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Research Article

Transfersomes Mediated Transdermal Delivery of Curcumin: *In Vitro* and *In Vivo* Evaluation

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Abstract

Background: Curcumin is an herbal anti-inflammatory drug that, when administered orally, shows low bioavailability owing to its poor water solubility, instability in acidic and neutral pH, and rapid intestinal metabolism. In this study, we aimed to improve curcumin delivery through the skin and systemically deliver the drug using transferosomes as carriers.

Methods: The formulations were prepared via thin-film hydration using soya lecithin, cholesterol, and surfactants (plurol oleique/Tween 80). After optimization trials using a central composite design, particle size analysis, zeta potential, TEM, DSC, FTIR, entrapment efficiency, drug release profiles, and ex vivo drug permeation were studied. In vivo investigations were performed using a rat model.

Results: Drug entrapment was observed in 12 formulations (74–91%). Drug release from the formulations over a 24 h study period varied from 63% to 77%. FTIR and DSC studies confirmed the absence of appreciable interactions between curcumin and the excipients. The mean particle size of the optimized formula was 164 nm and the zeta potential was -41 mV. The TEM results revealed evenly distributed spherical vesicles. The transdermal flux exhibited by the transfersome-loaded gel was ~2.5 fold higher than that of the drug-loaded gel. When subjected to a pharmacokinetic study, a stable, non-irritant optimized curcumin transfersome gel formulation showed enhanced bioavailability (AUC) compared to oral curcumin suspension.

Conclusion: The developed transfersomes have the potential to improve skin permeation, ensure an enhanced systemic effect, and improve the therapeutic efficacy of curcumin.

Introduction

In recent years, the selection of the route of drug administration has been based on factors such as patient requirements, physicochemical properties of the drug, and nature of the disease or condition being treated. The transdermal route of administration has gained popularity as a convenient and patient-friendly method for the delivery of a wide range of systemically active drugs.[1] However, the barrier properties of the skin pose significant challenges for the delivery of drugs with different solubilities into and through the skin. Various techniques have been employed to overcome these challenges and to improve transdermal drug delivery. [2] Some techniques used to enhance drug delivery through the skin include iontophoresis, which involves the application of an electric current to facilitate drug penetration, and physical disruption of the skin structure to enhance drug permeation. Non-invasive approaches, such as permeation enhancers and vesicular drug delivery systems, have also been utilized for the transdermal delivery of various therapeutic substances. [3-5]

Among these, vesicular drug delivery is a promising strategy.[4] Vesicles such as liposomes and transfersomes are lipid-based carriers that can encapsulate drugs and enhance their penetration through the skin.[6-8] These vesicular systems offer advantages, such as improved drug stability, controlled release, and the ability to encapsulate hydrophilic and lipophilic drugs. They can also interact with the skin barrier, enhancing drug permeation and targeted delivery to underlying tissues.[8,9] A vesicular drug delivery system is a system in which active substances are enclosed within vesicular structures. This approach helps bridge the gap between ideal and existing forms of novel drug delivery systems. The inclusion of drugs within vesicular structures can extend their presence in the bloodstream and potentially reduce toxicity by facilitating targeted uptake.[10]

Lipid vesicles are among the several experimental models of bio-membranes that have proven effective as controlled

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delivery vehicles[9,11] Vesicular structures called "elastic liposomes" are distinct from conventional liposomes as they comprise a phospholipid bilayer and an edge activator.[12] These vesicles possess excellent elasticity compared to that of traditional liposomes. The elasticity of these vesicles is attributed to the presence of an edge activator, a surfactant with a smaller radius of curvature. This edge activator can weaken the lipid bilayers of vesicles, thereby enhancing their deformability and flexibility.[13] By utilizing vesicular drug delivery systems, researchers have aimed to overcome the limitations associated with transdermal drug delivery and enhance therapeutic outcomes. These approaches offer the potential to deliver a wide range of drugs, opening new possibilities for the treatment of various diseases and conditions.[14,15]

Rheumatoid arthritis (RA) is a complex and multifactorial autoimmune inflammatory disease that affects joints. Although the precise cause of RA remains uncertain, there is a clear association between RA and an increased likelihood of cardiovascular complications.[16] The clinical features of RA include inflammation of the synovial tissue, production of autoantibodies, and damage to the cartilage and bone.[17] Additionally, RA has implications for cardiovascular health and pulmonary and skeletal disorders.[18]

Curcumin, an alkaloidal herbal anti-inflammatory drug, is used to treat RA and osteoarthritis despite its limited solubility in water. Curcumin offers a range of beneficial effects, including antibacterial, hypoglycemic, antioxidant, wound-healing, and antineoplastic properties. In addition, it exhibits antirheumatic, analgesic, and immunomodulatory properties. Curcumin exhibits potent anti-inflammatory and anticarcinogenic properties by modulating molecular signaling. It exerts its effects by suppressing several crucial components of cellular signal transduction pathways. Its mechanism of action includes suppression of protein kinases, inhibition of c-Jun/AP-1 activation, reduction in prostaglandin biosynthesis, and modulation of both the activity and expression of the cyclooxygenase (COX)-2 enzyme. After oral administration, curcumin undergoes significant first-pass metabolism in humans, with more than 90% being metabolized, and a short biological half-life of 1.5 to 2.0 h. One of the challenges associated with curcumin is its poor gastrointestinal absorption and extensive hepatic effect, and therefore, its low bioavailability.[19,20] In this regard, a transdermal route can be considered for effective drug delivery. The obstacle in conventional transdermal therapy of curcumin is its limited permeability through the skin barrier stratum corneum.[21]

