ORIGINAL

FORM-2

THE PATENTS ACT, 1970
&
THE PATENTS RULES, 2003

COMPLETE SPECIFICATION

ETHOSOME COMPOSITIONS OF CURCUMIN FOR TRANSDERMAL DELIVERY

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The following specification particularly describes and ascertains the nature of this invention and the manner in which it is to be performed

FIELD OF THE INVENTION

The invention relates to ethosome compositions for transdermal drug delivery. It specifically relates to transdermal drug delivery compositions in the form of ethosomes of curcumin. More specifically, it relates to transdermal drug delivery ethosome compositions of curcumin in the form of gel and process for preparation of ethosomes and their gel forms.

BACKGROUND OF THE INVENTION

One of the major advances in vesicle research was the finding that some modified vesicles possessed properties that allowed them to successfully deliver drugs in deeper layers of skin. Transdermal delivery is important because it is a noninvasive procedure for drug delivery. Further, problem of drug degradation by digestive enzymes after oral administration and discomfort associated with parenteral drug administration can be avoided. It is the most preferred route for systemic delivery of drugs to several diseases. Hence, transdermal dosage forms enjoy being the most patient compliant mode of drug delivery. The principle of transdermal drug delivery system is that they could provide sustained drug delivery (and hence constant drug concentrations in plasma), over a prolonged period of time. The perceived advantages for transdermal drug delivery include:

1. Avoids vagaries associated with gastro-intestinal absorption due to pH, enzymatic activity, drug-food interactions etc.
2. Substitute oral administration when the route is unsuitable as in case of vomiting, diarrhoea, etc.
3. Avoid hepatic "first pass" effect.
4. Avoid the risk & inconveniences of parenteral therapy.
5. Reduces daily dosing, thus improving patient compliance.
6. Extends, the activity of drugs having short plasma half-life through the reservoir of drug present in the therapeutic delivery system and its controlled release characteristics.
7. Rapid termination of drug effect by removal of drug application from the surface of the skin.
8. Rapid identification of the medication in emergencies, eg. Non-responsible, unconscious or comatose patient.
9. Enhance therapeutic efficacy, reduce side effects due to optimization of the blood concentration – time profile and elimination of pure entry of drugs into systemic circulation.

10. Provide predictable activity over extended duration of time & ability to approximate zero-order kinetics.
11. Improved control of the concentration of drug with small therapeutic indices.
12. Minimize inter and intra-patient variation.
13. Provide suitability for self – administration.

Difficulty of permeation through human skin: In addition to physical barrier, human skin functions as a chemical barrier. The outer most layer of skin, the stratum corneum is an excellent barrier to all chemicals including drugs. If a drug requirement is more than 10 mg. per day, the transdermal delivery will be difficult. Only relatively potent drugs can be given through this route.

Skin irritation: Skin irritation or contact dermatitis due to excipients and enhancers of the drug delivery system used for increasing percutaneous absorption.

Clinical need: It has to be examined carefully before developing a transdermal product.

Most of transdermal preparations are meant to be applied to the skin. So, basic knowledge of skin and its physiology function and biochemistry is very important. The skin is the heaviest single organ of the body, combines with the mucosal lining of the respiratory, digestive and urogenital tracts to from a capsule, which separates the internal body structures from the external environment. The pH of the skin varies from 4 to 5.6. Sweat and fatty acids secreted from sebum influence the pH of the skin surface. It is suggested that acidity of the skin helps in limiting or preventing the growth of pathogens and other organisms.

"Ethosomes are soft malleable vesicles composed mainly of phospholipid, ethanol (relatively high concentration) and water. These soft vesicles represents novel vesicular carrier for enhance delivery to / through skin. The size of ethosome vesicles can be modulated from tens of microns to nanometres." Typically, ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), unsaturated PC, alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols). Such a composition enables delivery of high concentration of active ingredients through skin. In comparison to other transdermal & dermal delivery systems the advantages of ethosomes are:

1. Ethosomes are enhanced permeation of drug through skin for transdermal and dermal delivery.

2. Ethosomes are platform for the delivery of large and diverse group of drugs (peptides, protein molecules).
3. Ethosome composition is safe and the components are approved for pharmaceutical and cosmetic use.
4. Low risk profile- The technology has no large-scale drug development risk since the toxicological profiles of the ethosomal components are well documented in the scientific literature.
5. High patient compliance- The Ethosomal drug is administrated in semisolid form (gel or cream), producing high patient compliance by is high. In contrast, Iontophoresis and Phonophoresis are relatively complicated to use which will affect patient compliance.
6. High market attractiveness for products with proprietary technology. Relatively simple to manufacture with no complicated technical investments required for production of Ethosomes.
7. The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
8. Various application in Pharmaceutical, Veterinary, Cosmetic field.

Ethosomes can be used for many purposes in drug delivery. Ethosomes are mainly used as replacement of liposomes. Mainly the transdermal route of drug delivery is preferred. Ethosomes can be used for the transdermal delivery of hydrophilic and impermeable drugs through the skin.

Curcumin (CUR), a constituent of *Curcuma longa* (Family-*Zingiberaceae*), chemically known as diferuloylmethane. It is used in cancer; inflammatory disease, arthritis, oxidative disease, diabetes, multiple sclerosis, Alzheimer disease, HIV, septic shock, cardiovascular disease, lung fibrosis, liver disease, kidney disease, and angiogenic disease can be cured by curcumin. Some of the novel formulations developed using curcumin include liposomes, solid lipid nanoparticles, transdermal film, microspheres, nanoemulsion, etc. Following oral administration (up to 8 g per day), it is poorly absorbed, and only the traces of compound appear in blood. It undergoes extensive first-pass metabolism, and hence is a suitable candidate for ethosomal gel formulation.

At present, the most common form of delivery of drugs is the oral route. While this has the notable advantage of easy administration, it also has significant drawbacks – namely poor bioavailability due to hepatic metabolism (first pass) and the tendency to produce rapid blood level spikes (both high

and low), leading to a need for high and/or frequent dosing, which can be both cost prohibitive and inconvenient. To overcome these difficulties there is a need for the development of new drug delivery system; which will improve the therapeutic efficacy and safety of drugs by more precise (i.e. site specific), spatial and temporal placement within the body thereby reducing both the size and number of doses. One of the methods most often utilized has been transdermal drug delivery – meaning transport of therapeutic substances through the skin for systemic effect. Closely related is percutaneous delivery, which is transport into target tissues, with an attempt to avoid systemic effects.

