RESEARCH PAPER

# Formulation and Evaluation of Novel Colchicine Liposomal Transdermal Gel for Gout

**Sushmita Yelwe[1], Deepak Gautam[4], Vijay Gokarn[3], Geeta Bhagwat[2*]**

1. Department of Pharmaceutics, H.K College of Pharmacy, Jogeshwari, India.
2. Department of Pharmaceutics, D Y Patil University, School of Pharmacy, Navi Mumbai, India.
3. Abhinav Healthcare Products Pvt Ltd. Andheri West, Mumbai.
4. Shobhaben Pratapbhai Patel School of Pharmacy and Technology Management, SVKM's NMIMS, Vile Parle, Mumbai, India.

***Correspondence Author**

Geeta Bhagwat
Associate Professors
Department of Pharmaceutics
D Y Patil University, School of Pharmacy, Navi Mumbai, India
Email: geeta.bhagwat@dypatil.edu

**Abstract**

**Background:** Colchicine is an effective first-line therapy for acute gout; however, its oral administration is associated with gastrointestinal intolerance and systemic toxicity, limiting long-term clinical use. The present study aimed to develop and evaluate a liposome-based transdermal colchicine formulation to improve localized drug delivery while minimizing systemic exposure.

**Methods:** Colchicine-loaded liposomes were prepared using ethanol injection and thin-film hydration methods. The optimized liposomal formulation was characterized for vesicle morphology, particle size, zeta potential, entrapment efficiency, drug content, and stability. The optimized formulation was subsequently incorporated into a Carbopol-based transdermal gel and a Methocel-based transdermal patch. Both dosage forms were evaluated for physicochemical properties, *in vitro* drug release, *ex vivo* permeation through goat and human cadaver skin, and accelerated stability.

**Results:** The optimized liposomal formulation (DCF1) exhibited spherical vesicles with a particle size of 651.4 nm and a zeta potential of −1.19 mV. It demonstrated the highest entrapment efficiency (81.71%) and drug content (99.65%). The liposomal gel showed acceptable physicochemical characteristics, including a skin-compatible pH (6.2 ± 0.53), viscosity ranging from 27,160 to 29,400 cps, spreadability of 277 g·cm/s, satisfactory extrudability, and drug content of 99.65%. The transdermal patch exhibited uniform thickness (0.143 ± 0.0039 mm), average weight (33.88 ± 0.23 mg), folding endurance (112 ± 0.23), and adequate tensile strength. The liposomal gel achieved 96.39% cumulative drug release within 12 h and followed the Higuchi release model ($R^2$ = 0.9178), indicating diffusion-controlled release. Ex vivo permeation studies demonstrated enhanced colchicine permeation across both goat and human cadaver skin, with cumulative permeation reaching 94.94% and 96.39% after 12 h, respectively. Stability studies under accelerated conditions showed satisfactory retention of drug content over the study period.

**Conclusion:** The developed liposomal transdermal colchicine formulation demonstrated favorable physicochemical characteristics, sustained drug release, effective skin permeation, and acceptable stability. These findings suggest that the formulation has potential as a topical delivery system for gout management and warrants further preclinical and clinical evaluation.

***Keywords****: Gout, Colchicine, Liposomes, Gel, Transdermal Patch*

**How to cite this article:** Yelwe S, Gautam D, Gokarn V, Bhagwat G. Formulation and Evaluation of Novel Colchicine Liposomal Transdermal Gel for Gout. Int J Drug Deliv Technol. 2026;16(70s): 1876-1887 DOI: 10.25258/ijddt.16.70s.203

## 1. Introduction

Gout is the most common form of inflammatory arthritis and is characterized by the deposition of monosodium urate (MSU) crystals in synovial fluid, articular cartilage, and periarticular tissues as a consequence of persistent hyperuricemia [1,2]. Although elevated serum uric acid (SUA) is a prerequisite for MSU crystal formation, hyperuricemia alone is insufficient for disease development, as only a small proportion of individuals with elevated SUA levels ultimately develop gout. Genetic predisposition, metabolic abnormalities, renal dysfunction, dietary habits, and other environmental factors contribute to disease susceptibility and progression. The deposition of MSU crystals initiates a robust inflammatory response mediated by activation of the NLRP3 inflammasome and subsequent release of pro-inflammatory cytokines, resulting in severe pain, erythema, swelling, and recurrent episodes of acute arthritis. Chronic uncontrolled disease may progress to tophi formation, joint destruction, nephrolithiasis, and chronic kidney disease. Definitive diagnosis is established by the identification of MSU crystals in synovial fluid or tophaceous aspirates, while long-term management focuses on lowering SUA below the saturation threshold through lifestyle

modifications and urate-lowering therapies to prevent crystal deposition and recurrent flares [3,4].

Colchicine, a natural alkaloid isolated from *Colchicum autumnale* (family *Liliaceae*), remains one of the first-line therapies for the treatment of acute gout owing to its rapid anti-inflammatory action. Its therapeutic effect is primarily mediated through inhibition of microtubule polymerization, which suppresses neutrophil chemotaxis, adhesion, activation, and migration toward MSU crystals. In addition, colchicine inhibits inflammasome activation, reduces the production of reactive oxygen species, and suppresses the release of inflammatory mediators, thereby limiting crystal-induced inflammation [5–7]. Despite its proven clinical efficacy, the therapeutic utility of colchicine is limited by its narrow therapeutic index and dose-dependent toxicity. Oral administration is frequently associated with gastrointestinal adverse effects, including nausea, vomiting, abdominal pain, and diarrhea, while prolonged systemic exposure may lead to bone marrow suppression, neuromyopathy, hepatotoxicity, and renal toxicity. Intravenous administration has largely been abandoned because of severe and potentially fatal adverse reactions, including cytopenia, tissue necrosis, disseminated intravascular coagulation, and multiorgan failure. These limitations underscore the need for alternative drug delivery strategies capable of delivering colchicine directly to inflamed joints while minimizing systemic exposure.

