RESEARCH PAPER

Preparation and Evaluation of Curcumin loaded Ethosomal Cream for Anti-inflammatory Activity in Animal Model

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ABSTRACT

The study was designed to evaluate the ethosomal Cream of Curcumin, its incorporation into cream formulations. The prepared cream were assessed for their anti-inflammatory activity against carrageenan induced oedema. Different formulations of ethosomes using lecithin and ethanol were prepared using different doses of Curcumin. In vivo anti-inflammatory activity studies were carried out using carrageenan-induced rat paw oedema model. Male Wistar rats weighing about 150-180 gms were divided into 4 groups of six rats (n=6) each. Curcumin was used as a standard drug. Three cream formulations (100, 200 and 300 mg) were used as test drugs. The pH of the cream formulations was found to be in the range of 5.2-7.7 and viscosities were between 2030-3206 centipoises. The drug content of cream ranged between 79.2-96.4%. From the study, it can be concluded that ethosomal cream served as an efficient drug delivery system for Curcumin with potential anti-inflammatory property.

Keywords: Ethosomal Cream, Curcumin, Inflammation, Carrageenan

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INTRODUCTION

Inflammation is a pathophysiological response of living tissues to injuries that leads to local accumulation of plasmatic fluid and blood cells. It is also a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is a protective attempt by the organism to remove the injurious stimuli and to initiate healing process [1]. During the development of inflammation, the concentrated actions of molecular signalling determine whether inflammatory cells undergo migration, activation, proliferation, differentiation or clearance [2]. Redness, heat, swelling, pain and loss of function are the five responses to tissue injury and are also called the cardinal signs of inflammation. [3] Though these signs and symptoms are necessary for tissue repair, it can be noxious to people thus must be treated with drugs known as anti-inflammatory agents. As the available anti-inflammatory drugs (steroidal and non-steroidal) present a wide range of side effects, many studies are being directed to find anti-inflammatory agents from natural sources. [4] In this context, medicinal plants are widely used in folk medicine in many countries to treat different inflammatory conditions and skin inflammation. [5]

Curcumin

The herb selected for the study was Curcuma longa (Zingiberaceae), which contains curcumin, demethoxycurcumin, and bisdemetho- xycurcumin, three major pharmacologically important curcuminoids, has been shown to possess antioxidant property and also have wound healing effect [6].

Curcumin has well established antioxidant property which supports its use as an antiwrinkle agent. The potential of ethosomes to deliver the antiwrinkle agent (curcumin) into the deeper layers of skin. Curcuma longa extract loaded ethosomes were incorporated into cream. The invivo studies revealed 10 to 50% improvement in skin viscoelasticity, total deformation, biological elasticity and sagginess. It was concluded that curcuma longa extract loaded ethosomal cream represents an efficient vehicle to deliver the antiwrinkle agent to the skin. Curcumin have shown its ability to significantly reduce the appearance of fine lines, wrinkles, and hyperpigmented macules [7].

Drug delivery through the skin has been a promising concept for long time because the skin is easy to access, has a large surface area with vast exposure to the circulatory and lymphatic networks and the route is non-invasive. Tropical preparations such as creams, ointments, and gels may be prepared where it can be spread to local inflammation sites. Transdermal drug delivery systems are gaining in popularity in this aspect. The main limiting factor of transdermal drug delivery i.e., epidermal barrier can be overcome by ethosomes when compared to transdermal dermal delivery [8].

Ethosomes are non-invasive delivery carriers that enable drugs to reach the deep skin layers and /or the systemic circulation. Plant drugs are considered safe of their natural origin. [9] Even after exhibiting the promising therapeutic effects, most of the phytoconstitutents fail to achieve bioavailability because of poor absortion. Large molecular sizes and low lipid solubilities are the prominent factors causing poor absorbtion of phytoconstituents resulting in reduced bioavailability. Incorporation of these plant actives or extracts into vesicular carriers vastly improves their absorption and consequently bioavailability. [10] Indirectly ethosomes has become an area of research in herbal formulations because of their enhanced skin permeation and improved entrapment efficiency. Therefore, the present study was aimed at formulating and investigating an effective antiinflammatory ethosomal cream formulation of Curcumin.

MATERIALS AND METHODS:

Materials:

Curcumin was purchased from Central Drug House Ltd.(Vardhan House,Daryaganj,New Delhi). Soya Lecithin, Propylene glycol was purchased from CDH Fine chemicals,New Delhi.Ethanol was purchased from Changshu Hongsheng Fine Chemicals Co. Ltd. Cetyl alcohol, Propyl paraben were obtained from CDH Fine chemicals, New Delhi. Stearic acid, Methyl paraben, Triethanolamine, Glycerol were obtained from Qualigens. All other solvent reagents utilized were of analytical grade and used as received.

