VITA SCIENTIA

volume 2 Issue 2 February 2026

www.vitascientia.net

Vita Scientia

Review Article

Read Online

Herbal Cough Syrups: Medicinal Plants, Formulation Approaches, Therapeutic Mechanisms and Recent Advances

Sonia Rambachan Kanojia[1*], Dnyaneshwar Vyavhare[1], Gita Mohire [1]

[1]B. R. Harne College of Pharmacy, Karav-Vangani, Thane, Maharashtra, India

*Correspondence: kanojiasonia67@gmail.com,

https://doi.org/10.65116/vitascientia.V2I2.rev.01

Summary

Cough is a common clinical manifestation of respiratory tract disorders and significantly affects quality of life across all age groups. Herbal cough syrups have been traditionally used for symptomatic relief and are increasingly incorporated into contemporary formulations due to their multi-component therapeutic potential. This review critically evaluates medicinal plants used in herbal cough syrups, their phytochemical constituents, formulation approaches, mechanisms of action, and recent advances reported in the literature. Published peer-reviewed studies were analyzed to clearly distinguish traditional use, preclinical pharmacological evidence, and available clinical validation. Several medicinal plants demonstrate antitussive, expectorant, anti-inflammatory, and antimicrobial activities in experimental studies; however, clinical evidence in human populations remains limited, inconsistent, and formulation-dependent.Variability in phytochemical composition, lack of standardization, potential herb–drug interactions, and limited comparative studies with conventional antitussive agents present important challenges for clinical translation. Recent developments, including standardized extracts, sugar-free formulations, and novel delivery approaches reflect progress in formulation science but require stronger clinical support. Overall, this review highlights both the therapeutic potential and existing limitations of herbal cough syrups, emphasizing the need for rigorous clinical studies, quality control, and regulatory harmonization to support their rational and evidence-based use in cough management.

Keywords

Herbal cough syrup; Medicinal plants; Phytochemical constituents; Formulation approaches; Antitussive activity; Clinical evidence

Introduction

Cough is a common clinical symptom associated with a wide range of upper and lower respiratory tract disorders, including infections, allergic conditions, and chronic inflammatory diseases (1). Although acute cough is generally self-limiting, persistent or recurrent cough can significantly impair quality of life by disturbing sleep, daily activities, and overall well-being (2). Due to its high prevalence across all age groups, cough remains one of the most frequent reasons for outpatient visits and self-medication worldwide (3).

Conventional management of cough primarily involves synthetic antitussive and expectorant agents, including opioid and non-opioid formulations. While these agents are effective in

specific clinical situations, their use is often limited by adverse effects, variable efficacy, and safety concerns, particularly in pediatric and geriatric populations (4). Sedation, gastrointestinal discomfort, and the risk of misuse have encouraged interest in alternative and complementary therapeutic approaches for cough management (5).

Herbal cough syrups have been traditionally used in systems of medicine such as Ayurveda, Unani, and other traditional practices for the symptomatic relief of cough and related respiratory conditions (6). These formulations commonly contain medicinal plants with reported antitussive, expectorant, anti-inflammatory, antimicrobial, and demulcent properties. Unlike single-target synthetic drugs, herbal cough syrups are believed to exert their effects through multiple bioactive constituents acting on different pathways involved in cough generation and airway irritation (7).

Despite their widespread use and general perception of better tolerability, the scientific validation of herbal cough syrups presents several challenges. Although numerous preclinical studies support the pharmacological activities of medicinal plants used in these formulations, clinical evidence from well-designed human trials remains limited and inconsistent (8). Variability in phytochemical composition, differences in extraction methods, lack of formulation standardization, and quality control issues further complicate the assessment of efficacy and safety (9). In addition, potential herb–drug interactions and regulatory gaps warrant careful consideration (10).

Therefore, the present review aims to provide a comprehensive and critical evaluation of herbal cough syrups by examining medicinal plants used, their phytochemical constituents, formulation approaches, mechanisms of action, evaluation parameters, safety considerations, regulatory aspects, and recent advances. By clearly distinguishing traditional use, preclinical evidence, and clinical validation, this review seeks to identify existing gaps and future directions to support the rational and evidence-based use of herbal cough syrups in cough management.

Pathophysiology of Cough

Cough is a protective reflex mechanism designed to clear the airways of irritants, secretions, and foreign particles. It involves a complex interaction between sensory receptors, neural pathways, and respiratory muscles. Depending on duration and underlying cause, cough may be classified as acute, subacute, or chronic, each associated with distinct pathophysiological mechanisms (16).

The cough reflex is initiated by the stimulation of specialized sensory receptors located in the respiratory tract, including the larynx, trachea, bronchi, and, to a lesser extent, the external auditory canal and esophagus. These receptors include rapidly adapting receptors (RARs), slowly adapting stretch receptors (SARs), and C-fiber nociceptors, which respond to mechanical, chemical, and inflammatory stimuli such as mucus, smoke, allergens, and infectious agents (17).

Upon activation, afferent signals are transmitted primarily via the vagus nerve to the cough center located in the medulla oblongata of the brainstem. This central processing integrates sensory input and generates an appropriate motor response. The efferent pathway then transmits signals through motor nerves to the diaphragm, intercostal muscles, abdominal muscles, and larynx, resulting in the characteristic cough sequence consisting of inspiratory, compressive, and expulsive phases (18).

Additionally, excessive mucus production and impaired mucociliary clearance contribute to persistent cough by increasing mechanical stimulation of airway receptors. Oxidative stress

and epithelial damage further exacerbate receptor sensitivity, leading to prolonged or recurrent cough episodes (20). Understanding these pathophysiological mechanisms is essential for the rational selection of therapeutic agents, including herbal cough syrups, which aim to modulate inflammation, reduce mucus viscosity, and soothe irritated respiratory mucosa.

Cough Reflex Arc

The cough reflex arc is a coordinated neural pathway responsible for initiating and controlling the cough response. It plays a vital role in maintaining airway patency by facilitating the removal of mucus, pathogens, and foreign particles from the respiratory tract. Dysregulation of this reflex is commonly associated with chronic and hypersensitive cough conditions (21).

Afferent Pathway

The afferent limb of the cough reflex begins with the activation of sensory receptors located in the upper and lower respiratory tract, including the larynx, trachea, and bronchi. These receptors are sensitive to mechanical stimuli such as mucus accumulation and foreign particles, as well as chemical stimuli including inflammatory mediators, pollutants, and infectious agents. Sensory impulses generated at these sites are transmitted primarily via the vagus nerve to the central nervous system (22).

Efferent Pathway

The efferent limb of the cough reflex involves motor nerve signals transmitted from the cough center to the respiratory muscles, including the diaphragm, intercostal muscles, abdominal muscles, and laryngeal muscles. This coordinated motor output produces the characteristic phases of coughing: deep inspiration, closure of the glottis with increased intrathoracic pressure, followed by forceful expiration that expels air and airway secretions (24).

Central Processing

The sensory signals from the afferent pathway are integrated in the cough center situated in the medulla oblongata of the brainstem. This center processes incoming stimuli and determines the initiation, intensity, and frequency of the cough response. Higher cortical centers may also modulate the cough reflex, allowing voluntary suppression or initiation of coughing under certain conditions (23).

Clinical Relevance

In respiratory diseases such as infections, asthma, and chronic inflammatory airway disorders, heightened sensitivity of the cough reflex arc may occur. Inflammatory mediators and epithelial damage lower the activation threshold of sensory receptors, resulting in excessive or persistent coughing. Understanding the cough reflex arc provides a physiological basis for therapeutic interventions aimed at suppressing excessive reflex activity and protecting airway mucosa (25).

Rationale for Herbal Cough Syrup

The increasing interest in herbal cough syrups is driven by the limitations associated with conventional synthetic antitussive therapies and the long-standing traditional use of medicinal plants in respiratory disorders. Synthetic cough

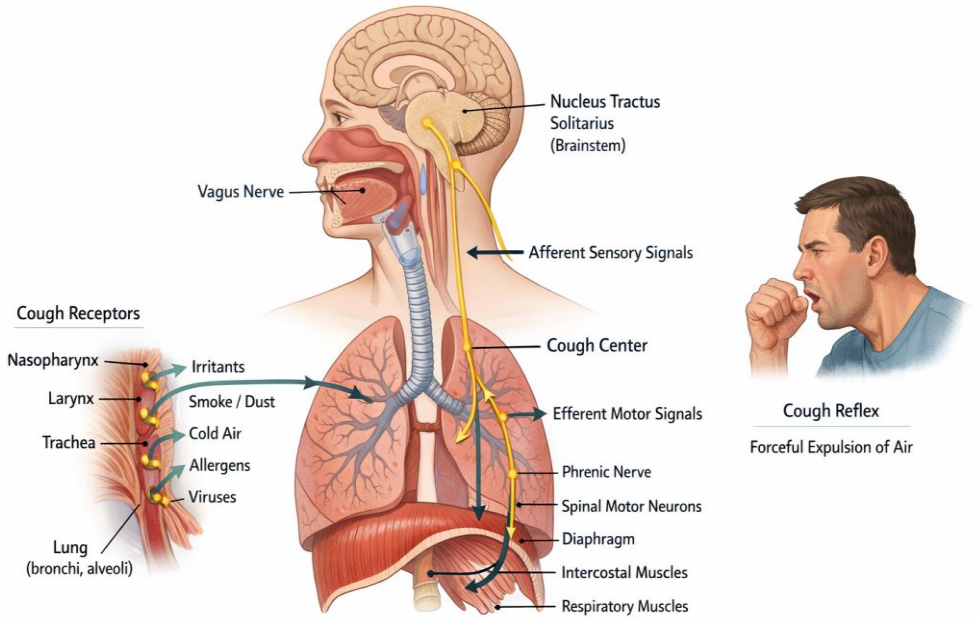


Figure 1. Pathophysiology of cough and the cough reflex arc.
(Author-generated schematic illustration based on published literature.Source: Dicpinigaitis PV, Chest, 2020; Chang AB et al., Chest, 2021.)

suppressants and expectorants, although effective in certain clinical conditions, are often associated with adverse effects such as sedation, gastrointestinal discomfort, and limited suitability for pediatric and geriatric populations. Moreover, concerns related to misuse, inappropriate self-medication, and variable clinical outcomes have highlighted the need for safer and more holistic alternatives (26).

Herbal cough syrups have been traditionally used in systems of medicine such as Ayurveda, Unani, and traditional folk practices for centuries. These formulations typically contain a combination of medicinal plants possessing antitussive, expectorant, mucolytic, anti-inflammatory, antimicrobial, and demulcent properties. Unlike single-molecule synthetic drugs, herbal cough syrups exert multi-target effects by modulating different components of the cough pathway, including airway inflammation, mucus viscosity, and cough receptor sensitivity (27).

Another important rationale for the use of herbal cough syrups lies in their potential to provide symptomatic relief while addressing underlying airway irritation. Several plant-derived constituents, such as flavonoids, saponins, alkaloids, and polysaccharides, are known to soothe inflamed mucosal surfaces and support mucociliary clearance. This multimodal action may be particularly beneficial in cases of mild to moderate cough associated with upper respiratory tract infections and allergic conditions (28).

Despite their widespread acceptance and perception of better tolerability, herbal cough

syrups are not devoid of challenges. Variability in phytochemical composition due to differences in plant species, geographical sources, harvesting conditions, and extraction methods can significantly influence therapeutic outcomes. Additionally, limited standardization, inconsistent clinical validation, and potential herb–drug interactions necessitate a cautious and evidence-based approach to their use (29).

Therefore, a critical evaluation of herbal cough syrups is essential to distinguish traditional claims from scientifically supported evidence. Understanding their pharmacological basis, formulation strategies, safety considerations, and regulatory aspects is crucial for promoting rational use and integrating herbal cough syrups into modern evidence-based respiratory care (30).

Medicinal Plants Used in Herbal Cough Syrups

Herbal cough syrups are formulated using a combination of medicinal plants selected for their traditional use and reported pharmacological activities relevant to cough management. These plants act through multiple mechanisms such as suppression of cough reflex sensitivity, facilitation of mucus expulsion, reduction of airway inflammation, and soothing of irritated respiratory mucosa. However, the extent of scientific validation differs among individual ingredients, necessitating a critical evaluation of available evidence.

Adhatoda vasica (Vasaka)

A. vasica is a key ingredient in many herbal cough syrups due to its expectorant and bronchodilatory properties. Alkaloids such as vasicine and vasicinone are known to enhance bronchial secretions and improve mucus clearance. Although extensively used in traditional medicine, clinical evidence supporting its antitussive efficacy remains limited and formulation-specific (31).

Glycyrrhiza glabra (Liquorice)

G. glabra is widely incorporated for its demulcent and anti-inflammatory effects. Glycyrrhizin and flavonoids help soothe inflamed mucosal surfaces and may reduce irritation-induced cough. Despite its long history of use, concerns

Figure2: Medicinal plants used in herbal cough syrup and their therapeutic role
(Author-generated conceptual illustration summarizing commonly reported medicinal plants. Source: WHO, 2020; Barnes J et al., Phytotherapy Research, 2021; ESCOP, 2020**.)**

regarding dose-dependent adverse effects highlight the need for standardized formulations and clinical evaluation (32).

***Ocimum sanctum* (Tulsi)**

O. sanctum exhibits antimicrobial, anti-inflammatory, and immunomodulatory properties. Traditionally used in respiratory infections, it may help reduce airway inflammation associated with cough. Most supporting evidence is derived from experimental and traditional sources, with limited data from controlled human studies (33).

***Zingiber officinale* (Ginger)**

Z. officinale is used in herbal cough syrups for its anti-inflammatory and antioxidant activities. Active constituents such as gingerols and shogaols are believed to relieve throat irritation and suppress cough associated with upper respiratory tract infections. Scientific evidence is largely preclinical, emphasizing the need for clinical validation (34).

***Piper longum* (Long Pepper)**

P. longum is traditionally used as an expectorant and bioavailability enhancer. Piperine present in long pepper may improve respiratory function and enhance the absorption of other herbal constituents in polyherbal formulations. However, its direct antitussive effects in humans are not well established (35).

***Mentha piperita* (Peppermint)**

M. piperita contains menthol, which produces a cooling sensation and provides symptomatic relief in cough by soothing irritated throat tissues. It may also exert mild bronchodilatory effects. Its use is primarily supportive, with limited clinical data specific to cough suppression (36).

Honey (Natural Demulcent)

Honey is frequently used as a natural demulcent in herbal cough syrups. It forms a protective coating over the pharyngeal mucosa, reducing irritation and cough frequency. Several clinical studies have suggested its benefit in reducing nocturnal cough, particularly in children, although variability in composition affects outcomes (37).

Phytochemical Constituents and Their Role

Alkaloids such as vasicine exhibit bronchodilatory and expectorant properties by relaxing smooth bronchial muscles and enhancing mucus clearance. Flavonoids and phenolic compounds reduce airway inflammation by inhibiting inflammatory mediators and oxidative stress, thereby decreasing cough reflex sensitivity. Saponins play a crucial role in enhancing bronchial secretions and facilitating expectoration, making them particularly useful in productive cough [7,8,12].

Tannins and mucilaginous compounds exert a soothing effect on irritated mucosal surfaces, reducing mechanical stimulation of cough receptors. Essential oils and terpenoids provide antimicrobial and mild anesthetic effects, which help in alleviating cough associated with upper respiratory tract infections. The synergistic interaction among these phytochemicals contributes to the overall efficacy of herbal cough syrups [5].

Herbal cough syrups exhibit their therapeutic activity due to the presence of diverse phytochemical constituents that act on different components of the respiratory system. These bioactive compounds contribute to antitussive, expectorant, anti-inflammatory, antimicrobial, and soothing effects, making herbal

formulations useful in the management of cough and related respiratory disorders (1).

Phytochemical Constituents and Their Therapeutic Role in Herbal Cough Syrups

Phytochemical class	Examples	Major source plants	Therapeutic Role in cough
Alkaloids	Vasicine, Piperine	Adhatoda vasica, Piper longum	Bronchodilator, antitussive, expectorant
Flavonoids	Quercetin, Luteolin	Ocimum sanctum, Glycyrrhiza glabra	Anti-inflammatory, antioxidant, cough suppression
Saponins	Glycyrrhizin	Glycyrrhiza glabra	Demulcent, expectorant, soothing effect
Tannins	Catechins, Gallic acid	Adhatoda vasica, Ocimum sanctum	Astringent action, reduction of throat irritation
Phenolic compounds	Eugenol, Rosmarinic acid	Ocimum sanctum, Mentha piperita	Anti-inflammatory, antimicrobial
Terpenoids	Menthol	Mentha piperita	Mild antitussive, decongestant
Essential oils	Ginger oil,Peppermint oil	Zingiber officinale, Mentha piperita	Expectorant, antimicrobial, soothing
Sugars & polysaccharids	Mucilage	Glycyrrhiza glabra, Honey	Demulcent, protective coating on mucous

Table 1. Phytochemical Constituents and Their Therapeutic Role in Herbal Cough Syrups

Alkaloids

Alkaloids such as vasicine and vasicinone, primarily obtained from Adhatoda vasica, are well known for their bronchodilatory and expectorant properties. These compounds help in loosening bronchial secretions and improving mucociliary clearance, thereby reducing cough frequency and intensity (2).

Flavonoids

Flavonoids present in medicinal plants such as Ocimum sanctum and Zingiber officinale exhibit significant anti-inflammatory and antioxidant activities. They reduce airway inflammation and oxidative stress, which play a key role in cough reflex hypersensitivity (3).

Saponins

Saponins, commonly found in Glycyrrhiza glabra and Hedera helix, act as natural expectorants. By decreasing the surface tension of mucus, saponins facilitate liquefaction of bronchial secretions and promote effective expectoration in productive cough conditions (4).

Tannins

Tannins possess astringent and mild antimicrobial properties. They help soothe inflamed mucosal surfaces of the throat and upper respiratory tract by forming a protective layer, which reduces local irritation and suppresses excessive cough reflex activity (5).

Essential Oils and Terpenoids

Essential oils and terpenoids present in herbs such as Zingiber officinale and Ocimum sanctum exhibit antimicrobial, anti-inflammatory, and mild bronchodilatory effects. These constituents also improve patient compliance by imparting a pleasant aroma and soothing sensation to herbal cough syrups (6).

Polysaccharides

Polysaccharides present in herbal ingredients such as honey and Glycyrrhiza glabra play a crucial role in cough management by exerting a demulcent effect. These compounds form a protective viscous layer over the irritated mucosal surfaces of the throat and upper respiratory tract, thereby reducing sensory nerve stimulation and cough reflex sensitivity. Polysaccharides are particularly beneficial in the management of dry and non-productive cough by providing soothing and moisturizing effects, which help in alleviating throat irritation and discomfort.

Mechanism of Action of Herbal Cough Syrups

Herbal cough syrups exert their therapeutic effects through multiple pharmacological pathways that collectively target the underlying mechanisms of cough. Unlike single-target synthetic antitussives, herbal formulations act in a multimodal manner by modulating the cough reflex, reducing airway inflammation, facilitating mucus clearance, and soothing irritated mucosal surfaces.

The combined action of diverse phytochemical constituents results in a synergistic effect,

Comparative Perspective: Herbal Cough Syrups vs Synthetic Antitussives

Parameter	Herbal cough syrup	Synthetic antitussives
Source	Medicinal plants and natural ingredients	Chemically synthesized drugs
Mechanisms of action	Multimodal: antitussive, expectorant, anti-inflammatory, demulcent	Primarily central or peripheral cough suppression
Effect on mucus	Promote mucus liquefaction and expectoration	Limited effect on mucus clearance
Safety profile	Generally perceived as safer; may vary with quality and composition	Associated with adverse effects such as drowsiness and gastrointestinal discomfort
Clinical evidence	Predominantly traditional use and preclinical studies; limited clinical trials	Supported by controlled clinical studies and standardized dosing

Use in pediatrics	Commonly used with caution under supervision	Restricted use due to safety concerns and guideline limitations
Risk factor	Variability in phytochemical content; potential herb–drug interactions	Risk of misuse, overdose, and adverse drug reactions
Regulatory control	Depends on herbal standardization and GMP compliance	Strict pharmaceutical regulatory oversight

Table 2-Comparative Perspective: Herbal Cough Syrups vs Synthetic Antitussives

contributing to symptomatic relief in both productive and non-productive cough conditions. The key mechanisms involved in the action of herbal cough syrups are discussed below.