Transdermal drug delivery has emerged as an attractive alternative to oral and parenteral administration because it bypasses gastrointestinal degradation and first-pass hepatic metabolism, provides sustained and controlled drug release, minimizes fluctuations in plasma drug concentrations, improves patient compliance, and reduces systemic adverse effects [8]. Topical delivery is particularly advantageous for localized inflammatory disorders such as gout, where therapeutic concentrations can be achieved directly at the affected site while limiting systemic drug distribution. Among various topical formulations, transdermal gels and patches are widely employed because of their ease of application, patient acceptability, and ability to provide prolonged drug release [9].

Liposomes are biocompatible phospholipid vesicles capable of encapsulating both hydrophilic and lipophilic therapeutic agents. Owing to their structural similarity to biological membranes, liposomes enhance drug stability, improve skin penetration, increase local drug retention, and enable sustained drug release while reducing local irritation and systemic toxicity. As colchicine is a hydrophilic molecule, liposomal encapsulation offers an effective strategy for improving its topical delivery and therapeutic performance. Furthermore, liposomes can facilitate localized drug accumulation within inflamed tissues, thereby enhancing therapeutic efficacy while reducing the frequency of administration and the risk of dose-related adverse effects [10].

Therefore, the present study aimed to develop and optimize colchicine-loaded liposomes using ethanol injection and thin-film hydration techniques and to incorporate the optimized formulation into both transdermal gel and transdermal patch systems. The developed formulations were comprehensively characterized for their physicochemical properties, drug encapsulation efficiency, in vitro drug release, ex vivo skin permeation, and stability to evaluate their potential as a localized and sustained transdermal drug delivery system for the management of gout.

## 2. Materials and Methods

Colchicine was kindly provided as a gift sample by India Glycols Ltd., India. Cholesterol was generously supplied by VAV Lipids Pvt. Ltd., Mumbai, India. 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) was obtained as a gift sample from Lipoid GmbH, Germany. Methanol, ethanol, n-octanol, and isopropyl alcohol were purchased from Thermo Fisher Scientific, India. Methocel® K100M was procured from Colorcon (Sigma-Aldrich, USA). Propylene glycol and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich, USA. Carbopol® 940, glycerol, methyl paraben, triethanolamine, and all other chemicals and reagents used in the study were of analytical grade and were used as received without further purification.

### 2.1. Preparation of liposomal suspension

Colchicine-loaded liposomes were prepared using the ethanol injection method followed by probe sonication, with slight modifications to previously reported procedures [11,12] Briefly, the organic phase comprising 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) and cholesterol was dissolved in 5 mL of absolute ethanol and sonicated until a clear solution was obtained. To minimize solvent evaporation during preparation, the beaker containing the organic phase was covered with a lid. The aqueous phase consisted of distilled water maintained at 55–60 °C under continuous magnetic stirring at 800 rpm. The organic phase was then injected slowly into the aqueous phase using a syringe while maintaining constant stirring. Stirring was continued for 25–30 min to facilitate spontaneous liposome formation and ensure complete evaporation of ethanol. The elevated temperature was maintained throughout the process to keep the phospholipids above their phase transition temperature, thereby promoting uniform vesicle formation and minimizing structural deformation of the liposomes. The resulting

liposomal suspension was subsequently subjected to sonication to reduce vesicle size and obtain a homogeneous dispersion [13–15]. The composition of the different liposomal formulations prepared in this study is presented in Table 1.

**2.2. Thin-film hydration method**

Colchicine-loaded liposomes were also prepared using the thin-film hydration method based on the optimized lipid composition of formulation DCF1, with slight modifications to previously reported methods [16,17]. Briefly, predetermined quantities of colchicine, 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), and cholesterol were dissolved in a chloroform mixture (3:2, v/v). The resulting solution was sonicated to ensure complete dissolution of all components and then transferred into a round-bottom flask. The organic solvents were removed under reduced pressure using a rotary evaporator operated at 60 rpm and 60 °C until a thin, uniform lipid film was formed on the inner wall of the flask. The lipid film was subsequently dried at room temperature for 24 h to ensure complete removal of residual organic solvents. The dried lipid film was then hydrated with 10 mL of phosphate buffer under continuous agitation using a temperature-controlled shaking incubator, resulting in the formation of a homogeneous liposomal suspension. The hydration process facilitated swelling of the lipid bilayer and spontaneous formation of multilamellar liposomes encapsulating colchicine[14,29]. The composition of the formulation prepared by the thin-film hydration method is presented in **Table 2**.

**2.3. Preparation of Liposomal Gel**

Liposomal gel was prepared using Carbopol 940. Specified amounts of Carbopol 940 (2% w/v) solution and liposomal formulations were first allowed to hydrate in distilled water for 3–4 hours. Subsequently, 1 mL of the liposomal dispersion was incorporated into 5 g of Carbopol 940 containing glycerine and methyl paraben, with continuous magnetic stirring, until a uniform dispersion was formed. Triethanolamine was added to adjust the pH. The liposomal gel was sonicated for 15 min. The mixture was then kept overnight to remove air bubbles. Subsequently, the dispersion was gently mixed at room temperature until a consistent gel is formed [18,19].