Table 1: Formula for Cream Base

Preformulation studies:

Drug excipients compatibility study:

Compatibility screening of Curcumin with different excipients like cetyl alcohol, stearic acid, methyl paraben, propyl paraben, triethanolamine, glycerin, propylene glycol ,ethanol was investigated by recording the FTIR spectra of different excipients with drugs by KBr disc method and comparing the important peak positions with FTIR spectrum of pure drug.

Differential Scanning Colorimetry (DSC)

Differential scanning calorimetry (DSC) is used to determine the transition temperature of vesicular lipid system. Lipid bilayer exhibits various phase transitions that are studied for their roles in triggered drug release. Lipid bilayer can exist in a low-temperature solid ordered phase and above a certain temperature in a fluid-disordered phase. The temperature of this phase transition can be tailored by selecting the proper lipids. [11]

EXPERIMENTAL WORK:

Formulation of cream:

The base cream (25 g) was prepared through fusion and trituration methods. The composition of the cream base is shown in table 1. The oil phase containing cetyl alcohol and stearic acid was melted at 65°C, while the aqueous phase, containing propylene glycol, glycerin and triethanolamine was also maintained at 65°C. Methyl paraben and propyl paraben (1:0.5 ratios) were added as preservative. [12]

S. No	Ingredients (% w/w)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Cetyl alcohol (gm)	2	2	2	2.5	2.5	2.5	3	3	3
2	Stearic acid (gm)	9	10	11	9	10	11	9	10	11
3	Propylene Glycol (ml)	5	5	5	5	5	5	5	5	5
4	Glycerine (ml)	5	5	5	5	5	5	5	5	5
5	Methyl Paraben (gm)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
6	Propyl Paraben (gm)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
7	Triethanolamine (ml)	2	2	2	2	2	2	2	2	2
8	Double Distilled Water (ml)	Qs to 25	Qs to 25	Qs to 25	Qs to 25	Qs to 25	Qs to 25	Qs to 25	Qs to 25	Qs to 25

Evaluation of Cream:

Cream formulations were evaluated for organoleptic and physicochemical properties [13,14].

Physical appearance:

The cream base underwent visual evaluation for color, homogeneity, smoothness and consistency.

Grittiness:

To assess grittiness, a small portion of the cream base was placed between two grease-free glass slides and examined under diffused light to detect any foreign particles.

Determination of pH:

To determine the pH of cream base sample (5g±0.1g) was placed in a 100 ml beaker. Distilled water (45 ml) was added, and the mixture was heated to 45°C while constantly stirring for 15 minutes using a glass rod on a heating mantle. After filtration, the pH was measured at 27°C using digital pH meter.

Spreadability:

To evaluate spreadability, samples were weighed and placed on a custom-made spreadability instrument consisting of glass plates. The lower plate was fixed to a wooden platform, and a second glass plate (10×6 cm) with a weight of 50g was placed on the top for pulling towards upper plates, to slide from one designated point to another 7 cm away was recorded. The spreadability (S) was calculated using the equation:

$$S = M \times L/T$$

Where, 'S' is the spreadability of the cream formulation, 'M' is the weight (g) tied on the upper plate, 'L' is the length (cm) of the glass plates, and 'T' is the time taken (s) for the plates to slide the entire length.

Viscosity:

To assess viscosity (in cps), the cream base formulation was examined for viscosity using Brookfield rheometer (Cone and plate) (R/S Plus). One gram of sample, kept at maintained at 25±1°C, was placed on the plate, and the cone was rotated at 10 rpm.

Thermal Stability:

The cream formulation was spread along the internal wall of a 100 ml glass beaker using a spatula. The beaker was then placed in a stability chamber (Thermolab scientific equipment, Mumbai) maintained at 60-70 % RH and 45 ± 1°C for 48 hours. Following this period formulations were inspected for any separation of the oil phase.

Formulation of Ethosomes loaded cream:

On the basis of physicochemical parameters of cream base an optimized base was selected for loading of Curcumin ethosome. Different doses of ethosomes containing Curcumin for loading in cream base were selected on the basis of literature survey. Optimized suspension of ethosomes equivalent to 0.05%, 0.075%, 0.1% and 0.125% was added in aqueous phase shown in **Table No.2.**

Table 2: Formulation of Curcumin loaded ethosomal cream formulations

S. No	Ingredients (% w/w)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	F7 (equivalent to Curcumin) (mg)	0.05	0.75	1	1.25	0.05	0.75	1	1.25	0.05
2	Cetyl alcohol (gm)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
3	Stearic acid (gm)	9	9	9	9	9	9	9	9	9
4	Propylene Glycol (ml)	5	5	5	5	5	5	5	5	5
5	Glycerine (ml)	5	5	5	5	5	5	5	5	5
6	Methyl Paraben (gm)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
7	Propyl Paraben (gm)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
8	Triethanolamine(m)	2	2	2	2	2	2	2	2	2
9	Double Distilled Water (ml)	Qs to 25	Qs to 25	Qs to 25	Qs to 25	Qs to 25	Qs to 25	Qs to 25	Qs to 25	Qs to 25

Evaluation of Ethosome loaded cream:

The ethosome loaded creams were evaluated for physical appearance, grittiness, pH, spreadability, viscosity as per method reported in section 2.3.2. Creams were also evaluated for drug content, ex-vivo diffusion study, skin irritation study using rat skin, microbial examination and stability study.