As a potent agent, curcumin has been explored via the topical route for various manifestations, and few studies have focused on curcumin incorporation into ultardeformamble vesicular carriers (transfersomes). Complete Freund's adjuvant reagent induced rheumatoid arthritis inflammatory model (mice paw edema method studied by Sana *et al.*,[22] reported a promising anti-inflammatory effect of the curcumin transferosome gel to the inflamed joints, when given by topical route.Curcumin been explored via nasal route as transfersome in situ gel system against SARS-Cov-2 Virus and proved adequate drug concentration in plasma and efficacy in comparison to curcumin gel.[23] Also experimental proof on comparison of curcumin as transfersomes and ethosomes and its effect on cancer cell lines with excellent reduction of the proliferation of malignancy.[24] It is worth noting that the skin is a formidable barrier that demands a permeation-enhancing effect.[25] There are also studies reported on ultra-deformable vesicles of curcumin utilised both edge activators and permeation enhancer to understand the permeation properties of transfersomes across the skin.[25] Transfersomes of Curcuma comosa extract have been studied and proved its for regenerative effect of UV radiation damaged skin for the clinical effectiveness using human volunteers.[26] The curcuma comosa extract from rhizomes in transfersomes have been studied for estrogenic activity via transdermal route. An adequate drug concentration and prolonged drug effect were observed in this study.[27]

Transethosomes been developed as a carrier mechanism for topical administration of curcumin.[28] As reported by Singh P, curcumin transfersomes are also been explored to eye for diabetic retinopathy.[29] Combined effect of diclofenac diethylamine and curcumin in optimised nano-transfersomes for an effective delivery across the skin proved its permeation profile.[30]

Many of the above-mentioned studies for curcumin transfersomes been explored for topical anti- inflammatory effect. Few of the studies are limited to by ex vivo permeation level or skin retention studies to understand the permeation behaviour. Studies that involved anti-cancer effects of curcumin transfersomes is limited to cancer cell lines. Moreover, the composition of transfersomes varied from formulation to formulation.

Considering the facts and findings of above-mentioned literatures, we developed unique transfersomes consisting of lipids (a combination of soya lecithin and cholesterol), with edge activators (Tween 80 and polyglyceryl dioleate or plurol oleque (PO) CC 497 combination) via an experimental design (central composite design). The optimized transferosome-laden carbopol (934) gel was studied for systemic drug effects via the transdermal route.

Systemic drug delivery is preferred over topical administration to alleviate recurrent inflammatory conditions in the body, especially in cases of autoimmune diseases such as rheumatoid arthritis. Therefore, an auxiliary system that can overcome oral-related side effects, bioavailability, and stability issues and enhance therapeutic efficacy is needed. Hence, this study proposes the use of flexible vesicular drug delivery systems (transfersomes) as carrier systems for increasing transdermal flux and effective systemic drug delivery to understand pharmacokinetics via the transdermal route in comparison with the oral route.

Methods

Materials

Soy lecithin and Tween 80 were purchased from Central Drug House, New Delhi, India. Plurol® Oleique CC-497 (PO) and curcumin were received as gifts from Gattefosse, Mumbai, India and Cancer Herbalist Ltd, Bangalore, India, respectively. Cholesterol and Carbopol 934 were procured from Loba Chemie, Mumbai, India. All other reagents used were of analytical grade.

Preparation of drug loaded transfersomes

Curcumin-loaded transfersomes were prepared using the thin-film hydration method described previously.[31-33] Soy lecithin, cholesterol, surfactants (Tween 80: PO), and curcumin were mixed and completely dissolved in an organic solvent (a combination of methanol and chloroform at a 1:1 ratio). This organic solvent was evaporated at 30°C to obtain a thin film using a rotary evaporator (Roteva, Equitron, Germany) at 40 rpm. The thin film formed after drying was subsequently hydrated using phosphate-buffered saline PBS (pH 5.5) at 45°C for 2 h. To obtain transferosome suspensions containing curcumin, the resulting suspensions were sonicated for 10 min.

The formulation trials were optimized via central composite design in Design-Expert version 13. The factors considered for the design were phospholipid concentration (soy lecithin, mg), edge activator concentration (Tween: PO, mg), drug entrapment efficiency (%), particle size (nm), and zeta potential (mV).[34] The range of various factors was selected based on the results of preliminary trials. The design resulted in 12 experimental trials, which are listed in Table 1.

Characterization of curcumin loaded transfersomes

Drug content

To analyse curcumin content in the drug-loaded transferosome suspension, a measured volume of suspension was dissolved in methanol, making up a total volume of 10 ml, further diluted in methanol, and UV spectrophotometric analysis was performed at 424 nm.[35] To establish a baseline, the blank formulation was treated in the same manner as the reference sample.

Drug entrapment efficiency

A specific volume of the transferosome formulation was collected and centrifuged at 10,000 rpm for 30 min. The sediment (entrapped drug) and supernatant (unentrapped drug) were collected separately.[36] After dilution with methanol, the free curcumin content in the supernatant was analysed using a UV-visible spectrophotometer at a wavelength of 424 nm.

$$EE = \frac{Total\ Drug - Free\ Drug}{Total\ Drug} \times 100 \qquad \text{Eq. 1}$$

Particle size analysis

The particle size was measured by Dynamic Light Scattering using a Horiba SZ-100 (Kyoto, Japan). For the measurements, 10 ml of the suspension was diluted with distilled water. Each sample was measured in triplicate to ensure reliable and consistent results.[37]

Zeta potential determination

Zeta potential measurements were performed using an SZ-100 Horiba Scientific instrument (Kyoto, Japan). After diluting 10 ml of the nanovesicle formulation under an electric field of 150 mV, the electrophoretic velocity of the vesicles was observed. All measurements were performed in triplicate.[37]

In-vitro release of curcumin from transfersome suspension

In vitro studies were performed using a modified Franz diffusion cell assembly. A 10 ml volume of transfersomes (F1-F12) was placed on a dialysis membrane-70 with a pore size of (70 µm), which was soaked overnight in 0.1 N hydrochloric acid solution and placed between the donor compartment and receptor compartment of a diffusion cell assembly.[38] The receptor compartment was filled with phosphate buffer (pH 7.4) containing 0.5% tween-80 and 20% ethanol to maintain sink conditions.[39] The receptor compartment was continuously stirred using a magnetic

Table 1. Formulation composition of different batches generated via central composite design.

