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FORMULATION, EVALUATION AND STABILITY STUDY OF ETHOSOMES CONTAINING BETA SITOSTEROL FOR ATOPIC DERMATITIS

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ABSTRACT

Atopic dermatitis (AD) is a chronic inflammatory skin condition characterized by symptoms such as dry, itchy, reddish-brown, cracked, or scaly skin. Ethosomes, phospholipid nanovesicles, are used for dermal and transdermal delivery of molecules. S.aureus being a predominant microorganism in AD.

Objectives: This study was to formulate and evaluate of Beta Sitosterol loaded ethosomes and to check the critical parameters of vesicle size, PDI, Zeta Potential, % Entrapment efficiency, and vesicularmorphology. Beta Sitosterol drug loaded ethosomes incorporated into the gel base and to check various parameters of the gel,such as physical description, viscosity, pH, spreadibility, extrudability, drug content, *In-vitro*drugpermeation study(IVPT) and stabilitystudy as per ICH guidelines.

Materials and Methods: Ethosomes were prepared by cold method using Beta sitosterol, Soya-lecithin, Cholesterol, Propylene glycol, Methanol, Carbopol 940 and double distilled water respectively.

Results and Discussion: We developed a topicalformulation of ethosomes in which drug is incorporated into it and the selected formulations were tested for in-vitro drug release, stability and other critical parameters. The results showed that F4 batch had optimal vesicle size, PDI, Zeta potential, and entrapment efficiency, with the highest drug release profile even after 18 hours. On the basis of our results it confirmed that our prepared ethosome was found to be stable and reproducible. The formulated ethosome gel could be a good dosage form for the treatment of AD. For more authentications we need to asses efficacy and safety on human.

Keywords: Atopic Dermatitis, Beta Sitosterol, Ethosomes, *In-vitro* study, Nanogel, and Stability study.

INTRODUCTION:

The term "dermatitis" originates from the Greek terms "derma" means skin & "itis" means inflammation. Although the name "eczema" is occasionally used to refer to the disease's acute presentation, dermatitis & eczema are frequently utilised interchangeably. An edoema and Skin inflammation are common types symptoms of dermatitis. It may have a range of causes and manifestations, including dry skin and an itchy or rashy area. On the other hand, the

resultant skin can blister, crust, leak, or flake. There are various types of dermatitis like Atopic dermatitis, Contact dermatitis, Diaper dermatitis (rash), Dyshidrotic dermatitis, Neurodermatitis, Nummular dermatitis, Perioral/Periorificial dermatitis, Seborrheic dermatitis (dandruff, cradle cap) and Statis dermatitis(Abuabara et al.,2018).Atopic Dermatitis (AD) is a complicated condition that is often occurring. It is characterised by immunological abnormalities in the skin and persistent relapses. AD is a potentially debilitating skin condition that influences people all around the world (Kim et al., 2019).The identification of AD is achieved by the use of distinctive characteristics such as erythematous plaques, eruptions, flexural lichenification, severe pruritus, and hives (Gaspar et al. and 2016). Intense pruritus causes mechanical damage to the skin and the release of cytokines and chemokines. It encourages allergens to enter the skin and increases skin permeability (Montes-Torres A et al., 2015).

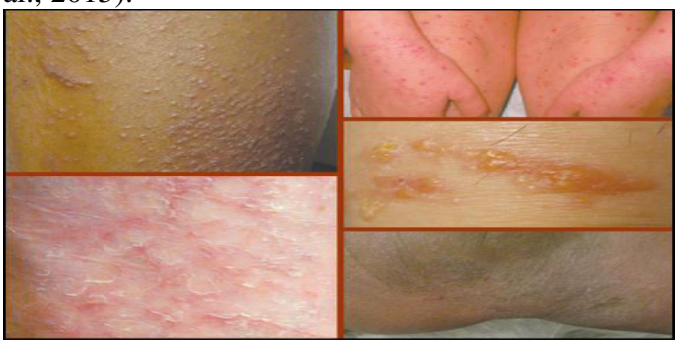


Figure.1 Atopic Dermatitis Skin (REBECCA BERKE et al., 2012)

