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Research Article

CURCUMIN ETHOSOMES FOR ENHANCED TRANSDERMAL DRUG DELIVERY: FORMULATION, CHARACTERIZATION, *IN VIVO* AND *EX VIVO* STUDIES

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ABSTRACT

Objectives: The current study aimed to prepare and optimize curcumin (CM) ethosomal gel for enhanced transdermal drug delivery to overcome the poor permeability barrier.

Methods: The cold method was employed to manufacture the CM-loaded ethosomes with varying quantities of soya phosphatidylcholine, propylene glycol (PG), and ethanol. Transmission electron microscopy was employed to evaluate the appearance of the formed ethosomes. Formulation parameters such as vesicle size and zeta potential, polydispersity index (PI), transition temperature, entrapment efficiency (EE), *in vitro* drug release, release kinetics, *ex vivo* studies on rat skin, *in vivo* pharmacokinetics, and stability were performed.

Results: The microscopy results showed that CM ethosomes have a smooth surface. The release of CM adhered to the zero-order release model. The optimized ethosomal gel formulation's (CM5) zeta potential and PI were determined to be −7.28±1.62 mV and 0.208, respectively, and it has 90.8±1.8% EE with a vesicle size of 437±2 nm. The maximum flux for the ethosome formulation (CM5) was 23.13±0.91 μg.h/cm^2. The bioavailability of the optimized formulation was 7.61 times higher than that of the control (oral suspension). The stability study revealed no significant change when stored at 4°C.

Conclusion: Based on current research, ethosomal vesicles may enhance transdermal dispersion without irritating the skin. Ethosomes infused with CM show significant potential for transdermal administration for the management of skin diseases.

Keywords: Ethosomes, Curcumin, Vesicle, Transdermal, Permeation, Bioavailability.

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INTRODUCTION

The rhizome from the East Indian plant Curcuma longa yields the polyphenol known as curcumin (CM). Anticancer, antibacterial, anti-inflammatory, antiangiogenic, antifungal, chemoprotective, and antioxidant properties are just a few of its many pharmacological activities [1]. Furthermore, it has shown promise in treating a wide range of skin disorders, such as vitiligo, psoriasis, oral lichen planus, vitiligo, alopecia, eczema, facial photoaging, pruritus, and acne [2]. However, because of its quick metabolism and excretion as well as its difficulty in absorption, it has a limited bioavailability. Their stability, activity, and pharmacokinetics are all enhanced by ethosomes [3]. To shield the skin from harm caused by diesel particulate matter, ethosomesin transdermal medication administration were examined for CM delivery in this work [4].

The primary constituents of ethosomes, which are vesicular systems, are phosphatidyl choline (PC), water, and ethanol (20–45% v/v). When PC self-assembles in water with ethanol present, double-layered multilamellar vesicles with a mean diameter of 150–200 nm are formed. Ethosomes, a type of well-known liposome, are distinguished by their superior solubility of lipophilic medicines, longer stability, and increased softness [5]. Indeed, a mixture of PC and ethanol has an enhanced penetration effect in this type of vesicular system, most likely as a result of partial disorganization and the opening of stratum corneum barrier pores [7]. We showed in recent work using transmission electron microscopy (TEM) that intact ethosomes may penetrate the skin of humans and reach deeper skin strata like the dermis [8]. Since they can increase the permeability of encapsulated medications via several skin layers, ethosomes might be seen in this way as transdermal delivery systems. As seen by the growing number of related study articles, the ethosome's straightforward and low-energy production process and the excipients' biocompatibility and antioxidants make these systems especially appealing for dermatological applications [9].

Because of their high entrapment efficiency (EE), increased drug delivery rate, and intense skin penetration, ethosomes have become a promising new vesicular approach to transdermal drug delivery in recent years [10]. Creating an ethosomal transdermal delivery system loaded with CM will enable the medication to penetrate the stratum corneum barrier and access the deeper layers of the skin, which will be extremely helpful in treating several skin diseases.

In this study PC functions as a surfactant and enhances penetration naturally, Propylene glycol (PG) acts as a gelling agent. By inducing structural rearrangement and intercalating with stratum corneum lipids, ethanol fluidizes the stratum corneum, improves CM solubility, and allows the gel to penetrate deeper into the skin. PC and PG organize a transdermally appropriate lamellar liquid crystalline gel by forming lamellar bilayer sheets that intervene with the aqueous layer within the kraft point temperature (Tc). When the gel gets applied topically, water that has been absorbed from the skin beneath several layers and the hydrophobic tail of PC's fatty acid ionize to produce negative zeta potential. As the concentration of hydrophobic acid increases, repulsion between the hydrophobic tails is generated, which increases vesicle stability. PG along with PC and ethanol improves the stability and in turn augmenting the EE.

MATERIALS AND METHODS

Materials

CM was procured from Molychem, Mumbai. The PG was supplied by Merck (Mumbai); Lipoid, Germany, sponsored Soya PC and Dr. Reddy's

Laboratories in Hyderabad gave a sample of CM. Every other chemical used in the investigation was analytical grade, including the solvents used in High-Performance Liquid Chromatography **(**HPLC). For the duration of the experiment, fresh double-distilled water was utilized.

Animals

Rats weighing between 200 and 300 g, male albino twisters were used for the animal examination. The institutional animal ethics committee of Chaitanya, Deemed to be University, Hyderabad, approved the study.

