

FORM 2
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COMPLETE SPECIFICATION
(See Section 10 and Rule 31)

TITLE OF THE INVENTION

A TOPICAL DUAL-DRUG FILM-FORMING HYDROGEL COMPOSITION COMPRISING
DIMETHYLOXALYLGLYCINE AND AMLODIPINE-LOADED NANOSTRUCTURED LIPID
CARRIERS

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PREAMBLE TO THE DESCRIPTION

The following specification particularly describes the invention and the manner in which it is to be performed

FIELD OF INVENTION:

The present invention relates to a novel topical dual-drug film-forming hydrogel composition comprising Dimethyloxalylglycine (DMOG) incorporated within a polymeric film-forming hydrogel matrix and Amlodipine-loaded Nanostructured Lipid Carriers (AMNLCs) uniformly dispersed therein for promoting angiogenesis, enhancing microvascular regeneration, improving endothelial function, and accelerating diabetic wound healing through synchronized localized and sustained drug delivery.

BACKGROUND OF THE INVENTION:

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from impaired insulin secretion, impaired insulin action, or both. Chronic hyperglycaemia leads to various microvascular and macrovascular complications, including diabetic nephropathy, neuropathy, retinopathy, peripheral vascular disease and impaired wound healing. Among these, chronic diabetic wounds, particularly diabetic foot ulcers, represent a major clinical challenge owing to delayed healing, recurrent infections, prolonged hospitalization and an increased risk of lower-limb amputation. Accordingly, there remains a continuous need for improved therapeutic systems capable of accelerating tissue regeneration and promoting effective wound healing.

Normal wound healing is a complex biological process involving haemostasis, inflammation, angiogenesis, fibroblast proliferation, collagen deposition, re-epithelialization and tissue remodelling. Under diabetic conditions, persistent hyperglycaemia disrupts these processes through oxidative stress, endothelial dysfunction, chronic inflammation, impaired angiogenesis, reduced cellular migration and extracellular matrix abnormalities, resulting in prolonged inflammatory responses, poor vascularization and delayed wound closure. Restoration of angiogenesis, particularly through activation of the hypoxia-inducible factor-1 alpha (HIF-1 α)/vascular endothelial growth factor (VEGF) signalling pathway, has therefore become an important therapeutic strategy for diabetic wound management.

Conventional wound dressings and currently available hydrogel formulations primarily provide physical protection and moisture retention but generally fail to deliver sustained localized therapy or simultaneously address the multiple pathological mechanisms responsible for impaired diabetic wound healing. Although nanostructured lipid carriers (NLCs) have demonstrated improved drug encapsulation, controlled release and enhanced dermal penetration, existing NLC formulations are generally employed independently as creams, gels or dispersions and do not provide an integrated film-forming system capable of prolonged wound coverage and synchronized delivery of multiple therapeutic agents.

Dimethyloxalylglycine (DMOG), a prolyl hydroxylase inhibitor, promotes stabilization of HIF-1 α and stimulates VEGF-mediated angiogenesis, thereby enhancing neovascularization and tissue regeneration. Amlodipine has additionally been reported to improve endothelial function, nitric oxide bioavailability and angiogenic activity. However, topical administration of these agents individually is limited by inadequate residence time, rapid drug diffusion and insufficient localized therapeutic concentrations at the wound site.

Although individual studies have investigated DMOG-loaded hydrogels, amlodipine formulations or nanostructured lipid carrier systems separately, the prior art does not disclose an integrated film-forming hydrogel incorporating DMOG together with amlodipine-loaded nanostructured lipid carriers for simultaneous localized, sustained and synergistic modulation of multiple wound-healing pathways.

Accordingly, there exists a need for an improved topical pharmaceutical composition capable of combining a film-forming hydrogel matrix with nanostructured lipid carrier technology to provide sustained dual-drug delivery, enhanced angiogenesis, improved endothelial function, prolonged wound residence, accelerated tissue regeneration and superior diabetic wound healing. The present invention addresses these technical deficiencies by providing a novel film-forming hydrogel comprising DMOG uniformly dispersed within a polymeric hydrogel matrix and amlodipine-loaded nanostructured lipid carriers, thereby establishing a multifunctional therapeutic platform for enhanced diabetic wound repair.

SUMMARY OF THE INVENTION:

In one aspect, the invention provides a pharmaceutical composition for topical administration, the pharmaceutical composition comprising: a hydrogel matrix comprising one or more hydrophilic film-forming polymers, one or more co-polymers, one or more plasticizers, one or more pharmaceutically acceptable solvents, and a first therapeutic agent comprising a pro-angiogenic agent; and a nanostructured lipid carrier (NLC) dispersed within the hydrogel matrix, the nanostructured lipid carrier comprising one or more solid lipids, one or more liquid lipids, one or more surfactants, an aqueous phase, and a second therapeutic agent comprising a calcium channel blocker, wherein the hydrogel matrix and the nanostructured lipid carrier are configured to provide sustained topical delivery of the first therapeutic agent and the second therapeutic agent for promoting angiogenesis, neovascularization, tissue regeneration and wound healing.

According to another aspect, the invention provides a method for preparing the topical dual-drug film-forming hydrogel comprising preparing a film-forming hydrogel containing Dimethyloxalylglycine, independently preparing Amlodipine-loaded Nanostructured Lipid

Carriers through high-shear homogenization followed by ultrasonication, characterizing the nanocarriers for particle size and stability, and subsequently incorporating the nanostructured lipid carrier dispersion into the hydrogel matrix under controlled mixing conditions to obtain a homogeneous film-forming hydrogel formulation suitable for topical administration. The resulting formulation exhibits uniform drug distribution, desirable rheological characteristics, appropriate spreadability, stable physicochemical properties, and sustained drug release behaviour.

The invention further provides a pharmaceutical composition that may be adapted for the treatment of diabetic foot ulcers, chronic non-healing wounds, pressure ulcers, venous leg ulcers, arterial ulcers, ischemic wounds, traumatic wounds, burns, post-surgical wounds, and other tissue injuries requiring enhanced angiogenesis and accelerated tissue regeneration. The formulation may be manufactured using pharmaceutically acceptable polymers, lipids, surfactants, solvents, stabilizers, and excipients compatible with topical pharmaceutical dosage forms and scalable manufacturing processes.