Antitussive Action

Several medicinal plants used in herbal cough syrups modulate the cough reflex by reducing the sensitivity of airway sensory receptors. Alkaloids such as vasicine from Adhatoda vasica and piperine from Piper longum are reported to suppress excessive cough reflex activity by acting on peripheral cough receptors and central cough centers. This action helps in decreasing cough frequency, particularly in chronic and irritative cough conditions (38,39).

Expectorant and Mucolytic Activity

Saponins present in plants like Glycyrrhiza glabra enhance bronchial secretions and reduce mucus viscosity, thereby facilitating easier expectoration. Improved mucus clearance helps remove irritants and pathogens from the respiratory tract, contributing to symptomatic relief in productive cough. Gingerols from Zingiber officinale further support mucolytic action by promoting smooth muscle relaxation and improved airflow (40,41).

Anti-inflammatory Effects

Airway inflammation is a key contributor to persistent cough. Flavonoids and phenolic compounds present in Ocimum sanctum, Glycyrrhiza glabra, and ginger inhibit the release of pro-inflammatory mediators such as prostaglandins, leukotrienes, and cytokines. Reduction of inflammation leads to decreased airway hypersensitivity and irritation, thereby indirectly suppressing cough (42,43).

Demulcent and Soothing Action

Polysaccharides and natural sugars, particularly from honey and licorice, exert a demulcent effect by forming a protective coating over the inflamed mucosal surfaces of the throat and upper respiratory tract. This protective layer reduces stimulation of sensory nerve endings and is especially beneficial in dry and non-productive cough. The soothing effect also improves patient comfort and compliance (44,45).

Antimicrobial and Antioxidant Effects

Essential oils from Mentha piperita and phenolic compounds from tulsi and ginger exhibit antimicrobial activity against common respiratory pathogens. Antioxidant properties further protect airway epithelial cells from oxidative stress, which may otherwise exacerbate inflammation and cough severity (46,47).

Overall Synergistic Effect

The combined presence of antitussive, expectorant, anti-inflammatory, demulcent, and antimicrobial activities results in a holistic approach to cough management. However, the

extent of therapeutic benefit depends on formulation composition, phytochemical concentration, and quality of manufacturing. Variability in these factors may lead to differences in clinical efficacy among herbal cough syrup formulations, highlighting the importance of standardization and quality control (48).

Difference in Therapeutic Approach

Synthetic non-opioid antitussives such as dextromethorphan primarily act by suppressing the cough reflex at the central or peripheral level, providing short-term symptomatic relief. In contrast, herbal cough syrups exhibit a multimodal therapeutic approach by combining antitussive, expectorant, anti-inflammatory, and demulcent actions, thereby addressing multiple components of cough pathophysiology rather than reflex suppression alone (49,50).

Effect on Airway Inflammation and Mucus Clearance

Synthetic antitussives have limited influence on airway inflammation and mucus viscosity. Herbal cough syrups, however, promote mucus liquefaction and expectoration through saponins and alkaloids, while flavonoids and phenolic compounds help reduce airway inflammation. This dual action may contribute to symptomatic relief in productive cough conditions (51,52).

Clinical Evidence and Validation

The clinical efficacy of synthetic antitussives is supported by controlled clinical trials and standardized dosing regimens. In contrast, most herbal cough syrups rely on traditional use and preclinical evidence, with limited high-quality human clinical trials available. Variability in formulation composition further contributes to inconsistent clinical outcomes among herbal products (53).

Safety Considerations and Pediatric Use

Synthetic antitussives are associated with adverse effects such as drowsiness, gastrointestinal discomfort, and restricted pediatric use.

Although herbal cough syrups are often perceived as safer, potential risks related to herb–drug interactions, variable phytochemical content, and poor manufacturing practices must be considered. Evidence-based pediatric cough guidelines recommend cautious use of both herbal and synthetic formulations (51).

Regulatory and Quality Control Aspects

Synthetic antitussives are subject to strict pharmaceutical regulatory frameworks, whereas herbal cough syrups fall under varying regulatory standards depending on regional guidelines. Lack of uniform standardization and quality control remains a key challenge for herbal formulations, emphasizing the need for Good Manufacturing Practices (GMP) and phytochemical standardization (48).

Formulation Approaches Reported in Literature

The formulation of herbal cough syrups involves careful selection of medicinal plant extracts, suitable solvents, syrup bases, and excipients to ensure therapeutic efficacy, stability, palatability,

and patient compliance. Literature reports emphasize that herbal cough syrups should be formulated in a manner that preserves the bioactive constituents of medicinal plants while meeting pharmaceutical quality standards. The formulation approach generally depends on the nature of the herbal ingredients, intended therapeutic effect, and target patient population [14].

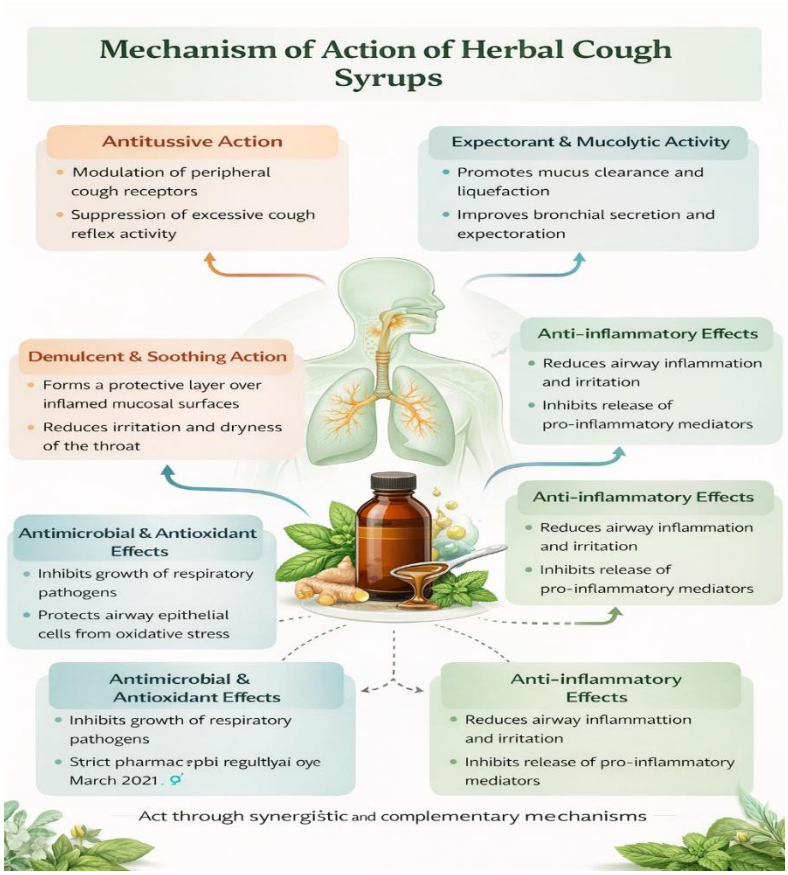


Figure 3: Mechanism of action of Herbal cough syrup

(Author-generated diagram illustrating antitussive, expectorant, anti-inflammatory, and demulcent mechanisms. Source: Heinrich M et al., 2020; Mukherjee PK, 2020.)

conditions should be used to avoid degradation of thermolabile constituents and to retain the therapeutic potential of herbal extracts [12,14].

Choice of Syrup Base

The syrup base plays a crucial role in the formulation of herbal cough syrups by providing viscosity, sweetness, and a soothing effect on the throat. Commonly used bases include sucrose syrup, glycerin, honey, or sugar-free alternatives such as sorbitol for diabetic patients. Honey and glycerin are particularly valued for their demulcent properties, which enhance the soothing effect of the formulation and improve patient acceptability [2,14].

Selection and Preparation of Herbal Extracts

The first step in the formulation of herbal cough syrups is the selection of appropriate medicinal plants based on their traditional use and documented pharmacological activities. Aqueous or hydroalcoholic extracts are commonly employed, as they are effective in extracting polar and moderately polar phytoconstituents such as alkaloids, flavonoids, and saponins. Literature suggests that mild extraction

Incorporation of Herbal Extracts

Once the extracts are prepared, they are incorporated into the syrup base under controlled conditions to ensure uniform distribution. Literature emphasizes the importance of gradual mixing and proper agitation to avoid precipitation or instability. Polyherbal formulations often require compatibility studies to ensure that the combined extracts do not interact adversely with each other or with the excipients used in the formulation [5].

Figure 4 General Formulation process of herbal cough syrup (Author-generated flow diagram depicting extraction, formulation, quality control, and packaging steps. Source: WHO, 2020; EMA, 2021; Mukherjee PK, 2020.)

Use of Excipients and Additives

Herbal cough syrups may contain preservatives, flavoring agents, and stabilizers to enhance shelf life, taste, and overall quality. Preservatives such as sodium benzoate or potassium sorbate are commonly reported in literature to prevent microbial growth, particularly in aqueous formulations. Flavoring agents derived from natural sources improve palatability, especially for pediatric use, while stabilizers help maintain formulation consistency during storage [14,15].

9.5 Standardization and Quality Considerations

Standardization of herbal cough syrups is a critical aspect highlighted in literature. This involves ensuring consistent levels of marker compounds in each batch to maintain therapeutic efficacy. Analytical techniques such as spectrophotometry, chromatography, and phytochemical screening are commonly employed for quality assessment. Literature also stresses adherence to good manufacturing practices (GMP) to minimize variability in herbal formulations [15].

Recent Trends in Formulation Approaches

Recent literature reports advancements in formulation approaches, including the development of sugar-free and alcohol-free herbal cough syrups to cater to specific patient groups. Improved extraction techniques and incorporation of bioavailability enhancers have also been explored to enhance the effectiveness of herbal formulations. These trends reflect ongoing efforts to align traditional herbal formulations with modern pharmaceutical standards [17].

Evaluation Parameters of Herbal Cough Syrups

Evaluation of herbal cough syrups is an essential step to ensure their quality, safety, stability, and therapeutic effectiveness. Literature reports emphasize that herbal

formulations should be evaluated using suitable physicochemical, microbiological, and stability parameters in accordance with pharmacopeial and regulatory guidelines. Proper evaluation helps in maintaining batch-to-batch consistency and consumer acceptability of herbal cough syrups [14,15].

Organoleptic Properties

Organoleptic evaluation involves assessing the color, taste, odor, and overall appearance of the herbal cough syrup. This is an important quality control parameter because it directly affects patient acceptability and compliance, especially in formulations intended for children or elderly patients. A uniform appearance and consistent taste also indicate proper formulation techniques and homogeneity of ingredients.

pH

The pH of the syrup is measured using a digital pH meter. Maintaining an appropriate pH is critical for chemical stability, as extreme pH values can degrade active herbal constituents or affect the syrup's shelf life. Proper pH also helps in reducing throat irritation and ensures that the formulation remains safe for regular consumption.

Viscosity

Viscosity determines the thickness and flow of the syrup and is measured using a viscometer. It plays a significant role in mouthfeel, pourability, and patient acceptability. Additionally, in herbal syrups, viscosity contributes to the demulcent effect, providing a soothing coating to the throat, which is particularly important in cough relief formulations.

Specific Gravity

Specific gravity is the ratio of the weight of the syrup to that of an equal volume of water. It provides information about the concentration of solids in the syrup and helps in assessing consistency and uniformity of the formulation. A consistent specific gravity indicates accurate formulation and quality control during manufacturing.

Refractive Index:

The refractive index, measured using a refractometer, reflects the sugar content and purity of the syrup. It is an indirect measure of total dissolved solids, which affects the taste, preservation, and stability of the syrup. Correct sugar concentration also prevents microbial growth while maintaining palatability.

Content Uniformity

Content uniformity involves estimating the amount of active herbal constituents in the syrup to ensure that each dose delivers a consistent therapeutic effect. Uniform distribution of herbal actives is critical for efficacy and safety, as variations in concentration can lead to under-dosing or overdosing.

Microbial Load

Microbial evaluation determines the total bacterial and fungal count in the syrup. This ensures the formulation is free from harmful microorganisms, which is essential for patient safety. Contamination can compromise both the safety and shelf life of the herbal syrup.

Stability Studies

Stability testing is performed by exposing the syrup to different temperature and environmental conditions over a specified period. This evaluation helps determine the shelf life, storage conditions, and long-term integrity of the herbal formulation. Stability studies also monitor any changes in color, taste, consistency, or active constituent levels, ensuring the product remains safe and effective throughout its intended shelf life.

Quality Control and Regulatory Aspects of Herbal Cough Syrups

Quality control and regulatory compliance are critical components in the development and commercialization of herbal cough syrups. Although herbal formulations are derived from

natural sources, their safety, efficacy, and quality must be ensured through scientifically validated procedures. Variability in raw materials, differences in cultivation conditions, and complex phytochemical composition pose significant challenges in maintaining consistent quality of herbal cough syrups. Therefore, robust quality control measures and regulatory oversight are essential to ensure the reliability and therapeutic effectiveness of these formulations [15].

Quality Control of Herbal Cough Syrups

Quality control of herbal cough syrups begins with the proper identification and authentication of medicinal plant materials. Botanical verification using macroscopic and microscopic evaluation helps prevent adulteration and substitution of raw materials. Literature emphasizes the importance of sourcing plant materials from reliable suppliers and ensuring proper documentation of their origin and quality [12].

Standardization of herbal extracts is another crucial aspect of quality control. This involves the quantification of marker compounds or characteristic phytoconstituents to ensure batch-to-batch consistency. Analytical techniques such as chromatographic and spectrophotometric methods are commonly reported for this purpose. Standardization helps in maintaining consistent therapeutic efficacy and minimizing variability among different batches of herbal cough syrups [15].

Physicochemical evaluation, including assessment of pH, viscosity, specific gravity, and refractive index, is routinely performed to ensure formulation stability and uniformity. Microbiological quality testing is equally important, as herbal syrups are susceptible to microbial contamination due to their aqueous nature. Compliance with acceptable microbial limits is essential to ensure patient safety [15].

Regulatory Aspects of Herbal Cough Syrups

Regulatory frameworks governing herbal medicinal products vary across countries but generally aim to ensure product safety, quality, and efficacy. International organizations such as the World Health Organization (WHO) have published guidelines emphasizing the importance of quality control, safety evaluation, and standardization of herbal medicines. These guidelines serve as a reference for national regulatory authorities in developing appropriate regulations for herbal products [3,15].

Recent Advances and Emerging Trends in herbal cough syrup

Recent literature indicates that the development of herbal cough syrups is gradually shifting from traditional empirical formulations toward more scientifically validated and standardized products. Advances reported in this field aim to improve formulation quality, therapeutic consistency, clinical relevance, and regulatory acceptance.

Standardization and Quality-Oriented Advances

Recent literature emphasizes the need for standardization of herbal cough syrups to improve consistency, safety, and therapeutic reliability. Marker-based standardization of key phytoconstituents such as vasicine, glycyrrhizin, and gingerols is increasingly reported as an important advancement. This approach aims to reduce batch-to-batch variability, which has traditionally limited the reproducibility of clinical outcomes associated with herbal formulations (18,24).

Evaluation Parameters of Herbal Cough Syrups

Parameter	Method/Description	Significance
Organoleptic Properties	Evaluation of color, odor, taste, and appearance	Ensures patient acceptability and uniformity
PH	Measured using digital pH meter	Maintains stability and minimizes throat irritation
Viscosity	Determined using viscometer	Influences pourability, mouthfeel, and demulcent action
Specific gravity	Ratio of weight of syrup to water	Indicates formulation consistency
Refractive index	Measured using refractometer	Indicates sugar concentration and purity
Content uniformity	Estimation of active constituents	Ensures uniform distribution of herbal actives
Microbial load	Total bacterial and fungal count	Ensures safety and absence of contamination
Stability studies	Evaluation under different temperature conditions	Determines shelf life of formulation

Table 3: Evaluation parameters of herbal cough syrup

Integration of Modern Drug Delivery Technologies

An emerging trend involves the application of modern drug delivery systems to herbal cough syrups. Technologies such as nanoformulations, phytosomes, and mucoadhesive systems have been explored to enhance bioavailability and prolong local action in the upper respiratory tract. However, most studies remain limited to preclinical investigations, and their clinical relevance has not yet been sufficiently established (21).

Shift Towards Clinical Validation

Recent reviews highlight a gradual shift from reliance on traditional use and preclinical evidence toward the need for well-designed human clinical trials. Although pharmacological mechanisms of herbal ingredients are increasingly understood, there remains a significant gap in robust clinical data supporting their efficacy in cough management. Addressing this gap is considered a key future direction for improving the acceptance of herbal cough syrups in evidence-based practice (15,21).

Pharmacovigilance and Safety Monitoring

Growing attention is being given to post-marketing surveillance and pharmacovigilance of herbal cough syrups. Recent literature acknowledges potential concerns related to herb–drug interactions, variability in phytochemical content, and quality of raw

materials. Strengthening safety monitoring systems is therefore recognized as an important emerging trend to ensure long-term patient safety (18,24).

Regulatory Harmonization and Global Acceptance

Another important trend is the effort toward regulatory harmonization and improved quality assurance. Adoption of Good Manufacturing Practices, clearer labeling requirements, and alignment with international regulatory frameworks are increasingly encouraged to enhance global acceptance of herbal cough syrups. Despite these advancements, inconsistent regulatory standards across regions continue to pose challenges (12,24).

Future Perspectives of Herbal Cough Syrups

Future development of herbal cough syrups is expected to focus on strengthening scientific validation, improving formulation quality, and enhancing clinical relevance. Addressing the existing gaps between traditional use, preclinical evidence, and clinical validation will be essential for broader acceptance of herbal cough syrups in evidence-based cough management (12,15).

Strengthening Clinical Evidence

Future research should prioritize well-designed human clinical trials to establish the efficacy and safety of herbal cough syrups. Comparative clinical studies evaluating herbal formulations alongside standard non-opioid synthetic antitussives may help clarify their therapeutic role and support informed clinical decision-making (15,21).

Improved Standardization and Quality Control

Advances in analytical techniques are expected to further improve marker-based standardization of herbal cough syrups. Consistent control of phytochemical composition, along with strict adherence to Good Manufacturing Practices, will be critical to minimize batch-to-batch variability and enhance reproducibility of therapeutic outcomes (18,24).

Integration of Pharmacovigilance Systems

Future perspectives also emphasize the need for robust pharmacovigilance systems for herbal cough syrups. Systematic monitoring of adverse reactions, herb–drug interactions, and long-term safety profiles is necessary to address concerns related to the generalized perception of safety associated with herbal medicines (18,21).

Rational Formulation and Pediatric Safety

Further research is required to optimize formulation strategies that ensure safety and efficacy across different patient populations, particularly in pediatric use. Rational selection of excipients, alcohol-free formulations, and age-appropriate dosing strategies are expected to gain increased attention in future developments (12,24).

Regulatory Harmonization and Global Acceptance

Harmonization of regulatory frameworks and alignment with international quality guidelines may facilitate the global acceptance of herbal cough syrups. Clear regulatory pathways, standardized quality benchmarks, and transparent labeling practices are expected to support their responsible integration into healthcare systems (12,18,24).

Conclusion

Herbal cough syrups remain an important component of cough management owing to their long history of traditional use and the presence of diverse bioactive phytoconstituents exhibiting antitussive, expectorant, anti-inflammatory, bronchodilatory, and demulcent properties. Unlike conventional antitussives that primarily suppress the cough reflex, herbal formulations act through multiple mechanisms, targeting various aspects of cough pathophysiology, including airway irritation, inflammation, and mucus accumulation.

This review highlights that although substantial preclinical evidence supports the pharmacological potential of commonly used medicinal plants, the clinical efficacy of herbal cough syrups remains inconsistent. Variability in phytochemical composition, lack of uniform standardization, and differences in formulation strategies significantly influence therapeutic outcomes. Moreover, the widespread perception of herbal cough syrups as inherently safer requires careful reconsideration in light of potential herb–drug interactions, quality-related issues, and limited pharmacovigilance data.

