Analysis and Metaphysics
ISSN: 1584-8574, e-ISSN: 2471-0849
Vol 20 (1), 2021
pp. 18–32

Transdermal Drug Delivery of Antihypertensive Drugs Using Ethosomal Gels

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Abstract

A naturally occurring polyphenolic flavonoid with limited water solubility and bioavailability, silymarin (SM) has lipid-lowering and antidiabetic properties. The goal of the current study was to create an anti-psoriatic gel formulation using silymarin. The goal of this research project is to lessen the suffering and distress experienced by psoriasis sufferers. To get over these issues, SM-incorporated ethosomes (ETO) were created and refined using the cold approach with a 32 complete factorial design in the present study. The physical appearance, size distribution, negative charge potential, morphological study, powder crystallinity, phase transition behavior, and percentage of drug entrapment were all assessed during the synthesis and evaluation of the SM-ETO. After optimization, SM-ETO was added to a carbapol 934p-containing gel and tested for drug content, rheology, pH, and in vitro drug release. The findings showed that at 2–8°C, SM-ETO batches did not exhibit phase separation. The batch E8 had a vesicular size of 168 nm, a 0.367 polydispersity index, a -0.49 mV zeta potential, and an 89.67% drug entrapment rate. A morphological analysis showed lengthy, round vesicles. X-ray diffraction analysis shows that SM powder is amorphous. A large pH range of 6.94 to 7.18 was found in a prepared gel. Additionally, it showed 96.32 to 98.45% drug content and 9.187 (cp) viscosity. 96, 97, 94, and 98 percent SM release from gel batches was shown by in vitro drug release. The thorough results examined the created gel's improved solubility and bioavailability, indicating that it may be used as a nanocarrier to deliver SM for upcoming therapeutic applications. In summary, it can be said that: Ethosomal gel formulation of Silymarin has been effectively created with the use of formulation development technique.
Keywords: topical application, gel, silymarin, ethersomes, and transdermal drug administration.

Introduction

For many years, transdermal drug delivery systems (TDDS) have been developed to provide pharmaceuticals. Once the transdermal bioactive substance has penetrated the epidermal layer, it enters the systemic circulation (1). After then, the medication travels throughout the body via the bloodstream to carry out its pharmacological function (2). Compared to other methods, the transdermal route of administration offers several benefits, such as avoiding first pass hepatic metabolism, extending the duration of the drug's action, minimizing side effects, improving pharmacological action, minimizing fluctuations in drug ratios, and improving patient convenience (3). Most notably, when long-term or chronic medication treatment is needed, TDDS may be employed effectively. As a result, developing TDDS to treat various pathological disorders, such as diabetes, is a feasible alternative. However, transdermal treatment works only with certain types of bioactive chemicals because the stratum corneum functions as a barrier to the penetrating molecules (4). Nano-formulations have emerged as a viable way to get around the limitations of transdermal therapy (5). Because of its advantages in terms of tiny particle size, enhanced drug absorption, and targeting, nano-formulations have been considered the best TDDSs. This has led to the development of many techniques that use nanoparticulate delivery systems including as liposomes, transferosomes, ETO, dendrimers, and microemulsions to increase the TDD of bioactive substances (6). Liposomes have been the subject of much research as one of the TDS since the 1980s. Yet liposomes do not penetrate deeply into the rat skin; they are limited to the outermost layer (7). In contrast, Ethosomes (ETO) are a novel kind of liposome that, when mixed with ethanol (20–45% v/v), contain phosphatidylcholine and a considerable quantity of water. The high ethanol level has many advantages, including increased malleability, solubility, longer stability, bioavailability, and the ability to entrap insoluble molecules (8). Because of their comparatively strong negative surface charge, ETO could be more stable and smaller.