The name "atopic dermatitis" was originally used in 1933 by Wise & Sulzberger to describe the disease's strong relationship to other respiratory atopies, including bronchial asthma and allergic rhinitis. This is how we currently refer to it (Hill LW et al., 1933).In 1892, Ernest Besnier first classified atopic dermatitis as a type of "prurigo diasthesique." (Besnier's EH). AD has been referred to by numerous names since the discovery of Besnier's prurigo, such as atopic eczema, dermatitis flexurarum and endogenous dermatitis, neurodermitis constitutionalis, and hay fever dermatitis (Sulzberger M.,1983).With a 95% probability range (UI) of 1.9-3.5, estimations place the global prevalence of AD at 2.6% & the number of affected individuals at 204.05 million. The prevalence rates of two percent (95 percent UI 1.4 to 2.6) and four percent (95 percent UI 2.8 to 5.3) indicate that, worldwide, there are about 102.78 million children and 101.27 million adults living with AD. Globally, AD was expected to affect 108.29 millions women & 2.8% (95 percent UI 2.0-3.7%) of men, while it afflicted 95.76 millions men & 2.4% (95 percent UI 1.7-3.3%) of both genders. AD was more common in women than in men (Jingru Tian et al., 2023).Since these early reports, the prevalence of AD has sharply expanded. Estimates of prevalence from seven decades ago in Scandinavia showed that 1.3 percent of peoples had AD (Nexmand PH). In Scandinavia, the percentage of people with AD had risen to 23% by 1993, a 20-fold increase (Larsen FS et al., 1996). Some of the most significant prevalence and trend statistics on AD have been published by the International Study of Allergies & Asthma in Childhood (ISAAC). The largest and only allergy study to adopt a genuinely worldwide approach is ISAAC, which includes almost 2 million childrens from 106 countries.

The research findings indicate that the prevalence of AD varies widely across the globe, with over 20% of youngsters affected in certain nations. Data revealed that the frequency of AD in children aged six to seven varied widely, from 0.9 percent in the country of india to 22.5 percent in Ecuador. Asian and Latin American values were high, according to recent data. Studies on adolescents and young adults revealed that the frequency of AD varied widely, from 0.2 percent in the country of china to 24.6 percent in Columbia. Africa, South America, Europe, and Oceania were among the nine locations examined where a prevalence of more

than 15 percent was discovered (Odhiambo JA et al., 2009).Studies involving both adult and paediatric patients in india found that the prevalence of AD ranged from 0.98-9.2 percent. According to reports, alterations in environmental elements are the reason behind the rising incidence of AD in India (Sarkar R et al. 2018). The majority of India's demographic data has come from research conducted in hospitals (Kanwar AJ., 2016). Planning management measures and comprehending the disease load across age groups depend on epidemiological data (Bylund S et al., 2020.).A pan-Indian investigation examining the disease burden of AD is currently lacking. It is crucial to comprehend the epidemiology and disease burden of AD in India for this reason. The range of treatments available for AD is expanding, and novel choices are becoming available. Given that AD is a chronic illness, it is critical that patients obtain a course of treatment that will aid in condition management.Staphylococcus aureus (S.aureus) is the predominant microorganism in AD. It is linked to a host transcriptome profile that is significant to the disease and is enriched for tryptophan metabolism, epidermal barrier function, and immune activation (Leyden JJ et.al.,1974 and Ring J et.al.,1992)
Phospholipid nanovesicles called ethosomes are utilised to transfer substances transdermally and onto the skin. (Touitou et al. (1997) created ethosomes, which are additional unique lipid carriers made of phospholipids, water, and ethanol. They are supposed to improve the skin-based delivery of several drugs. It is thought that ethanol works as an effective permeation enhancer by influencing the stratum corneum's intercellular area. Ethosomes are pliable, soft vesicles mostly made of water, ethanol & phospholipids. These soft vesicles are new vesicles carriers that will improve vesicle delivery via skin contact. It is possible to control the ethosomes vesicles' size from tens of nanometers to microns (Verma et al., 2010). Ethosomes consist mostly of several concentric layers of pliable phospholipid bilayers, containing a comparatively elevated amount of water, glycols, and ethanol twenty to fourty five percent. (Touitou E., 1996 & Touitou E., 1998). The general structure of them has been verified by DSC, EM, and 31P-NMR. Their great penetration of the skin's horny layer facilitates the penetration of medications that are encapsulated. The system's general characteristics are responsible for the permeation enhancement process (Dayan N et al., 2000 and Touitou E et al., 2000).The aim of the study was to formulate and evaluate ofBeta Sitosterolloadedethosomesand to check the critical parameters of vesicle size, PDI, Zeta Potential, %Entrapmentefficiency,andvesicularmorphology.Beta Sitosterol drug loaded ethosomes incorporated into the gel base and to check various parameters of the gel, such as physical description, viscosity, pH, spreadibility, extrudability, drug content, *In-vitro* drug permeation study(IVPT) and stabilitystudy as per ICH guidelines.