**2.4. Preparation of liposomal transdermal patch**

A liposomal gel was incorporated into a transdermal patch using the solvent-casting technique. The DCF1 liposomal formulation, prepared via the thin-film hydration method, was embedded into a methocel-based matrix [20]. Briefly, 1 g of Methocel K100M was dispersed in 50 mL of distilled water, followed by the addition of 1% propylene glycol and DMSO, serving as a plasticizer and penetration enhancer, respectively. The dispersion was allowed to stand for 24 hours to eliminate entrapped air. The liposomal preparation containing the required drug concentration was then mixed into the hydrated polymer solution. The final mixture was cast onto a glycerin-lubricated Petri plate and dried at 60 °C for nine hours. After drying, the formed transdermal patch was carefully peeled off the casting surface [21]. The compositions of the liposomal transdermal patch are listed in Table 4.

**2.5. Characterisation of liposomal suspension**

**2.5.1. Measurement of Vesicle Size and Shape**

The morphology of the liposomal vesicles was evaluated using an optical microscope. A drop of the liposomal suspension was placed on a glass slide, and observations were made at magnifications of 10X and 45X and pictograms were recorded. The average size and size distribution (Polydispersity index, PDI) of the liposome vesicles were measured by Horiba Scientific. Each sample was run 3 times, and the measurements were done at 25℃ at a scattering angle of 173 degrees.

**2.5.2. Scanning Electron Microscopy**

The surface morphology of liposomes was studied by SEM. The exact shape of liposomes was determined by SEM. The sizes of the vesicles were measured by scanning electron microscopy. A small amount of liposomal suspension was placed on the specimen stub, coated with carbon and then with gold vapour using a sputter coater. The samples were examined under a scanning electron microscope for vesicular shape, and images were captured.

**2.5.3. Determination of Zeta Potential**

Zeta potential measurements were performed at 25 °C using a Zetasizer (Horiba Instruments). The liposomal suspension was diluted 100 times with double-distilled water prior to analysis. The instrument voltage was set to 1.4 V, and the electrodes were immersed in the diluted dispersion for measurement. All analyses were carried out in triplicate at 25 °C using a scattering angle of 173°.

**2.5.4. Determination of entrapment efficiency**

The prepared liposomal suspension was reconstituted with 10 mL of isotonic phosphate buffer (pH 7.4) and sonicated for 10 minutes in a bath sonicator. Drug-loaded liposomes were then separated by centrifugation at 3000 rpm for 30 minutes at 20 °C. An aliquot of 1 mL from the supernatant was withdrawn, transferred to a 10 mL volumetric flask, and diluted to volume with the same buffer. The drug concentration in the resulting solution was quantified using a UV spectrophotometric method. The percentage encapsulation efficiency was calculated using the following equation:

$$\square\square\ (\%) = [Ct - Cr)/Ct] * 100$$

Where,

EE = Entrapment Efficiency,

$C_t$ = concentration of total drug, $C_r$ = concentration of free drug.

**2.5.5. Determination of Drug content**

A standard solution was prepared by dissolving 2.5 mg of colchicine in phosphate buffer (pH 7.4) to obtain 10 mL of suspension in a 25 mL volumetric flask, which served as the stock solution. From this stock, 1 mL was transferred into a 10 mL volumetric flask and diluted to volume, and the absorbance was recorded in triplicate. The sample solution was prepared by pipetting 1 mL of the test dispersion into a 10 mL volumetric flask and diluting to the mark with phosphate buffer. The absorbance of the sample was measured at 353 nm, and the percentage drug content was calculated using the following formula:

Drug Content (Dc): Sample Absorbance/Standard Absorbance*Dose

%Drug Content: Dc*100/Dose

**2.6. Characterization of liposomal Gel**

**2.6.1. Physical Appearance and Homogeneity**

The liposomal gel was evaluated visually for their colour, clarity, and overall appearance after setting in the container. Formulations were inspected for uniformity and checked for the presence of any aggregates, phase separation, or particulate matter to confirm homogeneity.

**2.6.2. Viscosity Measurement**

The viscosity of the prepared liposomal gels was measured using a Brookfield Viscometer (Model DV-E). Approximately 10 g of gel was placed in a beaker, and spindle number 64 was immersed into the formulation. Measurements were recorded at 20 rpm, as the gels belong to the high-viscosity (HA) category.

**2.6.3. Determination of pH**

The pH of the gel formulations was measured in triplicate using a calibrated digital pH meter. The glass electrode was immersed directly into each gel sample, and the recorded values were used to determine the formulation's pH suitability for topical application.

**2.6.4. Spreadability**

The spreadability of the gel formulations was evaluated using the parallel plate method. A 0.5 g sample of the gel was placed at the centre of a pre-marked 1 cm diameter circle on a glass plate, and a second glass plate was carefully positioned on top. A 50 g weight was then placed on the upper plate to allow uniform spreading. The increase in the diameter of the gel was measured, and the time required to separate the two plates was recorded as an indicator of spreadability. The study was performed in triplicate, and the mean diameter was used to calculate spreadability using the following formula:

$S = m \times L/t$

Where,
S: Spreadability,
m: weight of load
L: length travel by upper slide,
t: Time

**2.6.5. Extrudability**

Extrudability is a critical attribute that influences the ease of application and overall patient acceptability of topical gels. This parameter indicates whether the formulation can be expelled smoothly and uniformly from collapsible tubes during use. Highly viscous gels may not extrude adequately, whereas low-viscosity gels may flow too rapidly; therefore, an optimal consistency is essential. For evaluation, the gel formulations were filled into collapsible aluminium tubes, and each tube was compressed to extrude a 0.5 cm ribbon of gel within 10 seconds. The extrudability was assessed based on the quantity of gel expelled and was calculated using the following formula:

Extrudability = Applied weight to extrude gel from tube (in gm) / Area (in cm2)

**2.7. Characterization of liposomal patch**

**2.7.1. Physical Characterization**

**2.7.1.1. Thickness**

The thickness of the transdermal patch (batch T1) was measured using a Mitutoyo Digimatic Micrometer. Measurements were taken at five different points across the patch surface, and the mean thickness was calculated to ensure uniformity.