Drug content:

To determine the drug content, a 1g sample was placed in a 100ml volumetric flask and diluted with ethanol up to the mark. The flask was then shaken to dissolve the drug in the solvent. The solution was filtered through whatmann filter paper (#1), and the absorbance was measured using a UV-Visible spectrophotometer at 425 nm, against a similarly treated blank.

In-vitro drug release:

The in-vitro release study was carried out using a diffusion cell assembly consisting of a donor compartment, receptor compartment (20 mL), and a sampling port. A dialysis membrane with a molecular weight cut-off (MWCO) of 12–14 kDa was placed between the donor and receptor compartments. The MWCO indicates the pore size of the membrane; molecules larger than this range are retained, while smaller molecules such as curcumin (molecular weight 368 Da) are able to diffuse through. The receptor compartment was filled with 20 mL phosphate buffer (pH 5.5) and maintained at 37 ± 0.5 ◦C with continuous stirring at 50 rpm. A total of 5 g of the prepared ethosomal cream (equivalent to ~2 mg of curcumin) was applied uniformly onto the membrane in the donor compartment. At predetermined intervals (1, 2, 3, 4, 5, 6, 7, 8, and 24 h), 2 mL samples were withdrawn from the receptor compartment and replaced with fresh buffer to maintain sink conditions. The samples were diluted appropriately and analyzed using a UV–visible spectrophotometer (λmax 425 nm) to determine curcumin release [15].

Drug release kinetics and mechanism:

Drug release kinetics and mechanism were analyzed by fitting cumulative percent release data to various equations: Zero order (Cumulative percentage drug release vs time), first order (log cumulative percentage drug release vs time), Higuchi (cumulative percentage drug release vs √time), and Peppas exponential equation (log cumulative percentage drug release vs log time). The release exponent (n) from the Peppas exponential equation characterizes the drug transport mechanism. A value of 'n' ≤ 0.5 signifies Fickian diffusion-controlled release. 'n'> 0.5 < 1.0 indicates anomalous transport, while 'n' = 1 or > 1 indicates case-II transport, or super case-II transport, respectively [16, 17, 18].

Microbiological Evaluation:
Microbial examination of the formulated formulations followed Indian Standards methods IS 11648; 1999. Total viable counts, encompassing bacterial, yeast and mould counts were assessed using a colony counter. Bacterial counts were determined with soyabean casein digest agar, while mould and yeast estimation employed peptone agar medium as per Indian Pharmacopoeia standards. Formulations were diluted 1: 10 in sterile broth with appropriate neutralizers. 1 ml of each diluted sample was plated onto soyabean agar for bacterial growth and onto sabouraude dextrose agar for fungi, yeast, and mold. Incubation was carried out at 20-35°C for 48 hours for bacterial growth and for 5 days for fungal, yeast, and mould growth [19].

Stability study:
Stability studies involved filling the prepared cream formulations into collapsible tubes and storing them at ambient temperatures (30°C and 40°C). After one month, the cream formulations were assessed for phase separation, physical appearance, and drug stability [20].

Acute Skin Irritation activity:
Skin irritation test was performed following OECD guidelines 404. In skin irritation test, total 9 rats were taken of either sex weighing between 150-180 gm. Animals were divided into three groups of 3 each. Hairs were depleted from the back of the rats with the help of depilatories and area 4 cm2 was marked on both the sides. One side served as control while the other as test. Test substance of 50 gm was applied and the substance should be attached to the skin. The animals were observed for 14 days for signs of oedema and erythema [21].

Acute In Vivo anti-inflammatory activity:
A well-established model of acute inflammation, the carrageenan-induced paw edema is generated by a range of inflammatory mediators and has been widely used to assess the anti-edematous activity of natural products. Carrageenan-induced paw edema method: in vivo studies were performed under prescribed conditions as per guidelines of IAEC and approval of the IAEC committee. For in vivo studies, curcumin (pure drug) was employed as the standard drug (100mg/kg) while the equivalent dose (100mg/kg) and half dose (50mg/kg) of ethosomal cream were employed to study anti-inflammatory activity. Starving of rats was done overnight. Rats were divided into 4 groups [22,23]. The values of reduction of paw volume in each group, were found respectively after 5 hour of carrageenan administration [24].