Recent advances in marker-based standardization, formulation technology, and regulatory awareness reflect a positive shift toward improving the quality and scientific credibility of herbal cough syrups. However, the gap between traditional claims and robust clinical validation persists. Addressing this gap through well-designed clinical trials, improved quality control measures, and rational regulatory frameworks will be essential for integrating herbal cough syrups into evidence-based cough management. Overall, herbal cough syrups hold promise as complementary therapeutic options, provided their development and use are guided by scientific validation and standardized manufacturing practices.

Acknowledgement

I would like to express my heartfelt gratitude to Mr. Dnyaneshwar S. Vyavhare, my guide, for his continuous support, valuable guidance, and constructive suggestions throughout the preparation of this review article. His encouragement and insights were instrumental in completing this work successfully.I am also sincerely thankful to Ms. Gita Mohire, Principal, B. R. Harne College of Pharmacy, for providing the essential academic environment, resources, and support needed for this study.

I extend my appreciation to the faculty members and library staff for assisting me with relevant scientific literature and reference materials. Lastly, I am grateful to my family for their constant motivation, cooperation, and support during the completion of this article.

Author Contributions

1)Sonia Rambachan Kanojia (Main Author): Carried out the conceptualization, literature review, data interpretation, preparation of figures and graphical abstract, manuscript writing, and final editing.

2)Dnyaneshwar S. Vyavhare (Corresponding-author 2): Provided minor assistance in literature search and reviewed the draft manuscript.

3)Gita Mohire (Co-author 3): Offers supportive inputs and general feedback during manuscript preparation.

All authors have read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this review article.

References

1.Morice AH, et al. The diagnosis and management of chronic cough. European Respiratory Journal. 2020.
2. Song WJ, et al. Impact of cough on quality of life. Allergy, Asthma & Immunology Research. 2021.
3.Dicpinigaitis PV. Cough epidemiology and mechanisms. Chest. 2020.
4.Smith SM, et al. Over-the-counter cough medicines: Efficacy and safety. BMJ. 2020.
5.Paul IM. Therapeutic approaches to pediatric cough. Pediatrics. 2021.
6.Sharma PV. Classical uses of medicinal plants in respiratory disorders. Journal of Ayurveda and Integrative Medicine. 2020.

7.Barnes PJ. Mechanisms of cough and therapeutic targets. Pharmacology & Therapeutics. 2021.
8.Dhankhar S, et al. Pharmacological evaluation of medicinal plants used in cough. Journal of Ethnopharmacology. 2021.
9.WHO. Quality control methods for herbal materials. World Health Organization; 2020.
10. Izzo AA, et al. Herb–drug interactions: An overview. Drugs. 2020.
11.Dhankhar S, et al. A review on Adhatoda vasica: Pharmacological and therapeutic aspects. Journal of Ethnopharmacology. 2021.
12 Pastorino G, et al. Liquorice (Glycyrrhiza glabra): A phytochemical and pharmacological review. Phytotherapy Research. 2020.
13 Cohen MM. Tulsi (Ocimum sanctum) in traditional and modern medicine. Journal of Ayurveda and Integrative Medicine. 2021.
14 Mao QQ, et al. Bioactive compounds and health effects of ginger. Food Science & Nutrition. 2020.
15 Holzinger F, Chenot JF. Ivy leaf extract for respiratory diseases: A systematic review. European Journal of Pediatrics. 2021.
16.Morice AH, et al. Cough hypersensitivity syndrome: A distinct clinical entity. European Respiratory Journal. 2020.
17.Chung KF, Pavord ID. Prevalence, pathogenesis, and causes of chronic cough. Lancet. 2021.
18.Dicpinigaitis PV. Cough reflex physiology and pathophysiology. Chest. 2020.
19.Song WJ, et al. Mechanisms of chronic cough and cough hypersensitivity. Allergy. 2021.
20.Barnes PJ. Inflammatory mechanisms in cough and airway disease. Pharmacology & Therapeutics. 2020.
21.Dicpinigaitis PV. Neural control of cough reflex. Chest. 2020.
22.Mazzone SB, Undem BJ. Cough sensors and sensory pathways. Pharmacology & Therapeutics. 2021.
23.Morice AH, et al. Central mechanisms of cough regulation. European Respiratory Journal. 2020.
24.Chung KF. Physiology and pathophysiology of cough. Pulmonary Pharmacology & Therapeutics. 2021.
25.Song WJ, et al. Chronic cough and cough reflex hypersensitivity. Allergy. 2021.
26.Smith SM, et al. Over-the-counter medicines for acute cough: Systematic review. Cochrane Database of Systematic Reviews. 2020.
27.Barnes J, Anderson LA, Phillipson JD. Herbal medicines for respiratory diseases: A review. Phytotherapy Research. 2021.
28.Heinrich M, et al. Medicinal plants in respiratory disorders: Evidence-based evaluation. Frontiers in Pharmacology. 2020.
29.Posadzki P, et al. Herb–drug interactions: An overview. British Journal of Clinical Pharmacology. 2021.
30.World Health Organization. WHO guidelines on the safety and quality of herbal medicines. 2020.
31.Dhankhar S, et al. Pharmacological profile of Adhatoda vasica. Journal of Ethnopharmacology. 2021.
32.Pastorino G, et al. Liquorice (Glycyrrhiza glabra): Pharmacology and safety concerns. Phytotherapy Research. 2020.
33.Cohen MM. Ocimum sanctum (Tulsi): Evidence-based review. Journal of Ayurveda and Integrative Medicine. 2021.
34.Mao QQ, et al. Health effects of ginger and its bioactive compounds. Food Science & Nutrition. 2020.
35.Srinivasan K. Piperine and its biological effects. Critical Reviews in Food Science and Nutrition. 2021.
36.Eccles R. Menthol and respiratory sensations. Respiratory Physiology & Neurobiology. 2020.
37.Oduwole O, et al. Honey for acute cough in children. Cochrane Database of Systematic Reviews. 2021.
38.Dicpinigaitis PV. Cough reflex sensitivity and regulation. Chest. 2020.
39.Barnes PJ. Neural mechanisms of cough. Pulmonary Pharmacology & Therapeutics. 2021.

40.Heinrich M, et al. Herbal expectorants and mucolytics in respiratory care. Frontiers in Pharmacology. 2020.
41.Srinivasan K. Ginger and respiratory health. Critical Reviews in Food Science and Nutrition. 2021.
42.Gupta P, et al. Anti-inflammatory phytochemicals in respiratory diseases. Journal of Ethnopharmacology. 2021.
43.World Health Organization. WHO monographs on selected medicinal plants. 2020.
44.Oduwole O, et al. Honey for acute cough in children. Cochrane Database of Systematic Reviews. 2021.
45.Eccles R. Mechanisms of demulcent cough remedies. Respiratory Physiology & Neurobiology. 2020.
46.Burt S. Essential oils and antimicrobial activity. International Journal of Food Microbiology. 2020.
47.Rahman I, et al. Oxidative stress and airway inflammation. Antioxidants. 2021.
48.EMA. Quality and standardization of herbal medicinal products. European Medicines Agency. 2022.
49.Dicpinigaitis PV. Cough management and antitussive therapy. Chest. 2020.
50.Chang AB, et al. Pediatric cough guidelines. CHEST Guideline and Expert Panel Report. 2021.
51.World Health Organization. Traditional medicine and respiratory disorders. 2020.
52.Smith SM, et al. Over-the-counter cough medicines. BMJ. 2021.
53.NICE. Cough management guidelines. 2022.

Vita Scientia

Research Article

Read Online

The Role of Vericiguat in Heart Failure with Reduced Ejection Fraction: From Clinical Trials to Clinical Practice

Rudra Adhik Khanolkar [1], Dnyaneshwar Vyavhare[1*], Gita Mohire [1]

[1]Department Department of Pharmacy, Br. Harne College of Pharmacy, Thane 401107, Maharashtra, India

*Correspondence: dvyavhare108@gmail.com

https://doi.org/10.65116/vitascientia.V2I2.rev.02

Summary

Heart failure continues to exact a substantial global burden despite modern drug regimens. A persistent problem lies in the disruption of nitric oxide–soluble guanylate cyclase–cyclic GMP signalling, which promotes endothelial dysfunction, fibrosis and diastolic impairment. Vericiguat, an oral stimulator of soluble guanylate cyclase, restores cyclic GMP production independently of nitric oxide and thereby improves myocardial and vascular performance. This review integrates mechanistic, pharmacological and clinical evidence — including the SOCRATES and VICTORIA programmes — to evaluate the drug's place in contemporary therapy. Vericiguat provides a modest but meaningful reduction in the composite outcome of cardiovascular death or first hospitalisation for heart failure, with an excellent safety profile and compatibility with established guideline-directed therapy. Its once-daily dosing and minimal interaction potential facilitate adherence. Although the absolute survival gain remains limited, vericiguat represents a mechanistically novel and clinically practical option for high-risk HFrEF patients with recent decompensation.

Keywords

vericiguat; heart failure; soluble guanylate cyclase; cyclic GMP signalling; VICTORIA trial; pharmacotherapy

Introduction

Heart failure (HF) is a complex clinical syndrome rather than a single pathological entity and arises from diverse structural and functional abnormalities of the myocardium. Contemporary management of heart failure with reduced ejection fraction (HFrEF) relies on a combination of guideline-directed medical therapies, including β-blockers, renin–angiotensin system inhibitors, angiotensin receptor–neprilysin inhibitors, mineralocorticoid receptor antagonists, and sodium–glucose cotransporter-2 inhibitors. These therapies have substantially improved survival and reduced hospitalisation rates. Nevertheless, a considerable proportion of patients continue to experience persistent symptoms, recurrent decompensation, and frequent rehospitalisations despite optimal treatment [1–3].

Accumulating evidence suggests that this residual risk cannot be fully explained by neurohormonal activation alone. Instead, chronic endothelial dysfunction, oxidative stress, and impaired myocardial relaxation play central roles in disease progression. Oxidative stress reduces nitric oxide (NO) bioavailability and oxidises the haem moiety of soluble guanylate cyclase (sGC), leading to diminished conversion of guanosine triphosphate to cyclic guanosine monophosphate (cGMP). Deficiency

of cGMP signalling contributes to impaired myocardial

relaxation, increased ventricular stiffness, progressive fibrosis, and adverse vascular remodelling [4,5].

Targeting the NO–sGC–cGMP pathway therefore represents a mechanistically distinct therapeutic strategy in heart failure. Vericiguat is an oral stimulator of soluble guanylate cyclase that directly enhances cGMP production independently of endogenous nitric oxide availability. By restoring cGMP signalling even under conditions of oxidative stress, vericiguat offers a novel approach to addressing pathophysiological processes that are inadequately corrected by conventional neurohormonal therapies [6].

This narrative review summarises the pharmacological properties of vericiguat, evaluates evidence from key preclinical and clinical studies—including the SOCRATES and VICTORIA programmes—and discusses its role in contemporary management of patients with HFrEF.

Results and discussion

Mechanistic overview

Heart failure (HF) remains a major global health concern, characterized by high rates of morbidity, mortality, and recurrent hospitalizations, despite significant advancements in pharmacological therapies. A primary pathophysiological defect in HF is the dysfunction of the nitric oxide–soluble guanylate cyclase–cyclic guanosine monophosphate (NO–sGC–cGMP) signaling pathway, which results in endothelial dysfunction, myocardial fibrosis, impaired relaxation, and persistent ventricular remodeling.

Vericiguat, a novel oral stimulator of soluble guanylate cyclase, directly enhances cGMP production, independent of nitric oxide availability, thus targeting a disease mechanism that is inadequately addressed by conventional neurohormonal therapies.

This review synthesizes the current evidence regarding vericiguat, including its molecular mechanisms, pharmacokinetics, preclinical investigations, and clinical outcomes from pivotal trials, such as the SOCRATES and VICTORIA programs. Clinical results suggest that vericiguat offers a modest yet clinically meaningful reduction in the composite endpoint of cardiovascular death or the first hospitalization for heart failure in patients with worsening heart failure and reduced ejection fraction (HFrEF), along with a favorable safety and tolerability profile. Its once-daily oral dosing, minimal drug–drug interactions, and compatibility with guideline-directed medical therapy support its use in routine clinical practice. Although the absolute mortality benefits remain limited, vericiguat represents a vital mechanistically novel adjunctive option for stabilizing high-risk patients who have recently undergone decompensation.

Pharmacokinetics and pharmacodynamics

Vericiguat demonstrates advantageous pharmacokinetic characteristics that facilitate once-daily oral dosing. When ingested with food, its oral bioavailability reaches nearly 93%, and systemic exposure shows a slight increase compared to fasting conditions. Peak plasma levels are generally attained about one hour post-administration. The terminal elimination half-life is approximately 20 hours, allowing for stable plasma levels with daily intake. Metabolism primarily occurs through glucuronidation, facilitated by UGT1A9 and UGT1A1, with minimal participation from cytochrome P450 enzymes. As a result, the likelihood of clinically significant drug–drug interactions remains low. Roughly equal amounts of the administered dose are eliminated through urine and feces. Pharmacodynamic investigations reveal a dose-dependent rise in plasma cGMP levels, indicating effective target engagement. Notably,

vericiguat induces minimal clinically significant alterations

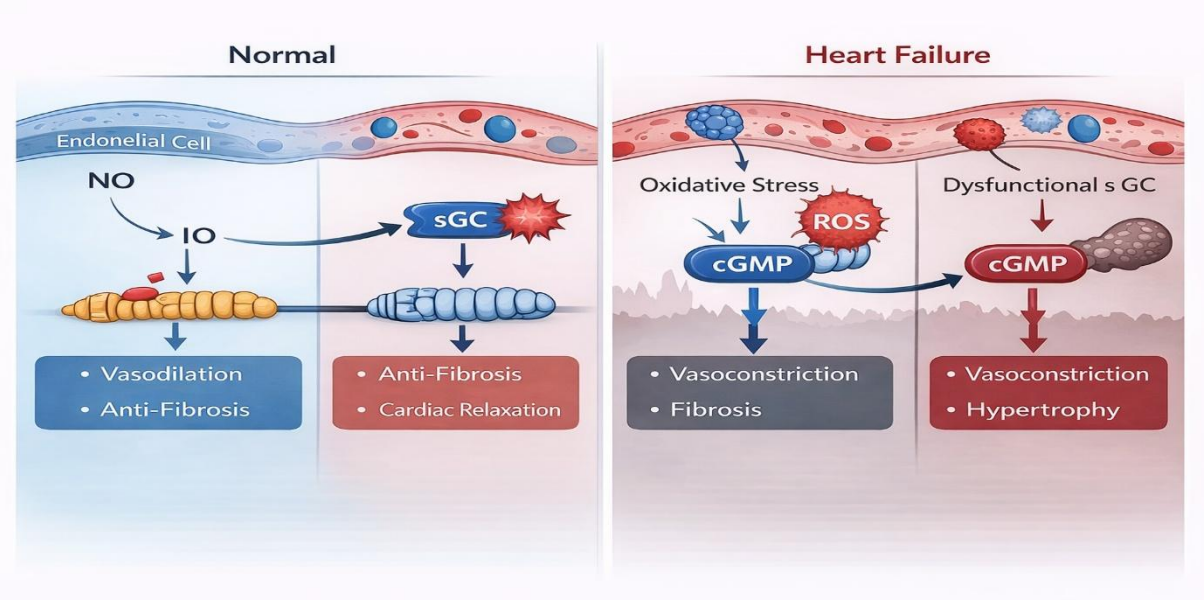


Figure 1. Schematic representation of the nitric oxide–soluble guanylate cyclase–cyclic guanosine monophosphate (NO–sGC–cGMP) signalling pathway under normal physiological conditions and in heart failure. Oxidative stress reduces nitric oxide availability and impairs sGC activity, resulting in diminished cGMP production and downstream myocardial and vascular dysfunction. *Figure created by the authors.*

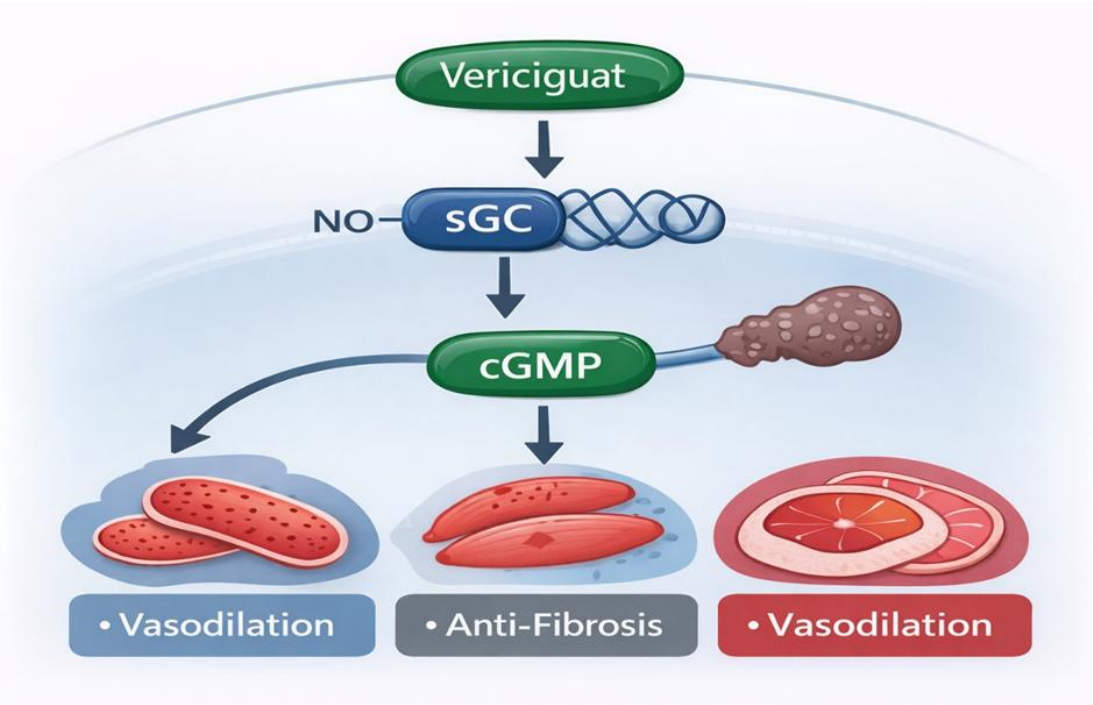


Figure 2. Mechanism of action of vericiguat. Vericiguat directly stimulates soluble guanylate cyclase independently of nitric oxide, restoring intracellular cGMP levels and improving myocardial relaxation and vascular function. *Figure created by the authors.*

in heart rate or systemic blood pressure, setting it apart from conventional vasodilators.

Pre-clinical and early clinical data

Preclinical studies have demonstrated that stimulation of the soluble guanylate cyclase–cGMP pathway by vericiguat leads to favourable structural and functional cardiac effects. In experimental models of heart failure, treatment with vericiguat reduced left ventricular hypertrophy and myocardial fibrosis while improving diastolic compliance and ventricular relaxation [18,19]. These findings provided early evidence that restoring cGMP signalling may attenuate maladaptive cardiac remodelling.

Phase I clinical studies conducted in healthy volunteers confirmed that vericiguat is well tolerated and exhibits predictable, dose-dependent pharmacokinetics, thereby supporting further clinical development [21]. Subsequent phase II evaluation in the SOCRATES-REDUCED trial enrolled 456 patients with HFrEF and demonstrated significant dose-related reductions in NT-proBNP concentrations, accompanied by modest improvements in left ventricular ejection fraction [22]. In contrast, the SOCRATES-PRESERVED trial, which included 477 patients with HFpEF, did not demonstrate significant changes in NT-proBNP levels, although some improvements in functional measures and patient-reported outcomes were observed [23].

Collectively, these early clinical findings highlighted the potential benefit of vericiguat in patients with reduced ejection fraction and informed the design of the pivotal VICTORIA outcomes trial.

Trial	Population	Design	Primary endpoint	Key result	Reference
SOCRATES-REDUCED	HFrEF (n = 456)	Randomised phase II dose-finding	Change in NT-proBNP	↓ NT-proBNP, ↑ LVEF	[22]
SOCRATES-PRESERVED	HFpEF (n = 477)	Randomised phase II	Change in NT-proBNP	Neutral biomarker trend	[23]
VICTORIA	HFrEF (n = 5050)	Randomised phase III outcomes trial	CV death or first HF hospitalisation	HR 0.90 (95 % CI 0.82–0.98)	[8]

Table 1. Major clinical trials of vericiguat in heart failure

Figure 3. Clinical development timeline of vericiguat, illustrating major preclinical studies and pivotal clinical trials leading to regulatory approval. Figure created by the authors.