MATERIAL & METHOD

Chemicals:

The Beta Sitosterol drug was obtained as a gift sample from Aurochem Laboratories Ltd, Solan. Carbopol 940 was obtained from Hi Media laboratories Mumbai. However, remaining chemicals utilized in the entire study was purchaged from Sigma Aldrich Chemicals, USA. Pure, analytical-grade substances were taken into consideration for the investigation.

Preparation of Beta Sitosterol Loaded Ethosomes

The preparation of Beta Sitosterolloaded ethosomes chemicals like Beta Sitosterol,Soy lecithin, cholesterol, propylene glycol was weighed in quantities mentioned in the Table number 1. Initially, Drug, Soya lecithin & cholesterol was dissolved in the mixture of methanol & propylene glycol at a temp. of 30 Degree Celsius on a water bath. Then, With the use of a syringe, double-distilled water was added to the resultant solution. After being transferred to a sealed borosil container, the entire mixture was agitated at a speed of 700 rpm using a magnetic stirrer. Finally, the fluid was sonicated using a probe sonicator. Three times,

a 5-minute sonication cycle was conducted, separated by another 5-minute pause (Touitou et al. 2000).

Table Number-1 Composition of Drug Loaded Ethosomes

Composition in (% w/w)	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8
Beta Sitosterol	1	1	1	1	1	1	1	1
Soya lecithin	2	2	2	2	2	2	2	2
Cholesterol	1	1	1	1	1	1	1	1
Propylene Glycol	1	1	1	1	1	1	1	1
Methanol	15	20	25	30	35	40	45	50
Double Distilled Water (q.s)	Up to 100 gm							

Preparation of Beta Sitosterol Loaded Ethosomal Gel Initially, Carbopol 940 0.75g was weighed and gradually dissolved in 100 millilitres of double-distilled water with the use of a magnetic stirrer. such that the polymer completely swells in the water. Moreover, 20 millilitres of drug-loaded ethosomes that had already been synthesised were added while it was being stirred. Until a uniform ethosomal gel-like texture was observed, stirring was maintained at 30 Degree Celsius and 700 rpm. In addition, 7.4 millilitres of gelling agent that isCarbopol 940was supplemented into it so, as to aid in the formation of gel. Lastly, Glycerine was incorporated into the gel to improve skin hydration, which in turn increased medication absorption. The eight remaining ethosomal formulations were prepared using the same procedures which are described above.

Characterization of Ethosomes incorporated with Beta sitosterol

Particle size, Polydispersity Index (PDI) & Zeta Potential determination.

Determining crucial parameters like zeta potential, particle size, and PDI is essential. Utilising the Malvern Zeta Sizer Nano ZS, which is based on dynamic light scattering, these parameters were assessed. One millilitre of the ethosomal mixture was diluted with HPLC water in order to begin the estimation process. Furthermore, data were determined at a 90 Degree scattering angle, with the medium's viscosity of 0.8862 & refractive index of 1. 36

Determination of (%) Entrapment Efficiency To estimate the amount of drug entangled in the colloidal system, entrapment efficiency Percent is computed. An Eppendroff tube containing a tiny amount of the formulation was first filled, and it was centrifuged for 15 minutes at 4 Degree Celsius & 14,000 rpm. The procedure was repeated until a cloudless type of supernatant was achieved using the Remi ultracentrifuge, which has a TLA-45 rotor. After obtaining the cloudless supernatant, the drug concentration was determined using a UV-visible spectrophotometer set to 299 nano meters. As a final step, the amount of drug entangled in the colloidal system was computed using the formula below.

$$EE(\%) = \frac{(\text{Quantity of incorporated drug} - \text{Quantity of free drug present in the supernatant})}{\text{Quantity of incorporated drug}} \times 100$$

pH Determination

A digital pH metre was used to determine the composition. After completely submerging the glass electrode in the ethosomal gel, the readings were recorded and shown.