Batch	Curcumin (mg)	Soy lecithin(mg)	Cholesterol (mg)	Tween:Plurol Oleique (1:1) (mg)	Ethanol: Chloroform (1:1) (mL)	Phosphate buffer (mL)
T1	100	250	10	10	20	10
T2	100	187.5	10	8.42499	20	10
T3	100	250	10	25	20	10
T4	100	187.5	10	17.5	20	10
T5	100	111.875	10	17.5	20	10
T6	100	187.5	10	17.5	20	10
T7	100	125	10	25	20	10
T8	100	187.5	10	26.575	20	10
T9	100	125	10	10	20	10
T10	100	187.5	10	17.5	20	10
T11	100	187.5	10	17.5	20	10
T12	100	263.125	10	17.5	20	10

stirrer at 100 rpm and was maintained at 37°C. The rate of drug release from the transfersomes was determined by withdrawal of 1 ml of receptor fluid. Drug concentration was analyzed using a spectrophotometric method after suitable dilution at a λmax of 424 nm.

Evaluation of experimental study

The responses of the experimental study were integrated into the design and assessed for model fit. The optimal formulation formulation (OTF) is derived from the design space using the desirability function.[39]

Evaluation of optimum formulation (OTF)

Optical morphology and transmission electron microscopy (TEM)

The formulation was observed and photographed at 40 × magnification using an optical light microscope (Leica Image; Wetzlar, Germany). For a more detailed analysis of the curcumin transfersomes, TEM (field electron and ion, Hillsboro, Oregon, US) was employed. A drop of diluted curcumin transfersomes was then added to the TEM grid. The grid was stained with 2% uranyl acid. Before the investigation, the grid was dried to ensure optimal imaging and clarity during TEM analysis.[40]

Fourier transform infrared spectrum (FTIR) spectroscopy

The FTIR analysis was performed using an instrument (Bruker 2, Ettingen, Germany). A small amount of the sample was placed on the sample holder, and the IR spectra were recorded using the mechanism of attenuated total reflection in the range of 400-4000 cm^{-1}.[11]

Differential scanning calorimetry (DSC)

A Perkin Elmer instrument (DSC 6000, Waltham, MA, USA) was employed to examine the thermal characteristics of the pure drug, physical mixture, and optimized formulation of the transfersome suspension (OTF). Approximately 5 mg of the sample, comprising pure and drug-equivalent formulations containing excipients, was precisely weighed, and placed into non-hermetically sealed aluminium pans. The pans were subsequently crimped for analysis. The samples were heated from 0 to 350°C at 10°C/min. Nitrogen gas was continuously purged at a flow rate of 40 ml/min, and DSC thermograms were recorded.[41]

Preparation of gel formulations

In a beaker containing 100 mL of distilled water, 500 mg of carbopol 934 was sprinkled on a magnetic stirrer.[42] Methyl and propylparaben sodium were added to the beaker while stirring the carbopol solution for 30 min to ensure the proper dissolution and dispersion of the parabens. Curcumin-incorporated gel was prepared by adding 1 g of pure curcumin to the carbopol gel and stirring until a consistent and even distribution of curcumin was obtained, identifiable by its smooth and uniform texture. In the case of transfersome-incorporated gel, 1g drug-equivalent curcumin transfersomes were added to the carbopol gel by constant stirring to obtain gel dispersion as per the formula given in Table 2. These formulations were further studied and evaluated for their intended application.

Table 2. Composition of curcumin loaded transferosomal gel.

Components	Quantity
Optimized formulation	1 g drug equivalent
Carbopol 934	0.5 w/v (%)
Polyethylene glycol	0.1 w/v (%)
Methylparaben	0.1 w/v (%)
Water to	100 ml

Evaluation of optimal formulation of curcumin loaded transfersome gel

Drug content

Transfersomes incorporated gel of 1 g containing a specific amount of curcumin were transferred into 50 ml volumetric flasks diluted with 50 ml of methanol. The solution was diluted with methanol and analyzed at 424 nm using UV spectrophotometry against the same blank.[42]

Rheological behaviour of gel

The viscosity of the prepared gels was measured using a Brookfield viscometer (LVD). Brookfield Engineering Inc., Germany, and equipped with an S94 spindle. The measurements (in CPs) were performed at 60 rpm and 25°C.[43]

Homogeneity and pH

A small amount of gel was positioned between the thumb and index finger, and a force was exerted to assess homogeneity. The texture and evenness of the gel were assessed by relying on the sense of touch for consistent and smooth composition, and the pH of the transfersome gel was measured using a digital pH meter. [43]

Spreadability

The test involved placing 0.5 g of the gel onto a glass plate that had a pre-marked circle of 2 cm diameter. Then, another glass plate was placed on top, and a weight of 0.5 kg was applied to the upper plate for 5 min. After removing the weight, the diameter of the gel circle spread was measured, as described previously.[44] Spreadability was calculated by the following eqution:

$$S = \frac{M \times L}{t} \qquad \text{Eq. 2}$$

where S = Spreadability coefficient, M = Weight applied, L = slide length, and t = time taken to separate the slides from each other.

Ex-vivo permeation studies

The abdominal region of healthy male Wistar rats was shaved using an electronic hair remover to ensure that the skin surface remained intact and undamaged. Following the completion of the experiment, the rats were euthanized, full-thickness abdominal skin samples were collected.[44] A

modified Franz diffusion cell assembly was used for ex vivo studies. Two different formulations, transfersomes loaded with gel and drug-loaded gel, each containing 5 mg of curcumin, were tested. Each formulation was applied to a separate rat abdominal skin sample with the stratum corneum facing the donor compartment of the diffusion cell. The receptor compartment was filled with phosphate buffer (pH 7.4) solution containing 0.5% tween-80 and 20 % ethanol to maintain sink conditions.[36] The medium was agitated at a speed of 100 rpm, and the temperature was maintained at 37°C. Drug transport from the transfersomes was assessed by collecting 1 ml of receptor fluid. Drug concentration in the receptor fluid was measured using spectrophotometry. The drug flux at the steady state (μg/h/cm^2) was determined by dividing the slope of the linear segment of the release curve by the surface area of the exposed skin. The steady-state flux and permeability coefficients were calculated using the equation described previously.[45]