Methods

Formulation of CM-loaded ethosomes

Ethosomes were prepared by adopting the previously proposed cold method (Touitou *et al*., 2000). A simple and convenient process. Precisely, various quantities of PC were solubilized in ethanol and PG in different ratios (1:1, 1:2, 1:3, 1:4, and 5:7) in the beaker, then CM was added and maintained the temperature at 40°C under continuous stirring (Table 1). Then add the 7.4 pH Phosphate Buffer Solution (PBS) to the above mixture and stir well until a homogenous ethosomal mixture is formed. Kept the preparation in vials at refrigerator temperature after sonication for 5 min [11].

Characterization of CM ethosomes

Fourier transform infrared spectroscopy (FTIR) studies on rat abdominal skin

Rat skin that had been prepared was treated for 6 h with the optimized ethosomal formulation, which is equivalent to 5 mg of CM. The untreated skin was utilized as a control, and the skin sample was subsequently cleaned using water and blotted dry. Using an FTIR-multipurpose spectrophotometer (Shimadzu, Japan), FTIR of the aforementioned treated skin sample has been analyzed in the 4000–400 cm^{-1} region in comparison to the untreated normal rat skin that was used as the control [12].

Differential scanning calorimetry (DSC)

The thermal properties and phase transition behavior of optimized ethosomes (CM5), soy PC, PG, and CM were examined using a DSC (Mettler DSC 821e, Mettler-Toledo, Switzerland). An aluminum pan that was hermetically sealed was used to heat a 5±2 mg sample to a temperature range of 20–300°C on average. Both a steady nitrogen gas flow of 30 mL/min and a heating rate of 10°C/min were maintained [13].

TEM

A tiny drop was applied to a TEM grid covered with form a film to negatively stain the CM ethosomes preparations for the TEM investigations [14]. After a minute, the excess drop was removed from the grid employing filter paper to preserve a thin layer of the substance on the supporting substrate. A very small amount of 2% phosphotungstic acid was used for 1 min and afterward removed using filter paper to surround and adhere to the surface of the nanosystems that were positioned on the grid. The grid was then seen using a TALOS L120C G2 TEM (Thermo Fisher Scientific, Eindhoven, Netherlands).

Vesicle size and surface charge

The ethosomes were soaked in PBS (pH 7.4) for 4 min to hydrate them. After sonication, the resultant ethosomal dispersion was used to determine the vesicle size using the dynamic light scattering approach. After appropriate dilution of each sample, size analysis was carried out under 25°C with a 90°C detection angle. Characteristics were analyzed by using an instrument Zeta sizer, nano ZS 90 (Malven instruments, Malven, UK) [15].

%EE

After centrifugation, the amount of unentrapped drug was subtracted from the total amount of drug injected to determine the %EE of the generated ethosomes [16]. The drug's concentration in the aqueous phase was measured using HPLC. The following formula, shown below [17], was used to determine the proportion of CM entrapment in ethosomes.

$$\% \text{ Drug entrapment} = \frac{\text{Total amount of drug added} - \text{Unentrapped drug}}{\text{Total amount of drug added}} \times 100$$

Determination of spreadability

Precisely 0.5 g of ready-to-use ethosomes were positioned inside the designated 0.5 cm radius circle on the glass plate, and another glass plate with the same measurements was placed on top of the first one. Around 500 g of weight was placed on the glass plate and allowed for 5 min. The weight was removed from the glass plate, and the increased gel diameter was noted as its spreadability [17].

Determination of rheological behavior

Using a controlled stress rheometer (Brookfield Programmable DVIII+ Digital Rheometer, MA, USA) with cone (24 mm) and plate geometry, the rheological behavior of ethosomal formulations was investigated. Before measuring, the sample was given 5 min to acclimatize to a torque sweep between 10% and 110%. The Rheocalc 32 programming was used to determine the rheological properties after making multiple observations at room temperature. The Ostwald power equation was used to study the rheological properties of a thixotropic fluid.

$$\eta = KS\text{-}n \quad (1)$$

After logarithmically transforming the equation, we obtain:

$$\text{Ln } \eta = \ln - n \quad \ln S \quad (2)$$

Where η indicates the thixotropic degree determined by the slope from the curve while ln η is plotted against ln S, K is a constant, S represents the shear rate, and η is the apparent viscosity [18].

In vitro drug release

The *in vitro* release studies were conducted using a synthetic cellophane membrane. 4.59 cm^2 was an efficient diffusion surface area, while the receptor cell volume was 17 mL. There was filling in the vertical Franz diffusion cells. Before the trial, the dialysis membrane was soaked, and the donor compartment was filled with the ethosome formulation or control (CM suspended in the same solvent as the formulations). PBS (pH 7.4) was added to the receptor compartment, which was continuously swirling and kept at 37±2°C for the day. Parafilm was used to cover the sample ports and donor chamber to stop the diffusion medium from evaporating. To keep the receptor's initial phase volume constant, the samples were routinely taken out and refilled with an equivalent volume. Following the conclusion of the study, the samples were suitably diluted, and the drug's concentration was determined

Table 1: Formulae of CM loaded ethosomes

Formulation	Curcumin (mg)	PG: Ethanol (mg)	Total ethanol content (mg)	PC (mg)	PBS (μL)
CM1	10	1:1	30	5.0	160
CM 2	10	5:7	35	4.5	160
CM 3	10	1:2	40	4.0	160
CM 4	10	1:3	45	3.5	160
CM 5	10	1:4	50	3.0	160

PC, PG, PBS-indicates soy phosphatidyl choline, Propylene Glycol and Phosphate buffer respectively

using HPLC was used to measure the drug's concentration. Release. The ethosomal formulations data mathematically fitted into first- and second-order kinetics along with the Higuchi model to determine the drug release pattern [19].