Anti-inflammatory activity of ethosomal cream
Experimental animal
Wistar albino rats (male and female; Young) weighing 150–200 g were procured in the animal house of the IFTM University, Lodhipur Rajput, Delhi Road (NH-24), Moradabad-244102 (Deemed to be) University, Moradabad (Reg no. 837/PO/ReBiBt/S/04/CPCSEA; 01/10/2004) and all animals were ethically approved as per the guidelines of the institutional animal ethical committee (IAEC) and (IAEC approval no. is IAEC/2025/27/16). Animals were maintained in well-controlled conditions, in a polycarbonate cage, with a 12-h light/dark cycle, a temperature of 22 ± 2 ◦C, and relative humidity of 45% ± 5%. During the experimental duration, the rats were supplied with food that had been approved by the ethical committee of the institute and were allowed to access water on ad libitum basis. The cages had been leveled correctly and all animals were taken care of in compliance with the Principles of Laboratory Animal Care (NIH,1985).

Carrageenan-induced rat paw edema
The anti-inflammatory effect of the ethosomal cream was evaluated in a study using a carrageenan-induced rat paw edema model. Ethosomal cream was topically applied to the rat paws after the induction of inflammation with carrageenan [134]. This well-established method is used to assess anti-inflammatory properties of the formulations. Rats weighing between 150g and 160 g, were randomly selected after a 12-h fast. They were divided into four groups, each consisting of six animals (n = 6/group). For in vivo studies, curcumin (pure drug) was employed as the standard drug (100mg/kg) while the equivalent dose (100mg/kg) and half dose (50mg/kg) of ethosomal cream were employed to study anti-inflammatory activity. Inflammation was induced by subcutaneously injecting 100 µl of carrageenan (1% v/v in saline water) into the sub-planter tissue of the right hind paw, and 100 µl of normal saline into the left hind paw of each animal. The negative control group received carrageenan without any drug administration. After 15 min from the induction of inflammation, the rats were treated with formulated cream and standard drugs as per the groups. Readings were recorded at 1-h intervals following the carrageenan injection, and paw diameter measurements were taken before and at 1, 2, 3, 4, and 5 h post-injection using the cotton thread method [25]. The anti-inflammatory activity of the treated groups was quantified by expressing the percentage reduction in edema compared to the positive control, calculated using the provided formula [26].

Percentage (%) inhibition = Mean difference in paw edema*:* the control - Mean difference in paw edema*;* treated X 100

Difference in edema*;* control

RESULT AND DISCUSSION:

FTIR SPECTRUM OF ETHANOL

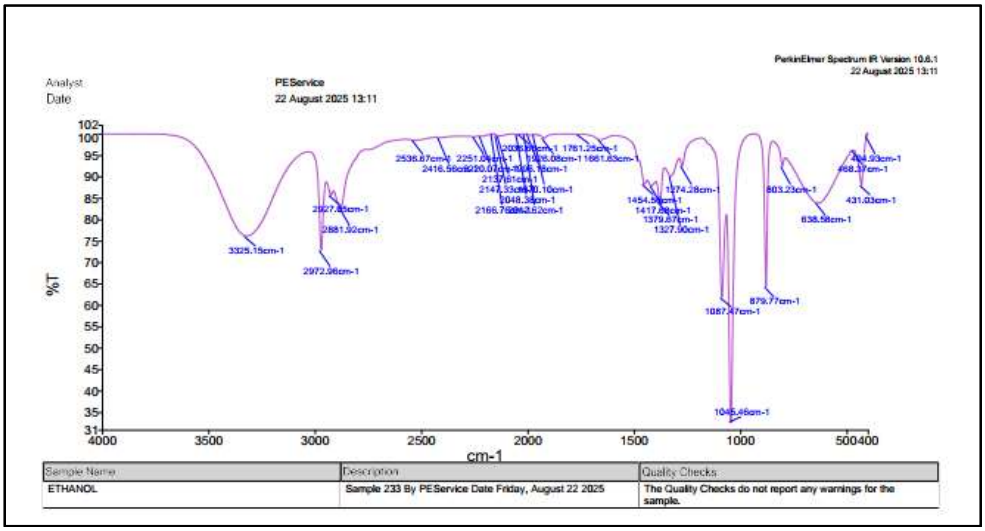

Figure 1: FTIR Spectra of Ethanol

Table 3: FTIR Spectral peaks of Ethanol

S. No.	Functional group	Observed peak (cm^{-1})	Standard Range (cm^{-1})	Inference
1	O-H stretching	3325.1 cm^{-1}	3320-3330 cm^{-1}	O-H stretching (indicative of hydrogen bonding)
2	C-H stretching	2972.9 cm^{-1}	2900-2980 cm^{-1}	CH_3 and CH_2
3	C-O stretching	1087.4 cm^{-1}	1090 cm^{-1}	Intense peak for C-O Stretching
4	O-H and C-H Bending	1274.2 cm^{-1}	1250-1380 cm^{-1}	Bending vibrations of O-H and C-H

