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Original Research Article

Development and Physicochemical Characterization of a Losartan Potassium Ethosomal Patch for Controlled Transdermal Delivery Applications

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Abstract: Losartan potassium (LP) is an angiotensin II receptor blocker (ARB) with established clinical utility in the management of hypertension and diabetic nephropathy. Oral delivery of LP is hampered by extensive hepatic first-pass metabolism and highly variable bioavailability (25–35%), making it a compelling candidate for transdermal administration. The stratum corneum, however, presents a formidable physicochemical barrier that restricts passive permeation of most drug molecules. The present study describes the development and comprehensive physicochemical characterization of LP-loaded ethosomes elastic, ethanol-enriched phospholipid vesicles—incorporated into a hydroxypropyl methylcellulose (HPMC K100M)-based matrix transdermal patch. Six ethosomal formulations (EP-1 to EP-6) were prepared by the cold-membrane extrusion method by systematically varying phosphatidylcholine (300–400 mg), cholesterol (75–100 mg), and ethanol content (20–40% v/v). The optimized formulation (EP-6) displayed a mean particle size of 176.3 ± 3.9 nm, a polydispersity index of 0.241 ± 0.01, a zeta potential of −34.8 ± 1.2 mV, and an encapsulation efficiency of 85.7 ± 1.5%. Ethosomal vesicles were incorporated into a solvent-cast HPMC matrix patch demonstrating acceptable physicochemical attributes including tensile strength (4.8 ± 0.3 N/mm^2), moisture content (3.2 ± 0.4%), and drug content uniformity (97.8 ± 0.9%). In vitro membrane permeation studies performed using Strat-M® synthetic membrane a well-validated, animal-free surrogate for human skin—demonstrated a steady-state flux of 62.4 ± 2.3 µg/cm^2/h for the EP-6 patch, representing a 4.3-fold enhancement over a conventional matrix patch. Release kinetics conformed to an anomalous (non-Fickian) diffusion model. Confocal laser scanning microscopy using fluorescently labeled ethosomes confirmed deep penetration through the Strat-M® membrane layers, validating the mechanistic basis of the observed permeation advantage. Stability evaluation under ICH Q1A(R2)-prescribed conditions demonstrated that the patch retained physicochemical integrity over six months. These results collectively support the viability of an LP ethosomal transdermal patch as a controlled, animal-free-validated delivery platform for antihypertensive therapy.

Keywords: Losartan potassium; Ethosomes; Transdermal drug delivery; Strat-M® membrane; Vesicular carriers; Controlled release; Stratum corneum; Permeation enhancement; Hypertension.

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INTRODUCTION

Hypertension remains among the most prevalent and clinically consequential non-communicable diseases globally, affecting an estimated 1.28 billion adults aged 30–79 years across low-, middle-, and high-income countries (WHO, 2023). Sustained elevation of systemic arterial pressure constitutes a leading modifiable risk factor for stroke, myocardial infarction, heart failure, and progressive renal impairment. The renin–angiotensin–aldosterone system (RAAS) plays a pivotal role in blood pressure regulation, and pharmacological interruption of this axis at the angiotensin II receptor level has become a cornerstone of modern antihypertensive therapeutics. Losartan potassium (LP), the prototype angiotensin type-1 (AT_1) receptor antagonist, selectively and competitively inhibits the vasoconstrictor and aldosterone-secreting effects of angiotensin II, promoting vasodilation, natriuresis, and cardiorenal protection without the bradykinin-mediated adverse effects commonly associated with angiotensin-converting enzyme (ACE) inhibitors.

Despite its proven therapeutic value, the oral pharmacokinetic profile of LP presents substantive clinical limitations. Following ingestion, LP undergoes extensive first-pass metabolism in the intestinal wall and liver, yielding an active carboxylic acid metabolite (EXP-3174) with considerably greater potency but with significant and unpredictable inter-individual variation. Absolute oral bioavailability of LP averages 25–35%, and the parent compound's plasma half-life of 6–9 hours necessitate twice-daily dosing in some patients (Kassler-Taub *et al.,* 1998). Non-adherence to chronic dosing regimens—a well-documented phenomenon in hypertension management—substantially undermines long-term blood pressure control and amplifies cardiovascular risk. Additionally, a subset of patients experiences gastrointestinal disturbances that reduce tolerability and further compromise adherence.

Transdermal drug delivery systems (TDDS) offer a conceptually attractive solution to these pharmacokinetic and compliance-related limitations. By circumventing the gastrointestinal tract and hepatic first-pass effect, TDDS provide more predictable systemic drug exposure, maintain sustained plasma concentrations, simplify dosing frequency, and are generally associated with improved patient acceptance (Prausnitz and Langer, 2008). The principal challenge of transdermal delivery lies in overcoming the stratum corneum (SC), the outermost layer of the skin, which serves as the body's primary physicochemical barrier. The SC consists of terminally differentiated corneocytes embedded in a lamellar matrix of ceramides, free fatty acids, and cholesterol, creating a densely organized, low-permeability domain. For LP—with a log P of approximately 3.74, a molecular weight of 461.01 Da, and moderate aqueous solubility—passive diffusion across intact SC is insufficient to achieve therapeutically meaningful transdermal flux without appropriate permeation-enhancement strategies.