The VICTORIA trial and clinical interpretation

The VICTORIA trial was a large, randomised, double-blind, placebo-controlled phase III study that enrolled 5,050 patients with symptomatic HFrEF who had experienced recent clinical worsening, defined by hospitalisation or the need for intravenous diuretics [8]. All participants received guideline-directed medical therapy, and vericiguat was initiated at a dose of 2.5 mg once daily, with gradual titration to a target dose of 10 mg based on tolerability.

Over a median follow-up period of 10.8 months, vericiguat significantly reduced the primary composite endpoint of cardiovascular death or first heart-failure hospitalisation compared with placebo (hazard ratio 0.90; 95% confidence interval 0.82–0.98; p = 0.02). The observed benefit was predominantly driven by a reduction in heart-failure hospitalisations, whereas cardiovascular mortality alone was not significantly different between groups.

Vericiguat demonstrated a favourable safety profile, with low rates of treatment discontinuation. Mild hypotension and anaemia were the most frequently reported adverse events, but these rarely necessitated cessation of therapy. Subgroup analyses indicated consistent efficacy across age groups, sex, renal function categories, and background use of angiotensin receptor–neprilysin inhibitors or SGLT2 inhibitors [24]. These findings support the role of vericiguat as a stabilising therapy in patients with high-risk HFrEF following recent decompensation.

Comparison with established heart-failure therapies

The contemporary management of HFrEF relies on a multidrug strategy that targets complementary pathophysiological pathways. Foundational therapies—including β-blockers, RAAS inhibitors, mineralocorticoid receptor antagonists, and SGLT2 inhibitors—have demonstrated robust mortality and morbidity benefits across a broad range of patient populations. In contrast, vericiguat primarily addresses impaired cGMP signalling and offers incremental benefit by reducing recurrent hospitalisations in selected high-risk patients. Its role is therefore best understood as additive rather than foundational, complementing existing therapies rather than replacing them.

Safety profile

The comprehensive safety profile of vericiguat has been thoroughly assessed throughout phase I to phase III clinical development programs, as well as through post-hoc analyses of significant trials. Across various studies, vericiguat has shown good tolerability, with adverse events typically being mild to moderate in severity and occurring at frequencies comparable to placebo [17]. Notably, the rates of discontinuation due to adverse events were low, indicating its appropriateness for prolonged outpatient use. The most frequently reported treatment-related adverse events consist of mild hypotension and anaemia. Hypotension seems to be dose-dependent but is generally asymptomatic and can be managed through careful titration and adjustment of accompanying diuretic therapy. The anaemia associated with vericiguat is believed to be a result of haemodilution rather than bone marrow suppression or haemolysis, and significant reductions in haemoglobin levels are rare [8,17]. Serious bleeding incidents have not been observed at rates exceeding those of placebo. Renal safety remains a critical issue for patients with advanced heart failure, many of whom also suffer from chronic kidney disease. In the VICTORIA trial, vericiguat did not elevate the incidence of acute kidney injury or lead to clinically significant declines in estimated

Drug class	Mechanism of action	Mortality benefit	Primary clinical advantage
β-blockers	Inhibit sympathetic drive	High	Reverse remodelling and improved survival
ACE inhibitors/ARNI	Block RAAS and enhance natriuretic peptides	High	Improved symptoms and outcomes
Mineralocorticoid antagonists	Counter aldosterone-mediated fibrosis	Moderate	Anti-fibrotic effect
SGLT2 inhibitors	Modify metabolism and osmotic diuresis	High	Reduced admissions and mortality
Vericiguat	Stimulates sGC → ↑ cGMP	Modest	Fewer rehospitalisation and improved stability

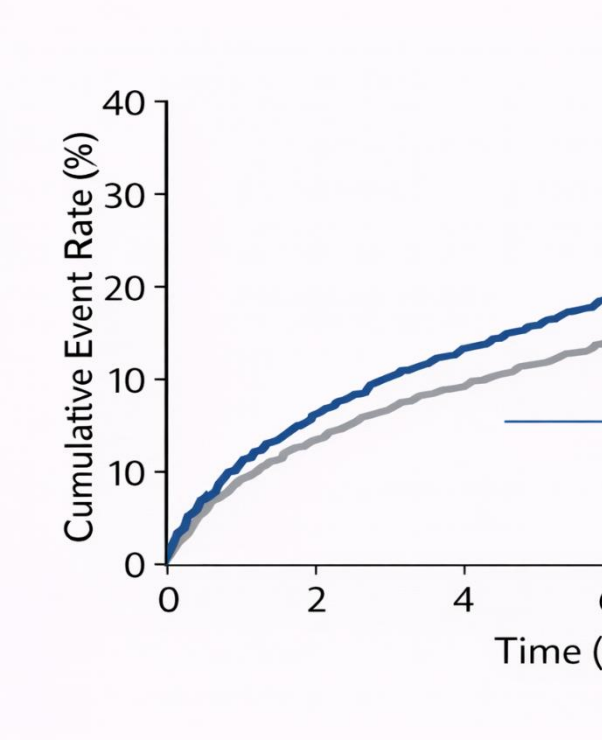


Figure 4. Kaplan–Meier curves from the VICTORIA trial showing the cumulative incidence of the composite endpoint of cardiovascular death or first heart-failure hospitalisation in patients receiving vericiguat versus placebo. Adapted from Armstrong et al. [8].

renal impairment at baseline [24]. Additionally, electrolyte imbalances, such as hyperkalaemia and hyponatraemia, were not significantly different between the treatment groups.

Economic and practical considerations

In addition to clinical effectiveness, the integration of any new heart failure treatment is significantly shaped by its economic implications and practical applicability within healthcare frameworks. Heart failure ranks among the top causes of hospital admissions globally, with repeated hospital stays constituting a considerable share of the total healthcare costs associated with this condition. Therefore, interventions aimed at decreasing rehospitalisation rates, even in the absence of substantial mortality improvements, may still present advantageous cost-effectiveness profiles [26]. Pharmacoeconomic evaluations

performed in Europe and North America have assessed the additional cost-effectiveness of vericiguat when used alongside guideline-directed medical therapy. These analyses suggest an incremental cost per quality-adjusted life-year (QALY) gained ranging from £17,000 to £20,000, which aligns with the accepted willingness-to-pay thresholds in numerous high-income healthcare systems [16,26]. Notably, the economic value of vericiguat is primarily influenced by its ability to reduce heart failure hospitalisations rather than by improvements in survival rates.

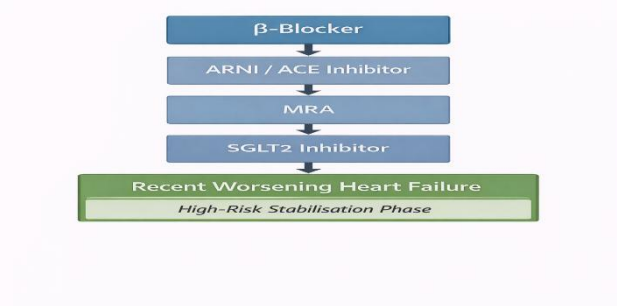


Figure 5. Proposed positioning of vericiguat within the contemporary treatment algorithm for heart failure with reduced ejection fraction as an adjunctive therapy in recently decompensated, haemodynamically stable patients. *Figure created by the authors.*

Practical selection criteria

Criterion	Rationale	Suggested action
Recent decompensation within three months	Benefit concentrated in recently decompensated cohorts	Start once haemodynamically stable
Optimised guideline therapy	Vericiguat is adjunctive	Maximise tolerated GDMT before initiation
Systolic BP ≥ 100 mmHg	Avoid symptomatic hypotension	Delay initiation or adjust diuretics if lower
Baseline NT-proBNP < 8000 pg/mL	Attenuated effect at very high levels	Prioritise patients below this threshold
eGFR ≥ 30 mL min^{-1} 1.73 m^{-2}	Limited data in severe renal impairment	Use cautiously and monitor renal function
No PDE-5 inhibitor use	Risk of excessive vasodilation	Contraindicated or advise against concurrent use

Appropriate selection of patients is essential for maximizing the therapeutic advantages of vericiguat while minimizing potential adverse effects. Findings from the VICTORIA trial and subsequent evaluations suggest that the drug's efficacy is most significant in patients experiencing recently exacerbated heart failure, especially those who have necessitated hospitalization or intravenous diuretic treatment within the last three months [8,24]. Haemodynamic stability is a crucial requirement for the commencement of treatment. Patients must exhibit a systolic blood pressure of no less than 100 mmHg to mitigate the risk of symptomatic hypotension. Vericiguat should be initiated only after the optimization of foundational guideline-directed medical therapy, as it is designed to serve as an adjunct rather than a replacement for established medications. Elevated levels of natriuretic peptides assist in identifying high-risk patients; however, extremely elevated NT-proBNP levels may indicate advanced disease, where the relative benefit of vericiguat may be diminished.

Clinical vignette

The subsequent clinical case exemplifies the practical use of vericiguat in standard care. A 68-year-old male with ischaemic cardiomyopathy and a left ventricular ejection fraction of 30% presented with exacerbating dyspnoea and peripheral oedema, which required hospitalisation and intravenous diuretic treatment. His medical background included chronic kidney disease (estimated glomerular filtration rate 42 mL min^{-1} 1.73 m^{-2}) and hypertension. In spite of the optimisation of guideline-directed therapy—including an ACE inhibitor, β-blocker, mineralocorticoid receptor antagonist, and SGLT2 inhibitor—he continued to experience symptoms at New York Heart Association (NYHA) class III. His NT-proBNP level was 4,200 pg/mL, and his blood pressure remained stable at 110/68 mmHg. In light of his recent decompensation and ongoing risk, vericiguat was commenced at a dose of 2.5 mg once daily and was gradually increased to 10 mg over several weeks.

Author's perspective and practical recommendations

From a clinical perspective, vericiguat should be considered a targeted therapy for patients with HFrEF who remain vulnerable to recurrent decompensation despite optimisation of guideline-directed medical therapy. Its greatest value lies in stabilising patients during the high-risk period following recent hospitalisation or intravenous diuretic therapy. Initiation should occur only after haemodynamic stability is achieved, with gradual dose titration and close monitoring during early treatment.

Clinicians should set realistic expectations, emphasising that the principal benefit of vericiguat is a reduction in heart-failure hospitalisations rather than dramatic symptom improvement or survival extension. Careful patient selection, combined with structured follow-up, is essential to maximise benefit while maintaining safety. As real-world experience grows, institutional protocols and registries may further refine its optimal use.

Future directions

Current studies look to figure out vericiguat's place in heart failure where ejection fraction stays preserved, along with how it pairs with other drugs that tweak cGMP pathways. References[27 - 30]cover those efforts. Down the line, one approach might combine stimulation of two pathways at once through both the soluble and particulate forms of guanylate cyclase. That could add up to better relaxation in heart muscle and more flexible blood vessels.

Limitations and Unmet Needs

Despite its mechanistically novel approach and favourable safety profile, vericiguat has several important limitations that warrant consideration. The magnitude of benefit observed in clinical trials has been modest compared with foundational therapies such as β-blockers, angiotensin receptor–neprilysin inhibitors, and SGLT2 inhibitors. In the VICTORIA trial, the reduction in the composite endpoint was primarily driven by fewer heart-failure hospitalisations, while a clear mortality benefit was not demonstrated. These findings indicate that vericiguat should be regarded as an adjunctive rather than disease-defining therapy.

Several unresolved questions remain. The optimal timing of initiation—whether during hospitalisation, immediately after discharge, or later during stable outpatient management—has not been conclusively established. Evidence supporting its use in patients with severe renal impairment, profound hypotension, or extremely elevated natriuretic peptide levels remains limited. Furthermore, access and affordability may restrict widespread use in resource-limited healthcare settings.

Future research should focus on pragmatic real-world studies, longer-term outcome

analyses, and improved phenotypic patient selection to clarify the populations most likely to benefit from vericiguat. Addressing these gaps will be essential to defining its long-term role in comprehensive heart-failure management.

Mechanistic and Translational Insights

Preclinical studies suggest that stimulation of the sGC–cGMP pathway by vericiguat may exert several potentially beneficial biological effects, including attenuation of inflammatory signalling, improvement of mitochondrial efficiency, reduction of myocardial fibrosis, and enhancement of endothelial function. Experimental models have demonstrated downregulation of pro-inflammatory cytokines, improved cellular energetics, and reduced fibroblast proliferation following restoration of cGMP signalling.

However, it is important to note that most of these observations are derived from preclinical or early translational studies. Direct evidence confirming long-term disease-modifying effects in humans remains limited. Consequently, these mechanistic findings should be interpreted as hypothesis-generating rather than definitive proof of structural myocardial reversal. Further clinical studies are required to determine whether these biological effects translate into sustained improvements in cardiac structure and long-term outcomes.

Materials and methods

Search strategy

We did a planned search through PubMed, ScienceDirect, and ClinicalTrials.gov. The time frame covered 2015 to 2024. Search terms included vericiguat. We also used sGC stimulator. Heart failure came up. SOCRATES and VICTORIA got included. Extra details came from European Medicines Agency or EMA documents. US Food and Drug Administration or FDA files added more. References [9,10] cover those.

Eligibility criteria

We picked studies on preclinical work with vericiguat. Phase one through three trials made the cut. Major reviews on its mechanism, pharmacology, or outcomes worked too. We left out reports not in English. Non-peer-reviewed stuff got skipped. Studies on unrelated drugs did not qualify.

Data synthesis

We pulled out info on study designs. Participant details came next. Interventions and endpoints followed. Safety results rounded it out. The evidence got put together narratively. We followed the IMRaD structure from Vita Scientia publications.

Conclusion

Heart failure with reduced ejection fraction remains a progressive and burdensome clinical condition despite advances in guideline-directed medical therapy. Although neurohormonal blockade has significantly improved outcomes, many patients continue to experience recurrent decompensation and hospitalisation. Vericiguat represents a mechanistically distinct therapeutic option by restoring impaired soluble guanylate cyclase–cGMP signalling, a pathway inadequately addressed by conventional therapies.

Evidence from the SOCRATES and VICTORIA programmes indicates that vericiguat provides a modest but clinically meaningful reduction in heart-failure hospitalisations, particularly among patients with recent decompensation who remain at high risk despite optimized background therapy. Its favourable safety profile, minimal drug–drug interactions, and once-daily dosing support its practical integration into clinical care.

Vericiguat should be viewed as an adjunctive therapy rather than a replacement for established treatments, with careful patient selection and realistic expectations regarding benefit. Ongoing and future

studies will be essential to clarify its optimal timing, broader applicability, and potential role in other heart-failure phenotypes. When used judiciously, vericiguat adds a valuable mechanism-based option to the modern heart-failure treatment landscape.

Acknowledgement

The author expresses deep gratitude for the academic guidance, institutional support, and scholarly resources offered by Br. Harne College of Pharmacy, Thane, Maharashtra, India, which played a crucial role in the successful completion of this review. The encouragement and assistance from the faculty members of the Department of Pharmacy are genuinely appreciated, especially for promoting a research-focused academic atmosphere and providing access to pertinent scientific literature and databases. Additionally, the author acknowledges the valuable contributions of researchers and clinicians whose published works laid the groundwork for this review. Their thorough investigations and clinical insights into the pathophysiology of heart failure and the modulation of soluble guanylate cyclase have greatly enhanced understanding in this area and facilitated the synthesis presented in this document.

Author contributions

Rudra Adhik Khanolkar conceptualised the review, designed the study framework, and drafted the manuscript. Dnyaneshwar S. Vyavhare contributed to literature screening, and critical revision of the manuscript. Gita Mohire assisted in refining the clinical datasets were created or analyzed specifically for this review. Therefore, there are no further data available for dissemination beyond the references mentioned in the manuscript.

Ethics statement

This is a narrative synthesis of previously published data and therefore, did not require ethical approval or participant consent.

interpretation, reviewing pharmacological content. All authors reviewed and approved the final manuscript and accept responsibility for the integrity and accuracy of the work.

Conflict-of-interest statement

The author asserts that there are no conflicts of interest, whether financial or non-financial, associated with the research, authorship, or publication of this manuscript. The author has not obtained any honoraria, consultancy fees, speaker payments, or research funding from pharmaceutical companies engaged in the development or marketing of vericiguat or related cardiovascular therapies. All interpretations and conclusions articulated in this review are derived exclusively from an independent and critical assessment of the published scientific literature and are devoid of any commercial or institutional influence

Funding statement

This review article did **not receive any specific grant** from funding agencies in the public, commercial, or not-for-profit sectors. The work was conducted as part of the author's academic activities at Br. Harne College of Pharmacy and was supported solely through institutional resources and personal scholarly effort.

Data-access statement

All data and information presented in this review were sourced from publicly available materials, such as peer-reviewed journal articles, clinical trial registries, and reports from regulatory authorities. No new

References

1. Paulus WJ, Tschöpe C. A novel paradigm for HFpEF. *J Am Coll Cardiol.* 2013;62:263–271. https://doi.org/10.1016/j.jacc.2013.02.092
2. Tsutsui H et al. Oxidative stress in cardiac failure. *Circ J.* 2020;84:1030–

1038. https://doi.org/10.1253/circj.CJ-19-0951
3. Sandner P et al. Soluble guanylate cyclase stimulators and activators. *Handb Exp Pharmacol.* 2021;264:355–394. https://doi.org/10.1007/164_2021_389
4. Gheorghiade M et al. Pharmacological modulation of cyclic GMP signalling. *Eur Heart J.* 2019;40:2900–2910. https://doi.org/10.1093/eurheartj/ehz273
5. Armstrong PW et al. Vericiguat in patients with heart failure and reduced ejection fraction. *N Engl J Med.* 2020;382:1883–1893. https://doi.org/10.1056/NEJMoa1915928
6. European Medicines Agency. *Verquvo (vericiguat) Assessment Report.* EMA; 2021.
7. US Food and Drug Administration. *Summary Review: Vericiguat Tablets.* FDA; 2021.
8. Stasch JP et al. Pharmacology of sGC modulators. *Circulation.* 2015;131:1074–1085. https://doi.org/10.1161/CIRCULATIONAHA.114.013540
9. Münzel T et al. Pathophysiology of oxidative stress and cGMP deficiency in HF. *Br J Pharmacol.* 2018;175:2310–2324. https://doi.org/10.1111/bph.14200
10. Evgenov OV et al. NO-independent activation of soluble guanylate cyclase. *Nat Rev Drug Discov.* 2011;10:755–769. https://doi.org/10.1038/nrd3562
11. Bender AT, Beavo JA. cGMP signalling: physiology and pharmacology. *Pharmacol Rev.* 2006;58:220–264. https://doi.org/10.1124/pr.58.2.3
12. Filippatos G et al. Vericiguat: a novel therapeutic option. *Eur Heart J.* 2022;43:451–458. https://doi.org/10.1093/eurheartj/ehab912
13. Wirtz J et al. Pharmacoeconomic evaluation of vericiguat. *Health Econ Rev.* 2022;12:37–48. https://doi.org/10.1186/s13561-022-00370-8
14. Butler J et al. Safety and tolerability of vericiguat. *Clin Cardiol.* 2019;42:636–643. https://doi.org/10.1002/clc.23178
15. Ghofrani HA et al. Role of cGMP signalling. *Br J Pharmacol.* 2017;174:1771–1784. https://doi.org/10.1111/bph.13654
16. Liu S et al. Vericiguat ameliorates cardiac fibrosis. *Cardiovasc Ther.* 2020;38:e12491. https://doi.org/10.1111/1755-5922.12491
17. McMurray JJV et al. Pathophysiological targets beyond neurohormonal blockade. *Nat Rev Cardiol.* 2022;19:706–722. https://doi.org/10.1038/s41569-022-00691-z
18. Stasch JP et al. NO-independent activation of sGC. *Trends Pharmacol Sci.* 2015;36:163–172. https://doi.org/10.1016/j.tips.2015.01.002
19. Armstrong PW et al. Predictors of outcome in the VICTORIA trial. *Eur J Heart Fail.* 2022;24:186–198. https://doi.org/10.1002/ejhf.2394
20. Solomon SD et al. VITALITY-HFpEF trial. *Eur J Heart Fail.* 2021;23:1264–1273. https://doi.org/10.1002/ejhf.2249
21. Lam CSP et al. Translating cGMP biology into clinical benefit. *Heart Fail Rev.* 2021;26:713–724. https://doi.org/10.1007/s10741-020-09999-0
22. Halliday BP et al. Future directions in heart-failure pharmacotherapy. *Nat Rev Cardiol.* 2024;21:201–218. https://doi.org/10.1038/s41569-023-00939-y
23. Pandey A et al. Economic burden and cost-effectiveness. *J Am Coll Cardiol.* 2021;77:2193–2205. https://doi.org/10.1016/j.jacc.2021.02.027

