregnancy. GDM usually resolves after giving birth, but women who have gestational diabetes have an increased risk of developing type 2 diabetes later in life [3].

Other rarer types of diabetes include monogenic diabetes and secondary diabetes, which can result from certain medications, diseases, or genetic syndromes [4].

Management of diabetes typically involves medication (such as insulin injections, oral medications, or other injectable drugs), lifestyle changes (such as diet modification, regular exercise, and weight management), and regular monitoring of blood sugar levels. Uncontrolled diabetes can lead to serious complications, including heart disease, stroke, kidney disease, nerve damage, and eye problems. Therefore, it is important for people with diabetes to work closely with their healthcare team to manage their condition effectively [5].

### **Diabetic Foot Ulcer**

Diabetic foot ulcers are common complications of diabetes, particularly in individuals with poorly controlled blood sugar levels or other risk factors. A sore or wound that develops on the foot, typically on the bottom of the foot or toes. These ulcers can be slow to heal and are prone to infection, which can lead to serious complications, including amputation if not properly treated [6].

The exact number of diabetic foot ulcer patients worldwide can be challenging to determine precisely due to variations in healthcare systems, data collection methods, and the dynamic nature of the condition. However, diabetic foot ulcers are a significant and common complication of diabetes, particularly in individuals with poorly controlled blood sugar levels or other risk factors [6].

As of my last update in January 2022, the International Diabetes Federation (IDF) estimated that approximately 15% of people with diabetes will develop a foot ulcer at some point in their lives worldwide. Considering the global prevalence of diabetes, which is approximately 422 million adults according to the World Health Organization (WHO), this translates to a substantial number of individuals affected by diabetic foot ulcers [7].

### **Several Factors Contribute to the Development of Diabetic Foot Ulcers Neuropathy**

Diabetes can damage nerves, leading to peripheral neuropathy, which reduces sensation in the feet. This means that individuals with diabetes may not feel pain or discomfort from a wound or injury, allowing it to worsen without them noticing [8].

### **Peripheral Arterial Disease (PAD)**

Diabetes can also lead to reduced blood flow to the feet and legs; this disease is known as peripheral arterial disease. Poor circulation can impair the body's ability to heal wounds and fight infection [9].

### **Foot Deformities**

Diabetes can cause changes in the structure of the foot, such as Charcot foot or hammertoes, which can increase the risk of developing ulcers due to abnormal pressure points or rubbing against footwear [10].

### **Poor Wound Healing**

Diabetes can impair the body's ability to heal wounds due to factors such as high blood sugar levels, reduced blood flow, and compromised immune function [11].

### **Wound Healing Mechanism**

Wound healing is a complex biological process that involves several overlapping phases, each orchestrated by various cells, growth factors, and signaling molecules. The process can be broadly categorized into four main phases: hemostasis, inflammation, proliferation, and remodeling [12].

### **Hemostasis**

When a wound occurs, blood vessels in the area constrict to reduce blood loss. Platelets then aggregate at the wound site, forming a temporary plug to stop bleeding. Platelets also release clotting factors and growth factors that initiate the next stages of wound healing [12].

### **Inflammation**

The inflammatory phase begins shortly after injury and is characterized by the influx of immune cells, primarily neutrophils and macrophages, to the wound site. These cells help clear debris, bacteria, and foreign substances from the wound and release cytokines and growth factors that promote tissue repair. Inflammation also plays a role in activating fibroblasts and endothelial cells, which are essential for the subsequent phases of wound healing [12, 13].

### **Proliferation**

During the proliferation phase, new tissue is generated to fill the wound space. Fibroblasts migrate to the wound site and produce collagen, a protein that provides structural support to the healing tissue. Endothelial cells proliferate to form new blood vessels (angiogenesis), supplying oxygen and nutrients to the growing tissue. Epithelial cells at the wound edges also migrate and proliferate to cover the wound surface (epithelialization) [12, 13].

### **Remodeling**

The final phase of wound healing and remodeling can last for months to years. During this phase, the newly formed tissue undergoes maturation and remodeling, characterized by the realignment and cross-linking of collagen fibers to increase tensile strength. Excess collagen is degraded, and the wound contracts, reducing its size. The end result is scar tissue that may differ in appearance and function from the surrounding tissue [12, 13].

### **Prevention and Management of Diabetic Foot Ulcers Involve Several Strategies Proper Foot Care**

Regularly inspecting the feet for any signs of injury, keeping the feet clean and moisturized, and wearing comfortable, well-fitting shoes can help prevent ulcers from developing [14, 15].

### **Control of Blood Sugar Levels**

Maintaining tight control of blood sugar levels through medication, diet, and lifestyle changes can reduce the risk of complications associated with diabetes, including foot ulcers [15].

### **Regular Foot Exams**

Individuals with diabetes should have regular foot exams performed by a healthcare professional to monitor for any signs of ulcers or other foot problems [15].