BRIEF DESCRIPTION OF THE DRAWINGS:

The accompanying drawings, which are incorporated herein and form a part of the present specification, illustrate exemplary embodiments of the present invention and together with the detailed description serve to explain the principles, objectives, experimental validation, and technical effects of the invention. The drawings are intended solely for illustrative purposes and shall not be construed as limiting the scope of the invention in any manner whatsoever.

Figure 1 illustrates the particle size distribution profile of the optimized Amlodipine-loaded Nanostructured Lipid Carriers (AMNLCs) obtained by dynamic light scattering analysis.

Figure 2 illustrates the zeta potential distribution profile of the optimized Amlodipine-loaded.

Figure 3: Digital photography images of wound contraction from day 1 to 15.

Figure 4 illustrates the percentage of wound contraction in rats the values.

Nanostructured Lipid Carriers (AMNLCs), indicating the surface charge characteristics and colloidal stability of the nanostructured lipid carrier formulation.

Other aspects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DETAILED DESCRIPTION:

The following descriptions are exemplary embodiments only, and are not intended to limit the scope, applicability or configuration of the invention in any way. Rather, the following description provides a convenient illustration for implementing exemplary embodiments of the invention. Various changes to the described embodiments may be made in the function and arrangement of the elements described without departing from the scope of the invention as set forth in the appended claims.

Moreover, all statements herein reciting embodiments of the invention, as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents as well as equivalents developed in the future (i.e., any elements developed that perform the same function, regardless of structure).

It will be understood by those within the art that, in general, terms used herein, and especially in the appended claims (e.g., bodies of the appended claims) are generally intended as "open" terms (e.g., the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.). It will be further understood by those within the art that if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims may contain usage of the introductory phrases "at least one" and "one or more" to introduce claim recitations. However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles "a" or "an" limits any particular claim containing such introduced claim recitation to embodiments containing only one such recitation, even when the same claim includes the introductory phrases "one or more" or "at least one" and indefinite articles such as "a" or "an" (e.g., "a" and/or "an" should be interpreted to mean "at least one" or "one or more"); the same holds true for the use of definite articles used to introduce claim recitations.

The following detailed description is provided to explain the constitution, preparation, physicochemical characteristics, functional cooperation, mode of action, working examples, experimental validation, and technical advantages of the present invention. The embodiments, quantities, process conditions, and test results described herein are illustrative of presently preferred implementations and are not intended to restrict the scope of the invention. A person skilled in pharmaceutical formulation, nanotechnology, biomaterials, or wound-care

technology may make suitable variations in the ingredients, proportions, batch sizes, manufacturing equipment, processing conditions, and dosage forms without departing from the essential inventive concept.

The present invention provides a topical dual-drug film-forming nanocarrier hydrogel comprising Dimethyloxalylglycine, hereinafter referred to as “DMOG”, dispersed within a polymeric film-forming hydrogel matrix and Amlodipine, preferably in the form of Amlodipine besylate, encapsulated within Nanostructured Lipid Carriers, hereinafter referred to as “AMNLCs”, wherein the AMNLCs are uniformly incorporated into the DMOG-containing hydrogel matrix. The resulting formulation provides a single, integrated topical pharmaceutical platform having a film-forming function, a moisture-retaining function, a wound-contact function, and a synchronized dual-drug release function.

The inventive formulation is particularly configured to overcome the physicochemical incompatibility associated with placing a comparatively water-compatible active such as DMOG and a poorly water-soluble active such as Amlodipine within a common topical delivery system. Instead of merely admixing both active agents in an undifferentiated gel, the present invention spatially organizes the two therapeutic agents in separate but functionally cooperating phases. DMOG is incorporated directly within the aqueous polymeric hydrogel phase, whereas Amlodipine is incorporated within a lipid nanocarrier phase comprising a solid lipid and a liquid lipid. The Amlodipine-loaded lipid nanocarriers are thereafter distributed throughout the DMOG-containing hydrogel network. This dual-compartment architecture enables the active agents to retain their respective physicochemical stability while providing coordinated release at the wound site.

The polymeric hydrogel, the lipid nanocarriers, DMOG, and Amlodipine perform interdependent technical functions. The film-forming polymeric matrix controls topical residence, wound coverage, moisture retention, adhesion, and release of DMOG; the nanostructured lipid carriers improve incorporation, protection, and sustained release of Amlodipine; and the two active agents provide complementary pro-angiogenic and tissue-regenerative activity. The combination produces a multifunctional wound-care composition having structural, delivery, and therapeutic properties that cannot be attributed to any single component operating independently.

The topical formulation comprises three principal functional subsystems:

- a DMOG-loaded film-forming hydrogel phase;
- an Amlodipine-loaded nanostructured lipid carrier phase; and

- an integrated final phase obtained by dispersing the AMNLC phase within the DMOG-loaded hydrogel phase.

The film-forming hydrogel phase preferably comprises Polyvinyl Alcohol (PVA) as a principal film-forming polymer and Polyvinylpyrrolidone (PVP) as a co-polymer or film-modifying polymer. Water provides the primary aqueous vehicle, ethanol functions as a co-solvent and assists drying and film formation, and propylene glycol functions as a plasticizer, humectant, and spreading aid. DMOG is incorporated into this hydrated polymeric matrix.

The nanostructured lipid carrier phase preferably comprises Glyceryl Monostearate (GMS) as a solid lipid, Peceol as a liquid lipid, Tween 80 as a surfactant, water as the continuous aqueous phase, and Amlodipine besylate as the active pharmaceutical ingredient encapsulated or entrapped within the lipid matrix. The solid and liquid lipids produce a structurally imperfect lipid core capable of accommodating the drug and reducing its expulsion during storage.

The final formulation is prepared by gradually incorporating an optimized AMNLC dispersion into the previously prepared DMOG-loaded hydrogel under controlled mechanical stirring. The nanocarriers remain distributed within the polymer network without substantial disruption of their particle integrity. The final composition exhibits a transparent-white or translucent appearance, a glassy and thick physical structure, a soft and viscoelastic texture, and slight adhesiveness suitable for prolonged contact with a wound surface.