FTIR SPECTRUM OF ETHOSOMAL CREAM

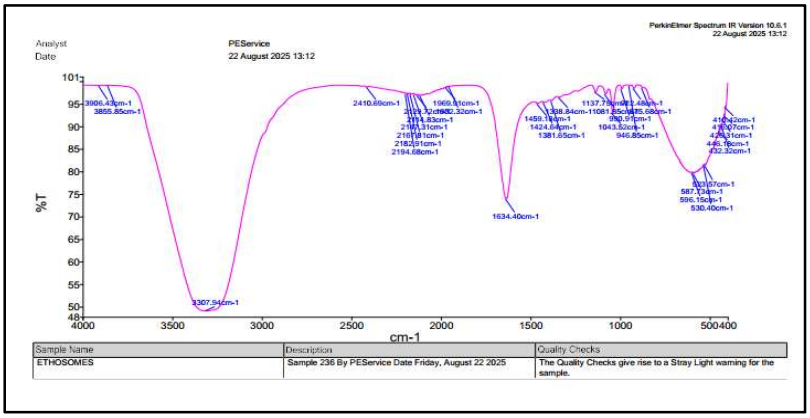

Figure 2: FTIR Spectra of Optimized Ethosomal cream

Table 4: FTIR Spectral peaks of Ethosomal Cream

S. No.	Functional group	Observed peak (cm^{-1})	Standard Range (cm^{-1})	Inference

1	O-H or N-H Stretching	3307 cm^{-1}	3300-3400 cm^{-1}	N-H stretching (often from phospholipids or alcohols)
2	C-H Stretching	2410 cm^{-1}	2400-2800 cm^{-1}	C-H stretching (hydrocarbon chains)
3	C=O or C=N Stretching	1634 cm^{-1}	1600-1700 cm^{-1}	C=O or C=N stretching
4	C-O-C or C-N stretching/Bending	1043 cm^{-1}	1000-1100 cm^{-1}	C-N stretching/Bending

DSC Thermogram of Curcumin The thermogram of pure, unformulated curcumin powder typically shows a sharp, distinct endothermic peak (melting point) around 183°C–185°C, confirming its crystalline nature. This loss of the crystalline peak is a key indicator of successful encapsulation or molecular-level dispersion of the drug within the ethosomal matrix, leading to an amorphous state. The resulting curve often shows a broad endothermic peak that may correspond to the melting or decomposition of the entire ethosomal system, demonstrating physical interaction between the drug and excipients

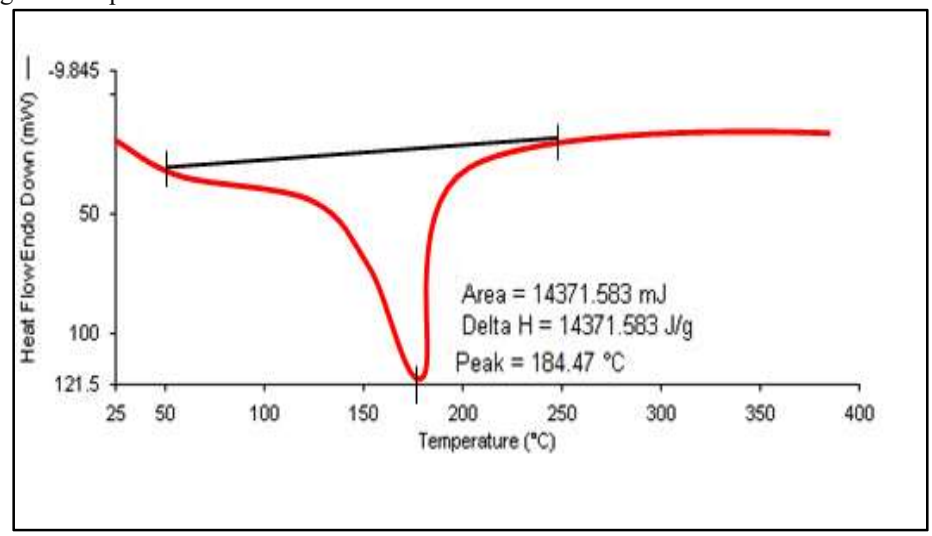

Figure 3: DSC of Curcumin

. **DSC Thermogram of Optimized Ethosomal cream:** The reduction in peak intensity or the shift in the melting point temperature demonstrates that the drug has been successfully entrapped within the ethosomal vesicles, often in an amorphous or a less crystalline state, rather than existing as a free crystalline form. The changes indicate that the lipid bilayers in the ethosomes are in a more fluid, less tightly packed state due to the high concentration of ethanol. This increased fluidity is crucial for enhancing the ethosomes' ability to penetrate the skin barrier.

