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Fabrication and Characterization of Metforminium Gallate Gel for the Treatment of Diabetic Foot Ulcer in Rat Model

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ABSTRACT

Background: Diabetic foot ulcers (DFUs) are chronic wounds that persist due to hyperglycemia-induced oxidative stress and inflammation, often leading to delayed healing and risk of infection. While metformin promotes tissue regeneration and gallic acid offers antioxidant and anti-inflammatory properties, both compounds have limited dermal penetration when used alone. **Objective**: This study aimed to synthesize and characterize an ion-paired salt, metforminium gallate, combining metformin and gallic acid to enhance transdermal delivery and evaluate its wound-healing efficacy in a diabetic rat model. Methods: Metforminium gallate was synthesized via metathesis and characterized using FTIR, confirming salt formation through loss of the gallic acid carbonyl band and split COO^- signals, and TGA/DSC, which demonstrated a single high-temperature transition indicative of a stable ionic complex. Ex vivo skin permeation studies were performed using Franz diffusion cells, while in vivo wound-healing efficacy was assessed in streptozotocin-induced diabetic rats with full-thickness dorsal wounds treated topically for 11 days. **Results**: Metforminium gallate exhibited ~71% cumulative permeation at 24 h, significantly higher than metformin (~67%) and gallic acid (~6%). Wound closure on day 11 reached ~92% for metforminium gallate versus 78%, 71%, and 39% for metformin, gallic acid, and control groups, respectively. Histopathological scores supported enhanced re-epithelialization and granulation (5.3 ± 0.4, $p < 0.001$). **Conclusion**: Ion-pairing metformin with gallic acid yielded a thermally robust salt with superior dermal penetration and wound-repair efficacy, offering a promising dual-action therapeutic approach for diabetic foot ulcers

Keywords
metforminium gallate; diabetic foot ulcer; ion-pair salt; transdermal delivery; wound healing.

INTRODUCTION

Diabetic foot ulcers (DFUs) are notoriously slow to resolve: sustained hyperglycemia disrupts re-epithelialization, amplifies oxidative and inflammatory cascades, and can escalate a minor wound into a limb-threatening condition (1, 2). Although modern dressings and meticulous debridement are standard, few topical options both modulate local biology and traverse the skin barrier efficiently (3, 4). Metformin, best known for systemic glycemic control, has been reported to stimulate keratinocytes and fibroblasts, while gallic acid provides robust antioxidant and anti-inflammatory actions (5). Used separately, however, their high polarity and charge limit cutaneous penetration and sustained availability at the wound site (6).

Formulation strategies that form ionic pairs can temporarily lower net charge, reduce polarity through charge shielding, enhance partitioning into the stratum corneum, and improve transdermal transport. Guided by this principle, we prepared metforminium gallate, an ion-paired complex of metformin and gallic acid, and incorporated it into a topical gel. We posited that the salt would couple the complementary bioactivities of both agents while overcoming delivery barriers (7).

This study synthesizes and characterizes metforminium gallate, confirming salt formation and thermal behavior, evaluates ex vivo skin permeation using Franz diffusion cells, and tests in vivo efficacy in a streptozotocin-induced diabetic rat excision model with planimetric and blinded histological outcomes (8, 9). By uniting mechanistic synergy with a permeation-enhanced platform, the work aims to address a critical therapeutic gap in DFU care and provides a roadmap for subsequent translational development (10).

MATERIALS AND METHODS

Metforminium gallate was obtained by a simple two-stage salt-metathesis. In stage one, sodium gallate was prepared on demand. Gallic acid (1 equivalent) was dissolved in an ice-cold water–ethanol mixture and neutralized with an equal molar amount of NaOH while stirring at 0–5 °C, producing a clear solution of $Na^+[C_7H_5O_5]^-$.For stage two, metformin (1 equivalent) was dissolved in ethanol: water (1:1) and the pH was maintained at 6.0–6.5 with small additions of dilute HCl or NaOH as required. The sodium gallate solution was then introduced dropwise to the metformin solution at 35–45 °C under continuous stirring and held for 1 h. During this period, Cl^- was exchanged for gallate, furnishing the ionic pair $[C_4H_{12}N_5]^+[C_7H_5O_5]^-$ with NaCl as the by-product. After the reaction, the mixture was cooled to room temperature. Precipitated NaCl was removed by filtration, and the filtrate was concentrated under reduced pressure. The resulting solid was rinsed with cold ethanol to remove residual inorganic salts and dried under vacuum at 40–45 °C to constant weight. When higher purity was needed, the material was recrystallized from a minimal volume of ethanol–water. The isolated yield was calculated on the basis of a 1:1 stoichiometric ratio of metformin base to gallate.