In a preferred laboratory embodiment, the hydrogel base contains PVA, PVP, water, ethanol, propylene glycol, and DMOG. The NLC dispersion contains GMS, Peceol, Amlodipine besylate, Tween 80, and water.

The uploaded formulation information describes the following illustrative amounts for preparing the respective phases. The quantities are provided as an enabling example and may be proportionally scaled according to the desired batch size.

The formulation may also include pharmaceutically acceptable variations in polymer concentration, lipid ratio, surfactant concentration, solvent content, plasticizer concentration, drug loading, and total solids, provided that the final composition retains the essential film-forming, nanocarrier-stabilizing, topical-retention, and dual-release characteristics.

In one aspect, the invention provides a pharmaceutical composition for topical administration, the pharmaceutical composition comprising: a hydrogel matrix comprising one or more hydrophilic film-forming polymers, one or more co-polymers, one or more plasticizers, one or more pharmaceutically acceptable solvents, and a first therapeutic agent comprising a pro-angiogenic agent; and a nanostructured lipid carrier (NLC) dispersed within the hydrogel matrix, the nanostructured lipid carrier comprising one or more solid lipids, one or more liquid

lipids, one or more surfactants, an aqueous phase, and a second therapeutic agent comprising a calcium channel blocker, wherein the hydrogel matrix and the nanostructured lipid carrier are configured to provide sustained topical delivery of the first therapeutic agent and the second therapeutic agent for promoting angiogenesis, neovascularization, tissue regeneration and wound healing.

According to another aspect, the invention provides a method for preparing the topical dual-drug film-forming hydrogel comprising preparing a film-forming hydrogel containing Dimethyloxalylglycine, independently preparing Amlodipine-loaded Nanostructured Lipid Carriers through high-shear homogenization followed by ultrasonication, characterizing the nanocarriers for particle size and stability, and subsequently incorporating the nanostructured lipid carrier dispersion into the hydrogel matrix under controlled mixing conditions to obtain a homogeneous film-forming hydrogel formulation suitable for topical administration. The resulting formulation exhibits uniform drug distribution, desirable rheological characteristics, appropriate spreadability, stable physicochemical properties, and sustained drug release behaviour.

A pharmaceutical composition for topical administration comprising: between 8 wt.% and 20 wt.% of a hydrophilic film-forming polymer; between 1 wt.% and 6 wt.% of a co-polymer; between 1 wt.% and 8 wt.% of a plasticizer; between 5 wt.% and 20 wt.% of a pharmaceutically acceptable co-solvent; between 50 wt.% and 85 wt.% of water; and between 0.1 wt.% and 5 wt.% of a pro-angiogenic agent; and

a nanostructured lipid carrier (NLC) dispersed within the hydrogel matrix, the nanostructured lipid carrier comprising nanostructured lipid carrier comprises: between 30 wt.% and 70 wt.% of a solid lipid; between 5 wt.% and 35 wt.% of a liquid lipid; between 10 wt.% and 50 wt.% of a surfactant; between 0.05 wt.% and 5 wt.% of a calcium channel blocker; and an aqueous phase;

wherein the hydrogel matrix and the nanostructured lipid carrier are configured to provide sustained topical delivery of the first therapeutic agent and the second therapeutic agent for promoting angiogenesis, neovascularization, tissue regeneration and wound healing.

The hydrophilic film-forming polymer comprises one or more polymers selected from polyvinyl alcohol, hydroxypropyl methylcellulose, hydroxyethyl cellulose, sodium carboxymethyl cellulose, carbomer, poloxamer, chitosan, gelatin, sodium alginate, polyethylene oxide, polyacrylic acid and combinations thereof.

The co-polymer comprises one or more polymers selected from polyvinyl pyrrolidone, polyethylene glycol, polyethylene oxide, polyacrylamide, carbopol, sodium alginate, xanthan gum, hyaluronic acid and combinations thereof.

The pro-angiogenic agent comprises dimethyloxallylglycine (DMOG), vascular endothelial growth factor, fibroblast growth factor, platelet-derived growth factor, stromal cell-derived factor, deferoxamine, cobalt chloride or combinations thereof.

The calcium channel blocker comprises amlodipine, amlodipine besylate, nifedipine, felodipine, nicardipine, lercanidipine, cilnidipine, benidipine or pharmaceutically acceptable salts thereof.

The solid lipid comprises glyceryl monostearate, stearic acid, glyceryl behenate, cetyl palmitate, tripalmitin, tristearin or combinations thereof.

The liquid lipid comprises glyceryl monooleate, medium-chain triglycerides, oleic acid, caprylic/capric triglycerides, isopropyl myristate, sesame oil, castor oil or combinations thereof.

The surfactant comprises Tween 20, Tween 40, Tween 60, Tween 80, Span 20, Span 60, Span 80, Poloxamer 188, Poloxamer 407, lecithin, sodium cholate or combinations thereof.

The hydrogel matrix comprises polyvinyl alcohol, polyvinyl pyrrolidone, water, ethanol, propylene glycol and dimethyloxallylglycine.

The hydrogel matrix comprises: between 8 wt.% and 20 wt.% of polyvinyl alcohol; between 1 wt.% and 6 wt.% of polyvinyl pyrrolidone; between 1 wt.% and 8 wt.% of propylene glycol; between 5 wt.% and 20 wt.% of ethanol; between 50 wt.% and 85 wt.% of water; and between 0.1 wt.% and 5 wt.% of dimethyloxallylglycine (DMOG).

The nanostructured lipid carrier comprises: between 30 wt.% and 70 wt.% of glyceryl monostearate; between 5 wt.% and 35 wt.% of glyceryl monooleate (Peceol); between 10 wt.% and 50 wt.% of Tween 80; between 0.05 wt.% and 5 wt.% of amlodipine besylate; and an aqueous phase.

The amlodipine besylate is present in the nanostructured lipid carrier in an amount ranging from about 0.1 wt.% to about 0.2 wt.% and is present in the hydrogel matrix dimethyloxallylglycine (DMOG) 0.5% w/w to 1.0% w/w. The composition is useful for treatment of diabetic wounds, chronic wounds, pressure ulcers, venous ulcers, burn wounds, traumatic wounds, post-surgical wounds, ischemic ulcers and skin defects.