Among the vesicular carrier systems explored for transdermal permeation enhancement, ethosomes—first conceptualized and described by Touitou et al. (2000)—represent a particularly sophisticated platform. Ethosomes are soft, deformable phospholipid vesicles enriched with relatively high concentrations of ethanol (20–45% v/v). Their defining advantage over conventional liposomes lies in the dual role played by ethanol: it simultaneously acts as a membrane fluidizer (intercalating into phospholipid bilayers and reducing the overall ordered packing of the SC lipid matrix) and as a driving force for vesicle deformability, enabling the vesicle to squeeze through lipid channels considerably narrower than its own diameter. The net result is substantially deeper deposition of drug-carrying vesicles into the SC and viable epidermis compared with rigid liposomal systems (Verma *et al.,* 2003). Critically, from a regulatory and ethical standpoint, the permeation performance of ethosomal systems can be rigorously assessed using validated synthetic membrane models, most notably Strat-M® (Merck KGaA), which closely replicates the multi-layered lipid architecture of human SC without the use of any animal-derived tissue.

While several prior investigations have examined transdermal delivery of antihypertensives—including amlodipine, captopril, and carvedilol—using ethosomal carriers (Ahad *et al.,* 2016; Garg *et al.,* 2021), a systematically optimized, comprehensively characterized LP ethosomal matrix patch evaluated exclusively through in vitro and synthetic membrane methods has not yet been described in the peer-reviewed literature. The present study addresses this gap. We report the preparation of LP-loaded ethosomes by cold-membrane extrusion, their integration into an HPMC K100M matrix patch by solvent casting, and thorough in vitro evaluation encompassing vesicle characterization, patch physicochemical testing, Strat-M® membrane permeation, release kinetics, confocal fluorescence microscopy, and ICH-compliant stability assessment—all conducted without any use of animal tissue or in vivo models.

MATERIALS AND METHODS

Materials

Losartan potassium was obtained as a gift sample from Sun Pharmaceutical Industries Ltd. (Mumbai, India). Soy phosphatidylcholine (SPC, purity ≥ 95%) was purchased from Lipoid GmbH (Ludwigshafen, Germany). Cholesterol (analytical grade), hydroxypropyl methylcellulose K100M (HPMC K100M), polyvinyl alcohol (PVA), propylene glycol (PG), and dibutyl phthalate were sourced from Sigma-Aldrich (St. Louis, MO, USA). Strat-M® synthetic membrane (a multi-layered polyether sulfone-based membrane designed to mimic the permeation properties of human skin) was procured from MilliporeSigma (Burlington, MA, USA). Absolute ethanol, acetonitrile (HPLC grade), methanol (HPLC grade), phosphate-buffered saline (pH 7.4, 0.01 M), and sodium dihydrogen phosphate were procured from Merck Specialities Pvt. Ltd. (Mumbai, India). Rhodamine B (fluorescent marker) was from Thermo Fisher Scientific (Waltham, MA, USA). All other chemicals and solvents were of analytical or pharmaceutical grade and used as received without further purification.

Preparation of Losartan Potassium Ethosomes

Ethosomes were prepared by the cold-membrane extrusion technique adapted from Touitou et al. (2000) with minor modifications. Soy phosphatidylcholine and cholesterol were dissolved in ethanol at the concentrations specified in Table 1, under gentle magnetic stirring at room temperature. LP (50 mg per batch) was co-dissolved in the ethanolic lipid solution and allowed to equilibrate for 10 minutes. A phosphate buffer (pH 7.4, 0.01 M) was then added dropwise to the ethanolic solution under vigorous mechanical stirring at 700 rpm, with the temperature maintained at 4°C using an ice bath. The resulting colloidal dispersion was probe-sonicated (Vibra-Cell VCX 500, Sonics & Materials Inc., USA) at 40% amplitude for 3 min in pulse mode (5 s on, 2 s off) and subsequently extruded sequentially through 400-nm and 200-nm polycarbonate membranes (Avanti Polar Lipids, USA) using a hand-held mini-extruder (LiposoFast, Avestin Inc., Canada) with a minimum of 11 passes per membrane, yielding narrow-size-distributed vesicle dispersions. The final ethosomal dispersions were stored at 4°C and used within 48 hours of preparation.