There are reports on the carrier systems of curcumin, few interested ones are **N. A. Patel et al.,** [Drug Development and Industrial Pharmacy. 2009; 35:234–242] developed a matrix-type transdermal therapeutic system containing herbal drug, curcumin (CUR), with different ratios of hydrophilic (hydroxypropyl methyl cellulose K4M [HPMC K4M]) and hydrophobic (ethyl cellulose [EC] polymeric systems by the solvent evaporation technique. Different concentrations of oleic acid (OA) were used to enhance the transdermal permeation of CUR. **N. A. Patel et al.,** [Pharmaceutical Development and Technology, 2009; 14:83–92] developed topical gel delivery of curcumin for its anti-inflammatory effects. Carbopol 934P (CRB) and hydroxypropylcellulose (HPC) were used for the preparation of gels. The penetration enhancing effect of menthol (0–12.5% w/w) on the percutaneous flux of curcumin through the excised rat epidermis from 2% w/w CRB and HPC gel system was investigated. There are reports on ethosomes of some drugs for transdermal delivery and of them the interested ones include **Ashoniya Sheer and Dr.Meenakshi Chauhan** [IJPI'sJournal of Pharmaceutics and Cosmetology, Vol 1:3 (2011); pages 1-14] reported the ethosomes as vesicular carrier for enhanced transdermal delivery of Ketoconazole. **Anitha et. al.,** [Int. J. Rev. Life. Sci., 1(1), 2011, 17-24] and **K Pavan Kumar et al.,** [International Journal of Advances in Pharmaceutical Sciences 1 (2010) 111-121] reported the Ethosomes as noninvasive vesicular carrier and priority for transdermal drug delivery along with their compositions. **Atul Kumar Garg et al.,** [International Journal of Pharmacy and Pharmaceutical Sciences Vol. 2, Suppl. 2, 2010; Pages 102-108] reported the gel containing ethosomal vesicles for transdermal delivery of aceclofenac. **Touitou E at al.,** [J Control Release. 2000 Apr 3;65(3):403-18] reported the ethosomes as novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. **V. Dubey et al.,**[European Journal of Pharmaceutics and Biopharmaceutics. 2007; 67: 398–405] developed the transdermal

potential of novel ethanolic liposomes (ethosomes) bearing Melatonin (MT), an anti-jet lag agent associated with poor skin permeation and long lag time. **Dave et al.,**[International Journal of Drug Delivery. 2010; 2: 81-92] developed the transdermal potential of novel vesicular carrier, ethosomes, bearing aceclofenac, Non-steroidal anti-inflammatory drugs (NSAIDs) agents having limited transdermal permeation. **Bhalaria et al.,**[Indian journal of experimental biology. May 2009; 47: 368 – 375] prepared and characterized fluconazol encapsulated ethosomes, incorporate it in suitable dermatological base, and asses its comparative clinical efficacy in the treatment in the candidiasis patients against liposomal gel, marketed product and hydroethanolic solution of the drug. Drug encapsulated ethosomes and liposomes were prepared and optimized by "hot" method technique and lipid film hydration technique. **M.M.A. Elsayed et al.,** [International Journal of Pharmaceutics. 2006; 322: 60–66] formulated deformable liposomes and ethosomes improve skin delivery of ketotifen under non-occlusive conditions. In vitro permeation and skin deposition behavior of deformable liposomes and ethosomes, having ketotifen both inside and outside the vesicles (no separation of free ketotifen), having ketotifen only inside the vesicles (free ketotifen separated) and having ketotifen only outside the vesicles (ketotifen solution added to empty vesicles), was studied using rabbit pinna skin. **Sheo Datta Maurya et al.,**[Indian J.Pharm. Educ. Res. 2010; 44(1): 102-108] developed transdermal delivery of stavudine, a hydrophobic drug used for the treatment of AIDS, from ethosomes. **Subheet Jain et al.,** [AAPS PharmSciTech. 2007; 8(4): E1-E9] developed ethosomal formulations using lamivudine as model drug and characterized in vitro, ex vivo and in vivo. **Mina I. Tadros et al.**, [AAPS PharmSciTech. 2007; 8(4): E1-E8] compared the transdermal delivery of salbutamol sulfate (SS), a hydrophilic drug used as a bronchodilator, from ethosomes and classic liposomes containing different cholesterol and diacetylphosphate concentrations.

Though there are reports but there is no report in the literature on the ethosomes of curcumin an herbal ingredient for transdermal delivery in the gel form. Curcumin is chemically (1E, 6E)-1, 7-bis (4-hydroxy-3-methoxyphenyl) hepta-1, 6-diene-3, 5- Dione. Curcumin is used for the treatment of anti-cancer, anti- oxidant, anti-inflammatory, hyperlipidemic, antibacterial, wound healing and hepatoprotective activities. Apart from its pharmacological actions, it has also been investigated as photostabilizing agent to protect photo-labile drugs in solution, topical preparations and soft gelatin capsules. Despite the presence of large number of pharmacological actions, the therapeutic efficacy of curcumin is limited due to its poor oral bioavailability. The poor oral bioavailability of curcumin

has been attributed to its poor aqueous solubility as its partition coefficient 3.2 and extensive first pass metabolism. The elimination half life of curcumin is 1.45 hrs. Transdermal administration of drugs that avoid first pass metabolism can improve the bioavailability and reduce the dosing frequency compared with the oral route. Accordingly, there is a real and continuing need for development of curcumin ethosomes in the form of gel and their compositions for transdermal delivery of curcumin. Hence, the present inventors aim is to develop a transdermal drug delivery system of curcumin in the form of gel.

OBJECTS OF THE INVENTION

The primary object of the present invention is the development of ethosomes for transdermal drug delivery to increase bioavailability and therapeutic action of the curcumin.

Another object of the invention is to develop the drug delivery compositions in the form of ethosomal gel containing curcumin.

It is yet another object of the invention is to develop a process for preparation of ethosomes loaded with curcumin for transdermal drug delivery by using phospholipid (phospholipon 90H), cholesterol, ethanol, propylene glycol, distilled water by cold method.