### **Prompt Treatment**

If a foot ulcer develops, prompt treatment is essential to prevent complications. This may include cleaning and dressing the wound, offloading pressure from the affected area, and possibly administration antibiotics if there is an infection [15].

### **Footwear and Orthotics**

Wearing properly fitting shoes and, if necessary, using orthotic devices can help prevent pressure points and reduce the risk of developing ulcers in individuals with diabetes [16].

### **Herbal Formulations**

Herbal formulations are products that are made from plant-based ingredients, such as roots, leaves, flowers, bark, or fruits, which are used for medicinal purposes. These formulations can take various forms, including teas, tinctures, capsules, powders, ointments, and creams. Herbal medicine has been practiced for centuries and is still widely used today in many cultures around the world for promoting health and treating various ailments [17].

### **There are Several Common Types of Herbal Formulations and their Uses:**

#### **Herbal Teas**

Teas made from dried herbs or herbal blends are often consumed for their therapeutic properties. For example, chamomile tea is known for its calming effects, while ginger tea is used to aid digestion and relieve nausea [18].

#### **Tinctures**

Tinctures are liquid extracts made by soaking herbs in alcohol or another solvent. These plants are typically taken orally and are concentrated forms of herbal medicine [19].

#### **Capsules and Tablets**

Herbal extracts or powders are often encapsulated or compressed into tablets for convenient consumption. These formulations may be standardized to contain specific amounts of active compounds [20].

#### **Ointments and Creams**

Herbal extracts or oils are sometimes formulated into topical preparations for application to the skin. These formulations may be used for wound healing, skin condition control, or pain relief [21, 22].

#### **Herbal Supplements**

These are dietary supplements containing one or more herbal ingredients, often in combination with vitamins, minerals, or other nutrients. They may be used to support overall health or to address specific health concerns [23].

### **Aloe Vera and its Medicinal Uses**

Aloe vera is a succulent plant species that has been used for centuries for its medicinal properties. It is native to the Arabian Peninsula but is now cultivated worldwide for its various uses. The leaves of aloe vera contain a gel-like substance amino acids, and other bioactive compounds, which contribute to its therapeutic effects [24].

### **The Medical Uses and benefits of Aloe Vera Include the Following Skin Care**

Aloe vera gel is perhaps most well-known for its topical application to the skin. It has moisturizing properties and is commonly used to soothe and hydrate dry or sunburned skin. Moreover, aloe vera gel can help reduce inflammation and promote wound healing. It is often included in skincare products such as lotions, creams, and gels [25].

#### **Wound Healing**

Aloe vera contains compounds that have been shown to accelerate wound healing by promoting cell proliferation and collagen synthesis. It also has antimicrobial properties, which can help prevent infection in wounds. Aloe vera gel or extracts may be applied topically to minor cuts, burns, or abrasions to aid in healing [26].

#### **Burn Treatment**

Aloe vera is frequently used to treat minor burns, including sunburns. Its cooling and anti-inflammatory properties can relieve pain and discomfort associated with burns. The application of aloe vera gel to the affected area can help soothe the skin and promote healing [27].

#### **Skin Conditions**

Aloe vera may be beneficial for treating various skin conditions, including eczema, psoriasis, and acne. Its moisturizing and anti-inflammatory properties can help alleviate symptoms such as itching, redness, and inflammation. Aloe vera gel may be applied topically to affected areas or incorporated into skincare products for daily use [28].

#### **Digestive Health**

Some people use aloe vera juice or supplements orally for digestive health. Aloe vera has been purported to have laxative effects and may be used to relieve constipation or promote regular bowel movements. However, it is essential to use aloe vera products orally with caution and under the guidance of a healthcare professional, as excessive consumption can lead to adverse effects [29].

#### **Oral Health**

Aloe vera may be beneficial for oral health when it is used in mouthwashes or dental products. It has been shown to have antibacterial properties and may help reduce plaque buildup and gingivitis. Aloe vera gel or juice can be diluted with water and used as a mouthwash or added to toothpaste for oral hygiene [30].

#### **Niosomes**

Like liposomes, niosomes are a type of lipid-based vesicle, but are formed from nonionic surfactants. They are used as drug delivery systems to encapsulate drugs or bioactive compounds and enhance their therapeutic efficacy. Herbal niosomes are niosomal formulations containing herbal extracts or bioactive compounds derived from plants [31]. Compared to other formulations, such as traditional herbal preparations or synthetic drug delivery systems, herbal niosomes offer several potential advantages:

#### **Enhanced Bioavailability**

Niosomes can improve the solubility and stability of poorly water-soluble herbal compounds, thereby enhancing their bioavailability and therapeutic efficacy. Encapsulation within niosomes protects herbal constituents from degradation in the gastrointestinal tract and facilitates their absorption into the bloodstream [32].