Physicochemical Characterization of Ethosomes

Particle Size, PDI, and Zeta Potential

Hydrodynamic particle size, polydispersity index (PDI), and zeta potential of all ethosomal formulations were determined by dynamic light scattering (DLS) and laser Doppler electrophoresis, respectively, using a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK) at 25°C. Samples were diluted 1:100 with freshly filtered (0.22 µm) deionized water prior to analysis. Measurements were performed in triplicate on independently prepared batches, and results are expressed as mean ± standard deviation (SD).

Encapsulation Efficiency

Encapsulation efficiency (EE) was determined by ultracentrifugation. A 1 mL aliquot of ethosomal dispersion was centrifuged at 100,000 × g for 60 min at 4°C (Optima L-90K, Beckman Coulter Inc., USA). The supernatant (containing unentrapped drug) was collected and assayed by HPLC as described in Section 2.7. EE was calculated as:

EE (%) = [(Total drug − Free drug) / Total drug] × 100

Vesicle Morphology by Transmission Electron Microscopy

The morphology and structural integrity of the optimized EP-6 ethosomes were examined by transmission electron microscopy (TEM). Vesicle dispersions were diluted 1:200 with deionized water, deposited onto 300-mesh carbon-coated copper grids (Ted Pella Inc., USA), and negatively stained with 1% (w/v) aqueous uranyl acetate for 45 seconds. After air-drying, grids were examined under a Tecnai G^2 Spirit TEM (FEI Company, Netherlands) operated at 120 kV.

Preparation of the Ethosomal Transdermal Patch

The optimized EP-6 ethosomal dispersion was incorporated into a polymeric matrix patch by the solvent-casting method. HPMC K100M (2% w/v) was dissolved in a water–methanol mixture (80:20 v/v) to produce a clear viscous gel. PVA (1% w/v), propylene glycol (20% w/v, plasticizer), and dibutyl phthalate (10% w/v) were added sequentially under gentle stirring. The LP ethosomal dispersion was then incorporated and stirred continuously for 30 min to achieve homogeneous distribution. The dispersion was cast onto pre-treated aluminum foil–lined polypropylene molds (10 cm × 10 cm) and dried in a hot-air oven at 40°C for 24 h. Dried patches were cut into 5-cm^2 discs, laminated with a Scotchpak™ 9733 impermeable backing membrane (3M, USA), and stored in sealed aluminum foil pouches at room temperature until evaluation. A conventional matrix patch containing an equivalent LP dose dispersed directly in the HPMC polymer without ethosomal encapsulation was prepared identically as a reference control.

Physicochemical Evaluation of the Patch

Patch thickness was measured at five equidistant points using a digital micrometer (Mitutoyo Corp., Japan). Weight uniformity was determined by weighing five patches cut to 5-cm^2 dimensions on an analytical balance (Mettler Toledo, Switzerland). Moisture content was assessed gravimetrically after 24-hour desiccation over

anhydrous calcium chloride. Moisture vapor transmission rate (MVTR) was measured using Thwing-Albert permeability cups filled with anhydrous calcium chloride, sealed with the patch, and stored at 32°C/75% RH for 24 h. Drug content was determined by dissolving a known patch area in methanol–water (60:40 v/v), sonicating for 20 min, filtering through a 0.45-µm membrane, and assaying by HPLC. Tensile strength and elongation at break were measured with a texture analyzer (TA.XT Plus, Stable Micro Systems, UK) at a crosshead speed of 10 mm/min using a 5 N load cell. Bioadhesive force was measured using a modified texture analysis protocol employing a gelatin disk substrate to simulate a soft tissue interface. The pH of a 24-hour aqueous extract of the patch was measured with a calibrated pH meter (Hanna Instruments, USA).

In Vitro Membrane Permeation Study Using Strat-M® Synthetic Membrane

In vitro permeation studies were conducted using Strat-M® synthetic membrane in Franz diffusion cells (diffusion area 1.77 cm^2, PermeGear Inc., USA). Strat-M® is a commercially available, multi-layered, animal-free membrane comprising a polyether sulfone top layer bonded to a series of polyolefin layers impregnated with skin-mimicking lipids, validated as a predictive in vitro surrogate for human skin permeation (Uchida *et al.,* 2015; Sintra *et al.,* 2019). Membranes were hydrated in phosphate buffer (pH 7.4) for 30 min prior to mounting. The receptor compartment was filled with phosphate buffer (pH 7.4) containing 10% PEG-400 to maintain sink conditions. Formulations were applied to the donor compartment, and the system was maintained at 32 ± 0.5°C (approximating skin surface temperature) under constant magnetic stirring at 200 rpm. Aliquots of 0.5 mL were withdrawn at 1, 2, 4, 6, 8, 12, 16, and 24 h and replaced with fresh receptor fluid. Drug concentrations were quantified by HPLC. Steady-state flux (Jss) was derived from the slope of the linear portion of the cumulative permeated amount per unit area versus time curve. The permeability coefficient (Kp) was calculated as Jss divided by the donor drug concentration, and the enhancement ratio (ER) was reported relative to the conventional patch.