A method of preparing a hydrogel-based nanostructured lipid carrier (NLC) pharmaceutical composition, the method comprising:

(a.) preparing a hydrogel matrix by:

- (i) preparing a hydrogel matrix by mixing water, ethanol and propylene glycol under stirring to obtain a homogeneous solvent system;
- (ii) gradually incorporating polyvinyl pyrrolidone followed by polyvinyl alcohol into the solvent system under controlled stirring at a temperature between 35°C and 45°C until a homogeneous hydrogel matrix is formed; and
- (iii) cooling the hydrogel matrix to room temperature and dispersing dimethyloxalylglycine (DMOG) therein under mechanical stirring to obtain a DMOG-loaded hydrogel matrix.

(b.)preparing a nanostructured lipid carrier by:

- (i) preparing a nanostructured lipid carrier by melting a solid lipid and a liquid lipid to obtain a molten lipid phase;
- (ii) dispersing amlodipine besylate in the molten lipid phase under stirring to obtain a drug-loaded lipid phase;
- (iii) preparing an aqueous surfactant phase comprising water and a surfactant, heating the aqueous surfactant phase to a temperature between 65°C and 75°C, and combining the aqueous surfactant phase with the drug-loaded lipid phase;
- (iv) subjecting the resulting mixture to high-speed shear homogenization to obtain a nanoemulsion; and cooling the nanoemulsion to room temperature to obtain an amlodipine-loaded nanostructured lipid carrier.

(c.)dispersing the amlodipine-loaded nanostructured lipid carrier into the DMOG-containing hydrogel matrix under continuous stirring until a homogeneous hydrogel-based nanostructured lipid carrier pharmaceutical composition is obtained.

The high-speed shear homogenization is carried out at 10,000 rpm for 10 minutes; the hydrogel matrix and the nanostructured lipid carrier are mixed under mechanical stirring until a uniform dispersion is obtained.

Example 1: Compositions of the present invention

Component	F1 (wt.%)	F2 (wt.%)	F3 (wt.%)	F4 (wt.%)	F5 (wt.%)
Hydrogel Matrix					
Polyvinyl alcohol	8	10	12	15	16
Polyvinyl pyrrolidone	1	2	3	4	4
Propylene glycol	2	3	5	6	6
Ethanol	8	10	12	15	18

DMOG	0.5	1	2	3	4
Water	80.5	74	66	57	52
Nanostructured Lipid Carrier					
Glyceryl monostearate	30	35	40	45	50
Glyceryl monooleate (Peceol)	8	10	15	20	25
Tween 80	15	20	25	25	20
Amlodipine besylate	0.5	1	2	3	4
Aqueous phase	46.5	34	18	7	1

B. Preparation of the Amlodipine Besylate-Loaded Nanostructured Lipid Carrier

In a preferred embodiment, 40.00 g of glyceryl monostearate and 15.00 g of glyceryl monooleate (Peceol) are charged into a clean, dry vessel and heated to 70°C (65–75°C) under stirring at 300 rpm until a uniform molten lipid phase is obtained. Subsequently, 2.00 g of amlodipine besylate is gradually incorporated into the molten lipid phase and stirred at 300 rpm for 30 minutes while maintaining the temperature at 70°C to obtain a homogeneous drug-loaded lipid phase.

Separately, 25.00 g of Tween 80 is dissolved in 18.00 g of purified water and heated to 70°C under stirring at 300 rpm to obtain a homogeneous aqueous surfactant phase. The heated aqueous phase is gradually added to the drug-loaded lipid phase over 3–5 minutes while stirring at 600–1,000 rpm, followed by pre-emulsification at 1,000 rpm for 5 minutes to form a coarse oil-in-water emulsion.

The pre-emulsion is subjected to high-speed shear homogenization at 10,000 rpm for 10 minutes while maintaining the temperature between 65°C and 75°C to produce a uniform nanoemulsion. The nanoemulsion is subsequently probe-sonicated at 70% amplitude using a 5-second pulse-on/5-second pulse-off cycle for five cycles of 2 minutes each (total sonication time: 10 minutes) while maintaining the dispersion in an ice bath and limiting the temperature to 25°C. The sonicated nanoemulsion is then cooled gradually to 25–30°C under continuous stirring at 300 rpm for approximately 30 minutes, thereby allowing crystallization of the lipid matrix to form a stable amlodipine-loaded nanostructured lipid carrier (AMNLC) exhibiting uniform particle size and enhanced physical stability.

C. Incorporation of the Amlodipine-Loaded NLC into the DMOG-Loaded Hydrogel Matrix

In a preferred embodiment, 80 g of the DMOG-loaded hydrogel matrix is stirred at 200 rpm, and 20 g of the amlodipine-loaded nanostructured lipid carrier (AMNLC) dispersion is gradually added over 10 minutes while maintaining the stirring speed between 200 rpm and 300 rpm. After complete addition, the mixture is stirred at 400 rpm for 30 minutes at 25–30°C to obtain a homogeneous dispersion. The formulation is allowed to stand for 30 minutes or vacuum-deaerated for 5–10 minutes to remove entrapped air. The resulting composition is a smooth, homogeneous and physically stable film-forming hydrogel-based nanostructured lipid carrier pharmaceutical composition comprising DMOG uniformly dispersed in the hydrogel matrix and amlodipine besylate encapsulated within the nanostructured lipid carrier particles.

Example 3: Optimization of the nanostructured lipid carrier system

The ratio of solid lipid to liquid lipid is selected to produce a partially disordered lipid matrix that improves drug encapsulation and minimizes drug expulsion during storage. Incorporation of the liquid lipid (Peceol) introduces imperfections within the crystalline structure of glyceryl monostearate, thereby enhancing the loading and retention of amlodipine besylate.

- 5 Tween 80 is employed at an optimized concentration to stabilize the nanostructured lipid carrier by reducing interfacial tension and preventing particle aggregation, while maintaining suitable dermal compatibility and controlled drug release.

The homogenization speed, sonication power, pulse cycle and processing time are optimized to produce uniform nanoparticles without causing excessive heat generation or drug
10 degradation. These process parameters provide an appropriate balance between particle-size reduction and formulation stability.