HPLC assay of CM

Perkin Elmer Series 200 HPLC Systems (PerkinElmer, Waltham, MA, USA) with a micropump, autosampler, and a UV detector that operates at 360 nm were used to conduct the HPLC investigations. A mobile phase of methanol/water 80:20 v/v was used to elute a stainless-steel C-18 reverse-phase column (15 × 0.46 cm) filled with 5 µm particle (Hypersil BDSC18 Thermo Fisher Scientific S.p.A., Milan, Italy) at a flow rate of 1 mL/min. The retention period was 2.3 min, and the injection's volume was 5 µL [20].

Study of *ex vivo* permeation

The study used male Wistar rats, weighing 180–200 g, that were purchased from SAINATH AGENCIES 1656/PO/BT/S12/CPCSEA in Hyderabad, India. The rats were allowed to have food and water until they were sacrificed for their skins. The animals were housed in individual cages with temperature controls. The Institutional Animal Ethical Committee at Chaitanya Deemed to be a University in Hyderabad, Telangana, India. Gave its prior approval before the study was carried out. PO/Re/S/17/CPCSEA; 0105/2024/1963).The rats were killed by inhaling too much ether. The hair was gently cut out of the exposed flesh of the abdomen so as not to rip it. The rat's abdomen skin was divided and removed, and its adherent subcutaneous fat, tissue, and capillaries were extracted.The entire abdomen was immersed in water at 60°C for 45 s to prepare the epidermis using the heat separation procedure. This is immersing the entire abdominal skin for 45 s in 60°C water. The prepared epidermis was kept for 2 weeks before being cleaned, wrapped in aluminum foil, and stored at −20°C to be used later [21].

Permeation study

For the *in vitro* permeation studies, unjacketed vertical Franz diffusion cells having a receptor cell volume of 17 mL and a successful diffusion surface area of 4.59 cm^2 were employed. As soon as the skin reached room temperature, it was positioned between the Franz diffusion cell's donor and receiver compartments, with the SC edge facing the donor compartment. The donor compartment was filled with limited dosages of the formulations and the control, which was a medication equivalent to 5 mg CM suspended in the same solvent after the skin had equilibrated for 30 min. For an entire day, the PBS pH 7.4 receptor compartment was continuously stirred and maintained at 37±2°C. To stop evaporation during the study, Para film was placed over the sampling port and donor chamber. At predefined intervals of time, samples were collected and analyzed using HPLC.

Drug content retained in the skin

After a 24-h permeation trial, the quantity of CM absorbed in the layers of the epidermis was estimated. After removing the skin via the diffusion cell after removing the adhering material with a quick methanol wash or ablution, the outer layer of skin remained dry for 10 min at 25°C. To extract the CM from the skin layers, the skin was cut into small pieces, homogenized with phosphate-buffered saline (pH 7.4), and then sonicated for 30 min (using a bath sonicator made by Sonica, Italy). A thin-film membrane filter (0.45 µm) was used to filter the samples, and HPLC was used for estimation [22].

Treatment of permeation data and statistical analysis

A graph was created by plotting the Cumulative Amount Penetrated (CAP) per unit area against time. The CAP versus time plot's terminal linear segment slope was used to compute the steady state flux (Jss). To calculate the Permeability Coefficient (Kp), divide the Jss by the drug's initial concentration in the donor compartment. Divide the Jss of the test formulation by the Jss of the control to get the enhancement ratio. A one-way Analysis of Variance was performed on the permeation parameters to ascertain the statistical significance. Using Instant Graph pad prism software, the student-Newman-Keuls (compare all pairings) method was used to determine the significance of the variations between the formulations. At $p<0.05$, the difference was deemed statistically significant [23].

Skin irritation test

The male Wistar rats were subjected to the skin irritation test to look for any signs of reddening or irritation. The rats were kept in individual cages with the prepared ethosome formulations applied to their dorsal sides, and they were observed every day for a week [24].

Stability analysis

To test the durability of the ethosome formulations, they were carefully packed in glass vials and kept in the refrigerator and at room temperature. The formulation was hydrated with PBS at predetermined intervals of 1, 3, 6, 9, and 12 months, and any indication of phase separation or aggregation was checked under optical microscopy. The samples were assessed for drug entrapment as well as vesicle size [25].

Pharmacokinetic Parameters

The plasma concentration versus time profile was directly used to determine the maximum concentration (Cmax) and time to reach it (Tmax). The trapezoidal rule approach was used for calculating the AUC (AUC0–t) (Lei *et al*., 2013). By dividing the plasma concentration for the last time point by an elimination rate constant (K), the AUCt–α was calculated. The AUC0–α of CM ethosomes was divided by the control oral solution to determine the relative bioavailability (F). Additionally, by contrasting the CAP (µg) with the AUC acquired *in vivo* from CM ethosomes, an *ex vivo* and *in vivo* correlation was carried out [26].

RESULTS AND DISCUSSION

Morphology of CM

The TEM indicates the morphology of vesicles, and the TEM images for CM5 were found to be spherical with a uniform surface. The vesicles were spherical with very flawless borders. The uniform surface and shape of vesicles have proved that there won't be any drug leakage happening from the formulations as shown in Figure1.