24. Papp Z et al. Emerging molecular targets for myocardial relaxation. *Br J Pharmacol.* 2020;177:2072–2090. https://doi.org/10.1111/bph.14960
25. Boehm M et al. Dual-pathway cGMP stimulation in heart failure. *Cardiovasc Drugs Ther.* 2023;37:447–458. https://doi.org/10.1007/s10557-023-07329-2
26. Butler J et al. Vericiguat in HFpEF. *Eur Heart J Suppl.* 2022;24:L33–L38. https://doi.org/10.1093/eurheartjsupp/suac053
27. Konstam MA et al. VITALITY-HFpEF results. *Eur Heart J.* 2023;44:1192–1201. https://doi.org/10.1093/eurheartj/ehad023

Review Article

Read Online

Advances in cosmetic science: A comprehensive review of lipstick formulation, ingredients, and emerging trends

Tanvi Ajit Kadam[1*], Dnyaneshwar Vyhvare[1], Gita Mohire [1]

[1]Department of Pharmacy, Faculty of Pharmacy, University of Mumbai, Vangani, Maharashtra, India

*Correspondence: kadamtanvi333@gmail.com

https://doi.org/10.65116/vitascientia.V2I2.rev.03

Summary

Lipsticks are among the most widely used cosmetic products, combining aesthetic appeal with essential functional performance. Effective lipstick manufacturing requires a precise balance of ingredients to achieve the desired colour, durability, texture, and user safety. This review outlines the fundamental principles of lipstick formulation, focusing on key components such as waxes, oils, pigments, colorants, emollients, and functional additives. These ingredients collectively influence important characteristics including hardness, melting behaviour, spreadability, gloss, and wear resistance. Safety and regulatory considerations are equally critical in cosmetic development. Strict quality control measures, systematic evaluation of ingredient safety, and cosmetovigilance practices in India play a major role in monitoring adverse reactions and ensuring consumer protection. Lipstick manufacturing also involves several evaluation parameters used to assess product quality, including melting point, breaking strength, ease of application, stability under varying conditions, and microbial safety. In addition, this review highlights emerging sustainability trends within the cosmetic industry, such as the adoption of ecofriendly raw materials, biodegradable formulations, and environmentally responsible packaging solutions. Despite ongoing advancements, challenges related to regulatory compliance, environmental impact, and ingredient safety remain significant. Future research and development efforts are expected to emphasize green chemistry approaches, safer alternative ingredients products.

Keywords

Lipstick formulation; Cosmetic science; Waxes and oils; Pigments and colorants; Quality evaluation; Safety and regulation; Cosmeto-vigilance; Sustainability; Green Chemistry; Cosmetic manufacturing

Introduction

Lipstick is one of the oldest and most popular cosmetic products which are used not only to add colour and enhance the appearance of the lips but also to keep them comfortable and protected [1].

The beauty and attractiveness of a person are enhanced as lipsticks colour the lips and protect them from the external surroundings. Currently lip care products not only focus on aesthetic appearance but also preferably have added medicinal value to the lip of consumers. This led to the emergence in the market of medicated lipsticks with active medicinal ingredients. The medicated lipsticks may provide protection against infections of bacteria due to the presence of an active medicinal ingredient in the formulation. This function adds on to the existing role of lipsticks, which provide moisture and emollient action to prevent cracking and chapping of the lips [2]. Recent progress in

cosmetic science has led to the creation of lipsticks that last longer, feel better, and are safer for consumers. As consumers become more aware, there is a growing interest in cruelty-free and vegan lipstick options that do not use animal products or harmful chemicals. [3].

In addition to looking good, safety checks are now essential in lipstick making, especially because of the chance of accidental swallowing while using it. As a result, regulatory bodies have set up strict rules to limit heavy metals and other harmful substances in lipstick to protect consumer health. [4].

History Of Lipsticks

The origins of lipstick can be traced back to prehistoric eras, during which early humans sought to improve their appearance by experimenting with natural materials. Historical records indicate that the practice of lip colouring began in ancient civilizations, with some of the earliest evidence discovered in Mesopotamia. These primitive cosmetic practices suggest that the desire for beautification was an integral part of human culture long before the advent of contemporary cosmetics.

As time progressed, the application of lipstick transformed across various cultures, mirroring the evolving cultural values and aesthetic tastes. The evolution of lip products persisted through different historical epochs, ultimately culminating in the creation of more sophisticated lipstick formulations in subsequent centuries. [5].

Lipstick formulation fundamentals

The process of creating lipstick is a specialized field within cosmetic science that involves mixing various types of ingredients to achieve the desired texture, colour, stability, and performance. A typical solid lipstick formulation includes a combination of waxes, oils, pigments, and emollients, each offering distinct functional properties to the final product. The quality of pigment dispersion in the wax-oil blend significantly influences the colour uniformity, strength, and visual attractiveness of the lipstick. Functional additives like antioxidants and preservatives may be incorporated to protect the formulation from oxidation and microbial contamination, thus enhancing shelf life and ensuring safety. Each lipstick formulation is usually optimized through repeated testing for melting point, hardness, texture, sensory feel, and colour performance to comply with safety regulations and meet consumer expectations. [6].

Oils and emollients like castor oil, coconut oil, and olive oil are added to improve spread-ability, moisture, and sensory experience while helping with pigment distribution. Natural oils and butters from plants not only enhance the feel but also offer extra skin conditioning benefits in herbal lipstick products.Reviews of herbal lipsticks emphasize the use of plant-based pigments, such as betalains and anthocyanins, which deliver colour along with antioxidant and skin-friendly properties. [7].

Advances in lipstick formulation technology

The formulation of modern lipstick has changed significantly, integrating improvements that boost not just colour and longevity but also provide functional advantages like hydration, antioxidant defence, and overall lip wellness, surpassing the typical cosmetic functions.[8]

A significant development is the use of natural bioactive ingredients and herbal extracts, which enhance skin conditioning, offer antioxidant benefits, and lessen the dependence on synthetic colorants in lipstick products. [9]

Current scientific formulation methods emphasize improving emollients, innovative wax mixtures, and natural oils to create the perfect texture, spread-ability, and stability, all while boosting the sensory qualities that today's consumers seek.[10]

Ingredient Type	**Function in Formulation**	**Examples / Notes**	**References**

Waxes	Provide structure, rigidity, and determine melting point; influence hardness and pay-off	Beeswax, Carnauba wax, Candelilla wax	[6]
Oils & Emollients	Improve spread-ability, moisture, sensory feel; aid in pigment dispersion	Castor oil, Coconut oil, Olive oil, Plant butters	[6][7]
Pigments & Colourants	Impart colour, opacity, and visual appeal; natural pigments may offer antioxidant benefits	Synthetic pigments, Plant-derived pigments like betalains, anthocyanins	[7]
Antioxidants	Protect oils and lipids from oxidation; enhance shelf life	Vitamin E (Tocopherol), natural extracts	[6]
Preservatives	Prevent microbial contamination and ensure product safety	Common cosmetic preservatives	[6]
Functional Additives	Enhance specific properties such as gloss, stability, and skin conditioning	Film-forming agents, UV filters, herbal extracts	[6][7]

Long Lasting and Transfer Resistant Systems.

Long-lasting and transfer-resistant lipstick formulas are made to form a strong film on the lips that sticks well and keeps its colour even when faced with activities like talking, eating, and drinking. Polymers and film-forming resins are key in ensuring the long-lasting hold of these products, as they create a semi-continuous layer that stays attached to the lip surface during everyday movements and contact.

In summary, today's long-lasting lipstick technologies depend on polymeric film-forming systems and carefully chosen ingredient interactions that ensure strong adhesion and less transfer, representing a major shift from traditional wax-based formulas to performance-driven cosmetic science. [11]

Natural and Herbal Lipstick Formulation

Natural lipsticks mainly consist of waxes, oils, and butters sourced from plants, including beeswax, carnauba wax, shea butter, and coconut oil. These ingredients contribute to the product's structure, emollience, and moisture retention, all while preserving appealing sensory characteristics. [6]

Nanotechnology in lipstick formulation

Nanotechnology involves manipulating materials at the nanoscale, specifically within the range of 1–100 nm. This manipulation leads to unique physicochemical properties that differ from those of bulk materials. [12]

In cosmetic science, nanotechnology is being used more and more to improve formulation stability, product performance, and sensory qualities. [13] Lipstick formulas now use nanotechnology to enhance pigment distribution, colour consistency, and stickiness

to the lips [14] Nanoparticles like silica, titanium dioxide, and zinc oxide are often added to lipsticks to boost opacity, shine, and feel. Silica nanoparticles are essential in lipstick recipes as they stop pigment clumping and allow for a smooth and even application [14].

Safety and regulatory consideration

In lipstick, safety checks are very important due to the risk of accidental oral exposure from licking, swallowing, and long contact with the lips. This way of exposure makes lipsticks different from other cosmetics and means that both skin and mouth toxicity need to be considered during safety evaluations [15].

Regulatory bodies stress that nanomaterials should not be considered safe just because their non-nano versions have been used in cosmetics before. In the European Union, cosmetics with nanomaterials are regulated by Regulation (EC) No. 1223/2009, which requires clear labelling of nanomaterials in the ingredient list with the term “(nano)”. This regulation also demands that manufacturers notify about nanomaterials through the Cosmetic Products Notification Portal (CPNP) at least six months before they are sold [16].

In the United States, cosmetics are regulated by the Food and Drug Administration (FDA), which assesses cosmetics that use nanotechnology under the current safety rules of the Federal Food, Drug, and Cosmetic Act. While nanomaterials do not need premarket approval for cosmetics, the FDA strongly advises manufacturers to perform extra safety tests when using nanoscale ingredients [17]

Evaluation parameters of lipstick formulation

Evaluating lipstick formulations is essential for maintaining product quality, stability, and consumer safety [18].

These evaluations include physical, mechanical, performance, and safety factors that affect usability and compliance with regulations [19].

Physical and Mechanical Parameters

The initial step in evaluation involves checking the appearance and surface features of the lipstick [20]. A visual inspection looks at uniformity, colour consistency, and surface flaws like cracks, air bubbles, or sweating [18]. Determining the melting point gives insights into thermal stability, ensuring the lipstick stays solid at room temperature [19]. Breaking point tests measure the force needed to break the lipstick, assessing its mechanical strength [20]. Hardness tests evaluate resistance to deformation, which affects how well the product spreads [18].

Performance Parameters

Spread-ability tests assess how smoothly the lipstick applies to the lips [19].
Pay-off tests measure how much product is transferred to the lips during application [20].
Smudge resistance tests check how well the lipstick stays in place when subjected to friction [18].
Longevity studies evaluate how long the colour and texture last while being worn [19].

Safety Parameters

pH testing ensures the lipstick matches the natural pH of the lips [20].
Irritation and safety tests identify any potential allergic or sensitizing reactions to the formulation [18]. Stability studies under various environmental conditions track changes in texture, colour, and smell over time [19].

Challenges and future directions in lipstick formulations

Finding a balance between long-lasting wear and comfort for lips is a major challenge in today's lipstick formulations. Translating lab

formulations to large-scale production presents challenges in maintaining batch consistency, reproducibility, and controlling costs [21]. Highly pigmented or long-lasting lipsticks can lead to dryness or friction, necessitating careful adjustment of wax-oil ratios and emollient systems [22]. Stability problems like phase separation, sweating, and cracking can happen at high temperatures, impacting product quality. Using nanotechnology-based pigments or delivery systems can enhance colour intensity and durability but also raises safety and regulatory issues [23].

Adding functional ingredients such as UV filters, antioxidants, and moisturizers can make it harder to ensure chemical compatibility and stability [24].

Different regulatory standards in global markets require careful adherence to ensure safety, labelling, and acceptable ingredients [25].

Conclusion

Lipstick is one of the most popular cosmetic products because it enhances appearance, provides comfort, and protects the lips [26]. Modern cosmetic science has evolved lipstick from basic pigment mixtures to advanced formulations that include moisturizing, antioxidant, and functional properties [27]
However, there are still challenges in formulation, such as finding the right wax-oil balance for texture and stability without losing effectiveness [28].

Recent developments in cosmetic science have greatly improved the effectiveness, safety, and sensory qualities of lipstick products.
New technologies in nanoscale delivery systems have enhanced pigment stability, moisturizing effectiveness, and the controlled release of bioactive ingredients, resulting in improved wear and greater consumer satisfaction.[29]

Acknowledgement

The authors would like to express their sincere appreciation to the faculty and management of B. R. Harne College of Pharmacy, Karav, Vangani, for providing the academic support and institutional facilities required for the successful completion of this review article. The guidance and constructive suggestions offered by the teaching staff were instrumental in improving the scientific depth and clarity of the manuscript.

The authors gratefully acknowledge the contributions of researchers and scholars whose previously published studies formed the scientific foundation of this review. Access to peer-reviewed journals, academic databases, and open-access scientific resources played a vital role in the collection, analysis, and interpretation of relevant literature.

The authors also acknowledge the academic environment that encouraged critical discussion and scholarly exchange, which contributed to refining the conceptual framework of the article. No specific funding was received for this work, and the authors declare that the preparation of this manuscript was carried out independently, without any external financial support.
The authors confirm that there are no conflicts of interest associated with this study.

References

1.Mawazi, S. M., Azreen Redzal, N. A. B., Othman, N., & Alolayan, S. O. (2022). Lipsticks history, formulations, and production: A narrative review. Cosmetics, 9(1), 25. https://doi.org/10.3390/cosmetics9010025
2.Afandi, A. S. R. U. L., Lazim, A. M., Azwanida, N. N., Bakar, M. A., Airianah, O. B., & Fazry, S. (2017). Antibacterial properties of crude aqueous Hylocereus polyrhizus peel extracts in lipstick formulation against gram-positive and negative bacteria. Malaysian Applied Biology, 46, 29–34.
3.Balaguru, R., Bhowmick, S., & Iyer, P. (2023). Formulation of chemical and cruelty-free vegan lipstick and analyzing its components. International Journal of Engineering Science

Technologies, 7(5), 37–53. https://doi.org/10.29121/ijoest.v7.i5.2023.507
4.U.S. Food and Drug Administration. (n.d.). Limiting lead in lipstick and other cosmetics. https://www.fda.gov/cosmetics/cosmetic-products/limiting-lead-lipstick-and-other-cosmetics
5.History of Lipstick. (2024). International Journal of Creative Research Thoughts (IJCRT), 12(11). https://www.ijcrt.org/papers/IJCRT2411417.pdf
6.Gaikwad, D. K., & Rathi, P. (2025). Review article on lipstick. International Journal of Innovative Research in Technology (IJIRT), 12(6). https://ijirt.org/article?manuscript=187816
7.Sadhu, P., Rathod, F., Kumari, M., & Shah, N. (2024). A comprehensive review on potential of natural ingredients in formulation of herbal lipsticks. Annals of Phytomedicine, 13(1), 158–166. https://doi.org/10.54085/ap.2024.13.1.15
8.Veludurthi, P. A., & Vadaga, A. K. (2024). A comprehensive review of formulations, ingredients, advances, and future perspectives in lipcare cosmetics. Journal of Pharma Insights and Research, 2(3), 123–128. https://jopir.in/index.php/journals/article/view/176
9.Aher, K. N., Shewale, V. V., & Mahajan, S. K. (2024). A review on formulation and evaluation of herbal lipstick from Opuntia (Ficus indica). International Journal of Pharmacognosy and Pharmaceutical Research, 6(2), 41–46. https://www.pharmacognosyjournals.com/archives/2024.v6.i2.A.77/a-review-on-formulation-and-evaluation-of-herbal-lipstick-from-opuntia-ficus-indica
10.Rigano, L., & Montoli, M. (2021). Strategy for the development of a new lipstick formula. Cosmetics (MDPI), 8(4), 105. https://doi.org/10.3390/cosmetics8040105
11.Alves, T. F. R., Morsink, M., Batain, F., Chaud, M. V., Almeida, T., Fernandes, D. A., da Silva, C. F., Souto, E. B., & Severino, P. (2020). Applications of natural, semi-synthetic, and synthetic polymers in cosmetic formulations. Cosmetics, 7(4), 75. https://doi.org/10.3390/cosmetics7040075
12.Gupta, V., & Mohapatra, S., et al. (2022). Nanotechnology in cosmetics and cosmeceuticals—A review of latest advancements. Gels, 8(3), 173. https://www.mdpi.com/2310-2861/8/3/173/pdf
13.Nanda, S., & Nanda, A., et al. (2024). Nanotechnology in the cosmetic industry: A review. Journal of Chemical Technology and Metallurgy, 59(1), 3–14. https://journal.uctm.edu/node/j2024-1/JCTM_2024_59_1_1_23-247_pp3-14.pdf
14.Meng, G. (2024). Application of nanoparticles in cosmetics. Transactions on Materials, Biotechnology and Life Sciences, 4, 69–75. https://www.researchgate.net/publication/383591299_Application_of_Nanoparticles_in_Cosmetics
15.Larese Filon, F., Mauro, M., Adami, G., et al. (2015). Human skin penetration of nanoparticles through cosmetics. Journal of Cosmetic Dermatology, 14(1), 15–22. https://onlinelibrary.wiley.com/doi/pdf/10.1111/jocd.12121
16.Gomes, M. J., Martins, S., et al. (2020). Nanomaterials in cosmetics: Recent updates. Nanomaterials, 10(5), 979. https://www.mdpi.com/2079-4991/10/5/979/pdf
17.European Commission. (2009). Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1223
18.U.S. Food and Drug Administration. (2014). Considering whether an FDA-regulated product involves the application of nanotechnology. https://www.fda.gov/media/88423/download
19.Sahare, S., Ankita, Prerana Sahu, G., Gyanesh Sahu, & Harish Sharma. (2024). Formulation and evaluation of herbal lipstick. International Journal of Novel Research and Development, 9(4), d581–d591.

https://www.ijnrd.org/papers/IJNRD2404364.pdf
20.Suryawanshi, M. R., & Yenegurkar, V. A. (2024). Formulation and evaluation of cosmeceuticals lipstick from product fixed oil of Moringa leaves Linn. International Journal of Pharmaceutical Research and Applications, 9(3), 2224–2228. https://ijprajournal.com/issue_dcp/Formulation%20and%20Evaluation%20of%20Cosmeceuticals%20Lipstick%20from%20Product%20Fixed%20Oil%20of%20Moringa%20Leaves%20Linn.pdf
21.Juma'at N, Rahmat NA, Hamidi SA, Adnan N. The production and stability evaluation of natural lipstick. Malaysian Applied Research and Innovation. 2021;2(2). doi:10.30880/mari.2021.02.02.028 https://publisher.uthm.edu.my/periodicals/index.php/mari/article/download/1789/655/10749
22.Soman, M., et al. (2024). Formulation and evaluation of herbal lipstick. Journal of Pharmaceutical Sciences and Research, 16(9), 63–68. https://www.pharmainfo.in/jpsr/Documents/Volumes/vol16issue09/jpsr16092401.pdf
23.F. T. S., et al. (2021). Preparation, characterization and evaluation of organogel-based lipstick formulations: Application in cosmetics. Gels, 7(3), 97. https://www.mdpi.com/2310-2861/7/3/97/pdf
24.Noble, J., et al. (2022). Evaluation of alkenones as a structuring agent for lipsticks. Foods and Raw Materials. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9291794/pdf/foods11XXXX.pdf
25.Reddy, V. S. (2024). Lip cosmetics — A formulator (or) an innovator to overcome a challenge in quest of research with its advances. Asian Journal of Pharmaceutics (AJP), 18(01). https://doi.org/10.22377/ajp.v18i01.5260
26.Prakash Kumar, A., Aradhya, S., Kumar, R., & Mishra, R. (2025). Formulation and evaluation of herbal lipstick by using Beta vulgaris (beet root). International Journal of Scientific Research in Science & Technology, 12(4), 139–150. https://doi.org/10.32628/IJSRST2512607
27.U.S. Food and Drug Administration. (n.d.). Guidance for industry: Considering whether an FDA-regulated product involves the application of nanotechnology. https://www.fda.gov/media/88423/download
28.A review on: Formulation and evaluation of lipstick. (2025). International Journal for Research in Applied Science & Engineering Technology (IJRASET). https://doi.org/10.22214/ijraset.2025.74158
29.Nano Beauty 2.0: Exploring nanotechnology in cosmetic delivery — A systematic review. (2025). Premier Science. https://doi.org/10.70389/PJS.100107
30.F. T. S., et al. Preparation, Characterization and Evaluation of Organogel Based Lipstick Formulations: Application in Cosmetics. Gels, 7(3), 97, 2021. https://www.mdpi.com/2310-2861/7/3/97/pdf

Vita Scientia

Research Article

Read Online

Formulation, Evaluation and optimization of Lenalidomide loaded niosomes

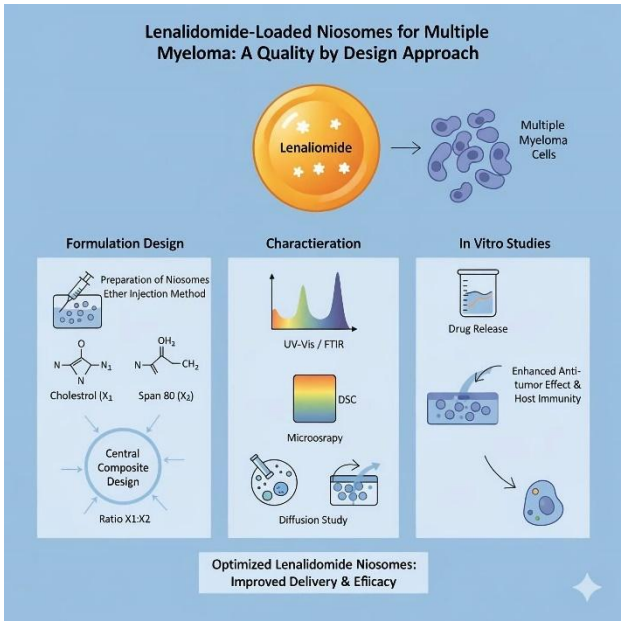
Sakshi Waware[1] , Pallavi Kaple[2*], Pratiksha More[3], Dipali Kamble[4], Shreeya Belwalkar[5]
Department of Pharmaceutics, Alard School of Pharmacy, Pune, India.