Stability studies

The stability of the optimum transfersome-incorporated gel formulation was assessed at 25±2°C/60% RH ±5% and 40±2°C/75% RH ±5% for three months. After the study period, the % drug release and % entrapment efficiency was evaluated.[46]

Skin Irritation study

All animal experiments complied with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and were carried out in accordance with the Prevention of Cruelty to Animal (PCA) Act, 1960. Wistar rats, divided into two groups, had their abdominal regions shaved and treated with 0.8% v/v aqueous formalin solution and optimized transfersome gel for Groups I and II, respectively. Applications were made in the hairless abdominal area. The rats were observed for erythema and edema, and their responses were monitored for one week.[47]

Pharmacokinetic study

Male Wistar rats weighing 140-160 g and maintained under controlled environmental conditions, were used for the pharmacokinetic study (Ref. No. KCP/IAEC/PCOL/PCEU/64/2020). The rats were separated into two groups: group I received a transdermal transfersome gel containing the drug equivalent to 4 mg (328.2 mg gel), and group II received curcumin as an oral suspension. The oral suspension was prepared by suspending 40 mg of pure curcumin in 10 ml of 1% methylcellulose, and 2 ml (8 mg of curcumin) of the suspension was administered to the animals (40 mg/kg body weight).[48] This study involved the application of a transferosome gel formulation and an oral curcumin suspension. Blood samples were collected at different intervals (0.5, 1, 2, 4, 6, and 12 h) via retro-orbital plexus and tail vein punctures. Blood samples were collected in Eppendorf tubes containing anticoagulant (sodium citrate). Plasma was separated by centrifugation (Remi, Mumbai, India) at 3000 rpm, stored at -80°C, and analyzed for drug concentration using HPLC. Briefly, the HPLC method consisted of a Shimadzu Prominence with a 50 μL sample loop coupled to UV-Visible detector (set to 420 nm and a computer system for data acquisition (Lab solutions) was used. The separation was achieved using a reversed phase Symmetry C18 column (4.6 × 250 mm, particle size 5 μm). A mobile phase consisting of methanol, acetonitrile, and 5% acetic acid (35:50:15, v/v) was used at a flow rate of 1.0 mL/min. Standard solutions were prepared in acetonitrile (1μg to 10 μg). The plasma samples were dissolved in acetonitrile and a volume of 20 μL was injected into the column. Pharmacokinetic parameters, such as Cmax, Tmax, and AUC, were determined and compared using suitable pharmacokinetic software.[49,50]

Statistical analysis

The data are presented as the mean ± standard deviation (SD) of independent experiments. Statistical significance was calculated using one-way analysis of variance (ANOVA) with Tukey's post hoc test.[50] Statistical significance was set at P value < 0.05.

Results and Discussion

The experimental design CCD was chosen as an alternate to already reported (Chaudhary H)[31] factorial designs, to understand the effect of concentration of soya lecithin and surfactant on the responses and therefore to lead an optimum formula**.** CCD is more economical method and a better alternate to the factorial design which maximise the runs within the operability ranges.

Evaluation of curcumin loaded transfersomes

Particle size, zeta potential and entrapment efficiency

The central composite experimental design generated a design matrix, leading to 12 trials targeting a particle size of 100-600 nm, zeta potential ≥ -30 mV, and drug entrapment efficiency of 70-90%, the results are presented in Table 3.

Particle size

The particle size of the formulation ranged between 128-503 nm, which varied significantly owing to the contribution of thin film formation and subsequent size reduction during sonication. The largest particle size was observed for formulations T12 and T1, and the largest concentration of phospholipids led to the formation of larger vesicles. At higher concentrations of soy lecithin, the vesicle size was larger owing to the inability of phospholipids to align spontaneously in the presence of the aqueous medium or may be the vesicles fuse, resulting in a larger structure or multi-lamellar structure.[51] However, most of them had a size of < 200 nm. The increased concentration of surfactant, carbon chain longitude, and HLB can contribute to the reduced particle size. Some influence of phospholipid and surfactant concentrations or competitive effect of the surfactants and cholesterol unlike

Table 3. Results of responses particle size, zeta potential and entrapment efficiency.

Batches	Particle size (nm)	Zeta potential (-mV)	Drug entrapment efficiency %
T1	462±1.21	38.4±0.98	90.6±0.19
T2	349±1.45	19.8±1.02	83.7±0.12
T3	222±1.09	54.5±1.1	84.7±0.42
T4	201±1.76	43.2±1.03	76.2±0.38
T5	328±2.01	23.1±1.21	85.5±0.08
T6	159±1.78	41.6±1.21	81.1±0.52
T7	138±3.12	38.8±1.23	88.9±0.05
T8	190±1.23	19.1±1.11	74.3±0.14
T9	146±1.78	36.5±1.07	89.9±0.05
T10	131±1.21	49.4±1.21	80.8±0.14
T11	128±1.89	44.4±0.97	86.2±0.05
T12	503±1.09	19.2±0.56	78.2±0.09

other curcumin transfersomes formulations[23] could have reduced the particle size to a lower range.[52]

Zeta potential

The zeta potential values for transfersome vesicles ranged from -19.2 -54.5 mV. Most of the formulations exhibited a zeta potential ≥ -30 mV, with exception of a few less than the limit, and the measurement of electrostatic charges is pivotal in assessing surface properties, providing insights into the repulsive potential and stabilization of vesicular systems. The net charge is the cumulative effect of the phospholipid and surfactant concentrations. The negative charge, particularly from cholate-based transfersomes, is believed to affect transdermal drug delivery. Increasing the surfactant concentration was correlated with an enhanced surface charge. Formulations T3 and T7 aligned with this pattern. The interplay between the positive charge from the surfactant and the negative potential from phospholipids led to a decreased charge density. In formulations T3, T4, T6, T10, and T11, where the phospholipid-to-surfactant ratio ranged from 10:1 to 11:1, the zeta potential values exceeded -40. Higher phospholipid concentrations influenced zeta potential, as phospholipids are amphiphilic, preferentially localized at lipid-aqueous interfaces, reducing interfacial tension, and enhancing transfersome vesicle stability.[53] Overall, all formulations demonstrated moderate to good stability independent of the phospholipid: cholesterol ratio. Additionally, the synergistic effect of non-ionic emulsifiers, such as PO and Tween 80, provides steric stabilization, where the surface-active agents create a protective barrier around particles, hindering their proximity and aggregation, thus providing colloidal stability of transfersome vesicles. Overall, the combination of electrostatic repulsion and steric stabilization effects provided by phospholipids and non-ionic emulsifiers ensures the stability and dispersion of transfersome vesicles in the formulation.[53]