**2.7.1.2. Average weight**

The average weight of the transdermal patch was determined by individually weighing the patches using a digital analytical balance. The mean weight was calculated to assess weight uniformity across the batch.

**2.7.1.3. Folding Endurance**

Folding endurance was assessed to evaluate the mechanical strength and flexibility of the transdermal films. The test was performed by repeatedly folding each film at the same location until it fractured. The number of successive folds a film could withstand without breaking was recorded as its folding endurance value.

**2.7.1.4. Tensile strength**

The mechanical properties of the biopolymeric film were evaluated by determining its tensile strength. An in-house assembly was used, wherein a pan was suspended using a strong thread, and the opposite end of the thread was attached to the centre of the film. The assembly was positioned on a beam

balance, and incremental weights were added to the pan until the film ruptured. The load required to break the film was recorded, and the tensile strength was calculated using the following formula:

Tensile Strength= [Weight added to break(kg)/Width (cm)]*Thickness of patch (cm)

**2.8. *In vitro* drug release study**

***In Vitro drug release from Transdermal gel***

The in vitro release of colchicine from the liposomal transdermal gel was evaluated using a dialysis membrane (70-µm pore size, HI Media) as the donor compartment. An amount of gel equivalent to 2.5 mg of drug was placed inside the dialysis bag. The membrane was pre-treated by soaking in phosphate buffer (pH 7.4) for 24 hours or alternatively in warm water for 10 minutes prior to use. After loading the formulation, both ends of the membrane were securely sealed with thread.

The dialysis bag containing the gel was suspended in the dissolution medium (500 mL phosphate buffer, pH 7.4) using a paddle apparatus operated at 50 rpm and maintained at 37 °C. At predetermined intervals over 12 hours, 3 mL samples were withdrawn and immediately replaced with an equal volume of fresh buffer to maintain sink conditions throughout the study. The collected samples were diluted appropriately and analyzed using a UV spectrophotometer at 353 nm to determine drug release.[31]

***In- Vitro drug release from Transdermal patch***

The in vitro drug release profile of the transdermal patch (T1) was evaluated using a CD14 dissolution test apparatus (Type V: paddle-over-disc, Teledyne Hanson). The study was performed in phosphate-buffered saline (PBS, pH 7.4) at a paddle speed of 100 rpm for 8 hours. The transdermal film was positioned within the transdermal sandwich assembly, which was subsequently placed at the bottom of the dissolution vessel containing pre-equilibrated PBS (pH 7.4).

At predetermined intervals, aliquots of 5 mL were withdrawn and immediately replaced with an equal volume of fresh dissolution medium to maintain sink conditions. The collected aliquots were filtered and analyzed spectrophotometrically to determine the amount of drug released. An identical procedure was performed for transdermal patches containing pure drug, which served as the control.

**2.9. *Ex-vivo* skin Permeation Study of transdermal gel**

*Goat skin*

Skin permeation studies were conducted using a Franz diffusion cell with an effective diffusion area of 1.5 cm². Freshly excised goat dorsal skin, obtained from a local slaughterhouse, was stored at −18 °C in physiological saline until use. Prior to the experiment, the skin was equilibrated in physiological saline at room temperature for 2 hours to remove residual biological material. The hair was carefully removed using a razor, and adhering subcutaneous fat on the dermal side was eliminated with isopropyl alcohol (IPA). The cleaned tissue was subsequently rinsed with distilled water. A circular section of skin (approximately 3 cm in diameter) was mounted between the donor and receptor compartments of the Franz diffusion cell. One gram of the drug-loaded liposomal gel was applied uniformly onto the epidermal (donor) side, ensuring intimate contact between the formulation and skin surface. The receptor chamber was filled with phosphate buffer pH 7.4, maintained at 37 ± 0.5 °C, and continuously stirred using a magnetic stirrer. At predetermined intervals (1, 2, 3, 4, 5, 6, 7, 8, and up to 12 hours), 3 mL aliquots were withdrawn from the receptor compartment and immediately replaced with fresh buffer to maintain sink conditions. The collected samples were analyzed spectrophotometrically (UV-Vis, Shimadzu-1800, Japan) at 300 nm using phosphate buffer pH 7.4 as blank. Permeation studies for the marketed gel were performed under identical conditions [22,23].

*Human Cadaver Skin*

Human cadaver skin was procured from the Skin Donation and Skin Banking Unit at Lokmanya Tilak Municipal General Hospital (LTMGH), Sion, Mumbai, India. The human skin cadaver tissue used for experiment included the epidermis and a part of the dermis with a total thickness of about 0.5-1 mm as required and preserved according to the tissue bank's validated protocols. The samples were transported to the laboratory in sterile, temperature-controlled containers. Prior to experimentation, the skin was rinsed and incubated in phosphate buffer saline (pH 7.4) and equilibrated to the required experimental conditions. All the studies were conducted within two months of the skin being cryopreserved [24,25].