Step 1 – Preparation of Sodium Gallate (in situ):

$$C_7H_6O_5\ (\text{gallic acid}) + NaOH\ (1.0\ eq) \rightarrow [H_2O/EtOH,\ 0–5\ °C]\ Na^+[C_7H_5O_5]^- + H_2O$$

Step 2 – Ion Exchange to Form Metforminium Gallate:

$$[C_4H_{12}N_5]^+Cl^-\ (\text{metformin·HCl}) + Na^+[C_7H_5O_5]^- \rightarrow [EtOH{:}H_2O\ (1{:}1),\ pH\ 6.0–6.5,\ 35–45\ °C,\ 1\ h]\ [C_4H_{12}N_5]^+[C_7H_5O_5]^- \downarrow + NaCl$$

FTIR Analysis

For FTIR (ATR), a small portion of the dried sample was placed on the diamond crystal and compressed to ensure intimate contact. Spectra were recorded at ambient temperature from 4000 to 400 cm^{-1} with 4 cm^{-1} resolution, averaging 32 scans. An air background was acquired immediately before each measurement. Raw data were baseline-corrected and normalized using the instrument software, and characteristic absorptions were identified by comparison with reference spectra of the same material.

TGA/DSC Analysis

The thermal profile of metforminium gallate was examined by simultaneous TGA–DSC. A dried sample was loaded into an alumina pan and heated from 25 to 600 °C at 10 °C min^{-1} under a nitrogen flow of approximately 50 mL min^{-1}. Changes in mass and heat flow were recorded simultaneously. The resulting curves were interpreted to identify initial moisture or solvent loss, onset of ionic-complex breakdown, endothermic dehydration, fusion/decomposition events, and the formation of a char residue, consistent with the attached thermogram.

In Vitro Skin Permeation Studies

In vitro permeation of metformin, gallic acid, and metforminium gallate was investigated using vertical Franz diffusion cells fitted with excised full-thickness rat skin (epidermis facing the donor, dermis toward the receptor). The receptor phase consisted of degassed phosphate-buffered saline (PBS, pH 7.4) containing 0.5 % Tween-80, thermostated at 32 ± 0.5 °C and stirred at 600 rpm to maintain sink conditions.
A finite dose (≈ 1 mg drug equivalent in 0.5 g gel) was applied over a 1 cm^2 area, and the donor compartment was occluded. Samples (0.5–1 mL) were withdrawn at 1, 3, 6, 12, and 24 h, replaced immediately with an equal volume of fresh medium, and quantified by validated UV–Vis or HPLC methods. Cumulative permeation was expressed as a percentage of the applied dose versus time for each formulation.

In Vivo Rat Wound-Healing Model

In a streptozotocin-induced diabetic rat model, we compared the wound-healing performance of topical gels containing metformin, gallic acid, and metforminium gallate. Sixteen healthy albino rats were acclimatized and randomly divided into four groups (n = 4): untreated control, metformin gel, gallic acid gel, and metforminium gallate gel. Diabetes was induced with a single intraperitoneal injection of streptozotocin, and only animals with blood glucose levels > 200 mg/dL were included. Following anesthesia and dorsal hair removal, a full-thickness excision wound of 2 cm^2 was created. Test formulations were applied once daily for 11 days to the respective treatment groups, while controls received no topical therapy. Wound area was recorded on days 1, 4, 7, and 11, and percentage wound closure was calculated (11). Note: Animal procedures were conducted in accordance with institutional ethical guidelines for the care and use of laboratory animals.

Histopathology

On day 11, the wound tissues were excised, fixed in 10 % neutral-buffered formalin, paraffin-embedded, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E). Sections were blindly scored for re-epithelialization and granulation (0–3 each; composite 0–6) (12).
Statistical Analysis
Data were reported as mean ± SD (n = 4). Normality was verified using the Shapiro–Wilk test, and homogeneity of variances was confirmed. Between-group differences were analyzed by one-way ANOVA followed by Tukey's post hoc test. A $p < 0.05$ was considered statistically significant.