HPLC Analytical Method

Quantification of LP was performed by a validated reversed-phase HPLC method. Separation was achieved on a Waters Symmetry C_{18} column (4.6 × 150 mm, 5 µm) at 30°C, with a mobile phase of acetonitrile and 10 mM ammonium formate buffer (pH 3.0) in a 40:60 v/v ratio at a flow rate of 1.2 mL/min. UV detection was at 254 nm, the injection volume was 20 µL, and irbesartan served as the internal standard. The method was validated per ICH Q2(R1) for linearity (R^2 = 0.9998, range 0.1–100 µg/mL), accuracy (% recovery 98.4–101.6%), precision (% RSD < 2%), and specificity.

In Vitro Drug Release and Release Kinetics

In vitro cumulative drug release was evaluated using a dialysis membrane diffusion method (cellulose dialysis membrane, MWCO 12,000 Da, Sigma-Aldrich). A 5-cm^2 patch was placed on the pre-soaked dialysis membrane mounted over the donor compartment of a Franz diffusion cell. Phosphate buffer (pH 7.4, 37°C) served as the receptor medium. Sampling and assay procedures were identical to those described in Section 2.6. To elucidate the underlying release mechanism, cumulative release data were fitted to zero-order, first-order, Higuchi matrix, and Korsmeyer–Peppas models using GraphPad Prism v9.5. Model discrimination was based on the coefficient of determination (R^2), adjusted R^2, and Akaike information criterion (AIC). The diffusion exponent n from the Korsmeyer–Peppas model was used to classify transport as Fickian ($n \leq 0.5$), anomalous ($0.5 < n < 1.0$), or Case II ($n \geq 1.0$).

Confocal Laser Scanning Microscopy (CLSM)

To visualize the depth of vesicular penetration through the Strat-M® membrane layers, fluorescently labeled ethosomes were prepared by incorporating 0.05% w/v Rhodamine B into the EP-6 formulation during preparation. Plain Rhodamine B solution and conventional Rhodamine B–loaded liposomes (prepared without ethanol) served as controls. Strat-M® membranes (1 cm^2) were mounted on Franz cell setups and treated with each formulation for 8 h under the standard permeation conditions described in Section 2.6. Following exposure, membranes were carefully removed, blotted dry, placed on glass slides, and covered with a coverslip using aqueous mounting medium. Confocal z-stack images were acquired at excitation/emission wavelengths of 543/580 nm using a Leica TCS SP8 confocal microscope (Leica Microsystems GmbH, Germany) with a 20× objective. Maximum intensity projection images and orthogonal cross-sections were generated using Leica LAS X software to quantify and compare the depth of fluorescence penetration through the membrane.

Stability Studies

Stability of the optimized EP-6 ethosomal patch was evaluated in accordance with ICH Q1A(R2) guidelines. Patches individually sealed in aluminum foil pouches were placed in stability chambers under accelerated conditions (40°C/75% RH) and long-term conditions (25°C/60% RH) for six months. Samples were withdrawn at 0, 1, 3, and 6 months and evaluated for particle size, PDI, zeta potential, drug content, EE, and in vitro Strat-M® membrane

permeation flux. Statistical analyses were carried out using one-way ANOVA with Tukey's post-hoc test (SPSS v26, IBM Corp.); $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Formulation Composition and Rationale

Table 1 presents the composition matrix for the six ethosomal formulations studied. The variables investigated—phosphatidylcholine concentration (300–400 mg), cholesterol content (75–100 mg), and ethanol proportion (20–40% v/v) were selected on the basis of their well-established influence on vesicle size, membrane deformability, encapsulation efficiency, and transdermal permeation performance. Phosphatidylcholine was chosen as the primary bilayer-forming lipid owing to its biocompatibility, favorable thermal properties, and capacity to interact with both hydrophilic and lipophilic drug domains. Cholesterol was included at concentrations sufficient to stabilize the bilayer without abolishing the ethanol-induced deformability that defines ethosomal systems. Ethanol serves a multiplicity of roles: it solubilizes the lipid components, reduces the surface tension at the lipid–aqueous interface to facilitate smaller vesicle formation, disrupts the lamellar order of the SC lipid matrix, and confers the characteristic softness and flexibility of ethosomal membranes.

A drug-to-lipid ratio range of 1:3 to 1:4 was examined to maximize encapsulation without saturation of the bilayer. The theoretical drug loading per 5-cm^2 patch was designed to deliver the transdermal dose equivalent of 50 mg oral LP (adjusted for the expected bioavailability advantage of bypassing first-pass metabolism) over a 24-hour period.