Determination of Viscosity

To determine the formulation's viscosity, a Brookfield viscometer (model LVDV-II Pro) spindle of S 96 was submerged in a beaker containing ethosomal gel at intervals of 10, 15, and 20 rpm at lower, middle, and higher case. However, throughout the entire process execution, room temp. was maintained (Sanjula Baboota et al., 2011).

Determination of Spreadbility

One gram of ethosomal gel was precisely weighed and kept between two 8-cm-long glass slides in order to estimate the spreadibility of the gel. Different weights were fastened to the pulley, and the mass at which the glass-slide moved was also recorded, as was the amount of time required to draw the upper slide and cause the gel to extend further to the bottom slide. After three measurements, the spreadibility readings were calculated using the following formula.

$$S = M*L/T$$

Whereas the symbols (S), (M), (L) & (T) stand for ethosomal gel's spreadibility, the weight in (gram) attached to the top slide, the slide's movement (centimetres), and the uppermost slide's downward movement time (seconds).

Determination of Extrudability

Twenty grams of prepared ethosomal gel were packed in a collapsible tube prior to extrudability, and regular pressure was then applied. In order to stop the content from flowing backward down the tube, a clamp was applied. Finally, the amount of gel that continued to extrude out of the tube while the pressure persisted was measured and noted (Md. Sarfaraz Alam et al., 2012).

Study of In-Vitro Permeation

Locally fabricated franz diffusion cells were taken for in-vitro permeation studies. In place of cadaver skin here dialysis membrane was taken for the release study. The activation of membrane was done by soaking in glycerin was allowed to soak in for 18 hours duration. The diffusion cell assembly was used to hold the cellophane membrane. Its effective permeation area measured 2.4 cm, and its receptor cell volume measured 200 ml. pH 7.4 phosphate buffer (30 millilitres) was added to the receptor section and the receptor fluid was maintained at 37 ± 0.5 degrees Celsius during the study. Beside the donor and receiver chambers was positioned a dialysis membrane. The membrane within the donor section was coated with the prepared mixture. At various periods, five millilitre samples were removed and promptly replaced with an equivalent volume.

Stability Studies Determination

Stability testing was used to assess the ethosomes' ability to store drugs. Three months were spent conducting the experiment at various temperatures. The two formulations, namely the lyophilized ethosomes and the ethosomal solution, were kept separate and in a sealed 10 millilitre vial at 4°C's/60 ± 5 RH & 25°C's/60 ± 5 RH. Following 0, 30, 60, and 90 days of hoarding, the samples were analysed as a function of time (MS Alam et al., 2012).

RESULT & DISCUSSION:

EvaluationofTrialBatchesofEthosomes

Figure number-2 Trial Batches of Ethosome F1 To F8

Microscopy

Microscopic study of the trial batches was performed for vesicle size, PDI, and zeta potential. Comparative results and graphs were presented in Table number-2 to 4

Vesiclesize

Tablenumber-2.–DeterminationofVesicleSizeofTrialBatches

Serial number	Ethosomes	
	TrialNo.	Vesiclesize(nm)
1.	F1	287.41±9.39
2.	F 2	284.74±21.45
3.	F 3	254.64±12.46
4.	F 4	157.32 ± 13.28
5.	F 5	221.17 ±31.67
6.	F 6	187.16±11.59
7.	F 7	109.82±12.60
8.	F 8	264.75±9.38

Polydispersity Index (PDI)

Tablenumber-3.DeterminationofPDIofTrial Batches

Serial number	Ethosomes	
	TrialNo.	PDI
1.	F 1	0.463±0.039
2.	F 2	0.491±0.025
3.	F 3	0.376±0.023
4.	F 4	0.286±0.032
5.	F 5	0.351±0.028
6.	F 6	0.511±0.026