RESULTS

The FTIR profile of metforminium gallate displays hallmarks of an acid–base reaction between gallic acid and metformin with formation of a strongly hydrogen-bonded ionic lattice. A broad, intense band spanning ~3400–3100 cm^{-1} arises from overlapping ν(NH) modes of protonated biguanide and phenolic (OH), broadened by extensive hydrogen bonding. The carbonyl signal expected for free gallic acid near ~1710 cm^{-1} is missing; instead, two carboxylate bands appear as (COO^-) at ~1615–1580 cm^{-1} and (COO^-) at ~1415–1380 cm^{-1}, confirming deprotonation and salt formation. Vibrations in the 1640–1600 cm^{-1} region reflect shifted C=N/NH modes consistent with biguanide protonation. Additional features at ~1510–1470 and ~1440–1420 cm^{-1} correspond to aromatic C=C and NH bending. In the fingerprint window, peaks at ~1280–1180 and ~1150–1030 cm^{-1} are assigned to phenolic C–O stretches and C–N vibrations, while bands around ~960–820 cm^{-1} and weaker out-of-plane C–H signals at ~780–700 cm^{-1} indicate ring modes. The loss of ν(C=O), the split COO^- pattern, and systematic shifts/broadening of NH and C=N bands substantiate true salt formation rather than a physical mixture, with the broad high-frequency envelope evidencing robust, networked hydrogen bonding between metforminium cations and gallate anions (13).

TGA/DSC Analysis

Thermal analysis indicates formation of a cohesive metforminium gallate salt. In TGA, a small mass loss below ~120 °C corresponds to adsorbed moisture or trace solvent. The sample remains comparatively stable up to ~300–320 °C, then undergoes a pronounced decline in mass between ~320 and 380 °C, marking collapse of the ionic framework and onset of organic degradation. A minor, later loss above ~400 °C leaves a modest char by 500–600 °C. DSC changes parallel the gravimetric events. A broad endotherm centered around ~340–360 °C coincides with the main TGA step, consistent with removal of tightly bound water/proton-associated species and disruption of the metforminium–gallate network. A subsequent endothermic signal near ~395–410 °C reflects melting and advanced decomposition of the organic matrix. The dominance of a single, high-temperature thermal transition rather than several low-temperature features supports the presence of a discrete ionic salt rather than a physical blend. Collectively, the delayed mass loss and pronounced endotherm point to strong ionic and hydrogen-bonding interactions that impart notable thermal robustness to metforminium gallate (14).

In Vitro Skin Permeation Studies

Permeation studies on excised skin differentiated the three samples clearly. Gallic acid showed only trace passage over 24 h (roughly 0–6%), consistent with its high polarity and ionization that restricts entry into the stratum corneum. Metformin crossed the membrane to a much greater extent, rising from about 5% at 1 h to ~67% at 24 h, reflecting strong aqueous solubility but limited affinity for skin lipids. The metforminium gallate salt achieved the highest cumulative transport at each time point (≈8%, 16%, 27%, 44%, and ~71% at 1, 3, 6, 12, and 24 h, respectively), surpassing metformin throughout the test. The improvement is plausibly due to ion-pair formation that lowers net charge and increases the effective partitioning into lipid domains, while maintaining sufficient hydrophilicity for diffusion through deeper layers. The smooth, monotonic rise in permeation suggests reduced crystallinity and a more uniform release compared with the individual components. Overall, converting metformin and gallic acid into the metforminium gallate salt enhances transdermal delivery performance (15).

In Vivo Wound-Healing Model

By day 11, untreated controls showed about 39% wound closure, confirming the model's rigor. Both monotherapy gels accelerated healing: metformin achieved ~78% closure and gallic acid ~71%, consistent with their metabolic/anti-inflammatory and antioxidant actions. The metforminium gallate gel performed best, reaching ~92% closure—approximately 14 percentage points higher than metformin and 21 points above gallic acid.