Table 1: Composition of LP Ethosomal Formulations (EP-1 to EP-6)

Formulation Code	Phosphatidylcholine (mg)	Cholesterol (mg)	Ethanol (% v/v)	Drug: Lipid Ratio
EP-1	300	75	20	1:3
EP-2	350	87.5	30	1:3.5
EP-3	400	100	40	1:4
EP-4	300	100	30	1:4
EP-5	350	75	40	1:3
EP-6 (Optimized)	400	87.5	20	1:4

All formulations contained 50 mg LP per batch; n = 3 independently prepared batches per formulation.

Physicochemical Characterization of Ethosomes

The vesicle characterization data for all six formulations are presented in Table 2. Mean particle size spanned a range of 176.3 ± 3.9 nm (EP-6) to 218.5 ± 6.3 nm (EP-3), with PDI values ranging from 0.241 ± 0.01 to 0.312 ± 0.02—indicative of monomodal, relatively narrow size distributions across all formulations. A clear and consistent trend emerged whereby increasing ethanol concentration from 20% to 40% v/v progressively reduced particle size when other compositional variables were kept constant. This observation is well-supported by existing literature and attributed to ethanol's ability to lower interfacial surface tension at the lipid–water boundary, thereby enabling the formation of smaller, more compact vesicles during the aqueous hydration phase (Bhalaria *et al.,* 2009). The lowest PDI values were uniformly recorded in formulations containing the highest ethanol fraction, confirming that higher alcohol concentrations promote more homogeneous vesicle populations during extrusion.

Zeta potential values were negative for all formulations (range: −28.6 to −34.8 mV), indicating sufficient surface charge to maintain physical colloidal stability through electrostatic repulsion between vesicles. The negative character of the surface potential arises primarily from the ionization of phospholipid phosphate headgroups at the measurement pH; cholesterol interactions within the bilayer contribute secondarily. Importantly, formulations with higher SPC concentrations consistently exhibited more negative zeta potential values, reflecting the increased density of ionizable headgroups on the outer leaflet of the bilayer—a finding consistent with prior reports on phosphatidylcholine-dominant vesicular systems (Verma *et al.,* 2003).

Encapsulation efficiency ranged from 72.3 ± 2.1% (EP-1) to 85.7 ± 1.5% (EP-6), with both higher phospholipid concentration and higher ethanol content contributing positively to EE. The amphiphilic nature of LP—bearing a lipophilic biphenyl-tetrazole moiety alongside a hydrophilic potassium carboxylate group—enables it to partition across different domains of the vesicular architecture. Ethanol, by fluidizing and laterally expanding the phospholipid bilayer, creates increased volumetric space for drug intercalation within the membrane, improving overall loading capacity. EP-6 (SPC 400 mg, cholesterol 87.5 mg, ethanol 20% v/v) achieved the most favorable combination of small size, low PDI, strongly negative zeta potential, and high EE among all formulations

and was therefore selected as the optimized formulation for subsequent patch development.

Table 2: Physicochemical Properties of LP Ethosomal Formulations (Mean ± SD, n = 3)

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)	EE (%)	Drug Content (%)	Flux (μg/cm²/h)
EP-1	187.4 ± 4.2	0.312 ± 0.02	−28.6 ± 1.4	72.3 ± 2.1	94.2 ± 1.8	34.2 ± 2.1
EP-2	201.8 ± 5.1	0.289 ± 0.03	−30.2 ± 1.7	76.8 ± 1.9	95.1 ± 1.4	41.7 ± 2.8
EP-3	218.5 ± 6.3	0.265 ± 0.02	−32.4 ± 2.1	80.4 ± 2.4	96.3 ± 1.6	48.6 ± 3.1
EP-4	195.2 ± 4.8	0.301 ± 0.02	−29.7 ± 1.5	74.6 ± 1.7	94.8 ± 1.3	37.4 ± 2.4
EP-5	209.6 ± 5.7	0.278 ± 0.03	−31.1 ± 1.9	78.2 ± 2.2	95.7 ± 1.9	44.9 ± 3.3
EP-6 (Opt.)	176.3 ± 3.9	0.241 ± 0.01	−34.8 ± 1.2	85.7 ± 1.5	97.8 ± 0.9	62.4 ± 2.3

PDI = polydispersity index; EE = encapsulation efficiency; Flux determined using Strat-M® synthetic membrane.

Vesicle Morphology

TEM examination of the EP-6 formulation revealed predominantly spherical, discrete vesicles with well-defined, electron-dense bilayer boundaries and electron-lucent aqueous interiors. No large aggregates, fused structures, or lamellar sheets were evident at the dilution employed, indicating that sequential membrane extrusion through 400-nm and 200-nm polycarbonate membranes effectively produced a predominantly unilamellar vesicle population. The mean diameter estimated from TEM micrographs (approximately 158 ± 12 nm) was marginally smaller than that determined by DLS (176.3 nm), an expected discrepancy arising because TEM images dehydrated specimens in vacuum whereas DLS captures the hydrodynamic diameter of vesicles in hydrated suspension—which includes the associated water shell and reflects dynamic behavior in solution. Collectively, TEM data confirmed the structural integrity and morphological uniformity of the ethosomal vesicles prepared at the optimized composition.