*Correspondence: pallavik.acp@gmail.com

https://doi.org/10.65116/vitascientia.V2I2.01

Graphical Abstract

Formulation, Evaluation and optimization of Lenalidomide loaded niosomes



Summary

The targeted and sustained delivery of anticancer drugs remains a major focus in pharmaceutical research to enhance therapeutic efficacy and reduce systemic toxicity. This study involves the formulation, evaluation, and optimization of Lenalidomide-loaded niosomes for improved drug delivery in cancer therapy. Niosomes were prepared using the ether method technique with non-ionic surfactants (Span 80 and cholesterol) in varying molar ratios. A series of formulations were developed and optimized based on particle size, polydispersity index (PDI), and entrapment
efficiency (EE%). The optimized formulation was characterized for morphology, zeta potential, in-vitro drug release, FTIR, DSC, and stability studies. Results indicated that the optimized niosomal formulation exhibited nanometric size, high entrapment efficiency, and controlled drug release over 8 hours. The findings highlight the potential of niosomal carriers to enhance the bioavailability and therapeutic performance of Lenalidomide, offering a promising approach for the effective and sustained management of multiple myeloma and related malignancies.

Keywords

Lenalidomide; multiple myeloma; niosomes; ether injection method

Introduction

Niosomes are multi lameller vesicular struc.ture of non-ionic surfactants, similar to lipos.omes and are composed of non-ionic surfactant instead of phospholipids which are the components of liposomes. [1,2] So, niosome or non-ionic surfactant vesicles are now widely studied as an alternative tool to liposome. Various types of surfactants have been reported to form vesicles, and have the capacity to entrap and retain the hydrophilic and hydrophobic solute particles [1-3]. Niosomes are vesicular nanocarriers that are stable, non-toxic, biodegradable, and reasonably priced. The instability, rapid disintegration, bioavailability,

and solubility of some drugs or natural substances may be improved by niosomes. When it comes to the targ.eted administration of antibacterial, antimicrobial, anti-inflammatory, antioxidant, and anticancer compounds, niosomes have the potential to be incredibly powerful systems. This essay will provide a summary of their makeup, the most popular methods for formulation, and their present application as delivery systems for cancer treatments. [4]

Niosomes mainly contain two types of components i.e., non-ionic surfactant and the additives. The non-ionic surfactants form the vesicular layer and the additives used in niosome preparation are cholesterol and the charged molecules.[3] The presence of the steroidal system (cholesterol) improves the rigidity of the bilayer and is important component of the cell membrane and their presence in membrane affects bilayer fluidity and permeability. This carrier system protects the drug molecules from the premature degradation and inactivation due to unwanted immunological and pharmacological effects.[5] By guaranteeing that noisome medications are distributed in a controlled manner that is customized to the needs of the patient, this innovation not only expedites the formulation process but also enhances therapeutic outcomes. [6]

In recent years, niosomes have been extensively studied for their potential to serve as a carrier for the delivery of drugs, antigens, hormones and other bioactive agents. Besides this, niosome has been used to solve the problem of insolubility, instability and rapid degradation of drugs.[7] Niosomes can be categorized into 3 groups based on their vesicle size, namely, small unilamellar vesicles (0.0.25–0.05 mm), multilamellar vesicles (>0.05 mm), and large unilamellar vesicles (>0.10 mm) [8].

Lenalidomide (prev.iously referred to as CC-.5013) is an imm.uno modul.atory drug with potent antineoplastic, anti-angiogenic, and anti-inflammatory prope.rties. It is a 4-amino-glutamyl analogue of [thali domide] and like thalidomide, lenalidomide exists as a racemic mixture of the active S(-) and R(+) forms However, Lenalidomide is much safer and potent than thalidomide, with fewer adverse effects and toxicities.[9] Thalidomide and its analogues, including Lenalidomide, are referred to as immuno modulatory imide drugs (also known as cereblon modulators), which are a class of immuno modulatory drugs that contain an imide group. Lenalidomide works through various mechanisms of actions that promote malignant cell death and enhance host immunity Available as oral capsules, Lenalidomide is approved by the FDA and EU for the treatment of multiple myeloma, myelodysplastic syndromes, mantle cell lymphoma, follicular lymphoma, and marg.inal zone lymphoma in selected patients. [10,11]

Multiple myeloma is a clonal B-cell malignancy associated with a monoclonal (M) protein in blood and/or urine, bone lesions, and immunodeficiency. It usually evol.ves from monoclonal gammopathy of undetermined significance (MGUS), with low levels of plasmacytosis and M protein without osteolytic lesions, anemia, hypercalcemia, and renal failure.[11] Multiple myeloma is characterized by genetic signatures, including frequent translocations into the immunoglobulin heavy chain switch region (IgH), oncogenes, and abnormal chromosome number[12,13]. Most patients with translocations have non-Hyperdiploid chromosome number (NHMM), while those patients lacking IgH translocations have Hyperdiploid chromosome number (HMM) with trisomies of chromosomes 3,5,7.,9,11,15,19, and 21. Importantly, patients with Hyperdiploid multiple myeloma have a better outcome with prolonged survival.[14,15].

Lenalidomide has a short plasma elimination half-life of about 3–4 hours, which necessitates frequent doses to maintain therapeutic levels and exposes patients to varying systemic exposure despite its excellent oral absorption

and bioavailability.[16] Additionally, dose-limiting toxicities including neutropenia and thrombocytopenia, which limit dose escalation and overall acceptability in multiple myeloma therapy, are correlated with increased systemic exposure to lenalidomide. These characteristics imply that, in comparison to conventional dosing, a sustained and targeted delivery platform such as niosomes, which have been demonstrated to improve drug stability, control release, and delivery to diseased tissues, may extend systemic exposure, lower off-target toxicity, and increase therapeutic efficacy [17].

Results and Discussion

Pre-formulation studies

a. Physical parameters of Lenalidomide

To determine Lenalidomide's fundamental physicochemical properties, its appearance and solubility were assessed. The substance was found to be a crystalline powder that was off-white to pale yellow in colour, indicating good purity and a constant physical form. Water, methanol, ethanol, and acetone were among the solvents used for solubility tests. Lenalidomide was found to be nearly insoluble in water, but it was more soluble in acidic (low pH) solutions and organic solvents such methanol, ethanol, and diethyl ether. Using the capillary tube method, the drug's melting point was found to be 272.36 °C, which is consistent with the range reported for pure Lenalidomide and validates the drug's identity and thermal stability.

b. Calibration curve

Calibr.ation cur.ve was plotted in meth.anol at 242 nm. The calibr.ation cur.ve was plot.ted as seen in figure1

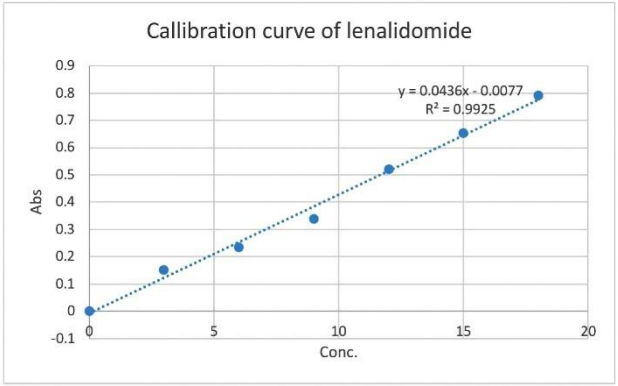


Figure1. Calibration curve of Lenalidomide in methanol

A calibration curve of Lenalidomide was prepared in methanol to determine its linearity and suitability for quantitative analysis using UV-visible spectrophotometry. The absorbance of standard solutions with concentrations ranging from 0 to 18 µg/mL was measured at 242 nm, which corresponds to the absorption maximum (λmax) of Lenalidomide in methanol. A linear relationship was observed between concentration and absorbance, indicating that the method follows Beer–Lambert's law within the tested range. The calibration curve, as shown in Figure1, exhibited a well-defined straight line with good correlation, confirming the reliability of the analytical method for determining the concentration of Lenalidomide in subsequent formulations and release studies.

Pre-formulation Screening of Surfactants

Pre-formulation trials (Table 5) comparing Span 80 and Span 60 indicated that Span 80 produced significantly smaller vesicles (106–254 nm) than Span 60 (348–488 nm) and achieved comparable drug entrapment efficiency. Therefore, Span 80 was selected for further optimization due to its lower hydrophilic–lipophilic balance (HLB = 4.3), which promotes the formation of stable, flexible bilayer vesicles with enhanced permeability. Subsequent results presented below focus on the optimization and characterization of Lenalidomide-loaded niosomes prepared using Span 80.

Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of the sample was recorded using a Shimadzu instrument. The analysis reveals characteristic absorption peaks which are consistent with the structural features of Lenalidomide.

The presence of these peaks confirms the functional groups expected in the compound and supports its identity. The FTIR of the API Lenalidomide are shown in the following figure 2a.

Table 4 presents the FT-IR interpretation of Lenalidomide, comparing the reported and observed wavenumbers (cm^{-1}) for various functional groups. It confirms the presence of characteristic stretches with observed peaks closely matching reported literature values, indicating the drug's structural integrity.

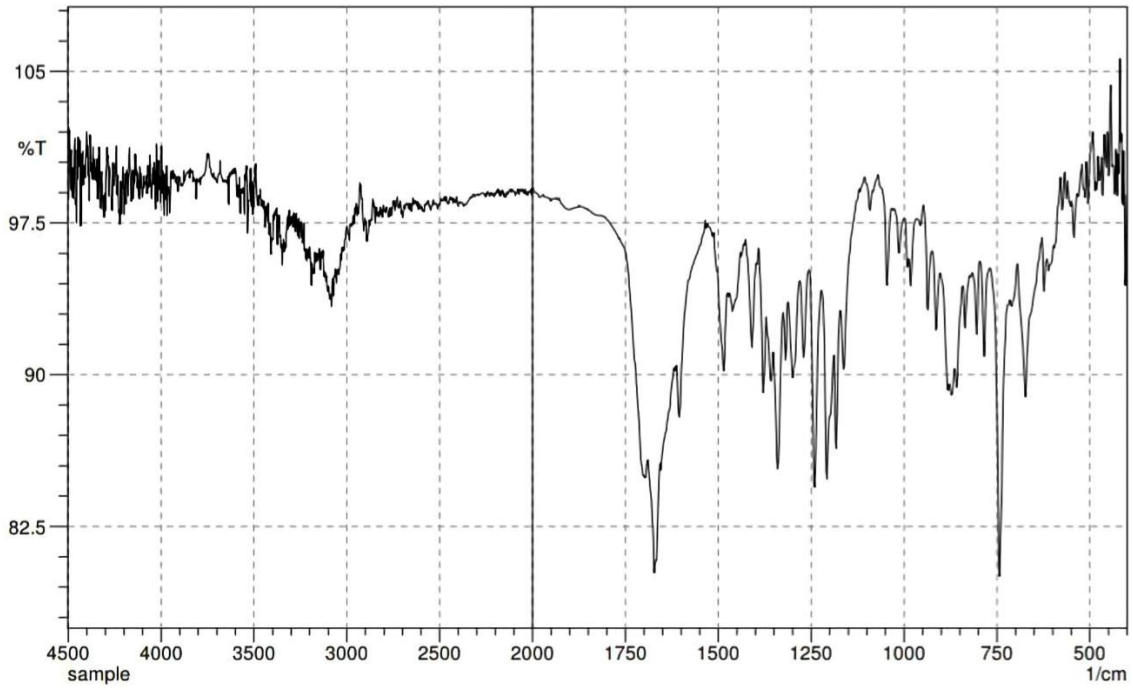


Figure2a. FTIR Spectrum of Lenalidomide

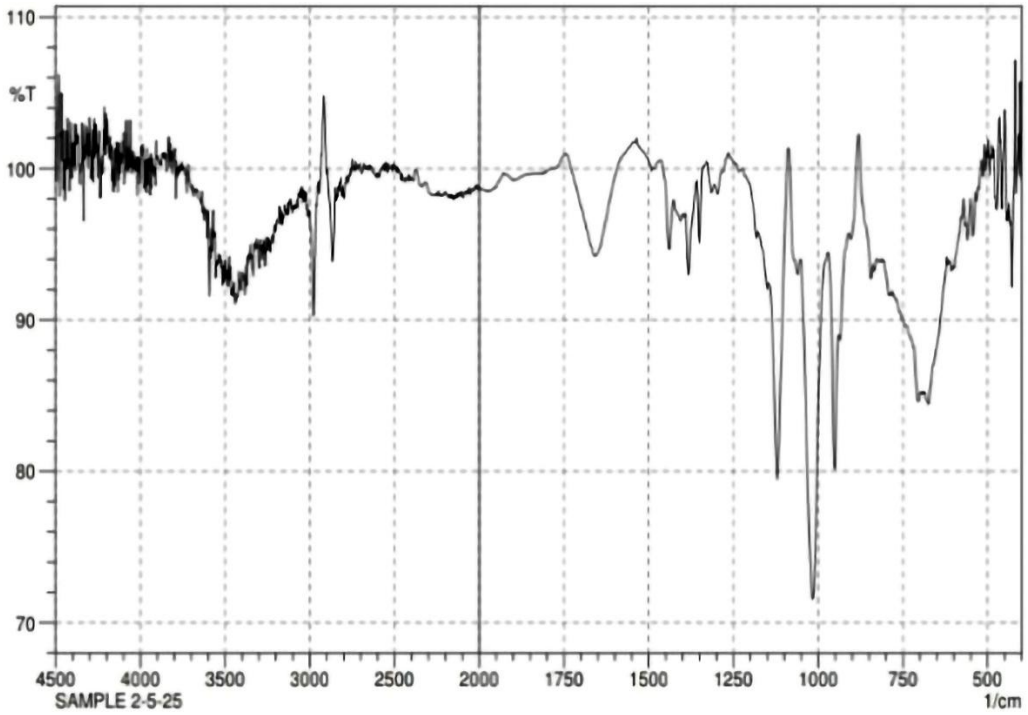


Figure2b. FTIR Spectrum of Lenalidomide loaded niosomes

Figureure. 2b illustrates Fourier-transform infrared (FT IR) spectroscopy of drug-loaded niosomes show the existence of fundamental functional groups and evidence successful incorporation of the drug into the vesicular formation.

Sr. no.	Stretching	Functional Group	
		Reported (cm^{-1})	Observed (cm^{-1})
1	C=O	1700-1750	1690
2	C=C	1450-1600	1500
3	C-O	1000-1300	1079.81
4	C-H	700-900	755.34
5	C-N	1200-1350	1311.59
6	N-H	3300-3500	3365.48

Table1 FTIR spectra readings

Sr. no.	Stretching	Functional Group	
		Reported (cm^{-1})	Observed (cm^{-1})
1	C=O (Amide)	1650-1750	1655
12	C=C	1450-1600	1595
3	C=O (Ester)	1720-1750	1732
4	C-H	2850-2950	2920
5	C-O-C	1050-1250	1100
6	N-H	3300-3500	3410

Table 2. FTIR spectra readings

Tab.le 1 and 2 presents the FT-IR interpretation of Lenalidomide, comparing the reported and observed wavenumbers (cm^{-1}) for various functional groups. All these peaks combined indicate that the active chemical properties of Lenalidomide and the components of niosomes are intact and properly introduced, with no significant chemical or chemical interaction or shift in peaks and evidence supporting the physical entrapment mechanism process, as opposed to any sort of covalent processing of the formulation.

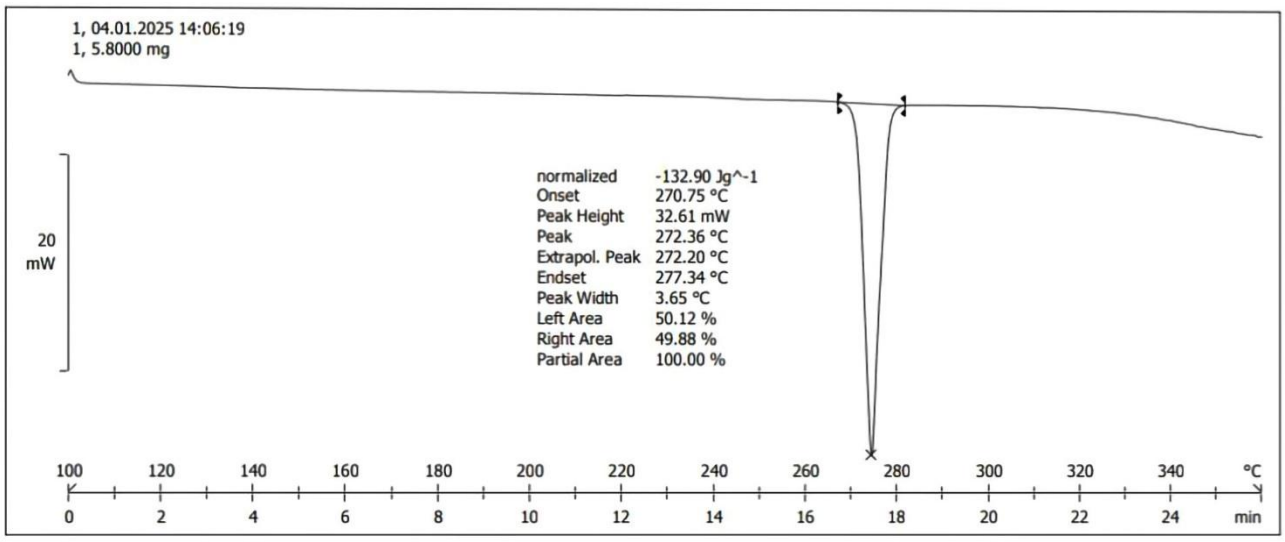


Figure3a shows the sharpness and narrow peak width (3.05 °C) suggest that the API is thermally pure with minimal impurities. The enthalpy value represents the energy absorbed during melting. A single, sharp peak with a narrow temperature range between onset and endset further confirms the homogeneity of the API. The sample exhibits a distinct and sharp melting endotherm at 272.36 °C, indicating high purity and crystalline nature. This information can be useful in confirming the identity of the compound and its suitability for formulation development.