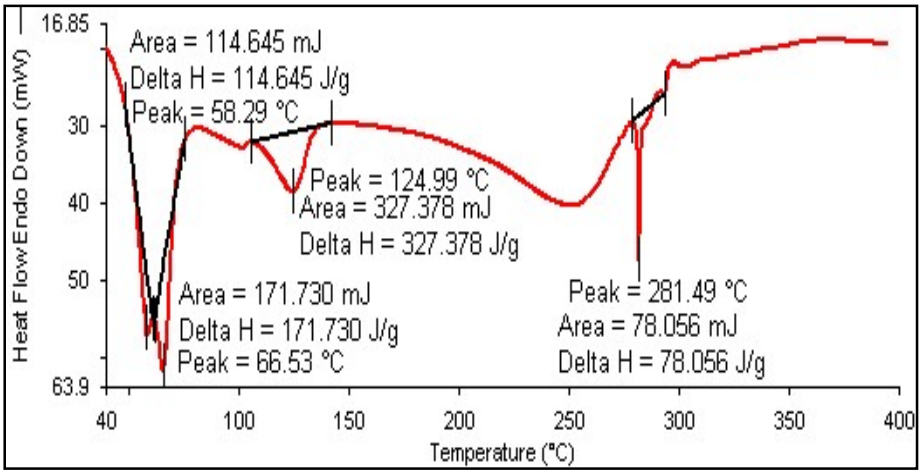

Figure 4: DSC of Optimized Ethosomal cream

Determination of sedimentation: Ethosomal dispersions were physically observed for turbidity and sedimentation at 0, 15, 30, 45, 60, and 90 days interval. The sedimentation rate results were reported based on bottom view observations of the containers having ethosomes.

Table 5: Results of sedimentation study of ethosomal formulations

Formulation code	0	15	30	45	60	90
F1	-	-	-	-	-	-
F2	-	-	-	-	-	+
F3	-	-	-	-	-	-
F4	-	-	-	-	-	+
F5	-	-	-	-	-	-
F6	-	-	-	-	-	+
F7	-	-	-	-	-	+
F8	-	-	-	-	-	+
F9	-	-	-	-	-	+

- No sedimentation, + easily redespersible

In F1, F3 and F5 no sedimentation was seen after 90 days shown in Table No.13. In F2, F4, F6, F7, F8 and F9 there was no sedimentation upto 60th days but, sedimentation was observed after 90th day. The sediment observed after 90th day was easily redespersible in F2, F4, F6, F7, F8 and F9. It indicated that with increased phospholipid concentration, the rate of sedimentation increased

Table 6: Results of entrapment efficiency of ethosomal formulations

Parameter	F1	F2	F3	F4	F5	F6	F7	F8	F9
Entrapment Efficiency (%)	46.2	55.7	61.4	67.2	69.5	71.2	75.5	84.2	89.09

F1 shows minimum Entrapment efficiency 46.2 % and F8, F9 shows maximum Entrapment efficiency 80.2 %, 87.9 % respectively. The Entrapment efficiency was increased on increasing ethanol content up to 35%, however efficiency decreased when the ethanol concentration was increased above 35%. This could be due to higher permeation enhancing property of ethanol that leads to leakage of lipid bilayer.

Table 7: Results of Drug Content of ethosomal formulations

Parameter	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug content (%)	79.2	82.4	84.8	87.4	88.6	91.4	93.5	95.3	96.4

F1 shows minimum Drug Content 79.2 % and F8, F9 shows maximum Drug Content 95.3 %, 96.4 % respectively. The Drug Content was increased on increasing ethanol content up to 35%, however efficiency decreased when the ethanol concentration was increased above 35%.

Table 8: Results of Microbiological study of ethosomal formulations

Parameter	F1	F2	F3	F4	F5	F6	F7	F8	F9

Microbial count	25	28	32	34	37	39	41	44	47

The microbial count result shown in Table No. 16 for all formulations was less than 1000 cfu/g and was in acceptable range.

Table 9: Evaluation parameters for selection of cream formula

Parameters	F1	F2	F3	F4	F5	F6
Consistency	Not good	Not good	Not good	Not good	Good	Good
Appearance	Light Yellowish	Light Yellowish	Light Yellowish	Dark Yellowish	Dark Yellowish	Dark Yellowish
Grittiness	-	-	-	Non greasy	Non greasy	Non greasy
Thermal stability	-	-	Less stable	Less stable	Stable	Stable
pH	7.6	7.5	7.7	5.7	6.2	5.8
Spreadibility (g.cm/sec)	13.41	14.31	13.20	9.65	11.03	12.98
Viscosity (cps)	2030	2119	2147	2405	2446	2553