### Targeted Delivery

Niosomes can be designed to target specific tissues or cells, allowing for site-specific delivery of herbal bioactive substances. Surface modification of niosomes with ligands or antibodies can enable them to selectively interact with receptors or biomarkers present on target cells, improving the precision and efficiency of drug delivery [33].

### Controlled Release

Niosomes can be engineered to control the release kinetics of encapsulated herbal compounds, providing sustained or prolonged drug release profiles. This controlled release feature can help maintain therapeutic drug levels in the body over an extended period, reducing the frequency of dosing and improving patient compliance [33].

### Biocompatibility

Niosomes are composed of biocompatible and biodegradable materials, which are well-tolerated by the body and reduce the risk of adverse effects or toxicity. Herbal niosomes formulated with natural surfactants and lipids are particularly attractive from a safety perspective, as they minimize the use of synthetic excipients and additives [34].

### Versatility

Herbal niosomes can accommodate a wide range of herbal extracts and bioactive compounds, offering versatility in formulation design. They can be tailored to encapsulate various types of herbal constituents, including hydrophilic, hydrophobic, and amphiphilic compounds, to suit diverse therapeutic applications [35].

Overall, the use of herbal niosomes represents a promising approach for the delivery of herbal medicines, offering advantages such as enhanced bioavailability, targeted delivery, controlled release, biocompatibility, and versatility.

## Materials

### Plant Profile

Aloe vera, also known as the Aloe barbadensis miller, is a succulent plant species that belongs to the genus Aloe. It is native to the Arabian Peninsula but is now cultivated worldwide for its medicinal, cosmetic, and ornamental uses. Aloe vera has been used for centuries in traditional medicine for its various therapeutic properties [36].

### Botanical Description

Aloe vera is a perennial, stemless plant that grows on rosettes with thick, fleshy, lance-shaped leaves.

The leaves of Aloe vera typically range from green to gray-green in color and are filled with a clear gel-like substance.

Mature plants can reach heights of up to 24 to 39 inches (60 to 100 centimeter), with leaves growing up to 12 to 20 inches (30 to 50 centimeter) long [37].

### Habitat and Cultivation

Aloe vera thrives in warm, arid climates and is commonly found in regions with sandy or well-drained soil.

It is often cultivated in tropical and subtropical regions worldwide, including Africa, the Mediterranean, Latin America, and parts of Asia.

Aloe vera is cultivated both commercially and as a household plant, primarily for its medicinal and cosmetic properties [38].

### Medicinal Uses

Aloe vera gel, extracted from the inner leaf pulp, is widely used for its therapeutic properties in traditional and modern medicine. It is commonly applied topically to soothe and moisturize the skin, promote wound healing, and alleviate symptoms of sunburn, burns, cuts, and abrasions.

Aloe vera gel is also used in various skincare and cosmetic products, including lotions, creams, ointments, and gels.

Some people consume aloe vera juice or supplements orally for digestive health, although this use is subject to controversy and should be approached with caution [39].

### Chemical Composition

Aloe vera gel contains a complex mixture of bioactive compounds, including polysaccharides (e.g., acemannan), glycoproteins, amino acids, vitamins (e.g., vitamin E, vitamin C), minerals (e.g., calcium, magnesium, zinc), enzymes (e.g., proteases, lipases), and antioxidants [40].

These compounds contribute to the various therapeutic effect of Aloe vera, including its anti-inflammatory, antioxidant, immunomodulatory, and wound healing effects.

### Cultivation and Harvesting

Aloe vera is typically propagated from offsets (pups) produced by mature plants or from seeds.

It requires well-drained soil and ample sunlight to thrive, with watering only when the soil is dry to avoid root rot [41].

Leaves are harvested by cutting them close to the base of the plant, and the gel is extracted from the inner leaf pulp for use in various applications [42].

### API

Spray-dried aloe vera powder. Aloe vera spray-dried powder is a form of aloe vera extract that is processed into a powdered form using the spray-drying technique. This process involved atomizing a liquid Aloe vera extract into a hot drying chamber, where it quickly dried into fine powder particles. The resulting powder retains the bioactive compounds and properties of Aloe vera, making it suitable for various applications in food, cosmetics, pharmaceuticals, and other industries [43].

### Some Key Points About Spray Dried Aloe Vera Powder Concentration

Aloe vera spray-dried powder typically contains a concentrated form of Aloe vera extract, with a high content of bioactive compounds such as polysaccharides, glycoproteins, vitamins, minerals, and antioxidants. The concentration of these compounds can vary depending on the extraction method and processing conditions [44].

### Stability

Compared with liquid aloe vera extracts, spray dried powder offers improved stability and shelf life, as it is less susceptible to degradation and microbial contamination. This makes it easier to store, transport, and incorporate various formulations without the need for refrigeration or special handling [44].

### Solubility

Aloe vera spray dried powder is often more soluble in water than are the other forms of Aloe vera extracts, increasing the ease of



incorporation into aqueous-based formulations such as beverages, cosmetics, and skincare products. It can also be reconstituted with water to produce aloe vera gel or juice for oral consumption [44].