It is yet another object of the present invention for the preparation of ethosomes loaded with curcumin which are physically and chemically stable transdermal drug delivery system, increase the patient compliance, minimize the frequency of dosing, maintain the plasma concentration of drug within the therapeutic window, reduces side effects of drug and increase safety and efficacy of drug.

STATEMENT OF THE INVENTION

Ethosome compositions for transdermal drug delivery comprising: a) therapeutically effective amount of curcumin; b) phospholipon 90H; c) cholesterol; d) propylene glycol and e) ethanol. The effective amount of curcumin is 0.1 to 2% (w/w) of composition. The amount of phospholipon 90H is 0.5 to 5%, cholesterol is 0.5 to 5%, propylene glycol is 5 to 25% and ethanol is 10 to 50% (w/w) of composition. The ethosome compositions for transdermal drug delivery in the form of gel comprising: a) ethosomes as in any of the preceding claims; b) carbopol 940; and c) triethanolamine. The composition comprises ethosomes equivalent to 1 to 5% of curcumin, carbopol 940 is 1 to 5% and triethanolamine is 0.1 to 2% (w/w) of composition. Process for preparation of ethosome

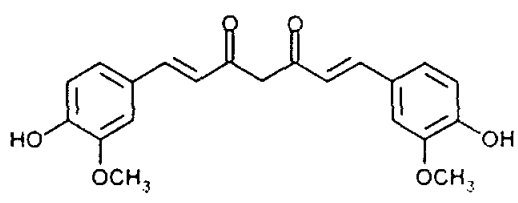
composition by cold method comprising: a) dissolving curcumin, cholesterol and phospholipids in ethanol and propylene glycol; b) addition of distilled water slowly in a fine stream with a constant mixing for 5 minutes at 30°C; c) cooling of ethosomes at room temperature and d) homogenised by using vertex shaker for 15 min to obtain uniform ethosome composition. The process for preparation of ethosome composition gel comprising: a) carbopol 940 powder was slowly added to ultrapure water at 100 °C for 20 min and triethanolamine was added to it drop-wise; b) ethosomal suspensions prepared according to process of claim 11 was then incorporated into gel base; and c) addition of water with continuous stirring until homogenous ethosomal gel compositions formed.

DETAILED DESCRIPTION OF THE INVENTION

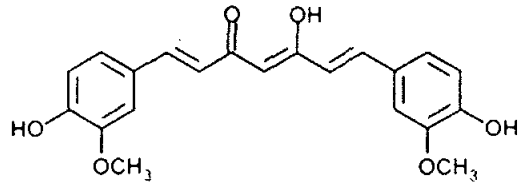
CURCUMIN

Synonym: Turmeric yellow, Indian saffron, Kurkum, Natural yellow. **Chemical name:** 1,7-Bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione = Diferuloylmethane; **CAS number:** 458-37-7; **Molecular formula:** $C_{21}H_{20}O_6$;

Structural formula:



Ketoform



Enol form

Molecular weight: 368.38 g/mol; **Appearance:** Bright yellow-orange powder; **Odor:** Odorless; **Melting point:** 183 °C (361 °F) (361 K); **Solubility:** methanol, ethanol, acetone, dimethyl sulfoxide (DMSO), dimethyl formamide; **Drug category:** Multiple action; **Purity:** ≥ 65%. **Ultraviolet spectrum:** curcumin has a maximum absorption (λ max) in methanol at 430 nm. It absorbs maximally at 415 to 420 nm in acetone. In toluene, the absorption spectrum of curcumin contains some structure, which disappears in more polar solvents such as ethanol and acetonitrile. The fluorescence of curcumin occurs as a broad band in acetonitrile (λ max = 524 nm), ethanol (λ max = 549 nm), or micellar solution (λ max = 557 nm), but has some structure in toluene (λ max = 460, 488 nm). **Beer's law range:** 0.5 to 5μg/mL; **P^{KA}:** Three acidity constants were measured for curcumin, as follows, pKA1 = 8.38 ± 0.04, pKA2 = 9.88 ± 0.02 and pKA3 = 10.51 ± 0.01. **Half life:** 28

minutes; **Bioavailability:** Bioavailability of curcumin is approximately 60-65% following oral administration. **Stability:** ≥ 2 years at room temperature

EXAMPLES:

Formulation of Ethosomes: The ethosomes of curcumin were prepared using cold method. The ethosomal system of curcumin was comprised of 1%w/w phospholipon 90H, 0.3%w/w of curcumin, 1%w/w of cholesterol, 10 – 50 %w/w of ethanol, 10%w/w of propylene glycol, and water up to 100 %w/w. Phospholipids, cholesterol and drug were dissolved in ethanol and propylene glycol. The mixture was heated to 30° C in water bath. In this solution distilled water was added slowly in a fine stream with a constant mixing (Mechanical stirrer, Remi equipment, Mumbai) at 700 rpm in a closed vessel. The temperature was maintained at 30° C during the experiment. The mixing was continued for 5 minutes. The final solution of ethosomes was left to cool at room temperature. The preparation was homogenised by using vertex shaker for 15 min. Finally, the formulation is stored under refrigeration.

Table 1: Ethosome Compositions

Sr. No	Formulation Code	Phospholipon 90H (%w/w)	Ethanol (%w/w)	Propylene Glycol (%w/w)	Cholesterol (%w/w)	Curcumin (%w/w)
1	ET-1	1	10	10	1	0.3
2	ET-2	1	20	10	1	0.3
3	ET-3	1	30	10	1	0.3
4	ET-4	1	40	10	1	0.3
5	ET-5	1	50	10	1	0.3

1. Determination of Entrapment efficiency percentage: Ethosomes entrapped curcumin was estimated by centrifugation method. The prepared Ethosome were placed in centrifugation tube and centrifuged at 15000 rpm for 2 hrs. The supernatant (1ml) was withdrawn and diluted with methanol. The unentrapped Curcumin was determined by UV spectrophotometer at 430 nm. The samples from the supernatant were diluted 100 times before going for absorbance measurement. The free Curcumin in the supernatant gives us the total amount of unentrapped drug. Encapsulation efficiency is expressed as the percent of drug trapped.

$$\% \text{ Entrapment} = \frac{\text{Total drug} - \text{Diffused drug}}{\text{Total drug}} \times 100$$

Entrapment efficiency of ethosomes formulations ranged from 81.36% to 86.96%. The drug

encapsulation efficiency of all five formulations is shown in **Table 3**. The data indicate that entrapment efficiency depends on ethanol concentration, as the concentration increases up to 30%, results in increase in entrapment efficiency of ethosomal formulation. With further increase in ethanol concentration entrapment efficiency decreases, owing to increase fluidity of membrane and vesicles become more permeable that leads to decrease in entrapment efficiency of ethosomal formulation.