Table 10: Cumulative percent release of Curcumin from ethosomes loaded creams

Parameters	F7	F8	F9
Consistency	Good	Good	Good
Appearance	Dark Yellowish	Dark Yellowish	Dark Yellowish
Grittiness	Non greasy	Non greasy	Non greasy
Thermal stability	Stable	Stable	Stable
pH	5.2	5.5	6.1
Spreadibility(g.cm/sec)	11.87	12.05	12.67
Viscosity (cps)	3206	3001	2807

The results of cream base formula are shown in Table No. 17. The consistency of formulation F1 to F4 was not good. Formulations showed flowable consistency. The consistency of formulation F5 to F9 was good. The color of formulations F4 to F9 was Dark Yellowish. All the formulations were having desired consistency for the creams. All the formulations were stable. The viscosity and spreadibility of the formulations increased with increase in concentration of cetyl alcohol and stearic acid. Critical chemical parameters to control in skin creams comprise pH (5.2 -7.7). The pH of formulations F4 to F9 was within the acceptable range.

Table 11: Evaluation parameters for selection of cream formula

Parameters	F1	F2	F3	F4	F5	F6
Consistency	Not good	Not good	Not good	Good	Good	Good
Appearance	Light Yellowish	Light Yellowish	Light Yellowish	Dark Yellowish	Dark Yellowish	Dark Yellowish
Grittiness	-	-	-	Non greasy	Non greasy	Non greasy
Thermal stability	-	-	Less stable	Less stable	Stable	Stable
pH	6.2	6.5	5.9	5.8	6.3	5.6
Spreadibility (g.cm/sec)	12.41	11.31	10.20	9.64	11.01	9.18
Viscosity (cps)	2405	2419	2446	2495	2501	2553

Parameters	F7	F8	F9
Consistency	Good	Good	Good
Appearance	Dark Yellowish	Dark Yellowish	Dark Yellowish
Grittiness	Non greasy	Non greasy	Non greasy

Table 12: Stability data of ethosomes loaded creams after 1 month

Thermal stability	Stable	Stable	Stable
pH	5.4	5.2	6.0
Spreadibility(g.cm/sec)	10.71	9.14	11.81
Viscosity (cps)	2720	2691	2645

Parameters	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Consistency	Not good	Not good	Good	Good	Good	Good
Appearance	Light Yellowish	Light Yellowish	Light Yellowish	Dark Yellowish	Dark Yellowish	Dark Yellowish
Grittiness	-	-	-	Non greasy	Non greasy	Non greasy
Thermal stability	Less Stable	Less Stable	Stable	Stable	Stable	Stable
pH	6.2	6.5	5.9	5.8	6.3	5.6
Spreadibility (g.cm/sec)	12.41	11.31	10.20	9.64	11.01	9.18
Viscosity (cps)	2405	2419	2446	2495	2501	2553

Stability study of formulations was done for 1 month. On the basis of comparison of data at 0 day and after 1 month it was concluded that there is no change in colour shown in Table 9, pH and viscosity of creams during storage. All the cream formulations were found thermally stable.

Skin irritation study

Table 13,14,15: Skin irritation study scores for primary irritation and primary irritation index measured at 24, 48 and 72 hrs after applying the cream formulation on the rat skin

Skin reaction	**Time (hrs)**	**Group 1 (control) Rat- 1**	**Group 1 (control) Rat – 2**	**Group 1 (control) Rat – 3**
Erythema	**24**	1	1	0
	48	0	0	1
	72	0	0	0
Edema	**24**	1	1	1
	48	0	0	0
	72	0	0	0
Score of primary irritation		0.5	0.33	0.50
Primary irritation index		0.44		

Skin reaction	**Time (hrs)**	**Group 2 (Ethosomes Loaded cream) Rat- 1**	**Group 2 (Ethosomes Loaded cream) Rat – 2**	**Group 2 (Ethosomes Loaded cream) Rat – 3**
Erythema	**24**	1	0	0
	48	0	0	0
	72	0	0	0
Edema	**24**	0	1	0
	48	1	0	0
	72	0	0	0
Score of primary irritation		0.00	0.33	0.16
Primary irritation index		0.16		

Skin reaction	Time (hrs)	Group 3 (Ethosomes Loaded cream) Rat- 1	Group 3 (Ethosomes Loaded cream) Rat – 2	Group 3 (Ethosomes Loaded cream) Rat – 3
Erythema	24	1	1	0
	48	0	0	1
	72	0	0	0
Edema	24	0	1	1
	48	0	0	0
	72	0	0	0
Score of primary irritation	0.33	0.16	0.33	
Primary irritation index	0.27			

Where: *Erythema/Edema Scores: '0'=no erythema/edema, '1'=very slight '2'=slight, '3'=moderate '4'= severe; ** PDI (Primary dermal irritation) score is obtained by adding average erythema and average edema scores; **PDII (Primary dermal irritation index) is obtained by adding the PDI scores for 24,48 and 72 hour scoring intervals and dividing by the number of soring intervals.