7.	F 7	0.722±0.037
8.	F 8	0.580±0.044

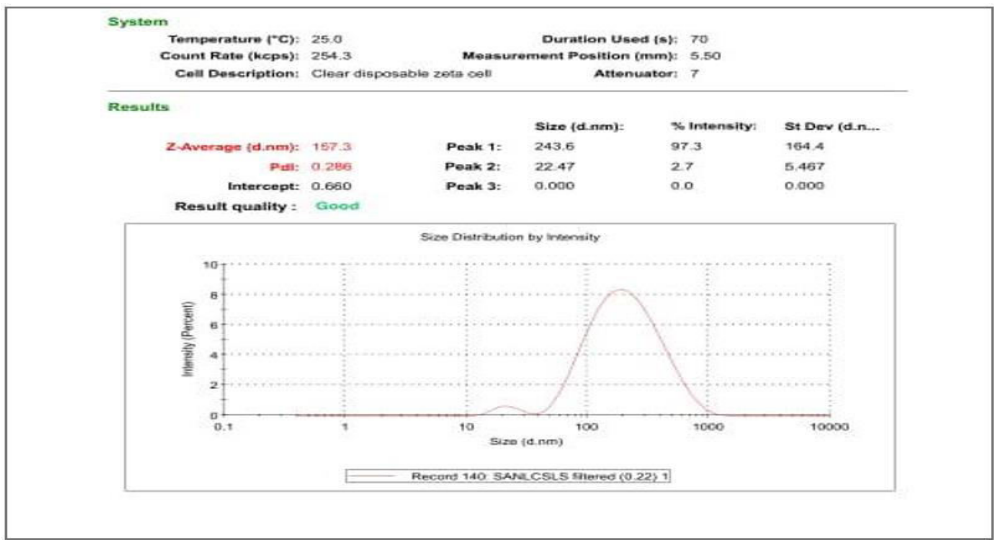


Figure number-3. Average Particle Size and PDI of Optimized Batch Report

Zeta Potential

Tablenumber-4.DeterminationofZetaPotentialofTrialBatches

Serial number	Ethosomes	
	Trial No.	ZetaPotential(mV)
1.	F 1	-28±1.52
2.	F 2	-30±1.03
3.	F 3	-26±1.18
4.	F 4	-31± 1.37
5.	F 5	-27±1.76
6.	F 6	-35±1.39
7.	F 7	-39±1.47
8.	F 8	-26±1.81

Drug (%)EntrapmentEfficiency

Drug (%) entrapment efficiency was determined by using Ultra vilot spectrophotometer (UV-1800 Shimadzu) for all trial batches. Comparative results were presented in Table number-5

Tablenumber-5.DrugEntrapment Efficiency(%)ofTrialBatches

Serial number	Ethosomes	
	TrialNo.	% Drug Entrapment Efficiency

1.	F 1	51.15±0.56
2.	F 2	64.69±1.68
3.	F 3	71.14±0.64
4.	F 4	73.05±0.79
5.	F 5	68.76±1.45
6.	F 6	61.32±1.66
7.	F 7	43.29±0.71
8.	F 8	65.56±1.07

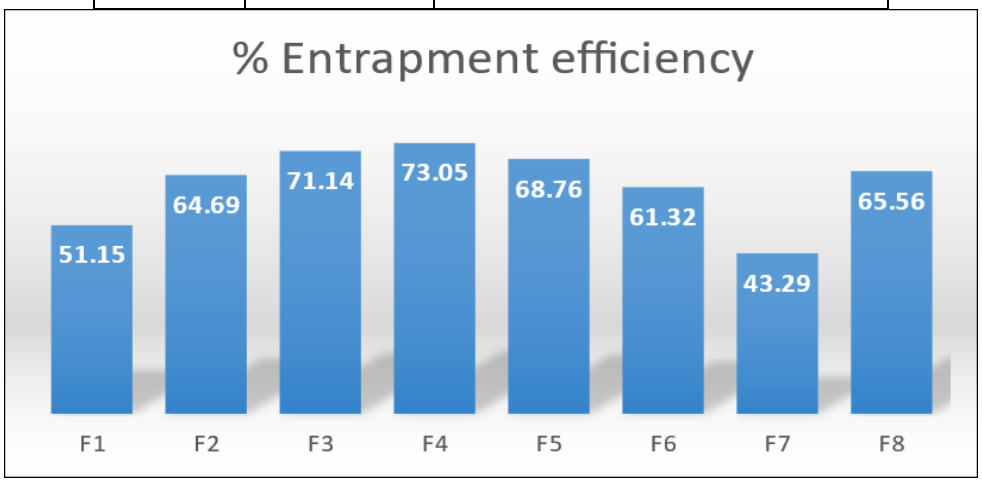


Figure number-19. Drug Entrapment Efficiency (%) of Trial Batches (F1 to F8)