2. Particle Size and Size distribution Analysis: Particle size of different batches of ethosomes was determined by dynamic scattering particle size analyzer (Nanotrac Particle Analyzer 150, Microtrac Inc., PA, USA). The range of the analyzer is 0.8 nm to 6.54 µm. Particles suspended in a dispersing fluid are subject to random collisions with the thermally excited molecules of the dispersing fluid resulting in Brownian motion. The velocity and direction of the resulting motion are random but the velocity distribution of a large number of mono-sized particles averaged over a long period will approach a known functional form, in this case the size distribution of the particles. In the Nanotrac, light from a laser diode is coupled to the sample through an optical beam splitter in the Nanotrac probe assembly. The interface between the sample and the probe is a sapphire window at the probe tip. The sapphire window has two functions! Firstly, it reflects the original laser back through the beam splitter to a photodetector. This signal which has the same frequency as the original laser acts as a reference signal for detection, offering heterodyne detection. Secondly, the laser passes through the sapphire window and is scattered by the particles which are in suspension but moving under Brownian motion. The laser is frequency shifted according to the Doppler Effect relative to the velocity of the particle. Light is scattered in all directions including 180 degrees backwards. This scattered, frequency shifted light is transmitted through the sapphire window to the optical splitter in the probe to the photodetector. These signals of various frequencies combine with the reflected signal of un-shifted frequency (Controlled Reference) to generate a wide spectrum of heterodyne difference frequencies. The power spectrum of the interference signal is calculated with dedicated high speed FFT (Fast Fourier Transform) digital signal processor hardware. The power spectrum is then inverted to give the particle size distribution.

Polydispersity index: PDI is an index of width or spread or variation within the particle size distribution. Monodisperse samples have a lower PDI value, whereas higher value of PDI indicates a wider particle size distribution and the polydisperse nature of the sample. PDI can be calculated by

the following equation as follows: **PDI = Δd/davg** . Where (Δd) is the standard deviation of particle size and (davg) is the average particle size. The usual range of PDI values is; 0-0.05 (monodisperse standard), 0.05-0.08 (nearly monodisperse), 0.08-0.7 (mid range polydispersity), > 0.7 (very polydisperse). In the ethanol concentration range of 10% to 50% the size of vesicle decreases with increase in ethanol concentration. This indicates that at higher ethanol concentration the membrane thickness reduced considerably, probably due to formation of a phase with interpenetrating hydrocarbon chain that will lead to decrease in size of ethosome vesicle on increasing concentration of ethanol. Polydispersity index of all five formulations is shown in **Table 3.** Formulations ET-1 to ET-4 indicates mid range polydispersity and ET-5 indicates very polydisperse.

3. Zeta Potential Determination: Zeta potential was measured by using Zetatrac. There are three ways by which a solid particle (colloid) dispersed in a liquid media can acquire a surface charge- By the adsorption of ions present in the solution, By the ionization of functional groups on the particle's surface and Due to the difference in dielectric constant between the particle and the medium. Attention should be paid to the formation of electric double layer at the solid-liquid interface. The zeta Potential is defined as the difference in potential between the surface of the tightly bound layer (shear plane) and the electro-neutral region of the solution. The potential gradually decreases as the distance from the surface increases. As the concentration of electrolyte increases in the medium, the zeta potential falls off rapidly due to the screening effect of the counter ions. It is easily measured because the charge of the potential will move as the suspension is placed between the two electrode that have D.C. voltage across them and the velocity will be proportional to the zeta potential of the particle. The technical term for this is electrophoresis. ASTM provides a table **(Table 2)** from which the stability of the colloidal dispersions cab be predicted based on the zeta potential.

Table 2: Zeta potential for colloids in water and their stability

Zeta Potential [mV]	Stability behavior of the colloid
0 to ±5	Rapid coagulation or flocculation
From ±10 to ±30	Incipient instability
From ±30 to ±40	Moderate stability
From ±40 to ±60	Good stability
More than ±61	Excellent stability

Zeta potential of all five formulations is shown in **Table 3**. Data indicate that zeta potential tends to be more negative as the concentration of alcohol increases.

4. Vesicle Morphology: Shape and surface morphology of ethosomes was studied using scanning electron microscopy (SEM). The ethosomes were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument. The photographs were taken using a Jeol scanning electrom microscope (JEOL-JSM-AS430, Japan). Scanning electron micrograph of formulations ET-1 to ET-5 are as shown in **Figure 1** which indicate that ethosomes has three dimentional nature, and this confirms the existence of vesicular structure at higher concentration of ethanol.

5. **Compatibility studies of Formulation ET-5 by FTIR-Spectroscopy**: The FT-IR spectra of Formulation ET-5 were compared with the FT-IR spectrum of the pure drug. The IR spectra of formulation ET-5 is shown in **Figure 2** which indicates no interaction between curcumin and phospholipid when compared with IR spectra of pure drug as all functional group frequencies were present.

6. **Compatibility studies of Formulation ET-5 by DSC**: Differential scanning calorimetry was performed by using DSC-60. The instrument comprised of calorimeter (DSC 60), flow controller (FCL 60), Thermal analyzer (TA 60) and operating software TA 60 from (Shimadzu Corporation, Japan.) The samples were placed in aluminium pans and were crimped, followed by heating under nitrogen flow (30 ml/min) at a scanning rate of 5°C/min. Aluminium pan containing same quantity of indium was used as reference. The heat flow as a function of temperature was measured for formulation ET-5. The results of DSC studies are given in **Figure 3**. The formulation ET-5 showed a sharp endotherm at 52.28°C corresponding to its melting point/transition temperature. The DSC thermograms obtained for the formulation showed no significant shift in the endothermic peaks confirming the stability of the drug in the formulations and only polymer peak was observed, which revealed that drug is in amorphous state in the formulations.