Anti-inflammatory activity:

Table 17: Paw volume data of animals

Treatment Group	Treatment	Paw volume (cm)0.5 hr	1 hr	2 hr	3 hr	4 hr	5 hr	Percentage (%) of inhibition
Group 1	Carrageenan	2.54	2.61	2.68	2.76	2.79	2.85	82.43
Group 2	Control group	1.99	1.85	1.68	1.57	1.51	1.44	85.65
Group 3	Curcumin loaded Ethosomal cream (Low)	0.59	0.54	0.50	0.45	0.43	0.41	85.95
Group 4	Curcumin loaded Ethosomal cream (High)	0.39	0.33	0.27	0.25	0.23	0.19	88.55

Skin irritation assay in rat

The skin irritation assay was performed by applying ethosomal cream on rat skin and observed for 72 h. The moderate to severe erythema was observed in terms of Primary irritation index (PII) for both the test compounds i.e. ethosomal cream. For control group PII was found as 0.44 which enhance the suitability and recipients. In contrary, caused less irritation is commensally rarely with encrusted directly by the ethosomes should be due to the increased the drug. Therefore, reliably used in dermal considered safe for Ideally, the Curcumin tolerability by the curcumin loaded cream with PII of 0.16. Curcumin associated with redness and skin. So it cannot be used patients. Curcumin loaded able to reduce the irritation encapsulation efficiency of Ethosomal cream can be issues and can be topical formulation. containing ethosomes could

Parameters	F7	F8	F9
Consistency	Good	Good	Good
Appearance	Dark Yellowish	Dark Yellowish	Dark Yellowish
Grittiness	Non greasy	Non greasy	Non greasy
Thermal stability	Stable	Stable	Stable
pH	5.4	5.2	6.0
Spreadibility(g.cm/sec)	9.01	9.14	11.81
Viscosity (cps)	2645	2691	2720

be able to reduce irritation and improves patient acceptability.

Skin Irritation study images of Group 1, Group 2, Group 3

Anti-inflammatory activity

Table 18: Paw volume data of animals

Treatment Group	Treatment	Paw volume (cm)0.5 hr	1 hr	2 hr	3 hr	4 hr	5 hr	Percentage (%) of inhibition
Group 1	**Carrageenan**	2.54	2.61	2.68	2.76	2.79	2.85	82.43
Group 2	**Control group**	1.99	1.85	1.68	1.57	1.51	1.44	85.65
Group 3	**Curcumin loaded Ethosomal cream (Low)**	0.59	0.54	0.50	0.45	0.43	0.41	85.95
Group 4	**Curcumin loaded Ethosomal cream (High)**	0.39	0.33	0.27	0.25	0.23	0.19	88.55

Carrageenan induced paw edema

As shown in Table 13, Curcumin loaded ethosomal cream formulation at 50 mg/kg inhibited the increase in paw edema (0.59 ± 0.04 to 0.41 ± 0.05 hr) from 0.5 hr to 5 hr. Similarly, Curcumin loaded ethosomal cream formulation at 100 mg/kg significantly inhibited paw edema (0.39 ± 0.06 to 0.19 ± 0.05 hr) from 0.5 hr to 5 hr. A dose dependent inhibition in paw edema was observed for both the formulations at 50 and 100 mg/kg. Carrageenan induced paw edema is a widely used model to assess anti-inflammatory capacity of any compound. A biphasic response is usually achieved after parentarally administered carrageenan, i. e. first phase of 15–45 min includes release of mediators like bradykinin, serotonin and histamine while second phase of 60–90 min implicates release of prostaglandins only. Carrageenan-induced rat paw edema model was used to investigate anti-inflammatory activity of Curcumin loaded ethosomal cream.

Rat **No Carrageenan Injection** **Carrageenan injection given for inducing inflammation via right hind paw**

CONCLUSION

The preformulation study provides a profile of drug and other excipients added to the formulation development, confirming their adherence to standard specifications. Consequently, these ingredients were utilized for the subsequent development of both cream base and ethosomes. The formulation F7 was further selected for loading in the optimized cream base to

obtain a topical cream containing ethosomes of Curcumin. A base cream was formulated by fusion method. Formulated creams were evaluated for organoleptic and physicochemical properties of creams. From evaluation, composition F4 (cetyl alcohol and stearic acid 2.5%w/w and 9%w/w, respectively) was selected for further loading of ethosomes. Optimized suspension of ethosomes equivalent to 0.05%, 0.075%, 0.1% and 0.125% of Curcumin were loaded in cream base F4. The ethosomes loaded cream formulations were evaluated for physical appearance, grittiness, thermal stability, pH, spreadability and viscosity. The creams were also evaluated for drug content, Anti-inflammatory study using rat skin and skin irritation study, Ethosomal cream formulations were evaluated for thermal stability, physical appearance, viscosity and drug stability after 1 month. Stability study indicates that all the cream formulations were stable after a time period of one month.

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