**2.10. Diffusion study**

Experiments were conducted using Franz-type diffusion cells (20 mL capacity, 1.5 cm² effective diffusion area), a widely used in vitro system in which a semi-permeable membrane or skin preparation separates the donor and receptor compartments [26]. The equilibrated skin was mounted onto the receptor compartment, and the temperature of the diffusion cells was maintained at 37 °C using a circulating water jacket. The receptor solution was continuously stirred with a magnetic bead throughout the experiment. At predetermined intervals (1, 2, 3, 4, 5, 6, 7, 8, up to 12 hours), 3 mL aliquots were withdrawn from the receptor compartment via a sampling port and immediately replaced with an equal volume of fresh phosphate buffer saline (pH 7.4) to maintain sink conditions. The collected samples were analysed spectrophotometrically at 300 nm using phosphate buffer (pH 7.4) as a blank. The cumulative amount of drug permeated per square centimetre at each

time point was calculated and plotted against time to obtain the permeation profile [27].

### 2.11. Ex-vivo Permeability Study of transdermal patch

*Ex vivo* permeation studies of the liposomal transdermal patch were performed using excised goat skin. The hair was carefully removed using a razor, and the skin was kept hydrated in phosphate-buffered saline (PBS) with continuous aeration throughout the preparation process. The skin surface was then cleaned with isopropyl alcohol and cut into 2 × 2 cm² sections.

Franz diffusion cells were employed for the permeation study. The goat skin was positioned between the donor and receptor compartments, and the transdermal patch was placed on the epidermal side. The receptor compartment was filled with 50 mL PBS (pH 7.4), maintained at 37 ± 0.5 °C, and continuously stirred at 200 rpm using a magnetic stirrer. At predetermined time intervals (0, 1, 2, 3, 4, 5, 6, 7, and 8 hours), 1 mL aliquots were withdrawn from the receptor compartment and immediately replaced with an equal volume of fresh buffer to maintain sink conditions. The amount of colchicine permeated was quantified using a UV-Vis spectrophotometer at 275 nm. All experiments were performed in triplicate. For comparison, the same procedure was carried out using transdermal patches containing pure colchicine as the control [28].

### 2.12. Stability Study

The stability of the liposomal gel formulations was evaluated according to ICH guidelines over a period of six months. Adequate quantities of the gels were filled into 5 g collapsible aluminium tubes in triplicate and sealed. The formulations were stored under accelerated conditions (40 ± 2 °C / 75% RH ± 5%) for six months. Samples were withdrawn at 1, 2, and 6 months and analysed for drug content, entrapment efficiency, and *ex vivo* permeability using human cadaver skin [32].

### 2.13. Statistical Analysis

All experiments were performed in triplicate (n = 3), and the results are expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism version XX (GraphPad Software, San Diego, CA, USA) (or equivalent statistical software). The normality of the data distribution was assessed using the Shapiro–Wilk test. Comparisons between two groups were performed using the unpaired Student's *t*-test, whereas comparisons among three or more groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Differences were considered statistically significant at $p < 0.05$.

The cumulative drug release and ex vivo permeation data are presented as mean ± SD. Drug release kinetics were evaluated by fitting the in vitro release data to zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetic models, and the model with the highest coefficient of determination ($R^2$) was considered to best describe the release mechanism.

## 3. Result and discussion

### 3.1. Characterization of Colchicine loaded liposomes

In the present study, colchicine-loaded elastic liposomal formulations were developed to enhance drug loading, entrapment efficiency, and stability, addressing limitations observed in previous formulations. As summarized in **Table 1**, the compositions of the liposomes were optimized with respect to lipid and cholesterol content, which are known to influence vesicle size, zeta potential, drug content, and entrapment efficiency. Cholesterol plays a crucial role in modulating the physicochemical properties of liposomal systems, although its effect on vesicle size, surface charge, and drug encapsulation can vary depending on the formulation.

**Table 1: Composition of colchicine liposome**

| Formulations | DSPC (mg) | Cholesterol(mg) | Ethanol (ml) | API (mg) |
|---|---|---|---|---|
| DCF 1 | 70 | 30 | 5 | 2.5 |
| DCF 2 | 70 | 30 | 5 | 5 |
| DCF 3 | 70 | 30 | 5 | 7.5 |

The morphology and size of the liposomal vesicles were characterized using optical and scanning electron microscopy. Optical microscopy confirmed the formation of spherical colloidal vesicles **(Figure 1).** The DCF1 formulation exhibited a particle size of 568-634 nm and a zeta potential of −17.5 mV, indicating negative surface charge. The zeta potential contributes to vesicle stability by providing electrostatic repulsion, minimizing aggregation, and preventing premature fusion, while the negative charge may facilitate faster systemic clearance.

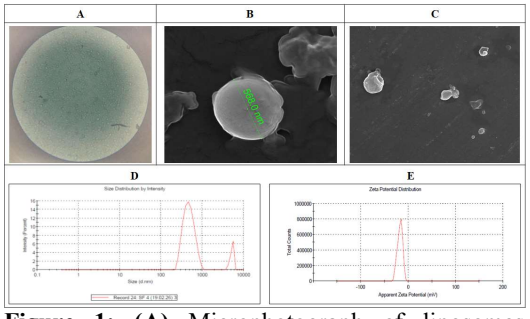



**Figure 1: (A)** Microphotograph of liposomes observed under optical microscope, **(B-C)** Liposomes observed under SEM, **(D)** Particle size of Liposomes and **(E)** Zeta Potential of Liposomes.