Drug percententrapmentefficiency

Drug percent entrapment efficiency of ethosomes was found almost same but ethosomal batch F4 has 73.05±0.79 % which is just greater than batch F3 i.e. 71.14±0.64 %. Comparative data was shown in Table number-6

Tablenumber-6.Drugentrapmentefficiency(%)ofreproduciblebatches

Serial number	ReproduciblebatchNo.	Drugentrapmentefficiency(%)
1.	F 3	71.14±0.64
2.	F 4	73.05±0.79

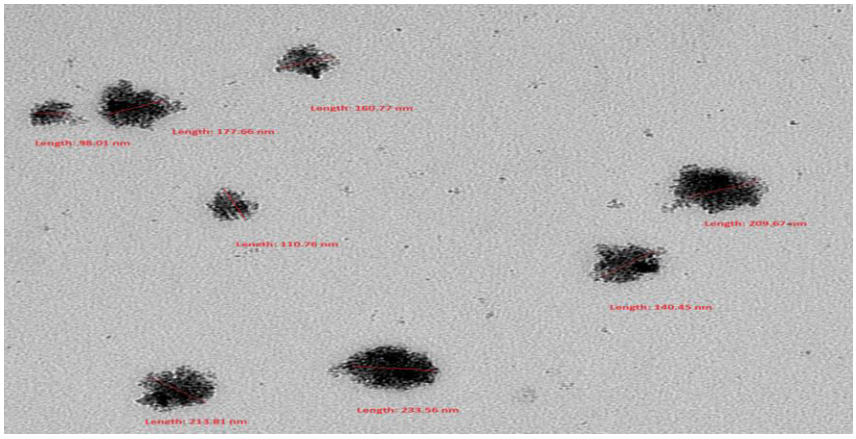
Vesiclemorphology

Microscopicstudy

The reproducible batch of ethosomes was evaluated for microscopic study by using inverted microscope (Lobomed TMC 400). This study revealed that formulations contained uniform vesicles shape and size of ethosomes suspended in off-white to light yellow suspension.

TEMofEthosome

Vesicle morphology was characterized by using TEM (Hitachi H-7500) and image of ethosomes revealed that ethosomal vesicles were when viewed at a 200 nm scale, it had a spherical shape, and the drug was confined inside the ethosome (Figure number-20).



Figurenumber-20.TEMImageofEthosome (F4)

Evaluation of Ethosomal Gel:

PhysicalDescription

Beta Sitosterol ethosomal gel was physicallyobserved for odour & colour of formulation and It was discovered to be an odourless, transparent gel with a colour ranging from off-white to bright yellow. Beta Sitosterol ethosomal gel also noted for physical characteristics, there was no difference found in their colour and odour to that of ethosomal gel formulation.

pH Determination

Ethosomal gels were characterized for pH & comparative results were shown Table number-15

Tablenumber-15.pHofEthosomalGel

Serial number	EthosomalGel Batch	pH
1.	F 4	6.23±0.019

Viscosity

Viscosity of the gels, checked on plate and cone Brookfield Viscometer. Cone spindle CPA-41Z was used for the model of HADV-II+ pro and the obtained results were tabulated in Table number-16

TableNumber-16.ViscosityofEthosomalGel

Serial number	EthosomalGel Batch	Viscosity (cps)
1.	F 4	4369.25± 25.38

Spreadibility

Spreadibilityofgels was characterized &results obtainedweretabulatedin Table number-17.

Tablenumber-17.SpreadibilityStudyofEthosomalGel

Serial number	EthosomalGel Batch	Spreadibility (g.cm/sec)

1.	F 4	11.91± 1.12

Extrudability

Extrudability was found that the optimized batch F4 was very similar to the marketed preparations.