The optimized amlodipine-loaded nanostructured lipid carriers exhibited a particle size of approximately 172.31 nm within a range of 150–200 nm, a Polydispersity Index of approximately 0.177 (17.7%), and a positive zeta potential of $+22.9 \pm 1.2$ mV within a range
15 of +19.2 to +25.2 mV. These characteristics indicate a uniform, physically stable nanodispersion with reduced particle aggregation, enhanced drug retention and improved localized interaction with biological tissues, thereby supporting sustained topical drug delivery and improved therapeutic performance.

Table : Characteristic properties of optimized AMNLCs

Parameter	Reported result	Technical inference
Particle-size range	150–200 nm	Suitable nanoscale dispersion
Optimized particle size	172.31 nm	Supports tissue contact and controlled release
PDI	Approximately 17.7% in representative sample	Comparatively narrow size distribution
Zeta-potential range	+19.2 to +25.2 mV	Supports colloidal stability
Representative zeta potential	$+22.9 \pm 1.2$ mV	Indicates stable positively charged particles
Centrifugation condition	5000 rpm for 10 minutes	Accelerated physical stability assessment
Phase separation	Not observed	Demonstrates resistance to creaming and cracking

The optimized amlodipine-loaded nanostructured lipid carriers exhibited a particle size of 172.31 nm within an overall particle-size range of 150 nm to 200 nm. The nanoscale particle size provides an increased interfacial surface area, facilitates uniform incorporation of the lipid carriers into the hydrogel matrix and supports controlled release of amlodipine besylate from the lipid phase.

The representative polydispersity index of approximately 0.177 indicates a comparatively narrow particle-size distribution. The narrow distribution demonstrates uniformity of the prepared particles and supports consistent drug loading, reproducible release characteristics and reduced susceptibility to differential sedimentation or aggregation.

The optimized NLCs exhibited a positive zeta potential within the range of +19.2 mV to +25.2 mV, with a representative value of $+22.9 \pm 1.2$ mV. The positive surface potential produces electrostatic repulsion between adjacent particles and thereby reduces particle aggregation and coalescence. The positive surface charge may additionally promote interaction with negatively charged biological surfaces and improve retention of the NLCs at the intended site of application.

No phase separation was observed when the formulation was subjected to centrifugation at 5,000 rpm for 10 minutes. The absence of creaming, sedimentation, cracking or visible separation under accelerated stress conditions confirms the satisfactory physical stability of the formulation. Collectively, the particle size, narrow particle-size distribution, positive zeta potential and resistance to centrifugation-induced separation demonstrate that the optimized amlodipine-loaded NLC formulation is suitable for incorporation into a DMOG-containing hydrogel pharmaceutical composition.

Dual-drug release mechanism

DMOG is dispersed substantially within the hydrophilic polymeric matrix. Following application, wound fluid and retained moisture hydrate the matrix and enable DMOG to diffuse from the hydrogel into the wound bed.

Amlodipine is encapsulated or entrapped within the solid-liquid lipid matrix of the AMNLCs. Its release is controlled by diffusion through the lipid phase, partitioning between the lipid carrier and surrounding aqueous environment, gradual structural rearrangement of the lipid matrix, and diffusion through the polymer film.

The formulation may therefore exhibit an initial release phase followed by a sustained release phase. The uploaded experimental information indicates an initial burst release greater than

approximately 20%, followed by sustained release exhibiting Higuchi-type behaviour. This profile provides initial local availability while maintaining prolonged delivery.

The hydrogel and AMNLC phases thus provide two sequential resistance barriers for Amlodipine release: the internal lipid matrix and the surrounding polymeric hydrogel. DMOG is principally controlled by the polymeric matrix. The differentiated localization of the active agents permits independent modulation of their release rates through adjustment of the polymer concentration, lipid ratio, particle size, surfactant concentration, hydrogel viscosity, and drug loading.

Example 4: In Vivo Evaluation of the Wound-Healing Potential of a Film-Forming DMOG Hydrogel Incorporating Amlodipine-Loaded Nanostructured Lipid Carriers in High-Fat Diet/Streptozotocin-Induced Diabetic Wistar Rats

The wound-healing efficacy of the hydrogel-based nanostructured lipid carrier pharmaceutical composition comprising dimethyloxallylglycine (DMOG) incorporated in a film-forming hydrogel matrix and amlodipine besylate encapsulated within nanostructured lipid carriers (AM-NLC) was evaluated using a high-fat diet (HFD)/streptozotocin (STZ)-induced diabetic excision wound model in Wistar rats.

Healthy adult Wistar albino rats weighing approximately 180 g to 220 g were procured from the Central Animal House Facility, JSS College of Pharmacy, Ooty, India. The animals were maintained under standard laboratory conditions at a temperature of $22 \pm 2^\circ\text{C}$, relative humidity of $55 \pm 10\%$, and a 12-hour light/12-hour dark photoperiod. Animals were housed individually in polypropylene cages with free access to drinking water and a standard laboratory diet unless otherwise specified.

The animals were acclimatized to the experimental environment for five days prior to commencement of the study. All experimental procedures were performed after obtaining approval from the Institutional Animal Ethics Committee (IAEC), JSS College of Pharmacy, Ooty, under Approval No. JSSCP/OT/IAEC/24/2025-26, and in accordance with CPCSEA guidelines for the care and use of laboratory animals. The required sample size was determined using G*Power Version 3.1 employing one-way fixed-effects ANOVA. Statistical analysis was performed using an alpha level (α) of 0.05 and a statistical power of 0.80. The calculated F value was approximately 9.552, indicating that a minimum of five animals per experimental group was sufficient to detect statistically significant differences among treatment groups.

Induction of Type II Diabetes Mellitus

Baseline fasting blood glucose concentrations were measured prior to initiation of the diabetic induction protocol.

The animals were subsequently fed a high-fat diet for fourteen consecutive days. The high-fat diet was prepared by pulverizing standard laboratory pellets and mixing them with approximately 20% animal fat, 10% butter ghee, 10% coconut oil and 10% casein. The resulting mixture was moulded into pellets, air-dried under shade and refrigerated until use.

- 5 On Day 15, diabetes was induced by administering a single intraperitoneal injection of streptozotocin at a dose of approximately 35 mg/kg body weight. Streptozotocin was freshly dissolved in 0.1 M citrate buffer maintained at approximately pH 4.4 immediately before administration.