FTIR analysis

Wavenumber shifts and new peaks in the treated skin spectrum suggest interactions between the gel's ingredients and the lipids in the skin. Changes in the membrane of lipids that improve drug penetration are indicated by an elevated shift in the 2800–3000 cm^{-1} region. In untreated skin, the hydrogen bond spectrum at 3291.04 cm^{-1} moved to 3368.42 cm^{-1}, indicating that the components of the ethosomal gel interacted with the skin proteins to break the hydrogen bond. This attests to the formulation's ability to reach the skin's deeper layers. New peaks seen in treated skin show lipid alteration and greater fluidity, which improves drug absorption by releasing the stiff lipid

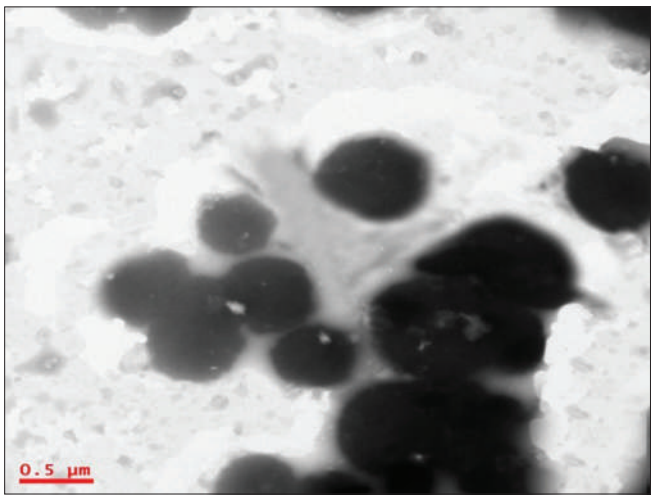

Fig. 1: Morphology of curcumin ethosomal vesicles (×500)

bilayer. C-H stretching associated with lipids and fatty acids was seen in untreated skin at 2924.36 cm^{-1} and 2841.50 cm^{-1} shift, respectively. The untreated skin's amide peak at 1643.50 cm^{-1} and 1545.88 cm^{-1} were moved to 1645.84 cm^{-1} and 1455.94 cm^{-1} in the treated skin, indicating an alteration of protein structures that may have been brought on by the medication and ethosomal carriers penetrating the deeper layers of the skin.

FTIR of CM incorporated ethosomal gel and untreated skin as control shown in Figure 2

DSC thermogram

PC and CM both displayed distinct endothermic peaks at 148.42°C and 183.65°C, respectively. The loss of the cholesterol's prominent endothermic peak in the DSC thermogram is caused by the pure lipids' phase transition behavior changing to a liquid crystalline state in the ethosomal formulation depicted in Fig. 3.

Vesicles' size and zeta potential

The vesicle mean size and zeta potential of CM ethosomal gel have been analyzed using a zeta sizer, and the outcomes given in Table 2 indicate that the ethosomal formulations range in size from 334±52 to 634±58 nm. This was comparatively less than the ethosomes made by Saraswathi *et al.*, which had a vesicle size of 706–1433 nm [27]. It was contemplated that both PG and ethanol significantly affect the size of the ethosomal vesicle. It was observed that as the concentration of ethanol increased, the size of the vesicle also increased, and this was due to the formation of the vesicle wall by the lipid, which further increased the thickness of the vesicle wall, thereby increasing the vesicle size. As the concentration of ethanol increases the zeta potential increased significantly to −9.46±2.06 from −4.34±2.12 mV indicating better stability due to electrostatic repulsion preventing aggregation. CM5 shows better stability compared to CM1.

Polydispersity index (PI)

The PI used as a measure of an unimodal size distribution was within the acceptable limits for all the CM ethosomes. A population is considered homogeneous if its PI value is tiny (<2.0), whereas it is considered heterogeneous if its PI value is more than 0.3. The PI of the optimized formulation was 0.208. This was comparatively less than the ethosomes made by Momekova *et al.*, which had a PI of 0.31±0.05 [28]. Every formulation's PI falls within permitted limits.

EE%

The EE was discovered to vary between 76.8±2.2 and 90.8±1.8. This was comparatively less than the ethosomes made by Maurya *et al.*, which had a EE of 43.39±2.5 [29]. It was found that the formulation with less ethanol and more PC (CM-1) had an EE of 76.8±2.2. Ethanol has been found to significantly increase the EE. The optimized formulation of CM5 ethosomes has an EE of 82.4±2.6%. All the formulation's EE was depicted in Table 2. The reason for the increased EE is the bilayer formed in the presence of PC ethanol and PG, due to increased solubility of CM in the core, as ethanol was distributed throughout the vesicles, ethanol caused the lipid bilayer to fluidize excessively. An increase in cholesterol concentration was observed to reduce EE; this could be because the increased cholesterol concentration could compete with the medication for the area within the bilayer.

Spreadability and rheological behavior

One of the most important factors to consider for excellent patient compliance is spreadability, which increases the gel's absorptivity and penetration by increasing the contact area. All ethosomal gel formulations were found to have spreadability between 5.78±0.18 and 7.20±0.44 cm. this was higher than the ethosomes prepared by Halagali *et al.*, The er preparation has a spreadability of 2.31 cm [30]. A significant reduction was observed in the spreadability of the ethosomes systems with higher PC content. All the formulations exhibited good skin feel and spreadability, ensuring ease of application and uniform distribution of the formulation on the skin (Fig. 4).