7. X-ray diffraction (XRD) Study: X-ray diffraction measurements of curcumin, phospholipon 90H, and formulation ET-5 were carried out with X-ray diffractometer in the diffraction range of 5–50°. A Cu-Ka radiation source was used, and the scanning rate (2θ/min) was 5 °C/ min. The diffraction pattern of the curcumin, phospholipon 90H and ET-5 formulation was analyzed **(Figure 4)**. From the diffraction patterns of both phospholipon 90H and ET-5 formulation, it was clear that less ordered crystals were majority, and thus, the amorphous state would contribute to the higher drug loading capacity. The diffraction pattern of curcumin showed remarkable difference from those

of ET-5 formulation. The sharp peaks of curcumin, indicating the crystalline nature, were not present in the diffraction pattern of ET-5 formulation indicating that curcumin is entrapped in the lipid core of ET-5 formulation and that too in amorphous or molecular dispersion form. Also, looking at the diffraction patterns of phospholipon 90H and ET-5 formulation, there was not much difference in the pattern, indicating that the addition of curcumin has not changed the nature of ET-5 formulation.

8. Degree of Deformability: The degree of deformability of ethosomes vesicles were measured by extrusion method. The ethosomal formulation were extruded through filter membrane (pore size diameter- 100 nm), using a stainless steel filter holder having 50 mm diameter, by applying a pressure of 2.5 bar. The quantity of vesicle suspension, extruded in 5 minutes was measured. The degree of deformability is calculated by using the following formula: $\mathbf{D = J^* (r_v / r_p)^2}$ Where, D is deformability of vesicle membrane, J is amount of suspension passed in 5 min, r_v is size vesicles (after passed), and r_p = pore size of barrier. Degree of deformability of all five formulations is shown in **Table 3**. Data indicate that degree of deformability depends on ethanol concentration, as the concentration increases, results in increase in degree of deformability of ethosomal formulation. Higher concentration of ethanol present in ethosomes perhaps provided deformability to vesicle membrane by reducing the interfacial tension of the vesicle membrane.

Table 3: Characterization of Ethosomes

Formulation Code	Entrapment Efficiency (%)	Particle Size (nm)	Polydispersity Index	Zeta Potential	Degree of Deformability
ET-1	81.36	200.4	0.599	- 30.71	24.64
ET-2	83.15	188.9	0.509	- 32.82	29.26
ET-3	86.96	179.2	0.536	-34.10	33.83
ET-4	85.79	168.6	0.188	- 35.55	36.60
ET-5	84.49	155.8	0.867	- 36.21	40.21

Formulation of Ethosomal and Free Drug Gels: The specified amount of Carbopol 940 powder was slowly added to ultrapure water and kept at 100 °C for 20 min. Triethanolamine was added to it drop-wise. Ethosomal suspensions equivalent to 2% of drug was then incorporated into gel base. Water q.s. was added with continuous stirring until homogeneous formulations were achieved. Gel containing free curcumin was prepared by similar method using 2% carbopol 940. The ethosomal gel and free drug gel compositions are given in **Table 4**.

Table 4: Composition of Ethosomal and Free Drug Gels

Gel Ingredients	Ethosomal Gel					Free Drug Gel
	G-1	G-2	G-3	G-4	G-5	G-6
Ethosomes	Eqv. to 2% of drug	Eqv. to 2% of drug	Eqv. to 2% of drug	Eqv. to 2% of drug	Eqv. to 2% of drug	-
Curcumin	-	-	-	-	-	2%
Carbopol 940	2%	2%	2%	2%	2%	2%
Triethanolamine	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
Distilled water	q.s.					

1. **Physical parameters of gels:** Ethosomal gel formulations (G-1 to G-5) and free drug gel formulation (G-6) were characterized for pH using pH meter, spreadability, consistency and homogeneity.

a. **pH:** The pH of the various gel formulations was determined by using digital pH meter. The pH value of ethosomal gel (G-1 to G-5) and free drug gel (G-6) was found to be 6.8.

b. **Spreadability:** It was determined by wooden block and glass slide apparatus. Weights about 10g were added to the pan and the time were noted for upper slide (movable) to separate completely from the fixed slides. Spreadability was then calculated by using the formula: **S = M.L/T** Where, S = Spreadability, M = Weight tide to upper slide, L = Length of glass slide, T = Time taken to separate the slide completely from each other. The value of spreadability of G-1, G-2, G-3, G-4, G-5, and G-6 was 13.5, 13.6, 13.8, 14.01, 14.3, and 13.4 (g.cm/sec) respectively. The values of spreadability indicate that the gel is easily spreadable by small amount of shear.

c. **Consistency:** The measurement of consistency of the prepared gels was done by dropping a cone attached to a holding rod from a fix distance of 10cm in such way that it should fall on the centre of the glass cup filled with the gel. The penetration by the cone was measured from the surface of the gel to the tip of the cone inside the gel. The distance traveled by cone was noted down after 10sec. The consistency reflects the capacity of the gel, to get ejected in uniform and desired quantity when the tube is squeezed. Consistency in terms of distance travel by cone was 10 mm for all developed batches.

d. ***Homogeneity:*** *All developed gels were tested for homogeneity by visual inspection after the* gels have been set in the container. They were tested for their appearance and presence of any aggregates. All developed gel showed good homogeneity with absence of lumps. All developed

preparations were clear and transparent.

2. *In Vitro* Drug Permeation Study: *In-vitro* release of curcumin from ethosomal formulation was studied using locally fabricated diffusion cell. The effective permeation area of the diffusion cell and receptor cell volume was 2.50cm^2 and 200 ml, respectively. The temperature was maintained at 37±1° C. The receptor compartment contained 200 ml of distilled water and was constantly stirred by magnetic stirrer at 100 rpm. The skin of mice was mounted between the donor and receptor compartments. Hydrogel formulation (equivalent to 10mg drug) was applied to the membrane. 2 ml sample were withdrawn through sample port of the diffusion cell at predetermined time interval over 24 hours and diluted it to 10 ml with methanol. The samples were analyzed spectrophotometrically at λ max 430 nm. The receptor phase was immediately replenished with equal volume of distilled water. Sink condition was maintained throughout the experiment. The *in vitro* release data of all the formulations are shown in **Table 6**. The cumulative percent drug release after 24 hrs was found to be 77.690%, 81.015%, 83.482%, 88.304%, 92.033%, and 35.111% respectively for the formulations G-1 to G-2. The *in-vitro* release study suggested that the penetration enhancing effect might be of greater importance in enhance skin delivery of curcumin by ethosomal vesicles under non occlusive condition, than intact vesicle permeation into the stratum corneum (SC). Possible interaction of vesicles with layers of SC, promoting impaired barrier function of these layers to the drug with less well packed intracellular structure forms, and with subsequent increased in skin partitioning of drug play a major role in increased skin delivery of drug. Ethanol used in the preparation of ethosome is a well known penetration enhancer and increase penetration of curcumin through skin was suggested of a synergistic mechanism between ethanol vesicles and skin lipid. Ethosomal formulations contain ethanol in their composition that interacts with lipid molecules in the polar head group regions, resulting in an increased fluidity of the SC lipids. The high alcohol content is also expected to partial extract the SC lipids. These processes are responsible for increasing inter and intracellular permeability of ethosomes. Propylene glycol used in formulation widely used as a penetration enhancer in topical formulation, either alone or in combination with other fatty acids. It will enhance solubility and partitioning of drug in SC, and increase the permeability of drug across SC. The data indicate that in vitro release of drug depends on the ethanol concentration. As the concentration of ethanol increases, release of curcumin increased. The permeability of drug from ethosomal gel was higher than free drug gel.