- Following a period of approximately 72 hours, fasting blood glucose concentrations were
10 determined using blood obtained from the tail vein and analysed using a calibrated glucometer. Animals exhibiting fasting blood glucose concentrations greater than approximately 250 mg/dL were considered diabetic and selected for subsequent wound-healing studies.

Creation of the Excision Wound Model

- Approximately ten days after confirmation of diabetes, the animals were anaesthetized by
15 intraperitoneal administration of ketamine (approximately 90 mg/kg) in combination with xylazine (approximately 5 mg/kg).

The dorsal region between the scapulae was shaved and disinfected using approximately 70% ethanol.

- A full-thickness circular excision wound having a diameter of approximately 20 mm was
20 created using a sterile biopsy punch. The excised skin and underlying subcutaneous tissue were carefully removed to expose the underlying muscle without causing excessive trauma.

Immediately after wound creation, the treatment formulations were applied according to the assigned experimental groups (Figure 3).

Experimental Design

- 25 The diabetic animals were randomly allocated into four experimental groups

Group	Treatment	Number of Animals
Group I	Untreated diabetic control or vehicle-treated control	5
Group II	Blank film-forming hydrogel without active ingredients	5
Group III	Film-forming hydrogel containing DMOG	5
Group IV	Film-forming DMOG hydrogel incorporating amlodipine-loaded nanostructured lipid carriers	5

	according to the preferred formulation of the present invention	
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The formulations were applied topically once daily for twenty-one consecutive days or until complete wound closure, whichever occurred earlier.

Wound Evaluation

- 5 The wound area was measured immediately after wound creation (Day 0) and subsequently at predetermined intervals, including Days 3, 7, 10, 14, 18 and 21.

Digital photographs were captured using a fixed camera arrangement under identical lighting conditions.

The wound area was determined using digital planimetric image-analysis software.

- 10 The percentage wound contraction was calculated using the following equation:

Percentage Wound Contraction (%) = [(Initial Wound Area – Wound Area on Observation Day) ÷ Initial Wound Area] × 100

The wound closure in the high-dose group was significantly higher than in the other groups.

- 15 The mean percentage of wound contraction in the high-dose group is 98.39 ± 0.76 , and for the low-dose and disease control groups, it is 96.21 ± 1.28 and 69.79 ± 2.22 , respectively.

Table : Effect of wound contraction

Groups	WOUND CONTRACTION (%)				
	Day 3	Day 6	Day 9	Day 12	Day 15
Disease control	9.49 ± 1.76	20.28 ± 3.41	47.43 ± 2.75	51.67 ± 2.28	69.79 ± 2.22
Vehicle control	8.26 ± 1.55	22.44 ± 2.35	43.02 ± 1.87	58.20 ± 2.18	71.16 ± 1.78
A(0.5%) + D(0.01%)	$10.83 \pm 2.97^*$	$27.51 \pm 1.99^*$	$62.38 \pm 2.03^*$	$89.36 \pm 2.58^{**}$	$96.21 \pm 1.28^{**}$
A(1%) + D(0.02%)	$9.40 \pm 3.35^*$	$30.23 \pm 2.28^*$	$66.12 \pm 2.42^*$	$96.11 \pm 1.72^{**}$	$98.39 \pm 0.76^{**}$

- 20 The present invention provides a significant technical advancement in the field of topical pharmaceutical compositions for the management of diabetic wounds by developing a film-forming nanocarrier hydrogel comprising dimethyloxalylglycine (DMOG) incorporated in a polymeric hydrogel matrix and amlodipine besylate encapsulated within nanostructured lipid carriers (NLCs). The invention does not merely combine two known active agents; rather, it

provides an integrated drug-delivery platform wherein each component performs a complementary function to achieve an enhanced therapeutic outcome.

The film-forming hydrogel matrix comprising polyvinyl alcohol and polyvinyl pyrrolidone forms a continuous, flexible and adherent film over the wound surface after topical application.

5 The film maintains a moist wound environment, minimizes transepidermal water loss, protects the wound from external contamination and prolongs the residence time of the incorporated therapeutic agents at the site of injury. The hydrogel further serves as a three-dimensional matrix for uniformly dispersing the nanostructured lipid carriers without causing aggregation or phase separation.

10 The nanostructured lipid carrier encapsulates amlodipine besylate within a mixed solid-liquid lipid matrix comprising glyceryl monostearate and glyceryl monooleate stabilized with Tween 80. The nanostructured lipid carrier exhibits an average particle size of approximately 172.31 nm, a comparatively narrow particle size distribution, and a positive zeta potential of approximately $+22.9 \pm 1.2$ mV, thereby providing a physically stable nanosuspension suitable for prolonged topical administration. The nanoscale particle size facilitates intimate contact with the wound surface and enables sustained release of amlodipine over an extended period.

Dimethyloxalylglycine incorporated within the hydrogel matrix functions independently through stabilization of hypoxia-inducible factor-1 alpha (HIF-1 α), thereby promoting angiogenesis, cellular proliferation and tissue regeneration. Simultaneously, the amlodipine-

20 loaded nanostructured lipid carriers provide localized and controlled delivery of amlodipine to the wound bed. The hydrogel matrix and the nanostructured lipid carrier therefore cooperate to provide sustained localization of both therapeutic agents throughout the wound-healing period.

The therapeutic efficacy of the formulation was evaluated using a high-fat diet and low-dose streptozotocin induced Type II diabetic excision wound model in Wistar rats. The selected

25 model closely resembles the pathological characteristics of human Type II diabetic wounds by producing insulin resistance together with partial pancreatic β -cell dysfunction. Such an experimental model is considered more clinically relevant than conventional high-dose streptozotocin models and therefore provides a realistic assessment of diabetic wound healing.