The entrapment efficiency (%EE) of colchicine in the elastic liposomes was evaluated using the centrifugation method. The effect of surfactant concentration and drug load on %EE is summarized in **Table 2**. Among the formulations tested, DCF1 (2.5 mg drug load) exhibited the highest entrapment efficiency of 81.71%, followed by DCF3 (7.5 mg, 79.20%) and DCF2 (5 mg, 75.57%). The superior entrapment efficiency of DCF1 can be attributed to its higher lipid and cholesterol content, which enhanced drug incorporation within the bilayer. These findings are consistent with previous studies demonstrating that increased lipid content and the presence of surfactants can significantly improve drug encapsulation in elastic vesicles.

**Table 2:** Entrapment efficiency of three different formulations with varying drug content

| Formulation | % Entrapment efficiency |
|---|---|
| **DCF 1(2.5mg)** | 81.71±1.23% |
| **DCF 2(5mg)** | 75.57±0.97% |
| **DCF 3(7.5mg)** | 79.20±0.78% |

The drug content of the optimized batch (DCF1) was 99.65±0.002%, confirming uniform drug distribution and proper mixing with the excipients. Stability studies conducted under accelerated conditions (40 °C, 75% RH) according to WHO and ICH guidelines showed that the drug content decreased slightly to 94.72% after 1 month and 93.67% after 2 months, indicating acceptable stability of the formulation over the study period **(Table 3)**.

**Table 3:** Degradation of colchicine in the optimized formulation at 40 ± 2 ˚C

| Time (months) | Drug content (mg) | % Remained |
|---|---|---|
| 0 | 2.491458 | 99.6543 ±0.00234 |
| 1 | 2.368126 | 94.72503 ±0.00332 |
| 2 | 2.341986 | 93.67946 ±0.00343 |

These results demonstrate that DCF1 possesses favorable physicochemical properties, including optimal particle size, zeta potential, high drug loading, and entrapment efficiency, making it suitable for further *ex vivo* and *in vivo* evaluation. The incorporation of surfactants and optimized lipid composition likely contributed to the improved entrapment efficiency and stability, supporting the selection of DCF1 as the lead formulation for subsequent studies.

### 3.2. Characterization of Liposomal Gel

Gels are semisolid formulations that provide stiffness to solutions or colloidal dispersions for topical application, offering advantages such as better site adherence, improved patient compliance, and the potential for controlled drug release. In the present study, all the prepared colchicine-loaded liposomal gel formulations exhibited a cloudy appearance, consistent with the incorporation of colloidal vesicles.

#### 3.2.1. Viscosity

The rheological properties of the gels were evaluated to assess their mechanical behaviour and applicability. Viscosity measurements indicated values ranging from 27,160 to 29,400 cps at 20 rpm using spindle S64. The apparent pH of the optimized drug-loaded formulation (DCF1) was 6.2±0.53, which lies within the normal skin pH range (5.5–7.0), suggesting minimal potential for irritation upon topical application **(Figure 2)**.

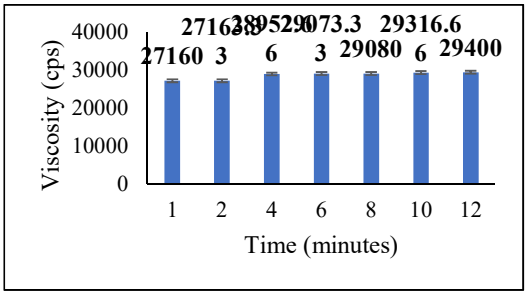



**Figure 2:** Viscosity of liposomal Gel Formulations DCF 1. Data represented in Mean ± S.D (n=3).

#### 3.2.2. Spreadability

The spreadability of the optimized gel was satisfactory, with a measured value of 277 g·cm/sec mentioned in below **Table 4** indicating ease of application. This was compared with the marketed Voligesic gel to confirm comparable application performance. The drug content of the selected batch was 99.69 ± 0.002, reflecting uniform distribution of colchicine and proper incorporation with the excipients.

**Table 4:** Spreadability of formulations

| Formulation | Weight tied to upper slide (g) | Length of glass slide moved (l) | Time taken (t) in (sec) | Average Spreadability S=m*l/t (g.cm/sec) |
|---|---|---|---|---|
| DCF 1 | 100 | 8.3 | 3 | 277 |
| MF | 100 | 7.9 | 5 | 158 |

#### 3.2.3. Extrudability

Extrudability is an important parameter influencing patient compliance, as gels must be easily dispensed from collapsible tubes. The DCF1 gel demonstrated good extrudability (i.e., 1), with the formulation readily extruding in uniform ribbons from the tubes. These results indicate that the optimized liposomal gel possesses suitable mechanical properties for topical administration, including viscosity, pH, spreadability, and extrudability, which together support both patient compliance and therapeutic efficacy.

The observed rheological and application characteristics can be correlated with the viscoelastic nature of the gel matrix. Semi-solid formulations such as these behave both as elastic solids and viscous fluids, with their viscoelastic properties affecting not only the physical appearance and patient perception but also the therapeutic performance. The incorporation of liposomal vesicles into the polymeric gel network likely contributes to the gel's structural stability and controlled drug release behaviour, like previously reported effects of deformable vesicles on gel rheology. Overall, the optimized DCF1 liposomal gel demonstrates a well-balanced combination of mechanical stability, ease of application, and formulation integrity, making it suitable for further in vitro and ex vivo evaluation.

### 3.3. Characterization of transdermal patch

The developed transdermal patch was evaluated for its physical and mechanical properties, including thickness, average weight, folding endurance, and tensile strength. The results of these assessments are summarized in **Table 5.** The patch exhibited a uniform thickness of 0.143 ± 0.004 mm and an average weight of 33.88 ± 0.23 mg, indicating consistent fabrication. The folding endurance, measured as the number of times the patch could be folded at the same location without breaking, was 112 ± 0.23, reflecting good flexibility. The tensile strength, representing the force required to break the patch, was determined to be $1.33\times10^{-5}\pm0.43$ N/mm², demonstrating adequate mechanical integrity suitable for handling and application.