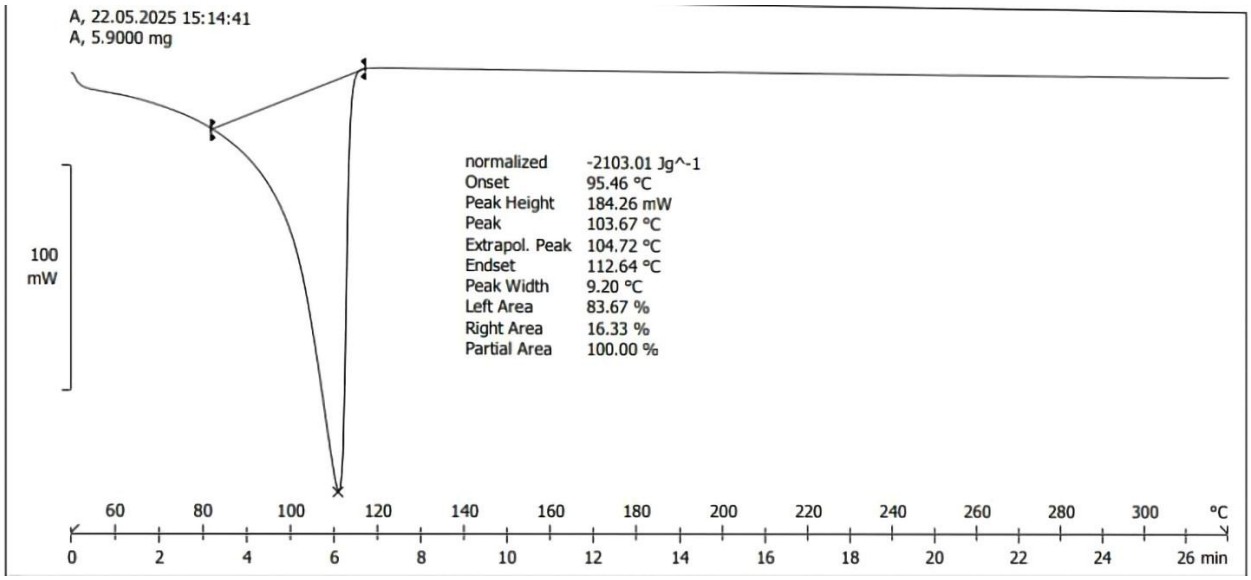


Figure3b. DSC of Lenalidomide loaded niosomes. The DSC thermogram of the niosomal formulation showed a completely different thermal behavior. The characteristic melting peak of pure Lenalidomide was absent, and instead, a new broad endothermic peak was observed at 103.57 °C, with an onset at 95.46 °C and an endset at 112.46 °C. The absence of the melting peak of pure Lenalidomide in the niosomal formulation suggests that the drug was successfully encapsulated within the niosomal bilayer. The shift in the thermal peak indicates that Lenalidomide may be present in an amorphous or molecularly dispersed form, rather than in its original crystalline state. The new thermal event around 103.5 °C could be attributed to the phase transition of the surfactant/cholesterol bilayer or interactions

between the drug and lipid matrix. The drug-excipient interaction may lead to improved solubility and bioavailability, which is often desirable in pharmaceutical formulations.

Evaluation of parameters of all batches

All prepared batches of niosomes were evaluated for parameters such as particle size, zeta potential and drug content. Results are shown in table 3.

FORMULATION	PARTICLE SIZE (in nm)	ZETA POTENTIAL (in mV)
F1	220.17	-30.71
F2	318.41	-32.87
F3	254.18	-31.69
F4	177.1	-30.5
F5	250.84	-31.23
F6	250.84	-31.23
F7	273.57	-30.55
F8	252.28	-29.73
F9	318.41	-32.87
F10	232.67	-30.87
F11	252.28	-29.73
F12	273.57	-30.55
F13	228.74	-28.23
F14	220.17	-30.71

Table 3. Evaluation of optimized batches

Above results indicates that batch B4 shows acceptable results, here selected as Optimized formulation.

3^2 Full factorial design:

Response 1: Particle size

The visualization of interactive effects of Cholesterol & Span 80 on particle size are shown in figure4a and 4b,

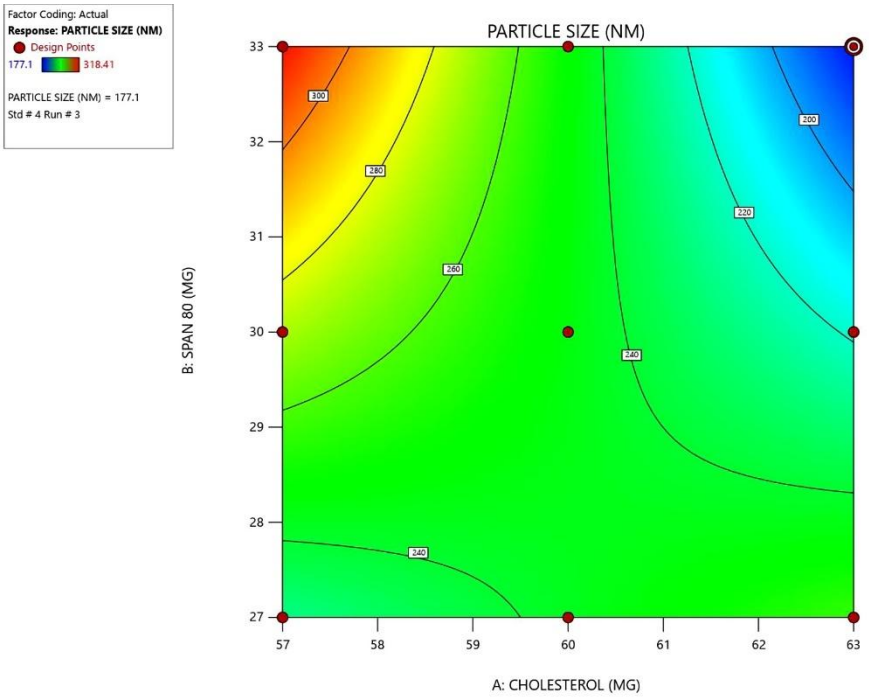
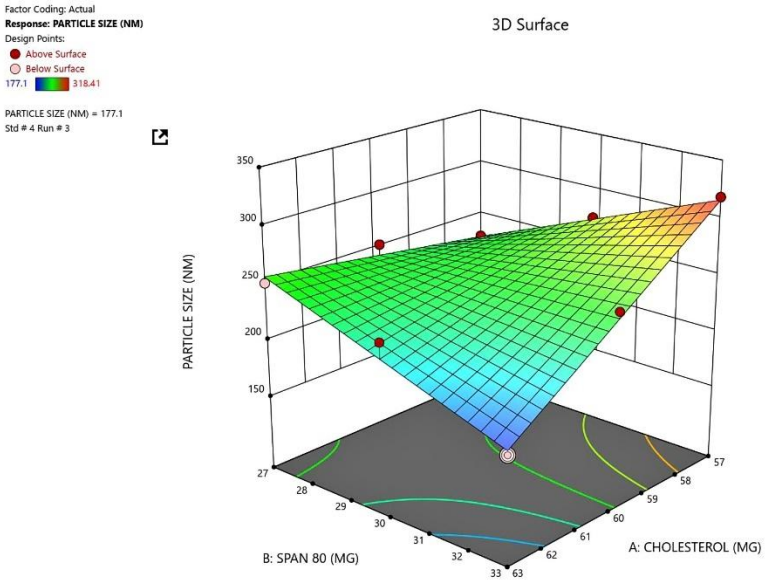


Figure4a Factorial design for response 1 particle size



Figureure4b 3-D plot for response 1 particle size

The visualization of interactive effects of Cholesterol & Span 80 on zeta potential are shown in figure5a and 5b

Response 2: Zeta potential

The visualization of interactive effects of Cholesterol & Span 80 on zeta potential are shown in figure5a and 5b

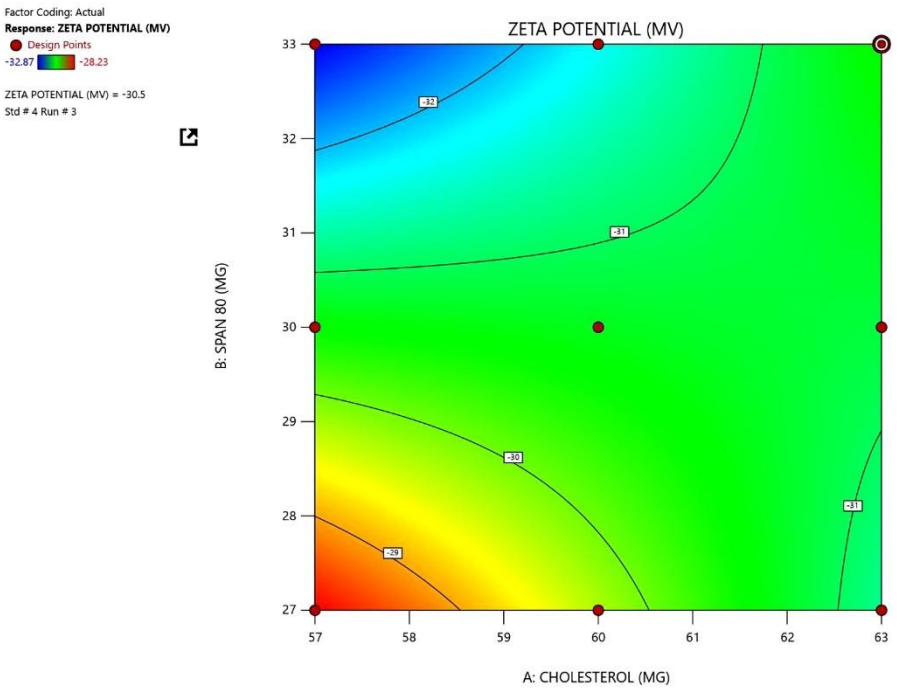
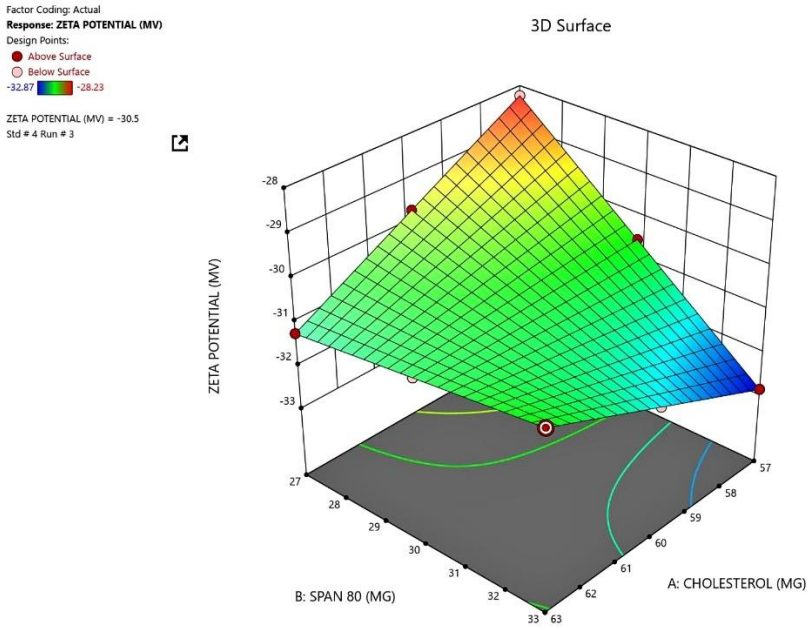


Figure5a Factorial design for response 2 zeta potential



Figureure5b 3-D plot for response 2 zeta potential

Optimization of Niosomal Formulation Using 3² Full Factorial Design

A **3² full factorial design** was utilized to study the combined influence of Span 80 and cholesterol concentrations on the physical characteristics of niosomes. The experimental outcomes for all nine formulations (F1–F14) are presented in Table 3.

Response 1: Particle Size

As shown in the contour and 3D response surface plots (Figure. 4a and 4b), an **increase in Span 80 concentration (X_1)** resulted in a **decrease in particle size**, suggesting that higher surfactant content enhances vesicle flexibility and reduces interfacial tension, thereby promoting the formation of smaller niosomes. Conversely, **increasing cholesterol concentration (X_2)** caused a **slight increase in particle size**, as the rigid bilayer formed by excess cholesterol limits curvature and expansion, producing larger vesicles. Therefore, an optimal Span 80-to-cholesterol ratio is necessary to maintain vesicle stability and uniform nanometric size.

Final equation in terms of coded factors is as follows

Particle Size = +245.33+28.24*A[1]-3.12*A[2]1.38*B[1]-3.19*B[2]-43.46*A[1]B[1]+11.45*A[2]B[1]+3.19A[1]B[2]-18.85*A[2]B[2]

Response 2: Zeta Potential

The response surface plots for zeta potential (Figure. 5a and 5b) indicated that **both Span 80 and cholesterol significantly influenced surface charge**. A moderate increase in Span 80 led to a more negative zeta potential, improving electrostatic stabilization of vesicles. Cholesterol also enhanced zeta potential up to an intermediate level, beyond which no substantial improvement was observed—likely due to charge shielding within the bilayer.

Among all batches, **formulation B4 (33 mg Span 80 and 63 mg cholesterol)** showed the **optimal combination**, achieving the **smallest particle size (177.10 nm)** and **high zeta potential (–30.50 mV)**, indicating excellent physical stability and bilayer integrity.

Final equation in terms of coded factors is as follows:

Zeta potential = -30.71+0.16*A[1]-1.11E-003*A[2]+0.98*B[1]-1.111E-003*B[2]+1.34*A[1]B[1]+1.111E-003*A[2]B[1]+1.111E-003*A[1]B[2]+1.111E-003*A[2]B[2]

Evaluation of Optimized batch:

Visual Appearance:

Dispe.rsion of niosomes was visually inspected and its appearance was seen as white turbid solution .

Particle size

Figure6 displays particle size measurement results of the prep.ared niosomes. The particle size of the samples ranged between 177.10 and 318.41 nm. The PS of niosomes was affected by many factors, such as the cholesterol amount in the formula. The vesicles formation and properties are well kno.wn to be affected by the HLB of the used surfactant.

Polydispersity index (PDI) of niosomes ranged between **0.2 and 0.6**, indicating that the produced niosomes were uniform in size and homogeneous. A PDI.≤0.5 is regarded as suitable for drug delivery applications because it demonstrates a relatively homogeneous and uniform distribution of nanocarriers. To provide proper particle distribution, the optimal formul.ation must have a PDI valu.e≤0.5.

Zeta potential

Niosomes were physically stable since the ZP values of all formulations ranged from **– 32.87 to – 28.23 mV**. Cholesterol had a sig.nificant eff.ect on ZP with a p val.ue of 0.0.094. Zeta

potential increased, as an absolute value, by increasing the cholesterol amount leading to enhancing niosomes stability. As a result, cholesterol is an important excipient in the preparation of niosomes because it enhances the stability of niosomes bilayers and reduces drug leakage due to the retarded solute permeability of these vesicles' aqueous core.

Entrapment Efficiency

After the vesicles were broken up with Triton X-100 (or propanol) and quantitative drug analysis was performed, the entrapment efficiency (EE%) of lenalidomide-loaded niosomes was found to be 80.20±2.53%. The data were expressed as mean ± standard deviation to account for experimental variability, and this value is the mean entrapment efficiency derived from several independent measurements (n = 3). The reliability of the niosome preparation procedure is confirmed by the comparatively low variability seen across duplicates.

The composition of the formulation is responsible for the high entrapment efficiency. The addition of Span 80, a non-ionic surfactant with a low hydrophilic–lipophilic balance (HLB), promotes the development of persistent, hydrophobic bilayers, which are especially useful for encasing drugs that are poorly soluble in water, such lenalidomide. Additionally, adding cholesterol to the niosomal membrane reduces drug leakage and membrane permeability by increasing bilayer stiffness and packing density. Additionally, cholesterol strengthens the bilayer's hydrophobic environment, which facilitates more lenalidomide sequestration and adds to the noted rise in EE%. (Mokale V. Niosomes as an ideal drug delivery system. J Nanosci Res Reports SRC/JNSRR-126. 2021.)

There are a number of reasons why about 20% of the medication was not caught. These include (i) lenalidomide's restricted solubility and partitioning during vesicle formation, which leaves a portion in the external aqueous phase; (ii) the bilayer domain becoming saturated, beyond which more drug cannot be added without compromising vesicle stability; and (iii) unanticipated process-related losses during the hydration, separation, or purification stages.

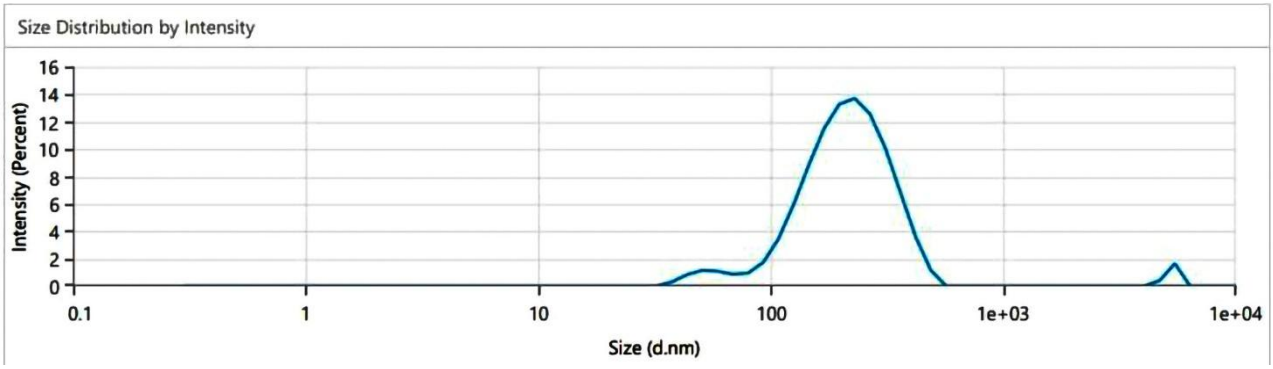


Figure6 Particle size distribution

For niosomal systems, an entrapment efficiency of about 80% is regarded as high, especially for hydrophobic medications. Depending on the kind of surfactant, cholesterol level, and preparation technique, reported EE% values for comparable niosome-based formulations usually fall between 60% and 90%. As a result, the EE% attained in this investigation is at the upper end of the reported range, suggesting that the developed formulation's efficiency is supported and drug loading is successfully enhanced by the optimised combination of Span 80 and cholesterol.[18]

Drug Content

Drug content was determined for all the batches. It was found in the range of

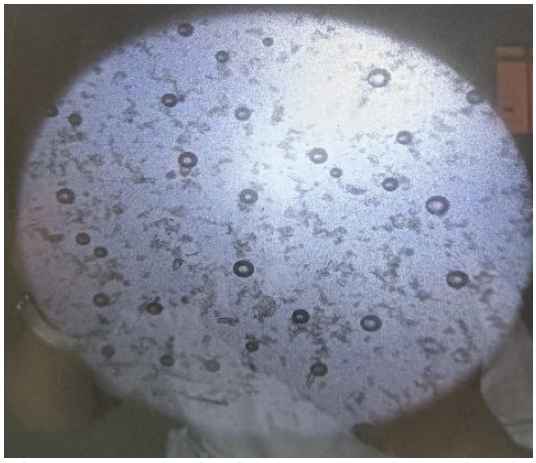


Figure7. Optical microscopy of LEN loaded niosomes

78.18±1.5 to 86.36±2.34. The maximum drug release was found to be 86.36 for F4 batch.

The micro.scopy ima.ges reve.aled from figure 7 that the nios.omes were predominantly spherical to slightly oval in shape, and they appe.ared as well-.formed, discrete vesi.cles. The vesicles showed uniform distribution without any noticeable aggregation, indicating good formulation stability. The spherical morphology is suggestive of successful vesicle formation and appropriate self-assembly of the surfactant and cholesterol components.