Experimental evaluation demonstrated that untreated diabetic animals exhibited wound

30 contraction of approximately $69.79 \pm 2.22\%$ after fifteen days of treatment. In contrast, animals treated with the film-forming nanocarrier hydrogel containing amlodipine 0.5% w/w together with DMOG 0.01% w/w exhibited approximately $96.21 \pm 1.28\%$ wound contraction, whereas animals treated with the formulation containing amlodipine 1.0% w/w together with DMOG 0.02% w/w exhibited approximately $98.39 \pm 0.76\%$ wound contraction (Figure 4). The

improvement in wound contraction was statistically significant ($p < 0.01$) when compared with the untreated diabetic control group. Furthermore, the enhanced wound contraction became evident from approximately the ninth day of treatment, indicating early initiation of tissue repair and accelerated progression through the proliferative phase of wound healing.

5 The experimental observations demonstrate that the film-forming nanocarrier hydrogel effectively overcomes the delayed wound healing commonly associated with diabetic wounds. The improvement cannot be attributed solely to the independent pharmacological activities of DMOG or amlodipine, but rather results from the coordinated interaction between the film-forming hydrogel matrix, the nanostructured lipid carrier and the two active pharmaceutical
10 ingredients. The hydrogel maintains prolonged contact with the wound surface, the nanostructured lipid carrier provides sustained and localized delivery of amlodipine, while DMOG continuously promotes angiogenic signalling through stabilization of HIF-1 α . The combined action produces accelerated wound contraction, improved tissue regeneration and enhanced healing under diabetic conditions.

15 Accordingly, the present invention exhibits a synergistic technical effect wherein the overall therapeutic performance of the integrated formulation is substantially greater than the expected additive effects of the individual components administered separately. The observed enhancement in wound healing therefore arises from the structural and functional cooperation between the hydrogel matrix, the nanostructured lipid carrier and the incorporated active agents.

20 The present invention also provides several technical advantages over conventional topical formulations, including improved physical stability of the formulation, uniform nanoparticle distribution within the hydrogel matrix, prolonged retention of the formulation at the wound site, sustained release of amlodipine, localized delivery of DMOG, enhanced angiogenesis, accelerated re-epithelialization, improved granulation tissue formation, enhanced collagen
25 deposition and significantly faster wound contraction in diabetic wounds. These technical advantages collectively contribute to improved therapeutic efficacy and demonstrate a substantial technical advancement over existing wound-healing formulations.

The experimental results further establish that the present pharmaceutical composition is not a mere aggregation or admixture of known ingredients. The individual formulation components
30 perform interdependent and complementary functions to produce an enhanced therapeutic response that is not predictable from the known properties of the individual constituents. The observed enhancement in diabetic wound healing, accelerated wound contraction, sustained local drug delivery and improved tissue regeneration collectively establish the presence of a synergistic technical effect arising from the claimed integrated pharmaceutical composition.

Accordingly, the present invention provides a pharmaceutical composition exhibiting enhanced therapeutic efficacy, improved physicochemical stability, sustained localized drug delivery and unexpected improvement in diabetic wound healing, thereby constituting a technical advancement over the existing art. The therapeutic efficacy of the present film-forming nanocarrier hydrogel was evaluated in a clinically relevant high-fat diet/streptozotocin-induced Type II diabetic excision wound model, which closely mimics human Type II diabetic wounds by reproducing insulin resistance together with partial β -cell dysfunction. The optimized formulation demonstrated significantly accelerated wound healing, achieving wound contraction of $96.21 \pm 1.28\%$ (low dose) and $98.39 \pm 0.76\%$ (high dose) by Day 15, compared with $69.79 \pm 2.22\%$ in the untreated diabetic control ($p < 0.01$). Enhanced wound contraction was evident from Day 9 onwards, indicating earlier initiation of tissue regeneration and a dose-dependent therapeutic response.

The improved wound healing is attributed to the synergistic interaction between the film-forming hydrogel matrix, DMOG and the amlodipine-loaded nanostructured lipid carrier. The hydrogel provides prolonged adhesion and moisture retention at the wound site, the nanostructured lipid carrier enables controlled and localized delivery of amlodipine with improved physicochemical stability, while DMOG promotes HIF-1 α -mediated angiogenesis and tissue regeneration. The coordinated action of these components enhances angiogenesis, collagen deposition, re-epithelialization and tissue remodelling, thereby overcoming the delayed healing associated with diabetic wounds.

Accordingly, the present invention provides a structurally integrated dual-drug nanocarrier hydrogel delivery system exhibiting enhanced therapeutic efficacy, sustained localized drug delivery, improved nanoparticle stability and accelerated diabetic wound closure. The experimentally demonstrated improvement is greater than the expected additive effect of the individual components and therefore represents a synergistic technical effect. The pharmaceutical composition is neither a mere new form of known substances nor a mere admixture, but an integrated formulation providing measurable technical advancement and unexpected enhancement in wound-healing efficacy.

WE CLAIM:

1. A pharmaceutical composition for topical administration comprising: between 8 wt.% and 20 wt.% of a hydrophilic film-forming polymer; between 1 wt.% and 6 wt.% of a co-polymer; between 1 wt.% and 8 wt.% of a plasticizer; between 5 wt.% and 20 wt.%
5 of a pharmaceutically acceptable co-solvent; between 50 wt.% and 85 wt.% of water; and between 0.1 wt.% and 5 wt.% of a pro-angiogenic agent; and a nanostructured lipid carrier (NLC) dispersed within the hydrogel matrix, the nanostructured lipid carrier comprising nanostructured lipid carrier comprises: between 30 wt.% and 70 wt.% of a solid lipid; between 5 wt.% and 35 wt.% of a liquid lipid; between 10 wt.% and 50 wt.%
10 of a surfactant; between 0.05 wt.% and 5 wt.% of a calcium channel blocker; and an aqueous phase;
wherein the hydrogel matrix and the nanostructured lipid carrier are configured to provide sustained topical delivery of the first therapeutic agent and the second therapeutic agent for promoting angiogenesis, neovascularization, tissue regeneration
15 and wound healing.
2. The pharmaceutical composition as claimed in claim 1, wherein the hydrophilic film-forming polymer comprises one or more polymers selected from polyvinyl alcohol, hydroxypropyl methylcellulose, hydroxyethyl cellulose, sodium carboxymethyl cellulose, carbomer, poloxamer, chitosan, gelatin, sodium alginate, polyethylene oxide,
20 polyacrylic acid and combinations thereof; the co-polymer comprises one or more polymers selected from polyvinyl pyrrolidone, polyethylene glycol, polyethylene oxide, polyacrylamide, carbopol, sodium alginate, xanthan gum, hyaluronic acid and combinations thereof; the pro-angiogenic agent comprises dimethyloxalylglycine (DMOG), vascular endothelial growth factor, fibroblast growth factor, platelet-derived
25 growth factor, stromal cell-derived factor, deferoxamine, cobalt chloride or combinations thereof.
3. The pharmaceutical composition as claimed in any one of the preceding claims, wherein the calcium channel blocker comprises amlodipine, amlodipine besylate, nifedipine, felodipine, nicardipine, lercanidipine, cilnidipine, benidipine or pharmaceutically
30 acceptable salts thereof; the solid lipid comprises glyceryl monostearate, stearic acid, glyceryl behenate, cetyl palmitate, tripalmitin, tristearin or combinations thereof; the liquid lipid comprises glyceryl monooleate, medium-chain triglycerides, oleic acid, caprylic/capric triglycerides, isopropyl myristate, sesame oil, castor oil or combinations thereof; the surfactant comprises Tween 20, Tween 40, Tween 60, Tween 80, Span 20,