Drug entrapment efficiency

The drug entrapment efficiency within the transfersomes ranged from 74.3±0.14 to 90.6±0.19%, with phospholipids, cholesterol, and surfactants collaborating to enhance drug loading. Three of the twelve formulations say T1, T7, and T9 displayed the highest entrapment efficiencies of 90.6±0.19%, 88.9±0.05%, and 89.9±0.05%, respectively, emphasizing the role of phospholipids in forming the vesicular bilayer structure conducive to drug entrapment. Relatively higher loading can be contributed due to lipophilicity of drug its potential to get incorporated into the lipid bilayers. [41,] Phospholipids contribute to the vesicle's structural integrity, whereas cholesterol enhances stability and inhibits drug leakage, thus promoting higher entrapment efficiency.[54] The incorporation of non-ionic surfactants, acting as edge activators, aimed to destabilize vesicles and enhance bilayer deformability, thereby accommodating a higher drug payload. Optimal surfactant and phospholipid levels in T9 resulted in a high payload. Conversely, T8, which had the highest surfactant concentration, exhibited the lowest entrapment efficiency, possibly because of mixed micelle formation at excessive surfactant levels, leading to fluidity changes and drug leakage.[55] The phospholipids contribute to the vesicle's structural integrity, whereas hydrophilic cholesterol fill the gaps between PL and surfactant, thus enhances stability and inhibits drug leakage, thus promoting higher entrapment efficiency.[54] There is an inverse relationship between hydrophilic lipophilic balance (HLB) entrapment efficiency.[25] The edge activators tween 80 is hydrophilic with a HLB of 15 and PO with a HLB of 3. Balance of these two HLB's might have maintained an acceptable entrapment efficiency. It is noteworthy that even with a low concentration of surfactant combination (4-7% w/w), the entrapment efficiency was high (46-76%) in comparison to the earlier works on curcumin transfersomes using Phospholipon 90 G and Tween 80 reported by Sana *et al.*[22] Also contribution from soy lecithin consisting of a mixture of three phospholipids; phosphatidyl choline, in addition to phosphatidylethanolamine (PE) phosphatidylinositol (PI), with surfactant combination might have had added effect.

In vitro drug release profile from transfersomes formulation

The drug release profiles of the 12 formulations ranged from ~62% to 77% over 24 h, as shown in Figure S1 in Supplementary Data. During the first 0.25 h, 7-12 % release occurred, possibly due to the presence of unentrapped drug. Subsequently, a gradual but constant release rate (52-69%) was observed for the next 5 h, which accounts for the drug entrapped in between lamellar structure might have released. The remaining drug release from accounts for the drug in aqueous core might have have partitioned gradually into the lipid bilayer structure, dissolved into the lipid leaflets and then slowly partitioned into the aqueous surrounding. The formulations T1, T3, and T15, with a larger concentration of phospholipids, maximum drug release was observed, which may be attributed to the low packing intensity of phospholipid: cholesterol. The remaining formulations exhibited moderate drug-release profiles. The impact of surfactant concentration

Table 4. Model fit summary of response's particle size, zeta potential and entrapment.

Source	Particle Size		Zeta potential		Entrapment efficiency%		
	F Value	P value	F value	P value	F value	P value	
Model	15.47	0.0023	11.02	0.0055	14.38	0.0027	Significant
A-Phospholipid	35.84	0.0010	7.55	0.0334	43.29	0.0006	
B-Surfactant	4.99	0.0670	0.6889	0.4383	1.92	0.2156	
AB	23.88	0.0027	16.68	0.0065	0.3220	0.5910	
A^2	8.64	0.0260	2.06	0.2016	18.88	0.0048	
B^2	3.99	0.0928	23.33	7.51	28.13	0.0018	
Residual	197.01	197.01	6	32.84	7.51	0.0337	
Lack of Fit	0.2256	0.8736	0.0363	0.9890	23.33	7.51	Not significant

Table 5. ANOVA of the response particle size, zeta potential and entrapment efficiency.

Response	Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Particle size	Quadratic	0.0334	0.8736	0.8680	0.8011	Suggested
Zeta potential	Quadratic	0.0046	0.9890	0.8200	0.8097	Suggested
Entrapment efficiency	Quadratic	0.0064	0.9571	0.8588	0.8262	Suggested

was slightly evident on the drug release profile; The two oleic acid ester-based surfactants (tween 80 and PO) with higher molecular weight results a flexible packing and the integrated effect of the two as seen in T3 might have resulted in a high drug release.

The drug encapsulation percentage is crucial in transfersome preparations, significantly affecting drug release. A higher entrapment efficiency ensures greater drug retention, enabling controlled and sustained drug release. Low entrapment efficiency is correlated with slower drug release, primarily from the internal lipid phase.[56]

Evaluation of the experimental design and model fit

The experimental matrix responses were scrutinized, and the model fit assessment revealed significance via ANOVA (P = 0.0027), validating the chosen experimental trials and responses. Phospholipid concentration significantly impacted all three responses, whereas surfactant concentration had no significant influence. Nonetheless, the interaction between phospholipids and surfactant concentration (AB) affected the particle size and zeta potential. Quadratic high-order interactions of surfactant concentration were evident for entrapment efficiency (P values of 0.0048 and 0.0018, Table 4). The ANOVA results for the response particle size, zeta potential, and entrapment efficiency are summarized in Table 5. ANOVA indicated a quadratic equation for all three responses with high-order interactions (P <0.05), validated by the proximity of the predicted and adjusted R^2 values. Particle size was found to have an inverse effect on phospholipid concentration. Conversely, an increase in surfactant concentration (from 10 mg to 25 mg) led to a decrease in particle size from 230 nm to 210 nm. Graphical optimization, as illustrated in Figure S2 in Supplementary Data, revealed that higher phospholipid concentrations led to a reduction in drug entrapment efficiency, while altering the surfactant concentration had minimal impact. The zeta potential results indicated no linear correlation with the surfactant or phospholipid concentrations.