Physicochemical Evaluation of the Patch

Table 3 summarizes the physicochemical evaluation parameters for the optimized EP-6 ethosomal patch alongside the conventional LP matrix patch prepared as a reference control. All parameters for both formulations fell within the pre-defined acceptance criteria, confirming pharmaceutical acceptability of the fabricated patch products.

Table 3: Physicochemical Evaluation of Ethosomal and Conventional LP Patches (Mean ± SD, n = 5)

Parameter	EP-6 Ethosomal Patch	Conventional Patch	Acceptance Criteria
Thickness (mm)	0.32 ± 0.02	0.31 ± 0.03	0.25–0.45
Weight uniformity (mg/cm²)	18.4 ± 0.9	18.1 ± 1.1	15–25
Moisture content (%)	3.2 ± 0.4	4.1 ± 0.6	≤5
Tensile strength (N/mm²)	4.8 ± 0.3	3.2 ± 0.4	≥3.0
Elongation at break (%)	62.4 ± 3.1	48.6 ± 4.2	≥40
MVTR (g/m²/24h)	128.4 ± 6.2	132.1 ± 7.4	80–160
pH of patch extract	6.9 ± 0.1	6.8 ± 0.1	6.5–7.4
Bioadhesive force (mN)	42.6 ± 2.4	38.1 ± 2.8	≥35
Drug content (%)	97.8 ± 0.9	96.4 ± 1.3	≥95

MVTR = moisture vapor transmission rate.

Thickness (0.32 ± 0.02 mm) and weight uniformity (18.4 ± 0.9 mg/cm²) were consistent across batches of the ethosomal patch, attesting to the reproducibility of the solvent-casting method. Low moisture content (3.2 ± 0.4%) is an important quality attribute that safeguards against microbial proliferation and premature drug release during storage. The MVTR value of 128.4 g/m²/24h falls within the clinically recommended range, balancing adequate local hydration of the skin surface—which itself facilitates permeation—against the risk of maceration under occlusive conditions. The tensile strength (4.8 ± 0.3 N/mm²) and elongation at break (62.4 ± 3.1%) of the ethosomal patch surpassed those of the conventional patch, indicating superior mechanical robustness and flexibility. This is clinically meaningful: a patch with greater elasticity conforms more effectively to the contours of non-planar body surfaces such as the forearm, upper arm, or chest, reducing the probability of delamination and ensuring consistent drug delivery over the wear period. The higher bioadhesive force measured for the ethosomal patch (42.6 ± 2.4 mN) compared with the conventional formulation (38.1 ± 2.8 mN) is likely attributable to a mild plasticizing effect of residual ethanol from the ethosomal dispersion on the HPMC polymer network, improving interfacial contact with soft tissue substrates. Drug content uniformity (97.8

± 0.9%) confirmed homogeneous spatial distribution of LP within the matrix, a critical quality attribute for consistent dose delivery.

In Vitro Membrane Permeation Using Strat-M® Synthetic Membrane

The Strat-M® membrane was selected for in vitro permeation evaluation because it constitutes the most extensively validated animal-free surrogate for human skin barrier function currently available. Its multi-layered polyether sulfone structure incorporates lipid blends that mimic the stratum corneum's intercellular lipid composition, yielding permeation characteristics closely correlated with those measured using human and cadaver skin across a broad range of physicochemical drug properties (Uchida *et al.,* 2015; Sintra *et al.,* 2019). Importantly, because Strat-M® is a fully synthetic, commercially manufactured membrane, it offers complete elimination of biological and ethical concerns associated with animal tissue procurement, while providing a high degree of batch-to-batch reproducibility unavailable with biological membranes.

Table 4 presents the permeation parameters derived from Franz diffusion cell experiments. The EP-6 ethosomal patch achieved a steady-state flux of 62.4 ± 2.3 μg/cm^2/h through the Strat-M® membrane, significantly exceeding the conventional patch (14.4 ± 1.3 μg/cm^2/h) by a factor of 4.3, and the plain drug solution (7.9 ± 0.8 μg/cm^2/h) by nearly 8-fold. The permeability coefficient was correspondingly highest for the ethosomal patch (4.16×10^{-3} cm/h).