### Applications

Aloe vera spray dried powder is used in a wide range of applications, including the following:

#### Food and Beverages

Added to juices, smoothies, yogurt, and other food products for nutritional and health benefits [45].

#### Cosmetics and Personal Care Products

These products are used in skincare creams, lotions, gels, shampoos, and conditioners for their moisturizing, soothing, and rejuvenating properties [45].

#### Pharmaceuticals

Formulated into tablets, capsules, and topical preparations for its medicinal properties, including wound healing, anti-inflammatory, and antioxidant effects [45].

#### Nutraceuticals

Used as a dietary supplement for its potential health promoting effects on digestion, immune function, and overall wellbeing [45].

### Quality and Purity

Aloe vera spray dried powder must be obtained from high quality sources and processed using good manufacturing practices to maintain its purity and efficacy. Products that are standardized for bioactive compounds and free from contaminants, additives, or adulterants should be identified [46].

### Chemicals and Solvents

The procurement details of the materials used in the formulation of the niosomes are as follows:

#### Span60 (Sorbitan Monostearate)

**Supplier:** Research-Lab Fine Chem Industries

**Location:** Mumbai-400 002, India

#### Cholesterol:

**Supplier:** MOLYCHEM

**Address:** 78/80, Babu Gane Road, Mumbai-400 002, India

#### Chloroform and Methanol

These materials were sourced from reputable suppliers in Mumbai, India, for the preparation of the niosome formulation. Proper procurement and quality assurance measures ensure the integrity and suitability of the materials for use in pharmaceutical and research applications.

### Equipment

The equipment used in the preparation and analysis of the niosome formulation includes the following:

#### Rotary Evaporator:

**Brand:** KNF Lab India Pvt. Ltd.

#### Ultracentrifuge:

**Model/Brand:** Not specified

#### Particle Size Analyzer:

**Model:** MALVERN PANALYTICAL

**Address:** Grovewood Road, Malvern, Worcestershire, WR14 1XZ, United Kingdom

#### UV Spectrometer:

**Model/Brand:** Not specified

This equipment is essential for various stages of the formulation process and characterization of the niosome formulation, including solvent evaporation, particle size analysis, determination of entrapment efficiency, and assessment of antimicrobial activity. The utilization of high-quality equipment ensures accurate and reliable results in the research and development of pharmaceutical formulations.

### Identification and Analysis of Spray-Dried Aloe Vera Powder (As Per the US Pharmacopeia)

**A:** Powdered aloe dissolves in nitric acid with effervescence and gives a reddish brown to brown or green solution [47].

**B:** one gram of finely powdered aloe was added to 25 mL of cold water in a flask, the mixture was shaken occasionally for 2 hours, filter the mixture was filtered, and the filter and residue were washed with sufficient cold water to ensure that the volume of the filtrate was 100 mL. The color of the filtrate, viewed in the bulb of a 100-mL volumetric flask, was a dark orange Color [47].

**C:** To 5 mL of the filtrate obtained in identification test B, 2 mL of nitric acid was added; the mixture was reddish-orange in color [47].

**D:** Mix 10 mL of the filtrate obtained in identification test B with 2 mL of ammonium hydroxide; the mixture emits an amber color [47].

### Chemical Evaluation

#### Quantitative Estimation of Polysaccharides by the Congo Red Method

The active polysaccharide acemannan can be identified and estimated using Congo red as a complexing agent. In this method, Congo red reacts with acemannan in an alkaline medium. When D-glucose and Congo red react with each other, the presence of acemannan in the spray-dried powder leads to the formation of a gel-like structure. This reaction is indicative of the presence of acemannan, allowing for its identification and estimation in the sample [48].

### Methods

#### Preparation of Aloe Vera Niosomes

##### Thin-Film Hydration Method

The thin-film hydration method is a technique used to prepare liposomes or niosomes, which are lipid-based vesicles capable of encapsulating drugs or bioactive compounds. This method is commonly employed in pharmaceutical and biotechnology research for drug delivery applications. Here is an overview of the thin-film hydration method [49].

##### Lipid Film Formation

Initially, lipid materials, such as phospholipids for liposomes or nonionic surfactants for niosomes, are dissolved in an organic solvent (e.g., chloroform, methanol) to form a thin lipid film. The solvent is then removed under reduced pressure or by evaporation, leaving behind a lipid film deposited on the walls of the reaction vessel [49].

##### Hydration

The lipid film is hydrated by adding an aqueous solution containing the drug or bioactive compound of interest. The addition of the aqueous phase causes the lipid film to swell and form vesicles spontaneously, driven by the hydrophobic-hydrophilic interactions between the lipids and water molecules [49].