The ethosome formulations' viscosity, which is reduced with an increase in shear rate, and rheological behavior determine the percutaneous application. The findings show that the viscosity of the formulations is influenced by the amount of PG, PC, and ethanol. For all formulations, the viscosity decreases as the shear rate rises. Because of their vesicular architecture and polymeric connections, ethosomal formulations frequently exhibit shear-thinning behavior. Create an organized network at low shear rates, increasing viscosity. The yellow triangle, or CM3, seems to have the highest initial viscosity, suggesting a larger lipid concentration or a stronger internal network. Stability can be preserved and the settling of active components during storage can be avoided with higher viscosity at low shear rates. Consistent with other

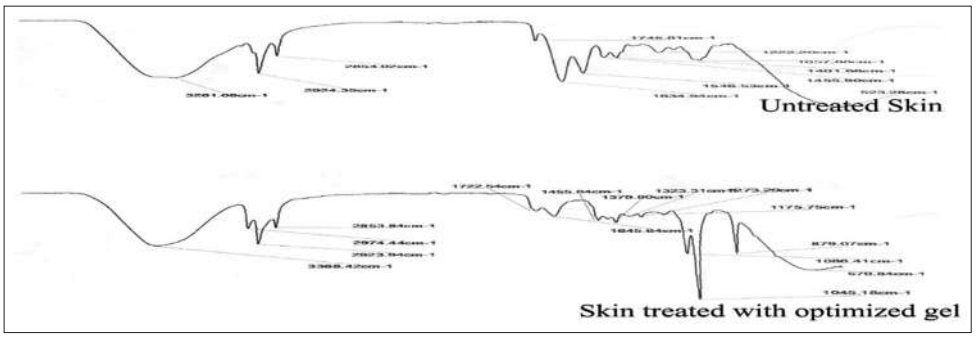

Fig. 2: Rat skin treated with optimized curcumin incorporating ethosomal formulation (CM5) and untreated rat skin (control) are shown in the Fourier transform infrared spectroscopy spectra

Table 2: Size, zeta potential, %EE, thixotropic degree, and spreadability of curcumin ethosomal gel formulations

F. code	Size (nm)	Zeta potential (mV)	PI	EE (%)	n	Spreadability (cm)
CM-1	324±17	−4.34±2.12	0.186	76.8±2.2	0.948	5.78±0.18
CM-2	423±20	−6.94±1.84	0.198	80.6±3.6	0.956	5.94±0.24
CM-3	437±28	−7.28±1.62	0.208	82.4±2.6	0.964	6.18±0.36
CM-4	483±16	−8.34±1.18	0.210	86.2±2.2	0.969	6.98±0.28
CM-5	492±12	−9.46±2.06	0.218	90.8±1.8	0.976	7.20±0.44

(Mean±SD, n=6); n and PI indicate thixotropic degree and polydispersity index

studies, the vesicular structures align and disintegrate as the shear rate rises, resulting in a drop in viscosity. Additionally, the thixotropic degree (n value), which was calculated using the rheological data, Thixotropy (n), ranges from 0.948 to 0.976, with a modest rise from CM1 to CM5, as shown in the accompanying Table 3. The formulation's spreadability and retention on the skin's surface can be enhanced by a higher (n).

In vitro drug release

It was investigated to comprehend CM's *in vitro* release behaviour from ethosomal gel. The percentage of drug released from various formulations is shown in Fig. 5. Within 24 h, the release of CM from the control group was almost 74.13±1.77%, indicating that thedrug in the control suspension is freely available for dissolution and diffusion, leading to rapid release. When using ethosome formulations, a standard biphasic release pattern (Initially, burst release followed by a gradual and prolonged release over time) was seen compared to the control.

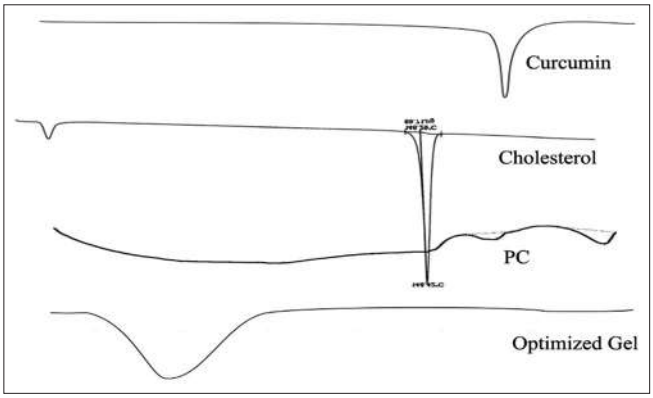

Fig. 3: Curcumin, cholesterol, phosphatidyl choline, and optimized ethosomal formulation (CM-3) of differential scanning calorimetry thermograms

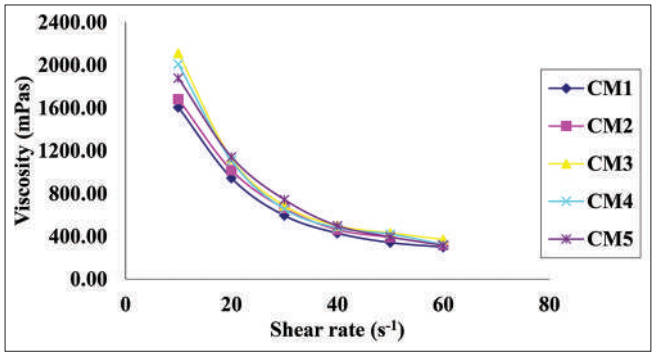

Fig. 4: Rheological behavior of curcumin ethosomal formulations. Data are given as a mean of 6 replicates