Table 6: *In Vitro* Release Profile of Ethosomal Gel Formulation G-1 to G-6 and G-5 after 30 days storage

Time in (Min)	Cumulative % of Curcumin Released (%CDR)							
	G-1	G-2	G-3	G-4	G-5	G-6	After 30 days Stored at 5°C±3°C for G-5	After 30 days Stored at 25°C±2°C for G-5
0	0	0	0	0	0	0	0	0
30	7.529	8.594	9.392	10.714	12.072	3.061	10.866	8.451
60	14.984	15.887	16.366	17.786	19.246	5.081	20.429	15.624
120	28.800	30.631	31.640	34.464	36.172	7.152	34.977	28.928
240	32.813	34.582	35.441	38.341	40.117	9.242	41.301	32.801
360	34.378	36.138	38.114	41.076	42.902	14.384	44.098	35.515
480	37.199	38.921	41.973	43.834	45.712	17.556	46.919	37.057
600	38.801	40.513	43.542	45.437	47.349	21.768	48.569	39.806
1440	77.690	81.015	83.482	88.304	92.033	35.111	92.069	79.637

3. Release Kinetics: To analyse the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted in to, Zero order, First order, Higuchi matrix, and Peppas model. In this by comparing the r-values obtained, the best-fit model was selected.

Zero Order Kinetics: Drug dissolution from Pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation: $\mathbf{Q_t = Q_o + K_o t}$, Where, Q_t = Amount of drug dissolved in time t, Q_o = Initial amount of drug in the solution and K_o = Zero order release constant.

First Order Kinetics: To study the first order release rate kinetics the release rate data were fitted to the following equation: $\mathbf{\log Q_t = \log Q_o + K_1 t / 2.303}$, Where, Q_t = Amount of drug released in time t, Q_o = Initial amount of drug in the solution and K_1 = First order release constant.

Higuchi Model: Higuchi developed several theoretical models to study the release of water soluble and low-soluble drugs incorporated in semisolids and or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The higuchi equation is: $\mathbf{Q_t = K_H \times t^{1/2}}$, Where, Q_t = Amount of drug released in time t and K_H = Higuchi dissolution constant.

Peppas Release Model: To study this model the release rate data is fitted to the following equation:

$\mathbf{M_t / M_\infty = K. t^n}$, Where, M_t / M_∞ = Fraction of drug release, K= Release constant, t = Drug release time and n = Diffusional exponent for the drug release that is dependent on the shape of the matrix dosage form. The results obtained from *in vitro* drug release studies were plotted adopting four different mathematical models of data treatment as follows: % Cum. Drug Release vs. Time (Zero order rate kinetics), Log % Cum. Drug Retained vs. Time (First order rate kinetics), % Cum. Drug release was plotted against $\sqrt{T}$ (root time). (Higuchi model) and Log % Cum. Drug Release vs. Log Time (Peppas exponential equation). In order to describe the kinetics of the release process of drug in all formulations, various equations were used, such as zero order rate equation, which describe the system where release rate is independent of the concentration of the dissolved species. The first order equation describes the release from the systems where dissolution rate is dependent on the concentration of the dissolving species. Higuchi equation describes the release from system where solid drug is dispersed in insoluble matrix, and the rate of drug release is related to the rate of diffusion. The Korsmeyer peppas equation is used to analyze the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomena could be involved. The applicability of all of these equations was tested **[Table No. 7]**. Drug release process was not zero order or first order in nature. To find out exact mechanism, dissolution data of all formulations were fitted in Higuchi equation & Korsmeyer - Peppas equation. All the formulations in this study were best expressed by Higuchi's classical diffusion equation, as the plots showed high linearity (r: 0.978 to 0.990). The linearity of the plot indicated that the release process was diffusion-controlled. Thus amount of drug released was dependent on the matrix drug load. To confirm the diffusion mechanism, the data were fitted to Korsmeyer-Peppas model. All formulations showed good linearity (r: 0.965 to 0.976), with slope (n) values ranging from 0.496 to 0.600. In Korsmeyer-Peppas model, n is the diffusional exponent indicative of mechanism of drug release. A value of n = 0.45 indicates Fickian or case I release; $0.45 < n < 0.89$ indicates non-Fickian or anomalous release; n = 0.89 indicates case II release; and n > 0.89 indicates super case II release. The case of the Fickian release mechanism, the rate of drug release is much less than that of polymer relaxation (erosion). So the drug release is chiefly dependent on the diffusion through the matrix. In the non-Fickian (anomalous) case, the rate of drug release is due to the combined effect of drug diffusion and polymer relaxation. Case II release generally refers to the polymer relaxation. The n values for formulations G1 to G6 ranged from

0.496 to 0.600, indicating that the release mechanism was non-Fickian or anomalous release (0.45 < n < 0.89). All formulations follow the Higuchi diffusion mechanism as shown in **Table 7**.