Drugcontent

Study of drug content of vesicular gel formulation was characterized by using Ultra vilot spectrophotometer to find out the amount (percentage) of drug present in the formulation. Obtained results of drug content of ethosomal gel were listed in the Table number-6.18

Table number-18.Drugcontent Percentofgelformulation

Serial number	EthosomalGel Batch	DrugContent Percent
1.	F 4	98.89 ± 0.95

In Vitro Permeation Study of Beta SitosterolEthosomal Gel

AnIn-vitro drug release study of ethosomal gel was performed by using (Locally Fabricated Franz Diffusion Cell). Samples were collected from different predetermined time intervals of 0.5 hr, 1 hr, 2 hr, 4 hr, 6 hr, 12hr, 18hr &analyzed using UV-Spectrophotometer. The obtained outcomes were shown in Table number-19.

Tablenumber-19.CumulativePercentDrugRelease

Time(Hrs.)	CumulativePercentDrugRelease
	F4
0.5	11.25 ± 1.29
1	26.65 ± 1.30
1.5	45.65 ± 2.46
2	68.89 ± 2.25
4	75.25 ± 3.40
6	78.65 ± 4.33
12	80.19 ± 5.02
18	80.27 ± 6.13

In-vitro drugreleasestudyofbatch F4of Beta Sitosterol ethosomal gelshowed maximum drug release at time point of 18 hours. Thus, these formulations were taken to perform the further studies.

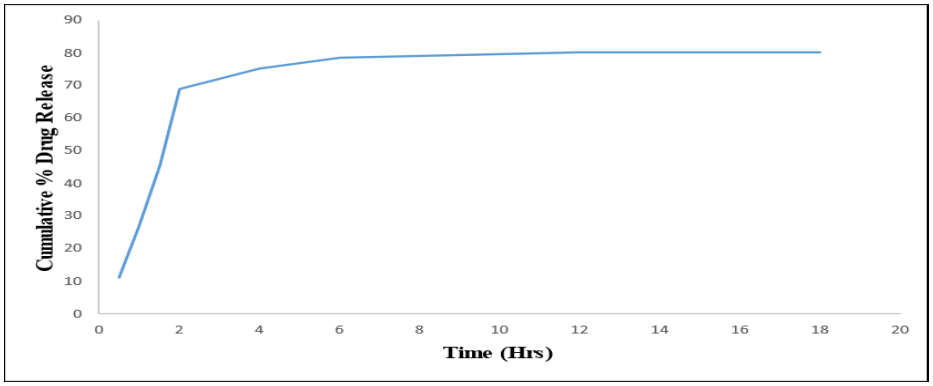


Figure number-22 CumulativePercentDrugRelease (F4)

Stability Studies

Table number-20 Droplet size, Viscosity, RI, pH and conductivity of optimized ethosome gel during storage.

Time (Days)	Temp (^{o}C)	Mean droplet size(nm) ± SD (n=3)	Mean viscosity (mP) ± SD (n=3)	RI ± SD (n=3)	pH	Conductivity (µs) ± SD
0	4.0 ± 0.5	90.32 ± 0.56	28.16 ± 1.06	1.410 ± 0.024	6.23 ± 0.019	346 ± 2.49
30	4.0 ± 0.5	90.46 ± 0.26	28.25 ± 1.03	1.465 ± 0.052	6.28 ± 0.027	349 ± 2.17
60	4.0 ± 0.5	90.48 ± 0.78	28.32 ± 1.08	1.432 ± 0.029	6.27 ± 0.046	352 ± 1.98
90	4.0 ± 0.5	90.58 ± 0.19	28.46 ± 1.53	1.474 ± 0.039	6.25 ± 0.064	350 ± 1.41

Table number-21

Time (Days)	Temp (^{o}C)	Mean droplet size (nm) ± SD (n=3)	Mean viscosity (mP) ± SD (n=3)	RI ± SD (n=3)	pH	Conductivity (µs) ± SD
0	25 ± 0.5	90.32 ± 0.56	28.16 ± 1.06	1.410 ± 0.024	6.23 ± 0.019	346 ± 2.49
30	25 ± 0.5	90.79 ± 0.65	28.56 ± 1.15	1.464 ± 0.078	6.27 ± 0.029	354 ± 2.11
60	25 ± 0.5	90.85 ± 0.48	28.73 ± 1.35	1.454 ± 0.064	6.26 ± 0.075	357 ± 2.19
90	25 ± 0.5	90.91 ± 0.95	29.84 ± 1.59	1.444 ± 0.035	6.39 ± 0.067	360 ± 2.24

SUMMARY AND CONCLUSION:

Atopic dermatitis (AD) is a inflammatory chronic skin disease. The most prevalent microorganism associated with atopic dermatitis is Staphylococcus aureus (S.aureus). It is associated with a host transcriptome profile that is enriched for immunological activation, tryptophan metabolism, and epidermal barrier function and is relevant to the disease.