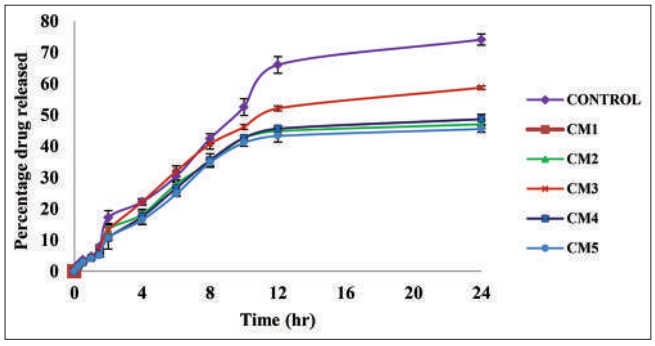

Fig. 5: Curcumin's *in vitro* release profile (mean±SD; n=3) across the cellophane membrane from different ethosomal formulation

Among the ethosomal formulations, CM1 shows the maximum drug release, suggesting a less compact vesicular structure with a poorer EE than CM5. The release profiles of CM2, CM3, and CM4 are comparable, exhibiting a gradual and regulated release. Longer drug retention results from CM5's slower drug release, which is indicative of a more stable vesicle shape and higher drug EE. The existence of a notable gradient in concentration at the early time points indicates the release of the unentrapped medication from the ethosomal gel formulae. The findings show that diffusion controls the release of drugs from ethosomal gel, which is consistent with the zero-order kinetics results shown in Table 4. The optimized formulation (CM5) has an *in vitro* drug release of 45.54±0.68; ($p<0.001$).

Ex vivo permeation study

The viability of transdermal distribution of CM ethosomes was assessed by conducting *ex vivo* permeation assays across excised abdomen skin taken from rats. Only after the medicine is liberated from the ethosomes that are created when transdermal ethosomes are hydrated with epidermal fluids can CM significantly penetrate. All of the formulations showed no lag time, indicating that ethosomes were forming. Additionally, the drug was found within 0.25 h, which clarifies how quickly the drug was released, the medication penetrated the skin earlier water in the receptor fluid infiltrated the epidermal membrane.

The entire amount of medication that entered the rat's abdomen skin during the *ex-vivo* permeation study is shown in Fig. 6. CM does not considerably penetrate until the drug is liberated from the ethosomes that form once the ethosomes are moistened with the skin fluids. There was no lag time seen in any of the formulations, which amply demonstrates the ethosomes formation process. Furthermore, the drug was found within 0.25 h, which states how quickly the drug was released from the skin membrane and how water diffused from the receptor fluid. The CM1 formulation had much less penetration, which could be the result of poor drug entrapment, less stability, and improper vesicle formation. When PG: Ethanol was added to the formulation, there was a noticeable increase in permeation.

The regulated delivery of CM from formulations is indicated by the flow, which was larger for the ethosomal formulations at all-time points compared to the control. A table was created and showed the permeation enhancement as determined by the permeation

Table 3: Rat skin penetration properties of CM from various ethosomal preparations

F. code	Q24	Flux (µg/cm²/h)	ER	PC
Control	1781.97±30.12	8.42±0.18	-	2.956±0.10
CM1	2725.63±103.9	14.85±0.81	1.77	2.974±0.16
CM2	2878.83±38.55	15.93±0.80	1.89	3.189±0.18
CM3	3187.61±63.05	16.96±1.62	2.02	3.40±0.32
CM4	3513.22±37.27	19.39±2.13	2.31	3.89±0.43
CM5	3970.01±62.92	23.13±0.91	2.75	4.63±0.18

(Mean±SD; n=6). $p<0.05$; Q24, ER, and PC represent the amount permeated in 24 h, steady state flux, enhancement ratio, and permeability coefficient, respectively

Table 4: *In vitro* release kinetics of curcumin from ethosomes

F. code	Zero-order		First order		Higuchi
	K_0 (h^{-1})	R^2	K_0 (h^{-1})	R^2	R^2
Control	0.427	0.871	0.438	0.576	0.786
CM-1	0.216	0.868	0.698	0.742	0.814
CM-2	0.178	0.918	0.534	0.624	0.836
CM-3	0.192	0.934	0.578	0.636	0.918
CM-4	0.215	0.942	0.563	0.601	0.849
CM-5	0.248	0.97	0.712	0.637	0.905

K_0 and K represent Zero and first-order release rate constants of respective formulations

Table 5: Rats' pharmacokinetic characteristics after receiving an oral suspension (control) and optimized ethosomes (CM 5)

F code	Cmax (µg/mL)	Tmax	AUC (0-t)	AUC (t-i)	AUC (0-i) (µg.h/mL^{-1})	K	F
Control	29.85±0.59	1.5	291.92±8.23	97.13±83.74	375.67±1716	0.032	
CM5	49.32±2.84	2	745.56±48.22	1593.56±316.67	2339.12±362.43	0.0138	7.61

Data is given as mean±SD of 6 replicates

parameters (flux, permeability coefficient, and enhancement ratio). The steady-state flux and permeability coefficient significantly increased for the ethosomes gel formulation (CM5). Improved permeation is indicated by an enhancement ratio that is well above 1, and our results show that all ethosomal formulations have an ER higher than 1 when compared to the control. A statistically significant improvement has been made (Table 3). Interestingly, the quantity of medication released through the cellophane membrane was much less than that which permeated through the skin, indicating the skin's barrier qualities. This implies that direct vesicle-skin contact and interaction are significant contributing factors to enhanced CM transdermal delivery. The reports and our results have a strong correlation (Fang *et al*., 2001). Overall, the data clearly show that CM permeation has significantly improved over ethosome formulations. The compositions can be arranged in a subsequent decreasing order according to the permeability parameters CM5->CM4>CM3>CM2>CM1> Control.