### Vesicle Formation

As hydration proceeds, the lipid molecules rearrange themselves into bilayer structures, with the hydrophilic head groups facing the aqueous phase and the hydrophobic tails oriented toward the interior of the vesicles. This results in the formation of liposomes or niosomes, which are spherical vesicles composed of one or more lipid bilayers encapsulating an aqueous core [49].

### Size Reduction

The size of the liposomes or niosomes formed by the thin-film hydration method can be controlled by various factors, including the composition of the lipid materials, the hydration conditions, and the use of sonication or extrusion techniques to reduce vesicle size and improve homogeneity [49].

### Characterization

The resulting liposomes or niosomes were characterized for their size, size distribution, morphology, encapsulation efficiency, and stability using techniques such as dynamic light scattering, transmission electron microscopy, fluorescence spectroscopy, and chromatography [49].

The thin-film hydration method offers several advantages for the preparation of liposomes or niosomes, including simplicity, reproducibility, and scalability. It allows for the encapsulation of hydrophilic, hydrophobic, or amphiphilic drugs or bioactive compounds within vesicles, offering potential applications in drug delivery, gene therapy, cosmetics, and food technology.

The thin-film hydration method is a widely employed technique for the creation of niosomes. In this procedure, a combination of surfactant and cholesterol at a 1:1 ratio was dissolved in a chloroform and methanol mixture in a round-bottomed flask placed in a rotary evaporator set at 100-110 RPM and a vacuum of 474. The organic solvent was then evaporated to generate a thin, dry film on the bottom of the flask. Subsequently, an aqueous medium, typically water or buffer, is added to the film at a temperature surpassing the surfactant transition temperature of 56–58°C. This mixture was subjected to mild agitation to induce the formation of multilamellar vesicles, followed by sonication for 1 to 2 minutes to yield unilamellar vesicles. After sonication, the sample was transferred to a centrifuge tube (e.g., Falcon tube) and centrifuged at the highest speed, such as 13,500 rpm, for 30 minutes. The resultant precipitate, or pellet, comprises the aloe vera drug-loaded niosomes. These pellets were resuspended in appropriate solvents (e.g., water or buffer) for subsequent characterization. The supernatant, which contains any untrapped drug, may be discarded or utilized for the determination of entrapment efficiency.

### Materials Used

Span60, cholesterol, chloroform, methanol and phosphate buffer were used in the formulation.

The formulation utilized in the preparation involved the following materials: Span60, cholesterol, chloroform, methanol, and phosphate buffer. These constituents were employed in the thin-film hydration method for the creation of niosomes, with Span60 and cholesterol serving as the main lipid components, while chloroform and methanol acted as the solvent systems for lipid dissolution. Additionally, phosphate buffer was employed as an aqueous medium to hydrate the lipid film, facilitating the formation of niosomes. This formulation strategy is commonly employed in niosome preparation due to the compatibility and efficacy of these materials in generating stable and biocompatible vesicles for drug delivery applications.

### Composition of Niosomes

The composition of the niosomes used in the formulation included the following ingredients:

#### Drug:

**Aloe vera powder:** 20 mg

#### Lipid Components:

**Span60:** 30 mg

**Cholesterol:** 30 mg

#### Solvent system:

**Chloroform and methanol (7:3 ratios):** 7 ml of chloroform and 3 ml of methanol

#### Aqueous medium:

**Buffer:** 5 ml

These ingredients were combined and processed using the thin-film hydration method to prepare a niosomal formulation containing aloe vera powder. Span60 and cholesterol served as the lipid components, while chloroform and methanol acted as the solvent for lipid dissolution. The buffer was used as the aqueous medium to hydrate the lipid film, leading to the formation of niosomes encapsulating the Aloe vera powder. This composition was chosen based on its compatibility, efficacy, and ability to produce stable niosomes for drug delivery applications.



**Figure 1:** Rotary Evaporator for Preparation of Niosomal Formulation

### Evaluation of Niosomes

#### Particle Size

Particle size analysis of the niosomes in the formulation was conducted using dynamic light scattering (DLS), a technique based on photon correlation spectroscopy. DLS measures the intensity fluctuations of scattered light caused by the Brownian motion of particles in suspension, allowing for the determination of the hydrodynamic size distribution and mean particle size. The mean diameter of the niosomes was determined using a MALVERN PANALYTICAL particle size analyzer. For analysis, a drop of sample from each formulation was diluted in 10 ml of dispersion medium (distilled water). Dynamic light scattering is particularly suitable for measuring particle sizes in the microscopic range, providing valuable insights into the size distribution and characteristics of the niosomal particles in the formulation.

#### Zeta Potential

The zeta potential is an important parameter used to characterize the surface charge of colloidal particles, including niosomes. This approach provides insight into the stability and behavior of colloidal dispersions, as well as their interactions with other particles or surfaces. In the context of niosomes, the zeta potential plays a crucial role in determining their stability, aggregation tendency, and interactions with biological systems.