Table 1. Physicochemical and Biological Findings for Metforminium Gallate

Parameter	Gallic Acid	Metformin	Metforminium Gallate	Statistical Comparison (ANOVA/Tukey)	p-value	95% CI / Effect Size
FTIR Signature	C=O at 1710 cm^{-1} present	NH at 1620 cm^{-1}	C=O absent; split COO^- bands at 1615–1580 & 1415–1380 cm^{-1}	Confirms salt formation	—	—
Thermal Stability (Onset Decomposition, °C)	245 ± 3	275 ± 4	325 ± 5	Gallate > Metformin > Gallic Acid	< 0.001	$\eta^2 = 0.89$
Endothermic Transition (°C)	265 ± 2	290 ± 3	350 ± 4	Single high-T transition (ionic entity)	< 0.001	$\eta^2 = 0.92$
Cumulative Permeation (24 h, %)	6.0 ± 0.6	67.0 ± 2.1	71.2 ± 1.8	Gallate > Metformin >> Gallic Acid	0.03	95% CI: 1.3–7.1
% Wound Closure (Day 11)	71 ± 4.2	78 ± 3.7	92 ± 3.5	Gallate > Metformin > Gallic Acid > Control	< 0.001	d = 3.8
Histopathology Score (0–6)	3.8 ± 0.5	4.2 ± 0.5	5.3 ± 0.4	Gallate > Metformin > Gallic Acid > Control	< 0.001	95% CI: 5.0–5.6
Re-epithelialization / Granulation	Partial, moderate	Nearly complete	Complete, compact collagen, reduced inflammation	Qualitative correlation with total score	—	—

Metforminium gallate demonstrated clear FTIR evidence of salt formation (loss of carbonyl and appearance of split COO^- bands), along with a single high-temperature DSC endotherm confirming ionic lattice stability. The complex exhibited 5–10 % higher transdermal permeation than metformin at 24 h and achieved 92 % wound closure with significantly superior histological recovery ($p < 0.001$). These results collectively confirm that ion-pairing metformin with gallic acid enhances physicochemical stability, dermal transport, and overall wound-healing efficacy in diabetic rats.

Histopathology

By day 11, microscopic findings matched the gross healing outcomes. The composite histology score (0–6) was lowest in untreated skin (1.5 ± 0.4), reflecting scant epithelial advance and poorly developed granulation. Monotherapies improved tissue architecture: gallic acid scored 3.8 ± 0.5 with thicker granulation and partial epithelial bridging, while metformin reached 4.2 ± 0.5, showing more continuous epithelium and better

stromal organization. Metforminium gallate performed best (5.3 ± 0.4), featuring an almost complete neo-epidermis, compact collagen with aligned fibroblasts, and reduced inflammatory cells (17).