There are various symptoms of AD such as reddish-brown patches, dryness, itchy, scaly or cracked skin. Some of the most significant prevalence and trend statistics on AD have been made available by the (ISAAC) International Study of Allergies and Asthma in Childhood.

AD affected 1-3% of adults and 15-20% of children globally. According to estimates, the number of people affected with AD and its prevalence worldwide are 204.05 million.
Currently AD has no known effective cure. Treatment is based on the kind and level of AD. Over-the-counter or topical medications are effective treatments for mild cases of AD. Moreover, the treatment of AD requires continuous treatment. Cost of drug and visit to doctors, amount to enormous financial losses.
Phospholipid nanovesicles are called ethosomes.which are used for dermal and transdermal delivery of molecules. Ethosomes were developed as additional novel lipid carriers composed of ethanol, phospholipids, propylene glycol and other glycol. They are reported to improve the skin delivery of various drugs. Ethanol or methanol is an efficient permeation enhancer that is believed to act by affecting the intercellular region of the stratum corneum. There are mainly two simple methods are available for the formulation of ethosomes like cold and hot method. In this work cold method were used for the preparation of ethosomes. There are various advantages of ethosome formulation such as reduction of toxicity, increase permeation, better solubility and stability and improvement in pharmacokinetics effects.

Beta sitosterol (BS) has little negative side effects and impacts. BS has anti-inflammatory, anticancer, immunomodulatory, angiogenic, antioxidant, and antinociceptive qualities without posing a serious risk. When AD is treated with a nano formulation, the drug delivery is more bioavailable to the skin's deeper layers.

- It was determined from the physical characteristics and identification test that the drug samples (Beta Sitosterol) were genuine, pure, and compliant with the requirements.
- The λmax of BS was determined by running the spectrum from 200 to 400 nm wavelength and measuring the absorbance of drug solution in an ultraviolet spectrophotometer against blank which was found 351 nm.
- FTIR and DSC was measured for compatibility study of drug and excipients and found to be no any interaction.
- To prepare the ethosomes, many components were chosen. The GRAS classification and pharmacological acceptability of the components were crucial selection criteria.

- The composition of optimized batch of ethosomes were found to be: Beta Sitosterol ethosomal gel was physically observed for colour and odour of formulation and it was found to be off-white to pale yellow in colour & an odorless translucent gel. Beta Sitosterol ethosomal gel also observed for physical characteristics, there was no difference found in their colour & odour to that of ethosomal gel formulation.
- Ethosomal gels of optimized batch were characterized for pH were found to be 6.13±0.109.
- Viscosity of the gels, checked on plate and cone Brookfield Viscometer. Cone spindle CPA-41Z was used for the model of HADV-II+ pro and were found to be 4369.25 ± 25.38 cps.
- TEM analysis was performed for optimized batch F4 formulation which revealed the spherical shape and uniform distribution of particles in ethosomes range.
- Spreadibility ofgel was characterized and theresults obtainedwere found to be 11.91 ± 1.12 (g.cm/sec).
- The extrudability of optimized batch found that was very similar to the marketed preparations.
- The drug content study of vesicular gel formulation was characterized by using UV spectrophotometer to find out the amount (percentage) of drug present in the formulation. Obtained results of drug content of ethosomal gel were found to be 98.89

± 0.95.

- Anin-vitrodrugreleasestudyofethosomal gel wasperformedbyutilising locally fabricated Franz diffusion cell. Samples were collected from different predetermined time intervals of 0.5 hr, 1 hr, 2 hr, 4 hr, 6 hr, 12hr, 18hr and analyzed using UV-Spectrophotometer. In-vitro drugreleasestudyofbatch F4of Beta Sitosterol ethosomal gelshowed maximum drug release at time point of 18 hours duration.
- Stability study of optimized ethosome was done as per ICH guidelines and it was found to be stable throught out the study period.

On the basis of our results it confirmed that our prepared ethosome was found to be stable and reproducible. The formulated ethosome gel could be a good dosage form for the treatment of AD. For more authentications we need to asses efficacy and safety on human.

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