**Table 5:** Physical parameters of transdermal patch

| Thickness (mm) | Average weight (mg) | Folding endurance | Tensile strength (T.S N/mm2) |
|---|---|---|---|
| 0.143±0.003 | 33.88±0.23 | 112±0.23 | $1.33\times10^{-5}\pm0.43$ |

### 3.4. *In vitro* drug release study

Formulation DCF1 demonstrated the highest drug release, achieving 96.38 ± 0.0005% over 12 hours. The incorporation of colchicine-loaded liposomal vesicles into the gel matrix resulted in a sustained release profile compared to the conventional gel, indicating controlled drug release from the liposomal system. The *in vitro* release studies were conducted using a synthetic dialysis membrane with MF (10 mg conventional gel), DCF1 (2.5 mg), and DCF2 (5 mg) formulations, employing a Franz diffusion cell to simulate topical application conditions **(Figure 3).**

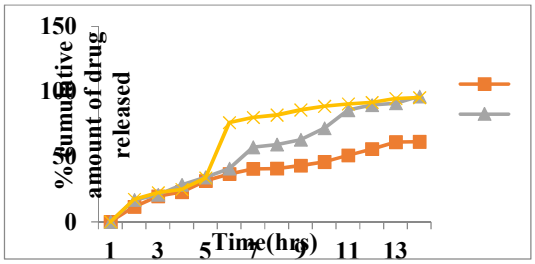



**Figure 3:** % Cumulative Drug Release of liposomal formulation

### 3.5. Drug Release Kinetics

To further analyse the release mechanism, kinetic modelling was performed on the in vitro release data of all three formulations, with MF serving as the reference standard. The results for DCF1 are presented in **Figure 4 & Table 6**. Among the models evaluated, the Higuchi model exhibited the best correlation ($R^2 = 0.9178$), suggesting that drug release from DCF1 followed a diffusion-controlled mechanism. These findings indicate that the liposomal gel matrix effectively modulates colchicine release, supporting its potential for sustained topical therapy.

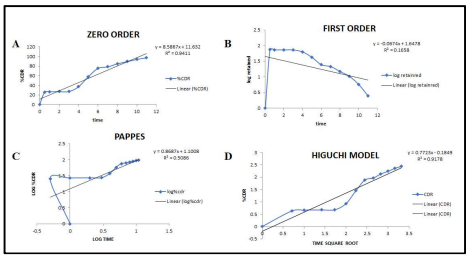
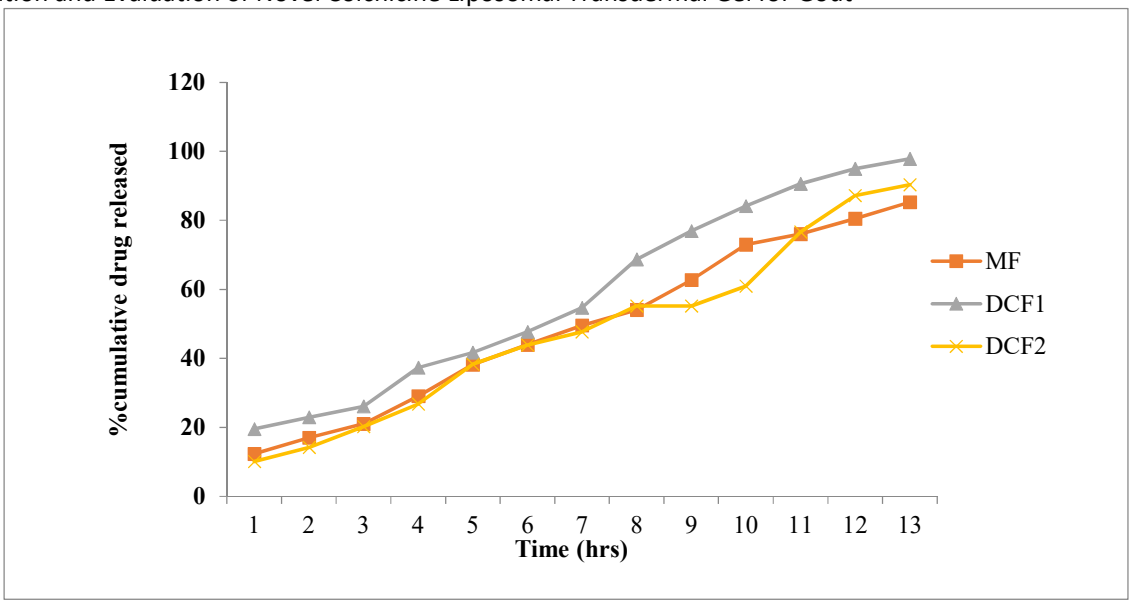



**Figure 4: (A)** Zero Order (% Cumulative Drug Release vs. time) for DCF 1, **(B)** First Order (log % Cumulative Drug Release vs. time), **(C)** Pappe's Equation (Log % Cumulative Drug Release vs. Log time), and **(D)** Higuchi model**.**

**Table 6:** Kinetic Release Study of Colchicine Liposomal Gel – DCF1

| Sr. No. | Kinetics model | Regression Coefficient |
|---|---|---|
| 1 | Zero order | 0.9411 |
| 2 | First order | 0.1658 |
| 3 | Higuchi model | 0.9178 |
| 4 | Peppa's model | 0.5086 |