The model fit summary suggested that among the responses studied, phospholipid concentration exerted the greatest impact, whereas surfactant concentration significantly affected only drug entrapment efficiency.

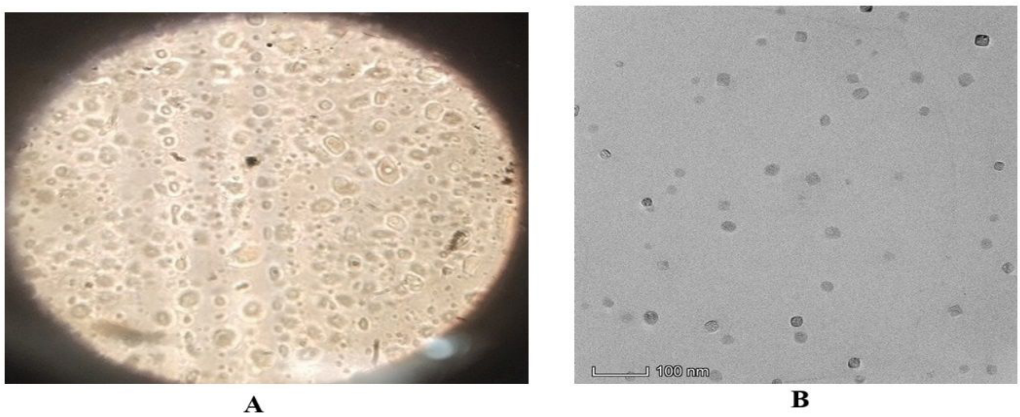

Figure 1. A) Optical microscopic image before Sonication B) Transmission electron microscopy images of optimized transfersomes formulation (OTF).

Interactive effects of phospholipids and surfactants were observed in all responses, except drug entrapment efficiency.

Evaluation of optimized formulation (OTF)

At a maximum desirability of 1, as indicated by the experimental design, the OTF involved a phospholipid concentration of 187.5 mg and a surfactant concentration of 17.5 mg, yielding a particle size of 220 nm, drug entrapment efficiency of 84%, and zeta potential of -46 mV. This formulation was prepared within these specified factor ranges and the responses were reassessed. Furthermore, the vesicles were characterized using TEM, in addition to the initial optical morphology analysis, as shown in Figure 1.

Microscopic analysis of the optimal transfersome formulation revealed well-defined almost spherical vesicular structures with distinct outer membranes. The vesicle size did not completely support the DLS results, as reported by Abdel-Hafez *et al.*[25] The selected factors and their concentrations are crucial for influencing the formation and integrity of these vesicles. Inspection of transfersomes highlighted conspicuous differentiation between the internal core and outer membrane, underscoring the unilamellar nature of vesicles within the size range of 50 nm to 200 nm. This distinction underscores the influence of the chosen PL and surfactant concentrations, ratios, and technique conditions on vesicle formation.[46] The well-defined structures, featuring clear internal and external compartments, validate the successful transfersome formation. Microscopic scrutiny affirms the crucial integrity for the desired functionality and drug-release properties, confirming the efficacy of the selected concentrations and conditions.[56]

The mean particle size 164.6 nm the zeta potential of the optimal formula was-43 mV, (Figure S3 in Supplementary Data) and a PDI of 0.365 indicated uniformity in the vesicle size distribution, without the absence of significant agglomeration and ensuring the stability of transfersomes.[47]

Table 6. Evaluation results of curcumin containing optimized transfersomes loaded gel.

Parameters	Results
Physical appearance	Yellow in colour
Homogeneity	Smooth and homogenous
Spreadability	32.19 ±1.09 g.cm/min
pH of the gel	5.9±0.12
Drug content	82.4±2.09%
Viscosity	1350±22.2 cP

Regarding DSC, the curcumin thermogram displayed an endothermic peak at 181.8˚C, (Figure S4 in Supplementary Data) while the physical mixture exhibited a peak at 175 °C. Notably, the optimized formulation showed no endothermic peak, suggesting an amorphous state and complete entrapment of the drug in the vesicles, as previously described.[46]

An overlay of the IR peaks is shown in Figure S5 in Supplementary Data. FTIR studies of curcumin exhibited characteristic molecular vibrations at 3507 (O-H) stretch, 1626 benzene ring stretch, 1514 C-O stretch, 1427 C=C stretch, and 1273 bending vibration of phenolic acid, corresponding to the chemical structure of curcumin, which is a physical mixture of drugs and lipids. The existence of these peaks in the physical blend ensured the compatibility of the drug with the excipients used.

Evaluation of gel formulation

A yellow, smooth gel with excellent spreadability was observed, indicating homogeneity, as shown in Table 6. The pH of the gel aligns with skin conditions, and its viscosity of 1350 cp implies easy application and effective retention on the skin. These results agreed with those reported by Tuntiyasawasdikul *et al.*[27] However, the crosslinked carbopol used in this study at the same polymer concentration had a lower viscosity than the Carbopol 934 gel.