## Measurement

Zeta potential can be measured using techniques such as electrophoretic light scattering (ELS) or laser Doppler velocimetry (LDV). These methods involve applying an electric field to the colloidal dispersion and measuring the velocity of particles as they move under the influence of the field. The zeta potential was subsequently calculated from the electrophoretic mobility of the particles using the Smoluchowski equation or the Henry equation.

## Interpretation

The magnitude and sign of the zeta potential provide valuable information about the surface charge and stability of niosomes:

Highly negative zeta potentials (below -30 mV) indicate strong repulsion between particles, resulting in good dispersion stability and resistance to aggregation.

Positive zeta potentials (above +30 mV) indicate repulsion between particles of the same charge, leading to dispersion stability.

Near-zero or low zeta potentials suggest weak repulsion and a greater propensity for particle aggregation or flocculation.

The zeta potential, a fundamental physical property exhibited by all particles in a preparation, plays a crucial role in understanding the electric double layer repulsion phenomenon. This repulsion can be quantified through phase analysis light scattering when an electric field is applied. In this process, charged particles within the preparation are drawn toward electrodes with opposite charges, while viscous forces acting on the particles oppose their movement. Once equilibrium is achieved, particles move at a constant velocity, known as electrophoretic mobility, allowing for the measurement of zeta potential. This parameter provides valuable insights into the stability and interactions of colloidal dispersions, guiding the formulation and optimization of various products, including niosomes, for diverse applications in pharmaceuticals, cosmetics, and materials science.

## Entrapment Efficiency

Entrapment efficiency is a crucial parameter used to evaluate the performance of niosomal formulations, particularly in drug delivery applications. The percentage of the drug or bioactive compound that is successfully encapsulated within the niosome vesicles relative to the total amount of the drug added during the formulation process. A high entrapment efficiency indicates efficient encapsulation and minimal loss of the drug during preparation. The way in which the entrapment efficiency of niosomes is determined and its significance follows:

### Determination of Entrapment Efficiency

The entrapment efficiency is typically determined by separating the encapsulated drug from the free (unencapsulated) drug using a suitable method such as ultracentrifugation, size exclusion chromatography, or dialysis.

The entrapment efficiency (%) was calculated using the following formula:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total drug} - \text{free drug}}{\text{total drug}} \times 100$$

The Entrapment efficiency serves as a critical indicator of the amount of drug encapsulated within the prepared formulation. To determine this parameter, one milliliter of the niosome formulation was withdrawn and dissolved in 10 ml of buffer. The resulting mixture was then transferred to Eppendorf tubes and subjected to centrifugation at 15,000 rpm and 4°C for 15 minutes in two cycles to separate the

unentrapped drug. The clear fraction obtained was utilized for the determination of the free drug concentration. The free drug content was calculated using the following equation derived from the respective portion. Absorbance measurements were conducted at 256 nm using a UV spectrophotometer to calculate the entrapment efficiency. This methodology enables the quantification of drug encapsulation within the niosomal formulation, facilitating the assessment of its efficacy and suitability for targeted drug delivery applications.

## Antimicrobial Activity

The antimicrobial activity of acemannan was assessed using *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* as test organisms. The agar diffusion method was employed for this study. Initially, sterile Petri plates were filled with prepared nutrient broth, which was subsequently dried and cooled to form a solid agar medium. Subsequently, each bacterial culture was evenly spread on separate sterile Petri dishes using a sterile cork borer to create 4 mm deep wells. Next, 3 drops of the aloe vera niosome solution were added to the wells. The inoculated plates were then incubated at 37°C for 48 hours to allow for bacterial growth. Following incubation, the plates were examined for the development of zones of inhibition, indicating the presence of antibacterial activity. The antimicrobial efficacy of the acemannan Niosomal formulation was assessed against the selected microbial strains, namely *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. This method enabled the evaluation of the potential of the formulation as an antimicrobial agent against clinically relevant bacterial pathogens.

## Results

### Identification And Chemical Evaluation of Powder

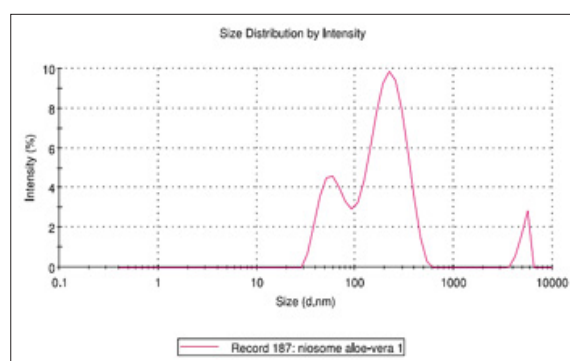
A species identification test was conducted on the Aloe vera spray-dried powder using various reagents, including nitric acid and ammonium hydroxide, which yielded appropriate results. The test confirmed that the spray-dried powder originated from the Aloe barbadensis Mill species.