The CM1 formulation had poorer penetration, which may have been caused by the drug's poor entrapment and decreased stability. The addition of ethanol resulted in a notable improvement in penetration. The high hydrophilicity of the vesicle, however, may have enhanced the partitioning promotion of CM into the skin bilayers, as seen by the increased penetration that followed a subsequent increase in ethanol concentration. For the control, the minimum flux was 8.42±0.18 µg.h/cm^2, while for the optimized ethosome formulation (CM5), it was 23.13±0.91 ($p<0.001$). Which was higher than that of the ethosomes made by Maurya *et al.*, which had a maximum flux of 17.54±0.78 [29]. Nonetheless, the flow was consistently larger for the ethosomal gel compositions than for the control, indicating that the ethosomal gel formulae delivered CM in a regulated manner. The table displays the computed permeation enhancement evaluated in terms of the permeability parameters (flux, permeability coefficient, and enhancement ratio). The ethosomes gel formulation (CM5) with a 1:4 PG: Ethanol ratio had a much higher steady-state flux and permeability coefficient. Improved penetration is indicated by an enhancement ratio considerably above 1, and our results showed that all ethosomes gel formulations had an ER >1 when compared to the control.

Drug content retained in the skin layers and skin irritation study

Fig. 7 shows the drug content retained in the skin layers after rat skin was treated with all of the ethosomes formulae and suspension of the drug. Given that all of the ethosomes formulations increased penetration, it follows that the drug's concentration in the epidermal layers rose as well. This makes sense because the higher drug deposit in the skin values could have resulted from the skin layers being saturated at the experiment's termination point, or 24 h in this case. The increased CM deposition in skin layers with ethosomes as compared to control suggests that these formulations may be able to better distribute the drug into the skin's viable regions while circumventing the stratum corneum's barrier function.The drug content retained in the rat's skin of the optimized formulation was 764.34 µg which was higher than that of the ethosomes made by Jukanti *et al.*, which had a drug content of 357 µg [16]. Control formulation containing the drug in suspension without ethosomes may have limited penetration, leading to lower drug accumulation in the skin ($p<0.001$). Results showed that as the concentration of ethanol increases the amount of drug retained in the skin decreases. This may be explained by the role of ethanol in formulation, which favors or enhances the permeation of the CM through SC and demonstrate that to permeate the skin, the drug must be released first. So optimized formulation (CM5) has the highest permeation rate among the ethosomal formulations. The application of

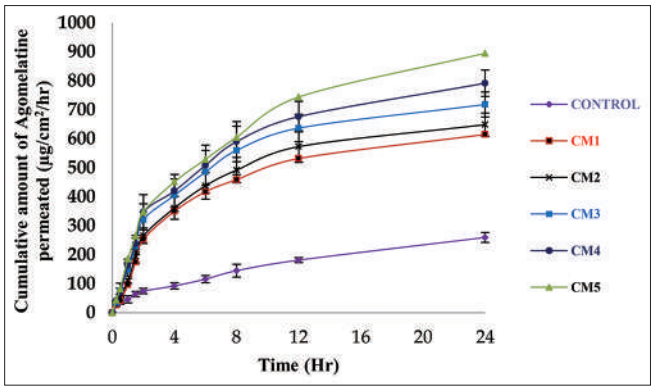

Fig. 6: Total curcumin that penetrated the rat skin from different ethosome formulations cumulative amount permeated per square. Centimeter per hour. Data is given as mean±SD of 3 replicates

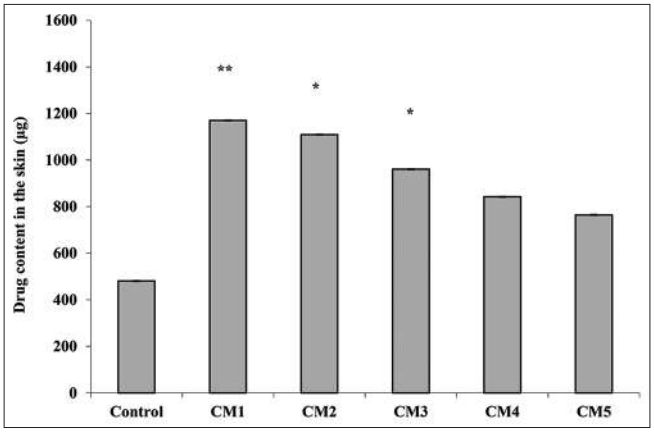

Fig. 7: After 24 h of treatment with various ethosomal gel formulations, the drug content remained in the skin layers (mean±SD; n=3)

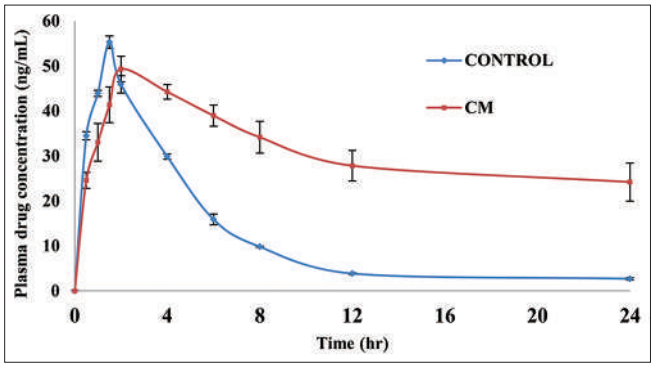

Fig. 8: CM5 optimized ethosomal formulation was used in the treatment, and the control group was administered after the CM's mean plasma concentration versus time profile. Data are given as mean±SD of 3 replicates