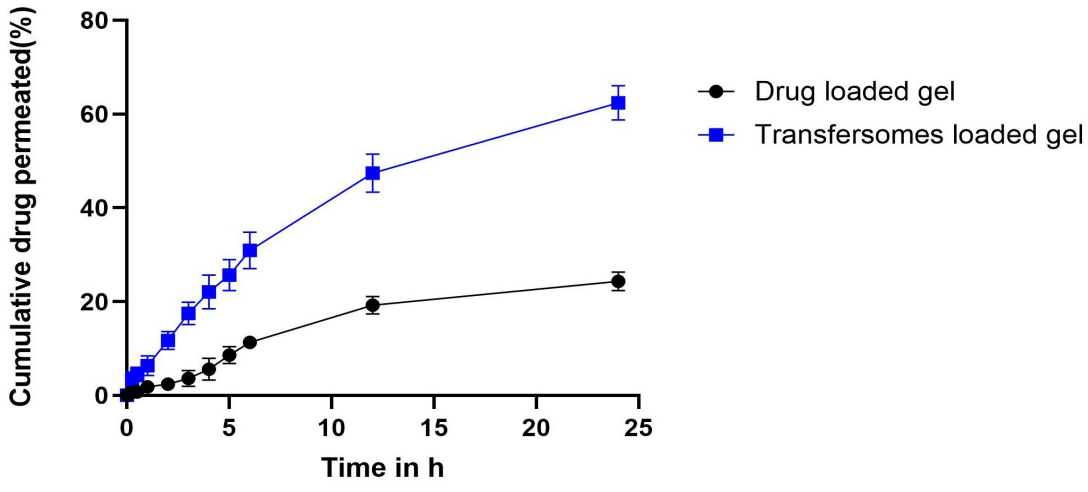

Figure 2. Comparative ex-*vivo* drug permeation profile.

Evaluation of transfersome gel

The optimized gel formulation exhibited a drug content of 82.2±0.09%, spreadability of 32.16±0.09 g.cm/min, pH level at 5.9 ± 0.02, and a viscosity of 1350±0.2 cP, indicating favorable shear thinning properties.

Comparative drug release profile and ex vivo permeation studies

Drug release from the drug-loaded gel and transfersome-loaded gel showed a release rate of 24.2% and 54.4%, respectively, in 24 h. In 24 h drug permeation studies, the profiles from the drug-loaded gel and transfersomes loaded gel exhibited rates of 24.3% and 62.4%, respectively. (Figure 2) The ex vivo permeation studies further supported this finding, revealing a threefold increase in flux and permeability coefficient for the transfersome-loaded gel compared to the drug-loaded gel. This enhancement is likely attributable to the deformable vesicles and hydrophilic lipid composition of transfersomes, enabling them to overcome the stratum corneum barrier in response to transcutaneous hydration gradients. Moreover, their surfactant content may act as a penetration enhancer, modifying the intracellular lipid content and increasing skin fluidity. [42,46,55] Ex vivo permeation profiles were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test to confirm statistical significance. One-way ANOVA results confirmed the statistical significance, with a P-value of 0.0282. Tukey's test indicated the ex vivo results of drug-loaded gel and transferosome-loaded gel; there was a considerable difference in the mean results of the study where the paired values were higher (15.39) than the Tukey's criterion value (13.87), indicating that there is a statistical difference between the comparative ex vivo permeation results, stressing the assurance of ANOVA results at the 5% alpha level.

Stability study

The optimal curcumin-loaded formulation underwent short-term stability studies for 90 days under two stability conditions, as shown in Table 7. The results showed that the drug release was not affected during storage. It seems that notable drug release occurred after 90 days, confirming the preservation of an amorphous drug matrix in the transfersome formulation. The amorphous drug form, which is more soluble with increased dissolution rates compared with its crystalline counterpart, remained intact during stability studies, minimizing drug expulsion during storage, as evidenced by entrapment efficiency data.

Table 7. Results of stability studies of optimized transfersomes loaded gel.

Days	% Drug release ± SD (n=3)		%Entrapment efficiency ± SD (n=3)	
	25 ± 2°C	40 ± 2°C	25 ± 2°C	40 ± 2°C
0	76.11±0.04	76.11±0.04	82.42±0.06	82.42±0.06
90	75.01±0.82	74.09±0.09	82.03±0.01	80.91±0.02

This suggests an extended shelf life of the formulation, indicating its suitability for long-term storage without substantial degradation or loss of efficacy.[52]

Skin irritation studies

Wistar rat skin irritation assessments were performed after the application of transfersomes. Skin samples, compared to the positive control, revealed no irritation from the transferosome gel, whereas the positive control exhibited noticeable redness after formalin application, as shown in Figure 3.

There were no signs of skin irritation, scored as erythema, edema, or inflammation on the skin treated with the formulation. The absence of redness in the skin treated with transfersomes-loaded gel suggests the friendliness of the formulation on the skin.[46]

Pharmacokinetic study

A few studies that support anti-inflammatory activity of curcumin transfersomes by Sana *et al.*[22] showed numerous promising results that support the topical anti-inflammatory activity of transferosome gel against curcumin-loaded gel. This study demonstrates the topical effect of curcumin gel, via the mouse paw edema method (CFA induced RA mice model), as evidenced by various scores of swellings and inflammation of the paws. In addition, biochemical analysis of excised paw and pro-inflammatory cytokine levels in excised and homogenized

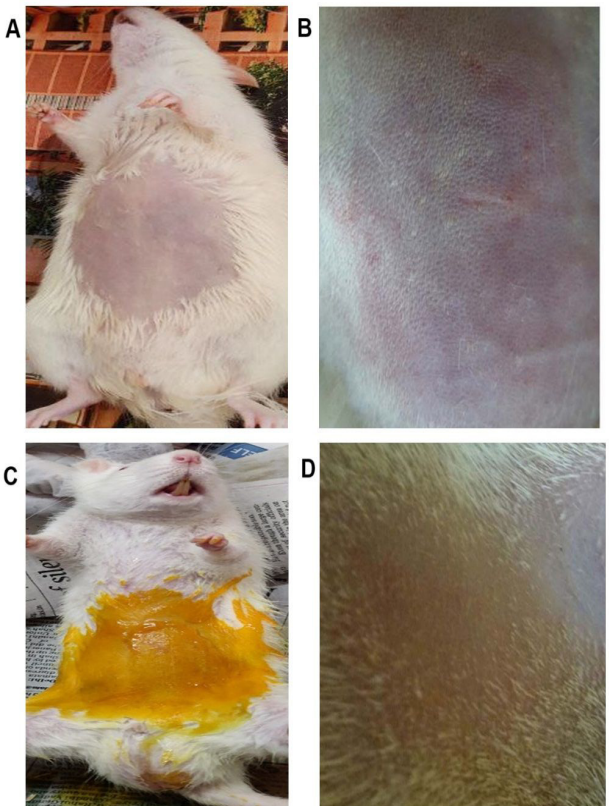

Figure 3. Rat abdominal skin A) before treatment with formalin B) After one week of treatment C) curcumin loaded transferosmes gel applied and D) After one week of transfersomes gel application.