Additionally, chemical evaluation was carried out using Congo red reagent, which forms a complex upon reaction with the powder solution. Based on the results of the Congo red method, it was concluded that the powder contained the polysaccharide acemannan.

These tests provided valuable insights into the botanical origin and chemical composition of the spray-dried aloe vera powder, facilitating its characterization and quality assessment for potential applications in various industries, including pharmaceuticals, cosmetics, and nutraceuticals.

### Particle Size

The particle size of the niosome formulation was determined to be 144 nm.

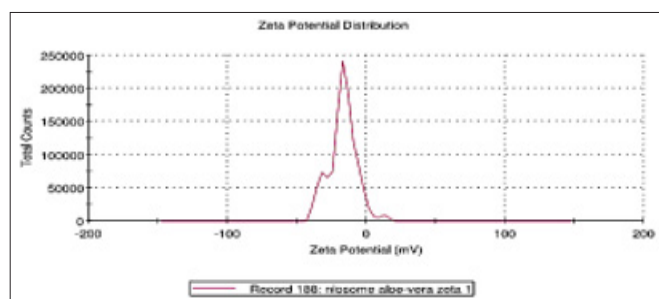


**Figure 2:** Particle Size of Niosomal Formulation



### Zeta Potential

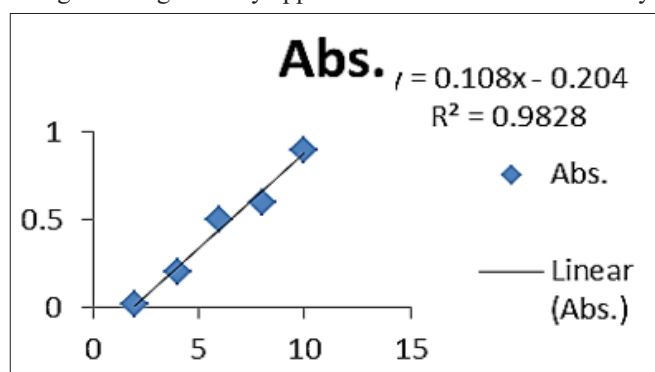
The zeta potential (ZP) of the niosomes was measured to be -17 millivolts (mV). The zeta potential is a key parameter that reflects the surface charge of colloidal particles, including niosomes. A negative zeta potential indicates that the niosomes are negatively charged, which can contribute to their stability by providing electrostatic repulsion between particles, thus reducing aggregation and enhancing dispersion. This information is crucial for understanding the colloidal stability and behavior of niosomal formulations, especially in applications such as drug delivery, where stability and controlled release are essential factors.



**Figure 3:** Zeta Potential of Niosomal Formulation

### Entrapment Efficiency

The entrapment efficiency of the niosomes was determined to be 73%. Entrapment efficiency refers to the proportion of the drug or active compound encapsulated within the niosomes relative to the total amount of drug added during the formulation process. A higher entrapment efficiency indicates efficient encapsulation and minimal loss of the drug during preparation. These findings suggested that a significant portion of the drug was successfully incorporated into the niosomes, making the formulation suitable for targeted drug delivery applications with enhanced efficacy.

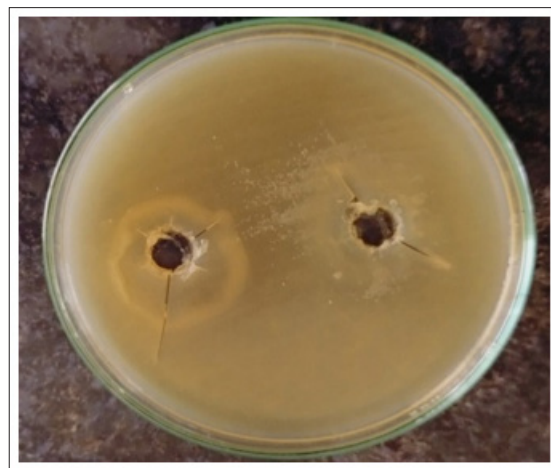


**Figure 4:** Absorbance of Niosomal Formulation

The antimicrobial activity of the aloe vera niosome formulation was assessed against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* using the agar diffusion method.

The zones of inhibition were measured using a Vernier caliper. The assay demonstrated significant antimicrobial activity, particularly against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Specifically, the Aloe vera niosome formulation exhibited a zone of inhibition of 20 mm against *Staphylococcus aureus* and 15 mm against *Pseudomonas aeruginosa*. Additionally, moderate activity was observed against *Escherichia coli*, with a zone of inhibition of 21 mm. These results indicate that the Aloe vera niosome formulation possesses notable antimicrobial effects against a range of bacterial strains, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Therefore, based

on the result of the zone of inhibition assay, it can be concluded that the aloe vera niosome formulation demonstrates promising antimicrobial efficacy, suggesting its potential utility in combating bacterial infections caused by these pathogens.