Span 60, Span 80, Poloxamer 188, Poloxamer 407, lecithin, sodium cholate or combinations thereof; the hydrogel matrix comprises polyvinyl alcohol, polyvinyl pyrrolidone, water, ethanol, propylene glycol and dimethyloxallylglycine.

4. The pharmaceutical composition as claimed in any one of the preceding claims, wherein the hydrogel matrix comprises: between 8 wt.% and 20 wt.% of polyvinyl alcohol; between 1 wt.% and 6 wt.% of polyvinyl pyrrolidone; between 1 wt.% and 8 wt.% of propylene glycol; between 5 wt.% and 20 wt.% of ethanol; between 50 wt.% and 85 wt.% of water; and between 0.1 wt.% and 5 wt.% of dimethyloxallylglycine (DMOG).

5. The pharmaceutical composition as claimed in any one of the preceding claims, wherein the nanostructured lipid carrier comprises: between 30 wt.% and 70 wt.% of glyceryl monostearate; between 5 wt.% and 35 wt.% of glyceryl monooleate (Peceol); between 10 wt.% and 50 wt.% of Tween 80; between 0.05 wt.% and 5 wt.% of amlodipine besylate; and an aqueous phase.

6. The pharmaceutical composition as claimed in any one of the preceding claims, wherein the amlodipine besylate is present in the nanostructured lipid carrier in an amount ranging from about 0.1 wt.% to about 0.2 wt.% and is present in the hydrogel matrix dimethyloxallylglycine (DMOG) 0.5% w/w to 1.0% w/w.

7. The pharmaceutical composition as claimed in any one of the preceding claims, wherein the composition is useful for treatment of diabetic wounds, chronic wounds, pressure ulcers, venous ulcers, burn wounds, traumatic wounds, post-surgical wounds, ischemic ulcers and skin defects.

8. A method of preparing a hydrogel-based nanostructured lipid carrier (NLC) pharmaceutical composition, the method comprising:

(a) preparing a hydrogel matrix by:

- i. preparing a hydrogel matrix by mixing water, ethanol and propylene glycol under stirring to obtain a homogeneous solvent system;
- ii. gradually incorporating polyvinyl pyrrolidone followed by polyvinyl alcohol into the solvent system under controlled stirring at a temperature between 35°C and 45°C until a homogeneous hydrogel matrix is formed; and
- iii. cooling the hydrogel matrix to room temperature and dispersing dimethyloxallylglycine (DMOG) therein under mechanical stirring to obtain a DMOG-loaded hydrogel matrix.

(b) preparing a nanostructured lipid carrier by:

- i. preparing a nanostructured lipid carrier by melting a solid lipid and a liquid lipid to obtain a molten lipid phase;
- ii. dispersing amlodipine besylate in the molten lipid phase under stirring to obtain a drug-loaded lipid phase;
- 5 iii. preparing an aqueous surfactant phase comprising water and a surfactant, heating the aqueous surfactant phase to a temperature between 65°C and 75°C, and combining the aqueous surfactant phase with the drug-loaded lipid phase;
- iv. subjecting the resulting mixture to high-speed shear homogenization to obtain a nanoemulsion; and
- 10 v. cooling the nanoemulsion to room temperature to obtain an amlodipine-loaded nanostructured lipid carrier.

(c) dispersing the amlodipine-loaded nanostructured lipid carrier into the DMOG-containing hydrogel matrix under continuous stirring until a homogeneous hydrogel-based nanostructured lipid carrier pharmaceutical composition is obtained.

15 **9.** The method as claimed in claim 9, wherein the high-speed shear homogenization is carried out at 10,000 rpm for 10 minutes; the hydrogel matrix and the nanostructured lipid carrier are mixed under mechanical stirring until a uniform dispersion is obtained.

Dated this the 05th day of August, 2026

To,

20 The Controller of Patent
The Patent Office, at Chennai.



Arokya Nithya. D (IN/PA-4159)

On behalf of the Applicant

ABSTRACT

The present invention relates to a topical dual-drug film-forming pharmaceutical composition for promoting healing of diabetic and chronic wounds. The composition comprises Dimethyloxallylglycine dispersed within a polymeric film-forming hydrogel matrix and
5 Amlodipine, or a pharmaceutically acceptable salt thereof, incorporated within Nanostructured Lipid Carriers comprising at least one solid lipid, at least one liquid lipid, and a surfactant. The Amlodipine-loaded Nanostructured Lipid Carriers are uniformly dispersed throughout the Dimethyloxallylglycine-containing hydrogel matrix. Upon topical application, the composition spreads over the wound and forms a flexible, adherent, breathable, and moisture-retentive film.
10 The hydrogel matrix provides localized release of Dimethyloxallylglycine, while the Nanostructured Lipid Carriers provide sustained release of Amlodipine. The integrated dual-delivery system promotes angiogenesis, improves endothelial function, supports collagen deposition and re-epithelialization, and accelerates wound closure. The invention also relates to a process for preparing the composition by hydrogel formation, nanostructured lipid carrier
15 preparation, and controlled incorporation of the nanocarrier dispersion into the hydrogel matrix.