Table 8. Pharmacokinetic parameters by PK solver for oral/ transdermal drug delivery of curcumin.

Pk Parameter	Units	Oral	Transdermal
t_{max}	H	1	2
C_{max}	µg/ml	4.001	3.823
$t_{1/2}$	H	1.5	4
AUC_{0-t}	µg/ml*h	13.46	24.30
$AUC_{0-inf\_}$ ob	µg/ml*h	14.11	30.69
$AUC_{0-t/0-inf\_obs}$	µg/ml*h	0.9539	0.7912
AUMC	$\mu gh^2/ml$	49.211	117.90
MRT_{0-12}—obs	H	2.89	4.85
MRT_{0-inf}—obs	H	3.48	7.777

tissues using commercially available ELISA kits supports this claim. In addition, radiological examination of the morphological features of the bone supported these findings.

Although studies have been published on the topical anti-inflammatory activity of curcumin transfersomes, studies on the pharmacokinetic evaluation of the drug are limited. For example, the work reported by Eleraky *et al.*,[23] a nasal in situ gel loaded with curcumin transfersomes (5 mg/ kg) in rabbits against curcumin gel exhibited improved pharmacokinetic profiles. Similar studies have reported for transfersomes of curcuma comosa rhizomes extract and their pharmacokinetic profile after topical administration. The findings supported the transdermal penetration and existence of plasma level at nanograms. However, no comparison of oral curcumin has been reported in either study.

Utilizing non-compartmental estimations with " Phoenix Certara winnonlin version 8.1," the plasma concentration-time profiles of optimized curcumin transfersomes gel and an oral curcumin suspension were analyzed, and various pharmacokinetic parameters such as AUC_{0-12}, $AUC_{0-\infty,}$ t_{max}, C_{max}, MRT, and $t_{1/2}$ for both groups were determined. After applying the transfersome gel to the skin vs. an oral curcumin suspension, the pharmacokinetic assessment showed an increase in $AUC_{0-\alpha}$ from 14.11 (oral) to 30.69 µg/ml*h (transfersomes gel) (Table 8). Additionally, there was an increase in tmax compared to the oral suspension. Meanwhile, a two-fold increase (3.89 to 7.77) in the mean residence time (MRT) was observed. (Figure 4) Furthermore, there was a fold increase in the maximum concentration (tmax) compared with the oral suspension. This suggests that the transdermal application of transfersome gel augments the bioavailability of curcumin, as evidenced by the elevated AUC and gradual drug concentration in plasma and a substantially higher MRT. These findings underscore the potential of transfersome gels for enhancing therapeutic efficacy and efficiency compared to conventional oral administration, providing valuable insights into optimizing curcumin delivery systems for improved clinical outcomes.[51]

Conclusion

Transfersomes provide an effective method for transdermal drug delivery, which is particularly suitable for inflamed or damaged skin owing to their biocompatible lipid composition. This study aimed to address the limitations associated with oral curcumin therapy, including its side effects and low bioavailability. Conventional transdermal curcumin therapy faces challenges due to its limited permeability to the stratum corneum. By incorporating soy lecithin, cholesterol, and Tween 80, Plurol Oeque as activators successfully converted curcumin into nanosized transfersomes via the rotary evaporation/thin-film hydration method. The formulation offered optimal entrapment efficiency and zeta potential. Pharmacokinetic studies supported the claims of both transfersomes and transdermal delivery based on the AUC results. In conclusion, these flexible vesicular carriers can offer clinicians an economical, safe, and stable drug therapy for various drugs without altering the structure of the skin.

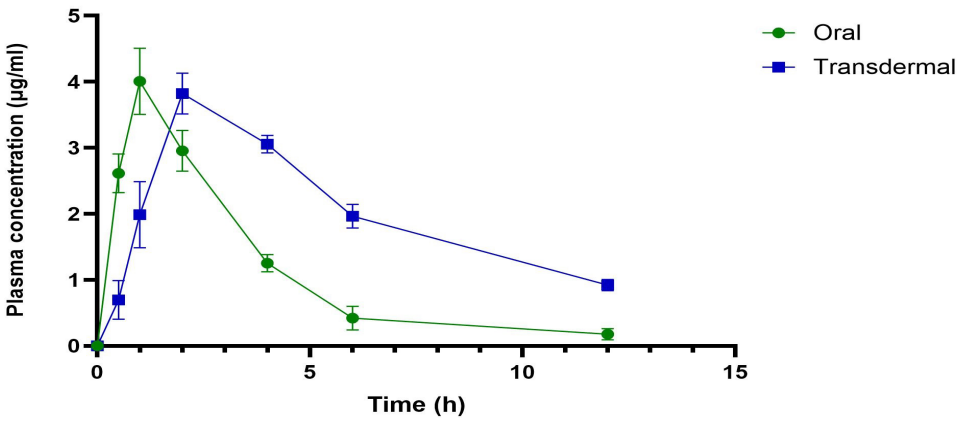

Figure 4. Plasma concentration profile of optimized transdermal transfersomes gel and oral suspension of curcumin.

Ethical Issues

The animal study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of the Krupanidhi College of Pharmacy. Ref No: Ref. No. KCP/IAEC/PCOL/PCEU/64/2020).

Author Contributions

Parameshwari Balasubramanyam: Development, Validation, Investigation, Writing the original draft. **Preethi Sudheer:** Conceptualization, methodology, software, Validation, Resources, Project administration, Writing, review and Editing. **Nagaraja Sreeharsha:** Software, Resources. **Anroop B Nair:** Validation, Software. **Darshan Petkar Ramachandra:** Resources.

Conflict of Interest

The authors report no conflicts of interest.

Supplementary Data

Figure S1-S5 are available at https://doi.org/10.34172/PS.025.40996.

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