### 3.6. *Ex vivo* Permeation across Goat Skin




*Liposomal Gel*

Goat skin permeation studies demonstrated a pronounced enhancement in drug flux from the liposomal gel formulation relative to the marketed formulation (MF) and conventional gel. The liposomal gel showed a flux of 225.44 µg/cm²·h, which was substantially higher than the MF (63.83 µg/cm²·h) and DCF2 (117.88 µg/cm²·h). Correspondingly, the permeability coefficients (Kp) exhibited the same trend, indicating superior permeation characteristics of the liposomal system. This enhancement is likely due to the elastic vesicular architecture, which allows liposomes to deform and pass-through skin pores smaller than their original size. The flexible bilayer structure, combined with possible lipid–lipid interactions, may reduce skin barrier resistance and promote drug diffusion. These observations clearly demonstrate the advantage of deformable liposomal incorporation over conventional gel matrices **(Figure 5).**

*Liposomal Patch*

The permeation performance of the liposomal patch across goat skin showed a sustained but controlled drug transport profile, consistent with the intended design of a prolonged-release transdermal system. Although the flux values were lower compared to the liposomal gel due to the patch's reservoir-like configuration, the permeation remained markedly enhanced relative to conventional delivery systems. The patch matrix facilitates gradual diffusion of liposome-entrapped colchicine, maintaining steady-state drug release while reducing peak-related fluctuations. The combination of deformable liposomes with a methocel-based transdermal patch provides dual benefits: enhanced vesicle penetration and extended release, thereby supporting its role as a promising platform for long-term topical colchicine therapy **(Figure 5).**

**Figure 5:** %Cumulative amount of colchicine permeated through goat skin

### 3.7. Ex-Vivo Permeation Across Human Cadaver Skin

*Liposomal gel*

The *ex vivo* permeation data obtained using human cadaver skin demonstrated the superior transdermal performance of the developed colchicine-loaded deformable liposomal gel formulations. The optimized liposomal gels exhibited flux values of 383.99 µg/cm²·h and 112.33 µg/cm²·h, significantly exceeding those of the conventional gel. This enhanced permeation can be attributed primarily to the elastic nature of the vesicles, which enables them to traverse the tightly packed intercellular lipid channels of the stratum corneum. The increased deformability likely reduces diffusional resistance, thereby improving thermodynamic activity and promoting greater drug transport to deeper skin layers. Furthermore, interactions between vesicular lipids and skin lipids may induce lipid fluidization, facilitating enhanced permeability. These findings confirm that deformable liposomal systems provide a more efficient pathway for transdermal delivery of colchicine compared to conventional gel systems **(Figure 6).**

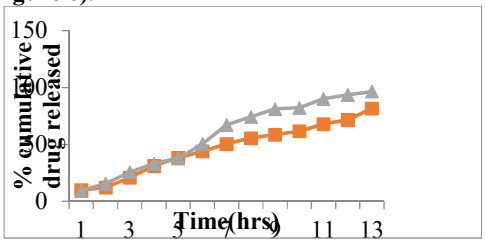



**Figure 6:** % Cumulative amount of colchicine permeated through human cadaver skin.

### 3.8. Stability Study

The stability of the optimized liposomal gel formulation (DCF1) was evaluated according to ICH guidelines under accelerated conditions (40 °C ± 2 °C / 75% ± 5% RH, Climatic Zone III) for a period of 2 months. Throughout the study period, the liposomal gel showed no signs of aggregation, fusion, or vesicle disruption, indicating that the multilamellar structure of the vesicles was well preserved.

The drug content of the formulation was monitored at predefined intervals to assess chemical stability. Initially, the drug content was 99.65%. After 1 month of accelerated storage, the drug content slightly decreased to 94.72%, and after 2 months, it further reduced to 93.67%, demonstrating acceptable stability of the formulation under accelerated conditions **Table 7.**

**Table 7:** Degradation of colchicine in the optimized formulation at 40 ± 2 ˚C

| Time (months) | % Drug Content |
|---|---|
| **0** | 99.65 |
| **1** | 94.72 |
| **2** | 93.67 |

## 4. Conclusion

Colchicine is a treatment option for gout, particularly when NSAIDs are unsuitable or ineffective [30] Colchicine-loaded elastic liposomal formulations were successfully developed and optimized for topical delivery via gel and transdermal patch. The optimized formulation (DCF1) exhibited favourable physicochemical properties, including appropriate vesicle size, high entrapment efficiency (81.71%), uniform drug content (99.65%), and stable multilamellar structure under accelerated conditions. *In vitro* and *ex vivo* studies demonstrated sustained drug release and significantly enhanced skin permeation compared to conventional gel, attributed to the deformable nature of the liposomes. The liposomal gel also showed suitable mechanical properties, pH, spreadability, and extrudability for topical application. Overall, the developed liposomal system provides a promising platform for sustained and effective transdermal colchicine delivery, with improved therapeutic potential and patient compliance.

## Funding

No funding has been received.

## Declaration of Competing Interest

There is no potential conflict of interest reported by any author.

## Acknowledgments

The authors gratefully acknowledge **Dr. D. Y. Patil Deemed to be University, Navi Mumbai, Maharashtra,** for providing the necessary infrastructure facilities. The authors also thank **PubWrite Academy** for manuscript formatting and submission support.

## Data availability

Data will be accessible upon request from the corresponding author.

## AI Use Statement

No artificial intelligence (AI) or AI-assisted tools were used in the writing, data analysis, or creation of figures for this manuscript.

## Ethics approval

Not Applicable

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