Table 4: In Vitro Membrane Permeation Parameters Through Strat-M® Synthetic Membrane (Mean ± SD, n = 3)

Formulation	Flux (μg/cm^2/h)	Permeability Coeff. (cm/h)	Enhancement Ratio	Lag Time (h)
EP-6 Ethosomal Patch	62.4 ± 2.3	4.16×10^{-3}	4.3	1.6 ± 0.2
Conventional Patch	14.4 ± 1.3	0.96×10^{-3}	1.0 (Reference)	3.4 ± 0.4
Plain Drug Solution	7.9 ± 0.8	0.53×10^{-3}	0.55	—

ER = enhancement ratio relative to conventional patch; lag time by extrapolation of steady-state linear region to time axis.

The markedly shorter lag time of the ethosomal patch (1.6 ± 0.2 h) relative to the conventional patch (3.4 ± 0.4 h) has direct clinical implications: a shorter lag time translates to a faster attainment of therapeutically meaningful drug concentrations in systemic circulation following patch application, which is particularly valuable in chronic antihypertensive therapy where consistent plasma drug levels are required throughout the day. The superior permeation performance of the ethosomal formulation is mechanistically attributable to two principal synergistic factors. First, the high ethanol content of the vesicles disrupts the lamellar ordering of lipids within the Strat-M® membrane (acting analogously to SC lipid disruption), increasing the diffusion coefficient of LP and reducing the diffusional resistance of the barrier. Second, the inherently deformable nature of ethosomes—arising from fluidized, ethanol-modified bilayers—enables intact vesicles to permeate through membrane pores significantly smaller than their equilibrium diameter, thereby delivering encapsulated LP deeper within the membrane layers and maintaining a sustained drug concentration gradient across the barrier.

It is noteworthy that increasing ethanol concentration beyond 40% v/v in preliminary experiments did not yield proportional gains in permeation enhancement. This is consistent with the threshold behavior described in the established literature for ethosomal systems, where excessively high ethanol concentrations may destabilize the vesicular membrane structure and precipitate aggregation or collapse, ultimately counteracting the intended permeation advantage (López-Pinto *et al.,* 2005). The ethanol concentration range of 20–40% therefore defines the optimal formulation window for this system.

In Vitro Drug Release and Kinetic Modeling

Cumulative in vitro drug release from the EP-6 ethosomal patch reached 82.4 ± 3.2% over 24 hours, compared to 71.6 ± 2.8% for the conventional patch over the same period. The release profile from the ethosomal patch was distinctly more sustained, with a near-linear release phase extending from approximately 2 to 18 h, consistent with the behavior of a well-functioning controlled-release matrix. Release kinetic parameters for both formulations are summarized in Table 5.

Table 5: In Vitro Drug Release Kinetic Parameters (n = 3)

Formulation	Zero-Order R^2	First-Order R^2	Higuchi R^2	Korsmeyer–Peppas R^2	n value
EP-6 Ethosomal Patch	0.9614	0.9483	0.9812	0.9927	0.68
Conventional Patch	0.9242	0.9361	0.9884	0.9776	0.49

Best-fit model in bold. n = diffusion exponent from Korsmeyer–Peppas model.

The Korsmeyer–Peppas model provided the best fit for the ethosomal patch (R^2 = 0.9927, n = 0.68), indicating anomalous (non-Fickian) transport. This composite release mechanism reflects the concurrent contributions of drug diffusion through the hydrated HPMC polymer matrix and matrix relaxation as water progressively permeates and swells the patch. The additional diffusional resistance introduced by the ethosomal phospholipid bilayer—which must be traversed before drug can partition into the polymer matrix—contributes to the higher n value observed relative to the conventional patch (n = 0.49, best described by the Higuchi model, R^2 = 0.9884). The Higuchi model's adequacy for the conventional patch confirms straightforward matrix diffusion as the dominant release mechanism in the absence of vesicular barriers. This mechanistic distinction is significant: it demonstrates that the ethosomal carrier does not merely enhance skin permeation at the membrane level, but also modulates the drug release kinetics from the patch matrix itself, providing an additional layer of release control.

Confocal Laser Scanning Microscopy

CLSM z-stack imaging of the Strat-M® membrane after 8-hour exposure to fluorescently labeled EP-6 ethosomes, conventional Rhodamine-loaded liposomes, and plain Rhodamine B solution provided direct visual confirmation of the penetration depth advantage conferred by the ethosomal formulation. Fluorescence from Rhodamine B-labeled EP-6 ethosomes was detected throughout the full depth of the Strat-M® membrane (approximately 120 µm), with strong signal intensity distributed evenly across all membrane layers in the z-stack projections. The fluorescence from conventional Rhodamine-labeled liposomes was largely confined to the upper membrane layers (~0–55 µm), with progressively attenuating signal in deeper strata, consistent with the known limitation of rigid liposomal vesicles in transiting the tightly organized lipid barrier layers. The plain Rhodamine B solution, in turn, showed signal concentrated exclusively in the topmost membrane layer (0–25 µm), with no significant penetration into deeper zones—a finding consistent with the relatively high aqueous solubility of Rhodamine B and its limited partitioning into the lipid-rich membrane interior.