Table 7: Drug Release Mechanism

Formulation Code	Zero Order (r value)	First Order (r Value)	Higuchi	Peppas		Best Fit Model
			r Value	r Value	n Value	
G-1	0.894	0.954	0.986	0.975	0.587	Higuchi
G-2	0.890	0.951	0.990	0.973	0.589	Higuchi
G-3	0.891	0.959	0.989	0.976	0.595	Higuchi
G-4	0.882	0.948	0.988	0.970	0.599	Higuchi
G-5	0.881	0.940	0.990	0.966	0.600	Higuchi
G-6	0.949	0.989	0.978	0.965	0.496	Higuchi

4. Drug Deposition Study: At the end of the permeation experiments (after 24hr), the skin surface was washed with distilled water. The skin was then cut into small pieces. The tissue was further homogenized with methanol: distilled water (1:1) and left for 6hr at room temperature. After shaking for 5 minutes and centrifuging for 5 minutes at 5000rpm, the curcumin content was analyzed by UV visible spectrophotometric method after appropriate dilutions with methanol at 430 nm. The percent of curcumin deposited in skin after 24 hrs in vitro drug release study was found to be 11.5%, 8.85%, 6.52%, 4.64%, 3.12%, and 16.23% respectively for formulation G-1 to G-6 and it is shown in **Figure 5**. The ethanol concentration was higher in G-5, so its drug deposition was lower 3.12% and the ethanol concentration was not present in G-6, so its drug deposition was higher 16.23%. The data indicate that drug deposition depends on ethanol concentration, as the concentration increases, results in decrease of deposition of drug.

5. *In vivo* Bioavailability Study:

Instruments: The HPLC system consisted of a Thermoquest Spectra system P1500 isocratic pump coupled with a Spectra System UV 6000 LP photodiode array detection system, A Spectra System AS 3000 auto sampler, a SCM 1000 vacuum membrane degasser, a SN 4000 system controller. The detector was set to scan from 200 to 500 nm.

Chromatographic conditions: µBondapak, C18 column (250x4.6 mm inner diameter, particle size of 10 µm; waters) was used at room temperature. The control of the HPLC system and data

collection was done by a computer equipped with spinchrome software. Methanol, 2% acetic acid and acetonitrile at a ratio of 5:30:65 were used as mobile phase with a flow rate of 1.0 ml/min at ambient temperature. The injection volume was 20 µL for all samples. The retention time was 8.4 min for curcumin and 11 min for β-17-estradiol acetate, the internal standard.

Plasma sample preparation procedure: A standard stock solution containing curcumin (1 mg/ml) was prepared methanol and stored at 4 ^{O}C. Working standard stock solutions were prepared from the stock solution by sequential dilution with methanol to yield final concentration of 100µg/ml. Then plasma standard solutions were prepared by spiking into drug-free rat plasma with different working standard solutions to give final concentrations between 0.1- 5.0 µg/ml for calibration curve.

Extraction of Drug from Plasma: An aliquot of plasma (200 µL) was mixed with water (80 µL) and β-17-estradiol acetate (internal standard, 20 µL) and vortex mixed for 30 s. The solution was then extracted thrice with 1 mL of ethyl acetate/methanol (95:5, v/v) as an extracting reagent and vortex mixed for 3 min. The samples were centrifuged at 3,000 rpm for 15 min at 4°C. The upper organic layer was collected. The organic phases from the three extractions were pooled and evaporated under a stream of argon at room temperature. All the extraction procedures were done under dim light to prevent the degradation of curcumin. The extracts were then reconstituted in methanol (100 µL) before HPLC analysis.

Application of the method: In vivo bioavailability studies were conducted on healthy male rabbits weighing around 2.5 kg. Two rabbits were devided into two groups and fasted for 24 hrs. Formulation G-5 applied to the rabbit skin of one group while formulation G-6 applied to the rabbit skin of second group. For each study, blood samples (1ml) were withdrawn from the marginal ear vein under anesthesia. Samples were withdrawn before dosing and 1, 2, 4, 6, 8, 10 and 24 hrs post dosing. The collected blood was harvested for 45 min at ambient temperature and centrifuged at 5000 rpm for 20 mins. The clear supernatant serum layer was collected and stored at -20 ^{0}C until analysis.

Pharmacokinetic Analysis: Pharmacokinetic parameters were derived from the plasma concentration vs. time plot. The area under the curve (AUC), the peak plasma concentration (C_{max}) and the time to attain peak concentration (T_{max}) were obtained from these plots. The elimination rate constant (K_{el}) was determined from the semilogarithmic plot of plasma concentration vs. time. Elimination half life ($t_{1/2}$) was calculated using the formula; $t_{1/2} = 0.693/ K_{el}$. AUC was statistically

analyzed applying one-way ANOVA at 0.05 levels in the GraphPad Prism version 5.01 software.

The *in vivo* evaluation of ethosomal gel of curcumin was conducted in one group of rabbit while comparing it with free drug gel conducted in second group of rabbit. Rabbit has been chosen as a model for study because the blood volume of the rabbit is sufficiently large (approximately 300 ml) to permit frequent blood sampling and allow a full characterization of the absorption and determination of the pharmacokinetic profile of the drug. **Table 8** shows the plasma concentration of the drug at each sampling interval for formulations G-5 (ethosomal gel) and G-6 (free drug gel).

Data Analysis: The maximum plasma concentration (C_{max}) and time (T_{max}) of it occurrence were directly computed from the plasma concentration vs. time plot. The elimination rate constant (K_{el}) was determined from the terminal phase of the log plasma concentration vs. time profile and was calculated as $K_{el} = 2.303 \times$ slope. The elimination half life was calculated using the formula 0.693/ K_{el}. The area under the curve (AUC) was calculated from the plasma concentration vs. time profile by trapezoidal method and was statistically analysed by applying one-way ANOVA **(Table 9).** The C_{max} of formulation G-5 was found to be 2.61µg/ml and the corresponding T_{max} was 6 hrs. The C_{max} of formulation G-6 was found to be 2.07µg/ml and the corresponding T_{max} was 4 hrs. The pharmacokinetic parameters of the formulations G-5 (ethosomal gel) and G-6 (free drug gel) were estimated and are given in **Table 9.** The relative bioavailability of the formulation G-5 was found to be 124.92% while the relative bioavailability of the formulation G-6 was found to be 80.04%. Thus, bioavailability of curcumin was found to have significantly increased by formulating the ethosomal gel as compared to free drug gel.