**Figure 5:** Antimicrobial Activity of Niosomes

### Conclusion

Based on the comprehensive data collected from various analyses and assays conducted on the Aloe vera niosome formulation, the following discussion and conclusions can be drawn:

#### Particle Size and Zeta Potential

The niosome formulation exhibited a particle size of 144 nm and a zeta potential of -17 mV. These parameters are crucial indicators of the formulation's stability and behavior in colloidal systems. The relatively small particle size of the niosomes suggested that they possess favorable characteristics for drug delivery applications, such as improved tissue penetration and cellular uptake. Negative zeta potential indicates good colloidal stability, suggesting minimal aggregation and enhanced dispersion of the niosomes in solution.

#### Entrapment Efficiency

The entrapment efficiency of the niosomes was 73%, indicating efficient encapsulation of the drug within the vesicles. High entrapment efficiency is desirable because it ensures maximum drug loading and minimizes waste, enhancing the therapeutic efficacy of the formulation.

#### Antimicrobial Activity

The aloe vera niosome formulation exhibited significant antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The zone of inhibition assay revealed prominent activity against *Staphylococcus aureus* and moderate activity against *Pseudomonas aeruginosa* and *Escherichia coli*. These findings highlight the potential of the aloe vera niosome formulation as an effective antimicrobial agent against clinically relevant bacterial pathogens.

#### Botanical and Chemical Characterization

The species identification test confirmed that the spray-dried powder originated from the *Aloe barbadensis* Mill species.

Chemical evaluation using Congo red reagent revealed the presence of the polysaccharide acemannan in the powder.

The aloe vera niosome formulation has promising potential for pharmaceutical and biomedical applications. Its small particle size,



negative zeta potential, high entrapment efficiency, and significant antimicrobial activity against bacterial pathogens underscore its potential as an effective drug delivery system for targeted therapy. Additionally, botanical and chemical characterization confirmed the presence of bioactive compounds such as acemannan, further supporting its therapeutic potential. Overall, the proposed aloe vera niosome formulation shows great promise as a versatile platform for the development of novel therapeutics, including antimicrobial therapy and wound healing agents, in various fields. Further research and clinical studies are warranted to explore its full potential and optimize its formulation for specific applications.

### Future Prospective

Based on the data obtained from the analyses and assays conducted on the Aloe vera niosome formulation, several future perspectives and potential research directions can be identified:

### Optimization of Formulation Parameters

Further optimization of the formulation parameters, such as lipid composition, surfactant concentration, and drug-to-lipid ratio, can be explored to enhance the properties and performance of the niosomes.

Investigating the effects of different preparation methods and processing conditions on particle size, zeta potential, and entrapment efficiency could lead to the development of more efficient and stable formulations.

### Characterization of Drug Release Kinetics

Future studies could focus on characterizing the drug release kinetics of the aloe vera niosome formulation, including the release profile, mechanism, and kinetics of drug release under physiological conditions.

Understanding the release behavior of encapsulated drugs from niosomes is essential for optimizing formulations for controlled and sustained drug delivery.

### In Vivo Evaluation of Therapeutic Efficacy

Further preclinical studies and animal experiments are needed to evaluate the therapeutic efficacy and safety of the aloe vera niosome formulation in vivo.

Assessing the pharmacokinetics, biodistribution, and tissue targeting capabilities of the formulation will provide valuable insights into its potential for clinical translation.

### Exploration of Alternative Applications

In addition to antimicrobial therapy, the aloe vera niosome formulation could be investigated for its potential in other therapeutic areas, such as wound healing, anti-inflammatory treatment, and cosmetic applications.

Exploring its potential synergistic effects with other therapeutic agents or natural compounds could broaden its application scope and therapeutic benefits.

### Development of Combination Therapies

Combination therapies involving the aloe vera niosome formulation and conventional antibiotics or other antimicrobial agents could be explored to enhance therapeutic efficacy and overcome antimicrobial resistance.

Investigating the potential synergistic effects of combining Aloe vera with other natural compounds or adjuvants could lead to the development of novel combination therapies with enhanced antimicrobial activity.

### Clinical Translation and Commercialization

Ultimately, clinical trials and regulatory approval will be necessary to validate the safety and efficacy of the Aloe vera niosome formulation for human use.

Collaborations with pharmaceutical companies or research institutions may facilitate the scale-up production, commercialization, and market introduction of the aloe vera niosome formulation as a novel therapeutic agent.

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### Credit Authorship Statement

Chaudhari Kedar: Methodology, protocol, instrument handling, software acquisition, original draft and writing and editing. Lohar Neha: Instrument handling, raw data treatment, sample preparation. Dr. Manoj Girase conceptualized the study, supervised the study, administrated the project generated, the results and discussion, Interpreted and remodeled the study.

### Conflict of Interest Statement

The authors have no conflicts of interest to declare with respect to the research, authorship, and/or publication of this article.

### Data Availability Statement

The authors confirm that the data supporting the finding of this study are available within the article and/or its supplementary materials.

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