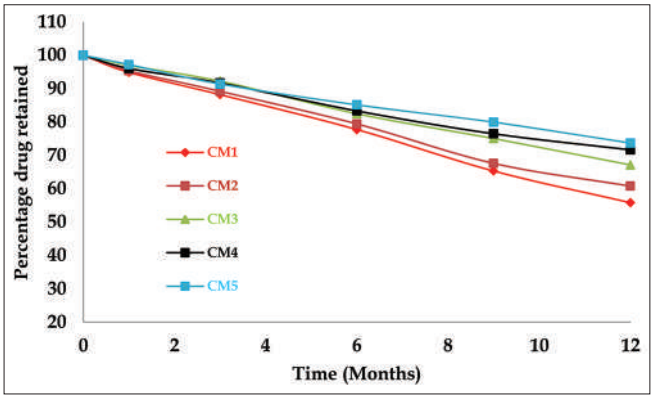

Fig. 9: Particle size changes in formulations containing ethosomes when stored at refrigerator temperature. Data are given as mean±SD of 6 replicates

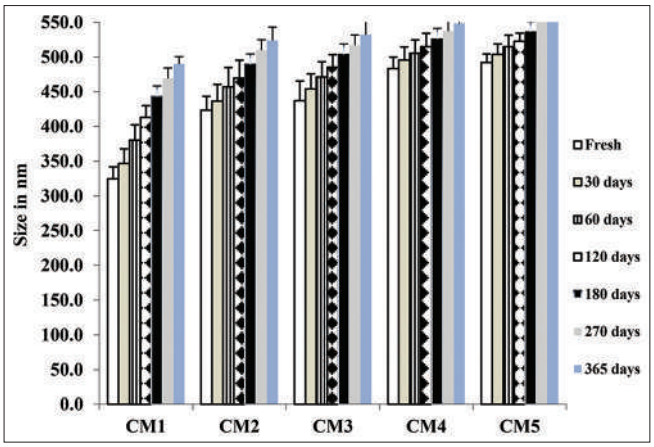

Fig. 10: The size of CM ethosome preparations changes as they are stored in a refrigerator

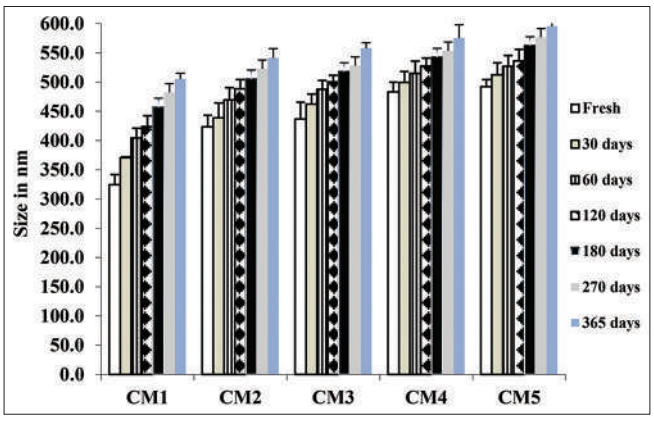

Fig. 11: The size of CM ethosome preparations changes as they are stored at room temperature

optimized ethosomes formulations (CM5) in three rats, failed to detect any noticeable erythema throughout seven days* ($p<0.05$).

Pharmacokinetic studies

Fig. 8 and Table 5 display the plasma concentration versus time profile after ethosomal formulation is administered orally and applied transdermally. Because of its greater vesicle size, higher entrapment effectiveness, and stable vesicular structure, CM5 was chosen as the formulation with the best-regulated release. The improved formulation's AUC and Cmax were determined to be 49.32±2.84 (µg/mL) and 2339.12±362.43 µg.h/mL^{-1}, respectively. When compared to the control (oral suspension), the improved formulation's bioavailability increased 7.61 times).

Stability studies

Over a year, the ethosomes formulations' physical characteristics, vesicle size, and drug leakage were investigated. The vesicles made from ethosomes were multilamellar, according to the microscopical investigations, and we were unable to detect any discernible alteration in the morphological behavior. The size and percentage of CM retained in the vesicles of the ethosomes that were produced following hydration were assessed. When CM formulations were kept in a refrigerator, we were unable to see any discernible change in medication size (Fig. 10). However, after 120 days, there was a noticeable increase in size and medication leakage ($p<0.05$). However, when the preparations were stored at room temperature, the size grew and the percentage of drug retention decreased (Fig. 11). The information shows how temperature affects ethosome stability. Conversely, the percentage of retention was decreased, indicating the significance of the vesicle composition. Comparatively speaking, the formulations kept at 4°C in the refrigerator were more stable than those kept at ambient temperature.

Ethamome-containing formulations' particle sizes alter when kept in a refrigerator are shown in Figure 9.

CONCLUSION

It was feasible to produce ethosomal gels containing CM. The ethosome preparations having good flow behavior have a high thixotropic degree, according to the rheological investigations. According to the permeation study, ethosomes can transport CM via the skin in a regulated way using diffusion with zero-order kinetics. The results indicate that CM5 formulation was optimized based on its greater vesicle size, higher entrapment effectiveness, thixotropic degree, and enhanced permeation kinetics. *In-vivo* study revealed a 7.61 times significant increase in bioavailability. Overall, the findings show that CM may be effectively delivered transdermally for skin care using the recommended ethosomal formulation.

AUTHORS CONTRIBUTIONS

The Ph.D. thesis of RajithaNallagandla, who carried out the preliminary study, gathered the data, completed the work, and wrote the full manuscript, is the basis for this publication, according to the authors. The supervisor, (Kothapally Daniel), edited the study's wording and verified its data.

CONFLICTS OF INTERESTS

All authors have none to declare.

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Nil.

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