These CLSM findings corroborate and mechanistically explain the permeation data presented in Table 4. The capacity of EP-6 ethosomes to traverse the entire depth of the Strat-M® membrane as intact vesicles—rather than simply releasing drug at the surface and relying on passive diffusion—is the definitive mechanistic signature of effective ethosomal permeation enhancement. The pronounced depth of fluorescent labeling achieved with ethosomes, and its absence with both liposomes and free dye, confirms that the flexibility and ethanol-modified bilayer composition of the ethosomal system are indispensable for membrane penetration rather than merely supplementary features.

Stability Studies

The stability evaluation demonstrated that the EP-6 ethosomal patch maintained acceptable physicochemical attributes over a six-month observation period under both storage conditions tested. Under long-term conditions (25°C/60% RH), no statistically significant changes were detected in particle size, PDI, drug content, EE, or Strat-M® membrane permeation flux at any time point throughout the study ($p > 0.05$ for all pairwise comparisons versus the initial time point), affirming the inherent robustness of the HPMC polymer matrix in shielding the ethosomal vesicles from environmental stress at ambient storage conditions.

Under accelerated conditions (40°C/75% RH), all parameters remained statistically stable through the 3-month time point. By month 6, a modest but statistically significant increase in mean vesicle size (from 176.3 ± 3.9 nm to 197.2 ± 6.4 nm) and a marginal reduction in EE (from 85.7 ± 1.5% to 81.6 ± 2.1%) were observed ($p < 0.05$), consistent with temperature- and humidity-driven vesicular reorganization—a recognized phenomenon in ethosomal and liposomal systems stored under thermal stress (Wissing *et al.,* 2004). Importantly, the Strat-M® permeation flux of the 6-month accelerated sample (58.1 ± 3.2 µg/cm^2/h) remained substantially superior to the conventional patch and statistically non-inferior to the initial EP-6 value ($p > 0.05$), indicating that the permeation-enhancement capability of the system was durably preserved despite modest vesicular reorganization. These data collectively suggest a satisfactory shelf-life profile for the formulation under recommended storage conditions, with appropriate packaging.

CONCLUSIONS

This study demonstrates the successful development and comprehensive physicochemical characterization of a losartan potassium ethosomal transdermal patch as a controlled-delivery platform free of any animal tissue or in vivo testing at the development stage. The optimized formulation (EP-6), prepared by cold-membrane extrusion using 400 mg soy phosphatidylcholine, 87.5 mg cholesterol, and 20% v/v ethanol, yielded vesicles of 176.3 ± 3.9 nm with a PDI of 0.241, a zeta potential of −34.8 mV, and an encapsulation efficiency of 85.7%. Integration of these vesicles into a solvent-cast HPMC K100M matrix patch produced a mechanically robust, flexible, and pharmaceutically acceptable

transdermal system with excellent drug content uniformity.

In vitro permeation studies through the Strat-M® synthetic membrane—a validated, animal-free human skin surrogate—yielded a steady-state flux of 62.4 μg/cm^2/h, representing a 4.3-fold enhancement over a conventional matrix patch and an 8-fold improvement over a plain drug solution. CLSM imaging through the Strat-M® layers provided mechanistic verification of deep, intact vesicle penetration unique to the ethosomal system. Drug release followed anomalous non-Fickian kinetics, reflecting dual control by matrix diffusion and vesicular bilayer resistance. Six-month ICH-compliant stability data confirmed physicochemical durability under both long-term and accelerated storage conditions.

Taken together, these findings establish a scientifically rigorous, ethically compliant proof of concept for the LP ethosomal transdermal patch as a superior alternative to conventional matrix patches—and a compelling candidate for further clinical advancement. The demonstrated permeation enhancement, controlled release, and colloidal stability profile position this system to potentially improve bioavailability predictability, reduce dosing frequency, and enhance adherence in hypertensive patients who are poorly served by oral pharmacotherapy. Future investigations should focus on computational skin permeation modeling to extrapolate in vitro Strat-M® flux data to anticipated in vivo performance, human volunteer skin permeation studies using tape-stripping or microdialysis methodology, and process scale-up feasibility assessments to advance this platform toward clinical translation.

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Declarations

Funding- None**.**

Conflict of Interest-None**.**

Ethical Statement: This study was conducted entirely using synthetic materials, commercially available synthetic membranes, and in vitro methods. No animal tissue, animal-derived biological materials, or in vivo animal experiments were used at any stage of this investigation. Accordingly, no institutional animal ethics committee approval was required or sought.

Data Availability: All datasets generated and analyzed during the present study are available from the corresponding author upon reasonable request.

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