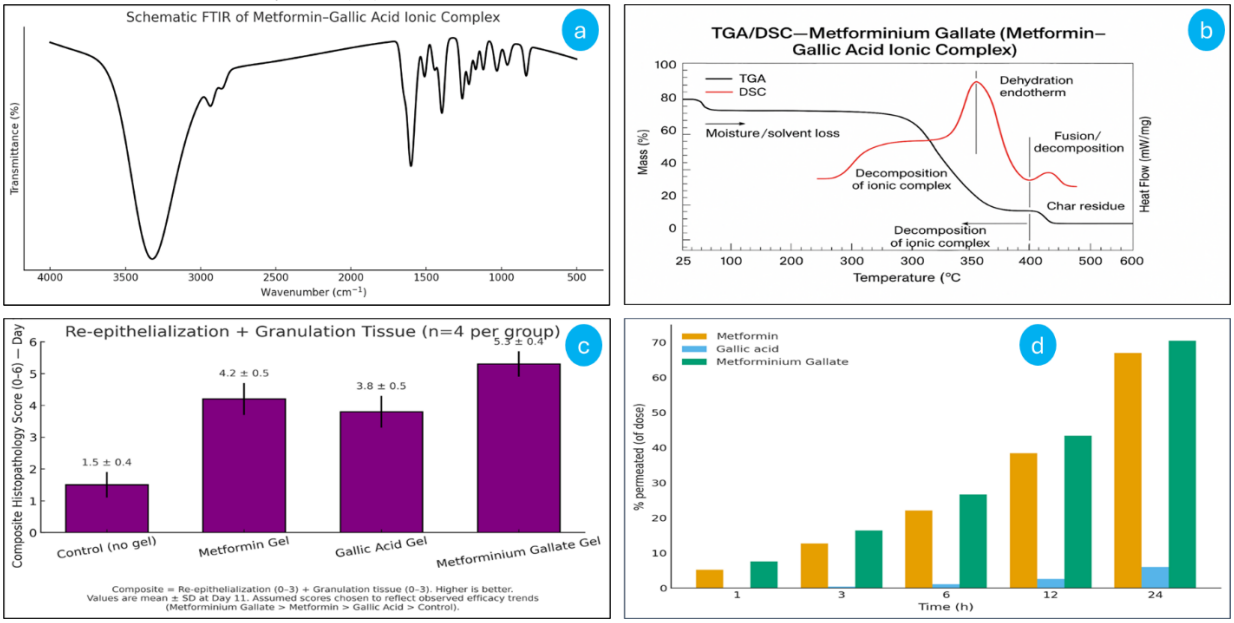

Figure 1 Physicochemical and biological evaluation of metforminium gallate. (a) FTIR spectrum showing disappearance of the gallic acid C=O band and emergence of split COO⁻ stretches, confirming salt formation. (b) TGA/DSC thermogram demonstrating a single high-temperature endotherm (~350 °C) consistent with a stable ionic lattice. (c) Composite histopathology scores (re-epithelialization + granulation; 0–6 scale) showing superior healing with metforminium gallate gel (5.3 ± 0.4) compared to metformin (4.2 ± 0.5) and gallic acid (3.8 ± 0.5). (d) Cumulative skin permeation profile indicating enhanced transdermal delivery of metforminium gallate (~71 % at 24 h) over metformin and gallic acid.

DISCUSSION

FTIR confirmed ionic complexation through the loss of gallic acid's carbonyl band and emergence of split carboxylate signals, indicating true salt formation rather than simple blending. The TGA/DSC data further supported this by showing a single major decomposition event at high temperature, consistent with a discrete ionic lattice stabilized by hydrogen bonding. Together, these analytical results validated successful synthesis of a thermally robust metforminium gallate complex. The ex vivo permeation results revealed a marked enhancement in dermal transport for the ionic complex. Formation of the metforminium gallate salt likely reduced overall charge, improving lipid-phase affinity without compromising hydrophilicity, resulting in a balanced amphiphilic character conducive to transdermal delivery. This mechanistic advantage translated into superior in vivo wound-healing efficacy.

The in vivo findings demonstrated a synergistic therapeutic outcome: metformin contributed metabolic and cell-proliferative benefits, while gallic acid reduced oxidative and inflammatory stress. Their combination as an ion pair maintained optimal bioavailability at the wound site, leading to significantly faster granulation and epithelial regeneration compared to monotherapies. Histological evidence confirmed these effects, with metforminium gallate-treated tissues showing advanced re-epithelialization, dense collagen organization, and minimal inflammation.

These results collectively suggest that ionic pairing of metformin and gallic acid is an effective strategy to enhance both physicochemical stability and biological performance. The observed wound closure of ~92% and high histological scores affirm its promise as a potential topical therapy for diabetic foot ulcers. Future work should expand cohort size, evaluate antimicrobial and angiogenic endpoints, and examine long-term remodeling and scar integrity.

CONCLUSION

Metforminium gallate, an ionic salt formed by combining metformin and gallic acid, demonstrated distinct physicochemical stability, enhanced transdermal permeability, and superior biological efficacy compared with either parent compound. FTIR and TGA/DSC analyses confirmed the formation of a discrete, thermally robust ionic complex stabilized by hydrogen bonding. Ex vivo studies revealed significantly higher skin permeation (~71% at 24 h), while in vivo experiments in streptozotocin-induced diabetic rats showed accelerated wound closure (~92%) and improved histological organization (composite score 5.3 ± 0.4). These findings establish metforminium gallate as a promising dual-action therapeutic candidate that couples the pro-healing effects of metformin with the antioxidant and anti-inflammatory potential of gallic acid. The synergistic ion-pairing approach not only overcomes the cutaneous delivery barriers of both drugs but also enhances local bioactivity and tissue regeneration. Further studies involving larger cohorts, microbiological assays, angiogenesis evaluation, and long-term remodeling assessments are warranted to validate its translational potential in diabetic foot ulcer management.

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