In vitro drug release studies

The lenalidomide-loaded niosomal formulation and plain lenalidomide differ significantly in the in vitro drug release profile (Figure8). The niosomal formulation had a higher and more sustained release, achieving virtually entire drug release by the completion of the investigation, whereas the plain drug showed a comparatively slower and partial release, reaching roughly 65–70% over 12 hours. As is typical of vesicular drug delivery systems like niosomes, the release pattern from the niosomes was biphasic, with an initial, quicker release followed by a longer, regulated phase [19,20].

The Higuchi and Korsmeyer-Peppas models showed a stronger correlation with the release data when fitted to different dissolution kinetic models than with zero-order kinetics, suggesting that the release of lenalidomide from the niosomes was primarily diffusion-controlled with a contribution from bilayer relaxation or reorganisation [21, 22]. This behaviour implies that the surfactant–cholesterol bilayer modulates drug release in a sustained way by acting as a diffusional barrier.

In vitro permeation studies

There is progressive and uniform increase in the amount of the drug permeation with time. Lenalidomide loa.ded niosomes sho.wed significantly greater permeation i.e. 66.83% than plain Lenalidomide at 8 hours, this indicates enhanced permeation profile. Figureure. 8 shows the graphical presentation of % cumulative percent permeability of Lenalidomide loaded niosomes.

The purpose of the in vitro permeation investigation was to assess the niosomal formulation's capacity to improve drug transport through a biological membrane model. Even though lenalidomide is mostly taken orally, measuring membrane permeation is important to investigate the possibility of using niosomes to enhance drug absorption across lipidic biological barriers and to look into other delivery methods, like transdermal or transmucosal administration, particularly for patients who might not tolerate oral therapy. When comparing the niosomal formulation to the plain medication, the penetration data showed a significant improvement.

Lenalidomide's cumulative permeation from the niosomes reached roughly 66.8% at 8 hours, while the plain drug's permeability was only 42.3%, suggesting a notable improvement in membrane transport. The presence of the non-ionic surfactant (Span 80), which is known to interact with membrane lipids, increase membrane fluidity, and decrease diffusional

resistance, thereby facilitating drug permeation, and the smaller particle size of the niosomes, which increases surface area and contact with the membrane, are both responsible for this enhancement [20, 23, 24].

But it's important to recognise the permeation model's limitations. The complex structure and barrier characteristics of human skin or intestinal mucosa are not fully represented by the egg membrane employed in this study, which functions as a simpler surrogate membrane. Therefore, additional research utilising more physiologically relevant membranes and in vivo models is necessary to confirm the clinical and translational relevance of these findings, even though the observed increase in permeation offers a strong preliminary indication of the ability of niosomes to enhance lenalidomide permeability [25].

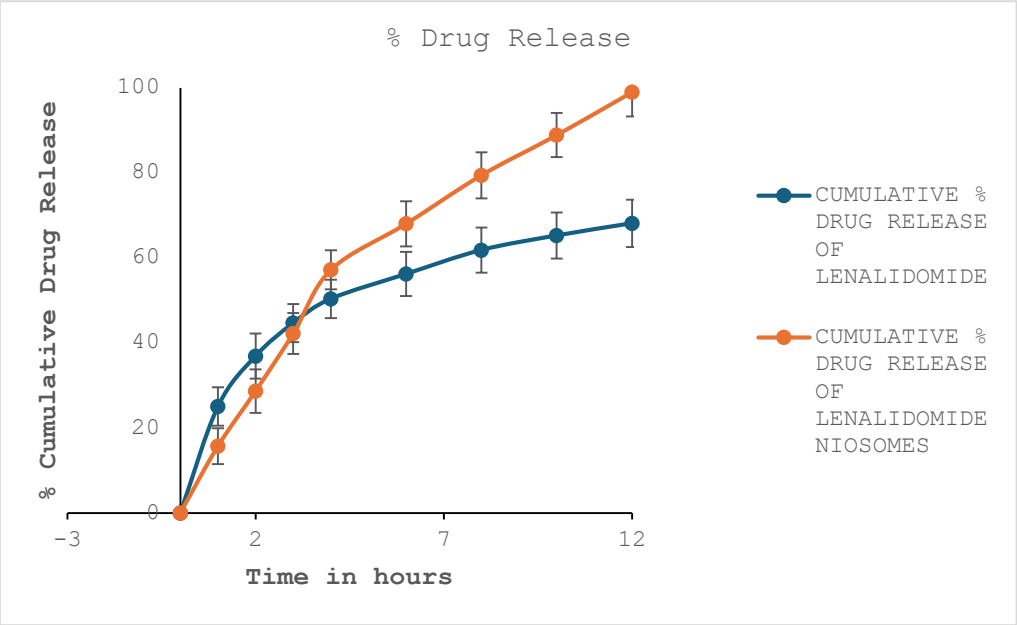


Figure8. Time vs Cumulative % drug release of plain Lenalidomide and Lenalidomide niosomes

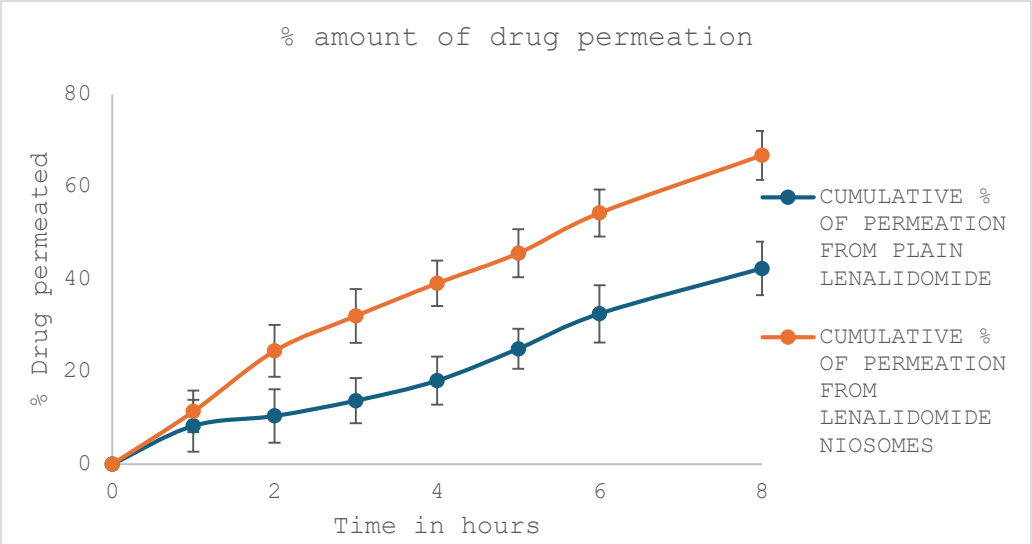


Figure9. Time vs Cumulative % Permeability of Lenalidomide niosome

Conclusion

The current investigation sought to develop, optimise and assess niosomes loaded with lenalidomide. The final formulation (F4) was chosen based on the design space generated through a factorial design approach for optimisation. In comparison to previous batches, the optimised formulation (F4) showed favourable properties such as ideal particle size and homogeneity, excellent entrapment effectiveness, and an improved release profile, suggesting its potential to increase bioavailability and decrease dosage frequency With a particle size of roughly 177.10 nm, niosomes made with Span 80 confirmed their nanoscale range and suggested increased bilayer flexibility that might aid drug penetration through biological membranes. Additionally, after niosomal encapsulation, the release profile showed a higher release rate.

In further research we will concentrate on in vivo studies, including pharmacokinetic and pharmacodynamic assessments in appropriate animal models of multiple myeloma, as the current study was restricted to in vitro evaluations. These investigations would be necessary to establish the Lenalidomide-loaded niosomal formulation's improved bioavailability, therapeutic efficacy, and safety, thereby validating its promise as a clinically feasible drug delivery system.

Materials And Methods

Materials

Lenalidomide was purchased from J. K. Chemicals Gujarat India Cholesterol span 60 and span 80 was purchased from Analab fine Chemicals The all other reagents were of analytical grade.

Pre-formulation studies

Pre-formulation studies involves the physical parameters (Appearance, solubility, melting point), Ultra-violet spectroscopy, FT-IR (Fourier transform- Infrared) studies and DSC (Differential scanning calorimetry) to check the physical parameters and compatibility of drug and excipients.

Formulation of Lenalidomide loaded niosomes

The drug was dissolved into an organic phase i.e. methanol, then it was mixed until compl.etely dissolved. Next, sorbitan mono stearate (Span 80), cholesterol, and lip.id were added into the solution and mixed using a magnetic spin bar in a 20 mL glass beaker. In a separate 50 mL glass beaker, purified water was heated at various temperatures using a hot plate with magnetic stirring. The temperature of the water phase was selected based on the design requirement. The organic phase was filled into a 10 mL syringe with a 26 G needle. The organic phase mixture was injected into the preheated aqueous phase using predetermined parameters based on the experimental design. Mixing was carried out based on the values identified from the design of experiment (DoE). In the last step of the process, the batch was cooled down to RT and the formulation was sto.red in a suit.able glass storage container.

Formulation trial batches of different ratios of span 80 and Span 60 were prepared as seen in table 4.

Sr. no	Surfactant type	Surfactant to drug ratio	Particle size (nm)	Drug content (in %)
1.	Span 80	1:0.5	189..20	82.26
2.	Span 80	1:1	106.0	85.23
3.	Span 80	1:2	254..40	79.18
4.	Span 60	1:0.5	435..77	84.49
5.	Span 60	1:1	348..03	81.86
6.	Span 60	1:2	488.0	83.76

Table 4. Trial batches formulation table

Factor	Symbol	Low (–1)	Medium (0)	High (+1)
Conc. of Span 80 (mg)	X_1	27	30	33
Conc. of Cholesterol (mg)	X_2	57	60	63

Table 5 Independent parameters and levels

The dependent variables (responses) were particle size (Y_1 , nm) and zeta potential (Y_2 , mV). The experimental design generated nine formulation trials (F1–F14), each representing a unique combination of the two independent variables (Table 6).

The statistical analysis, model fitting, and generation of contour and 3D surface response plots were performed using Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, USA). The optimized batch was selected based on achieving the smallest vesicle size, high absolute zeta potential, and satisfactory entrapment efficiency.

Different experimental runs F1 to F14 were prep.ared as shown in table 6

Table 6. Experimental runs

Sr. No.	Batches	Cholesterol Conc.	Span 80
1	F1	0	0
2	F2	-1	+1
3	F3	0	+1
4	F4	+1	+1
5	F5	+1	-1
6	F6	+1	-1
7	F7	-1	0
8	F8	0	0
9	F9	-1	+1
10	F10	+1	0
11	F11	0	-1
12	F12	-1	0
13	F13	-1	-1
14	F14	0	0

Visual Appearance

Dispersion of niosomes was visually inspected to determine its appearance, turbidity and to see the presence of flocculation and phase separation by taking dispersion in transparent container.

Drug content

10 mg equivalent was taken from prepared niosomal suspension and dissolved in 10 ml of methanol. These were kept for the sonication and to check absorbance in the UV spectrophotometer.

Optical Microscopy

The ultrastructure of the niosomal vesicles was observed using the microscope. A slide of a dispersion was applied on to a glass slide and this was covered with a cover slip. The eye piece magnification set to 10X and the objective lens to 40X was used.

Entrapment

The efficiency of entrapping (EE%), can be regarded as the percentage that represents that segment of the amount of the drug used and gets trapped within the niosomes. Centrifugation can be applied to eliminate free drug which has not been encapsulated in the niosomal solution by applying dialysis procedure. In the phase the vesicles destroyed enable the drug that is loaded in niosomes to be released. Niosomal suspension niosomes could be destroyed by addition of 0.1 per cent Triton X-100 or 50 percent propanol. The loaded and free concentration of the drug can be measured by the UV spectrophotometer. In order to measure the entrapment efficiency, it is calculated as follows:

$$\textbf{Entrapment Efficiency (EE\%)} = \frac{\text{Entrapped drug}}{\text{Total amount of added drug}} \times 100$$

Dispersion of size, Poly Dispersity Index and Measurement of Zeta potential:

To obtain a Size Dispersion, and Poly Dispersity Index (PDI), Dynamic light scattering was done. Zeta potential of dispersion value is noted by the exposure of dispersion into electric field. The zeta potential will have a proportionate relationship to the speed of the particles of the dispersion that will be inclined to stray to the electrode of opposite polarity.

***In vitro* release studies**

In vitro release of Lenalidomide from the niosomes was studied in phosphate buffer pH 6.8 for 8 h using United States Pharmacopeia (USP) type II Paddle type apparatus using volume 900 mL, at 100 rpm and 37°C. Samples (5 mL) were withdrawn through pipette at different time intervals and were assayed at 242 nm for Lenalidomide content spectrophotometrically.

***In vitro* permeation study:**

The in vitro permeation study of the prepared niosomes was carried out using Franz diffusion cell through egg shell membrane because the egg shell membrane resembles human stratum corneum as it consists mainly of keratin [26]. The membrane was accordingly prepared before use [27]. The water in the outer jacket of the cell was warmed and set at 37 ± 1◦C throughout the experiments to provide a skin surface temperature. Phosphate buffer solution of pH 6.8 was used as dissolution medium in the receptor compartment. A 10mg equivalent of niosomes was taken and applied over the mounted membrane in diffusion cell. After that, the samples were withdrawn from the receptor compartment at regulated intervals. The sampling schedule was at 0, 1 hour, 2 and then it was at every hour interval till 8th hour of release. One mL of the receptor solution was collected as sample each time and simultaneously one mL of phosphate buffer solution was added back to the receptor cell for maintaining the same initial volume of the receptor cell solution. The collected samples were analysed using UV-Vis spectrophotometer [28].

Acknowledgement

This work was supported by Alard School of Pharmacy, Alard University, Marunji Pune.

Author contribution

Conceptualization and experimental work: Sakshi wavare

Manuscript writing: Prof. Pallavi kaple, Dipali Kamble

Data analysis and interpretation (Results and Discussion): Dr. Shreeya Belwalkar

Supervision and overall guidance: Dr. Shreeya Belwalkar, Pratiksha More

Conflict Of Interest

The authors declare no conflict of interest

References

[1] Cosco D, Paolino D, Muzzalupo R, Celia C, Citraro R, Caponio D, Picci N, Fresta M. Novel PEG-coated niosomes based on bola-surfactant as drug carriers for 5-fluorouracil Biomedical microdevices. 2009 Oct;11(5):1115.

[2] Paolino D, Muzzalupo R, Ricciardi A, Celia C, Picci N, Fresta M. In vitro and in vivo evaluation of Bola-surfactant containing niosomes for transdermal delivery. Biomedical microdevices. 2007 Aug;9(4):421-33.

[3] Junyaprasert VB, Teeranachaideekul V, Supaperm T. Effect of charged and non-ionic membrane additives on physicochemical properties and stability of niosomes. AAPS pharmscitech. 2008 Sep;9(3):851-9.

[4] Waware SU, Singh S, Belwalker S, Kaple P. Role of Artificial Intelligence in Niosomes Development. J Pharm Res Rev Rep. 2025 Feb.;3(2).ISSN: 2583.-973X

[5] Vyas SP, Khar RK. Targeted and Controlled drug delivery: concepts and advances.

[6] Kuldharan RA, Belwalkar S, Singh S, Kaple P. Niosomes: A comprehensive review of their preparation, characterization, and applications

in drug delivery. Int J Creative Res Thoughts. 2025 Apr.;13(4).

ISSN: 2320.-2882

[7] Rajera R, Nagpal K, Singh SK, Mishra DN. Niosomes: a controlled and novel drug delivery system. Biological and Pharmaceutical Bulletin. 2011 Jul 1;34(7):945-53.

[8] Kazi KM, Mandal AS, Biswas N, Guha A, Chatterjee S, Behera M, Kuotsu K. Niosome: a future of targeted drug delivery systems. Journal of advanced pharmaceutical technology & research. 2010 Oct 1;1(4):374-80.

[9] https://go.drugbank.com/drugs/DB00480

[10] https://pubchem.ncbi.nlm.nih.gov/compound/Lenalidom

[11] Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. Nature Reviews Cancer. 2007 Aug;7(8):585-98.

[12] Chng WJ, Santana-Davila R, Van Wier SA, Ahmann GJ, Jalal SM, Bergsagel PL, Chesi M, Trendle MC, Jacobus S, Blood E, Oken MM. Prognostic factors for hyperdiploid-myeloma: effects of chromosome 13 deletions and IgH translocations Leukemia. 2006 May;20(5):807-13.

[13] Van Wier S, Braggio E, Baker A, Ahmann G, Levy J, Carpten JD, Fonseca R. Hypodiploid multiple myeloma is characterized by more aggressive molecular markers than non-hyperdiploid multiple myeloma Haematologica 2013 Oct;98(10):1586.

[14] Smadja NV, Fruchart C, Isnard F, Louvet C, Dutel JL, Cheron N, Grange MJ, Monconduit M, Bastard C. Chromosomal analysis in multiple myeloma: cytogenetic evidence of two different diseases Leukemia 1998 Jun;12(6):960-9.

[15] Fonseca R, Debes-Marun CS, Picken EB, Dewald GW, Bryant SC, Winkler JM, Blood E, Oken MM, Santana-Dávila R, González-Paz N, Kyle RA. The recurrent IgH translocations are highly associated with nonhyperdiploid variant multiple myeloma. Blood. 2003 Oct 1;102(7):2562-7.

[16] Chen N, Zhou S, Palmisano M. Clinical pharmacokinetics and pharmacodynamics of lenalidomide Clinical pharmacokinetics 2017 Feb;56(2):139-52.

[17] Teron C, Bhuyan A, Saikia P, Dutta SR, Gogoi H, Rongpi S. A Comprehensive Review on Niosomes in Drug Delivery and Recent Advancements. Journal of Drug Delivery & Therapeutics. 2024 Sep 1;14(9).

[18] Nguyen MH, Le TH, Nguyen TP, Le TN, Nguyen TY, Nguyen KA, Pham TT, Le MT. Niosomes: recent advances and applications in targeted drug delivery. VNUHCM Journal of Science and Technology Development. 2024 Sep 30;27(3):3507-34.

[19] Sharma, A., & Sharma, U. S. (1997). Niosomes: A Controlled and Novel Drug Delivery System. BioDrugs, 9(6), 397–407.

[20] Moghassemi, S., & Hadjizadeh, A. Niosomes as Nanocarriers for Drug Delivery: A Review of Fundamentals, Properties, and Clinical Applications. Journal of Controlled Release, 185, 22–36 (2014).

[21] Siepmann, J., & Peppas, N. A. (2011). Higuchi Equation: Derivation, Applications, and Limitations. International Journal of Pharmaceutics, 418(1), 6–12.

[22]Korsmeyer, R. W., Gurny, R., Doelker, E., Buri, P., & Peppas, N. A. (1983). Mechanisms of Solute Release from Porous Hydrophilic Polymers. International Journal of Pharmaceutics, 15(1), 25–35.

[23] Honeywell-Nguyen, P. L. & Bouwstra, J. A. (2005). Vesicles as a Tool for Transdermal and Dermal Delivery. Drug Discovery Today: Technologies, 2(1), 67–74.

[24] Touitou, E. & Godin, B. (2009). Enhancement in Drug Delivery Across the Skin Using Liposomes. Journal of Drug Delivery Science and Technology, 19(3), 185–196.

[25] Chandrasekaran, P., & Rajan, V. (2016). In vitro permeation studies using egg membrane for preliminary screening of formulations. International Journal of Pharmaceutical Investigation, 6(4), 224–229.

[26] Haigh JM, Smith EW. The selection and use of natural and synthetic membranes for in vitro diffusion experiments. European journal of pharmaceutical sciences. 1994 Dec 1;2(5-6):311-30.

[27] Shah V, Raval S, Peer S, Upadhyay UM. A comparative evaluation of different membranes for their diffusion efficiency: An in vitro study. Pharma Sci. Monit. 2010;1:41-9.

[28] Suryadevara PK. Formulation and evaluation of antiemetic patch comprising ondansetron hydrochloride [MS thesis]. KLE University, Belgaum, India. 2010.