Table 8: Plasma Concentration of Curcumin (µg/ml) at Each Sampling Interval

Formulation Code	Time (hrs)						
	1	2	4	6	8	10	24
G-5	0.59	1.23	2.23	2.61	2.37	1.98	0.52
G-6	0.32	0.85	2.07	1.94	1.87	1.73	0.27

Table 9: Pharmacokinetic Parameters of G-5 (Ethosomal Gel) and G-6 (Free Drug Gel)

Formulation Code	Pharmacokinetic Parameters				
	T_{max} (h)	C_{max} (µg/ml)	AUC (µg $ml^{-1}h$)	K_{el} (h^{-1})	$t_{1/2}$ (h)
G-5	6	2.61	36.34	0.297	2.33
G-6	4	2.07	29.09	0.445	1.55

6. Short-term Stability Study: Information on the stability of drug substance is an integral part of the systematic approach to stability evaluation. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under influence of variety of environmental factors such as temperature, humidity and light, and to establish a re-test period for drug substance or a shelf life for the drug product and recommended storage conditions. Stability is defined as the extent to which a product remains within specified limits throughout its period of storage and use. A drug formulation is said to be stable if it fulfills the following requirements: It should contains at least 90% of the stated active ingredient, It should contains effective concentration of the added preservatives, if any; It should not exhibit discoloration or precipitation, nor develops foul odor and It should not develop irritation or toxicity.

Procedure: Formulation G-5 (ethosomal gel) was tested for stability studies. Formulation G-5 (ethosomal gel) was divided into 2 sample sets and stored at: 5°C ± 3°C in refrigerator and 25° C ± 2°C. The G-5 was tested for drug release study at each temperature after 30 days of storage. **Table 6** shows the data for *In vitro* release studies, which were carried out after storing a selected formulation (G-5) at 5° C ± 3°C and 25° C ± 2°C for thirty days. *In vitro* release studies revealed that the formulation stored at 5° C ± 3 ° C showed 92.069 % release, the one which stored at 25° C ± 2°C showed 79.637 % release after 24 hours. It was observed that the formulation was more stable at 5° C ± 3°C.

The present study has been satisfactorily completed to formulate an ethosomes of a curcumin for transdermal delivery with a view of enhancing bioavailability of the drug. From the experimental results it can be concluded that, The IR spectra revealed that there was no interaction between phospholipid and drug, hence they are compatible. The DSC thermograms obtained for the pure drug and for the formulation showed no significant shift in the endothermic peaks confirming the stability of the drug in the formulations and only polymer peak was observed, which revealed that drug is in

amorphous state in the formulations. From XRD studies, it was observed that drug peak did not appear in formulation and only polymer peak was observed, which revealed that drug is in amorphous state in the formulations. % entrapment efficiency was higher for ET-3 formulation than the other formulation. The particle size analysis revealed that all formulations gave particles in the range of 150-250 nm which is suitable for transdermal delivery of formulation. SEM analysis of the ethosomes revealed that all the formulations were three dimensional natures. Zeta potential was more negatively charged for ET-5 formulation than the other formulation. Degree of deformability was higher for ET-5 formulation than the other formulation. *In vitro* release of ethosomal gel was higher than free drug gel. For the mechanism of drug release, all the formulations followed higuchi model with non-fickian release. As the concentration of ethanol increased, % drug deposition decreased. Assessment of AUC showed that the relative bioavailability of G-5 formulation (ethosomal gel) was higher than G-6 formulation (free drug gel). Stability study for one month revealed that the formulations were stable at 5°C ± 3° C. From all the parameters studied, it can be concluded that ethosomal gel is better than free drug gel for transdermal delivery of drug. Thus, the formulated ethosomes seems to be a potential candidate as transdermal drug delivery system which increases the bioavailability of drug.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: Scanning Electron Micrograph of (A) ET-1, (B) ET-2, (C) ET-3, (D) ET-4, (E) ET-5.

Figure 2: Infrared Spectrum of ET-5 Formulation

Figure 3: DSC Thermogram of ET-5 Formulation

Figure 4: X-ray Diffraction of (A) Curcumin (B) Phospholipon 90H (C) ET-5

Figure 5: Drug Deposited in % (After 24 hrs *in vitro* Drug Release Study)

We Claim,

1. Ethosome compositions for transdermal drug delivery comprising:
    a. *therapeutically effective amount of curcumin;*
    b. phospholipon 90H;
    c. cholesterol;
    d. propylene glycol and
    e. ethanol.
2. Ethosome compositions as claimed in claim 1, wherein the effective amount of curcumin is 0.1 to 2% (w/w) of composition.
3. Ethosome compositions as claimed in claim 1, wherein the amount of phospholipon 90H is 0.5 to 5% (w/w) of composition.
4. Ethosome compositions as claimed in claim 1, wherein the amount of cholesterol is 0.5 to 5% (w/w) of composition.
5. Ethosome compositions as claimed in claim 1, wherein the amount of propylene glycol is 5 to 25% (w/w) of composition.
6. Ethosome compositions as claimed in claim 1, wherein the amount of ethanol is 10 to 50% (w/w) of composition.
7. Ethosome compositions for transdermal drug delivery in the form of gel comprising:
    a. ethosomes as in any of the preceding claims;
    b. carbopol 940; and
    c. triethanolamine.
8. Ethosomal gel compositions as claimed in claim 7, wherein the composition comprises ethosomes equivalent to 1 to 5% (w/w) of curcumin.

9. Ethosomal gel compositions as claimed in claim 7, wherein the amount of carbopol 940 is 1 to 5% (w/w) of composition.

10. Ethosomal gel compositions as claimed in claim 7, wherein the amount of triethanolamine is 0.1 to 2% (w/w) of composition.

11. Process for preparation of ethosome composition by cold method comprising:

    a. dissolving curcumin, cholesterol and phospholipon 90H in ethanol and propylene glycol mixture;

    b. addition of distilled water slowly in a fine stream with a constant mixing for 5 minutes at 30°C;

    c. cooling of ethosomes at room temperature and

    d. homogenised by using vertex shaker for 15 min to obtain uniform ethosome composition.

12. Process for preparation of ethosome composition gel comprising:

    a. carbopol 940 powder was slowly added to ultrapure water at 100 °C for 20 min and triethanolamine was added to it drop-wise;

    b. ethosomal suspensions prepared according to process of claim 11 was then incorporated into gel base;

    c. addition of water with continuous stirring until homogeneous ethosomal gel compositions formed.

Dated 14th day of June 2011

Signature

M Patel...

PATEL MAHESHKUMAR KHODABHAI