The use of herbal remedies to treat a range of illnesses has grown in importance recently. Milk thistle seeds (Silybum marianum L.) are the natural source of silymarin (SM), a polyphenolic flavonoid whose main active ingredient is silibinin (Silybin) (9). SM is a very effective hepatoprotective medication that has shown anti-inflammatory/immunomodulatory, antifibrotic, and antioxidant properties in several in vitro and in vivo animal models (10, 11). According to recent studies conducted on animal models, SM may have potential lipid-lowering and anti-diabetic effects (13, 13). Numerous studies have shown that SM's low intestinal permeability, poor aqueous solubility/bioavailability, extensive metabolism, damage from gastric enzymes, and rapid excretion may all contribute to its ineffectiveness as a therapeutic agent for certain diseases (14). Therefore, efforts have been made to increase the solubility, bioavailability, and permeability of SM by synthesizing SM-ETO and combining them into gel formulation for maximal drug release in TDDS in order to address these study-related problems. 32 complete factorial design was used in the cold method's initial design and optimization of SM-ETO. The term "optimization" is derived from the verb "optimize," which implies to make anything as good, practical, or effective as might be. In the past, the word "optimized" was used to describe pharmaceutical preparations or procedures, suggesting that a product had been enhanced to satisfy a development scientist's intended objectives. One method for determining the optimal composition or experimental circumstances is optimization. Optimization is the term used to describe the use of systematic techniques to get the optimum possible combination of a process's and/or product's attributes under a given set of limitations. Another way to put it is that optimization entails choosing the optimal element from a variety of easily available options (15). Factorial design (FD), sometimes referred to as experimental designs for first degree models, is the most widely used method. The simplest way to build up a design of experiments (DOE) is to test two or more variables (n) at different levels. The number of trials is fn, where f is the factor and n is the level, and all factors are connected with each other on all levels in a complete factorial method. The 32 complete factorial design uses nine experiments, the 42 uses sixteen, and the 52 uses twenty-five. At level 3, the number of trials rises to 33, 43, and 53. Naturally, the number of trials increases and beyond what is deemed fair. Two levels are often taken into consideration in order to minimize the number of exams. If each component has the same number of levels—22, 33, etc.—the design is considered to be symmetrical. It is said that the design is asymmetrical if The factor determines the number of layers, which might be 23, 32, etc. (16). The physical appearance, size distribution, negative charge potential, morphological study, powder crystallinity, phase transition behavior, and percentage of drug entrapment of the formulated SM-ETO batches were then evaluated. The optimized batch was chosen for further research based on these assessments as well as the attractiveness value derived from Design-Expert software version 7. Pharmaceutical research and development has undergone a revolution thanks to the creation of innovative medication delivery methods. Ethosomal drug delivery systems are one such novel strategy that has attracted a lot of interest due to its potential to improve the therapeutic effectiveness of different medications. Milk thistle's silymarin has shown potential in the treatment of psoriasis and other skin disorders. Effective skin penetration is made possible by the special lipid-based nanocarriers of the ethosomal system, which provide better medication delivery and bioavailability. The formulation and development of silymarin-loaded ethosomal drug delivery systems for topical application are thoroughly examined in this introduction, which also highlights the research's potential advantages and contributions to the fields of dermatology and pharmaceutical science. The penetration of formulation from the application site is a crucial viewpoint when creating a transdermal delivery system. In order to assess the pH, rheology, drug content, and in-vitro drug release, the created ETO formulation was put into a gel formulation. The next section examines the specifics of the chosen technique and the study's findings.

Materials and Methods

Materials

Silymarin, Ethanol, Propylene glycol, Carbopol 934p were procured from (Fine Chem Industries, Mumbai, India). Soya-lecithin and Cholesterol were procured from (Hi Media Laboratories Pvt. Ltd., Mumbai, India). All the chemicals and excipients utilised in this investigation were of analytical grade.

Methods

Development of ETO

The nine batches of ETO were synthesized using a cold method consisting of dropwise integration of the aqueous phase into the lipid phase. Briefly, as shown in (Table 1) given concentration of soya lecithin (as a vesicle-forming Component) and cholesterol (to provide the vesicle membrane integrity) was primarily dissolved in ethanol solution (for providing softness to the vesicles and as a penetration enhancer) with continuous stirring at 1500 rpm on a mechanical stirrer (Remi Instruments Pvt. Ltd. Mumbai, India). Simultaneously to develop ETO, the drug SM (1%w/v = 10 mg/mL), and propylene glycol was incorporated into the above

solution. After the solubilization process, Distilled water (DW) was progressively included in the lipidic solution to get the desired final concentration. The ethosomal mixture was agitated constantly for 30 min to achieve the desired vesicle size of the formulation utilising probe sonicator. After that, formulated ethosome were cooled at room temperature and stored at 4-8 °C for further assessment [17].

Table 1. Formula of the batches of ETO.

Ingredients (%)	Formulation Codes								
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_8	E_9
SM (%w/v)	1	1	1	1	1	1	1	1	1
Soya lecithin (w/v)	1	2	3	1	2	3	1	2	3
Cholesterol (%w/v)	0.4	-	-	-	-	-	-	0.4	0.4
Propylene glycol (%v/v)	10	10	10	10	10	10	10	10	10
Ethanol (%v/v)	20	20	20	30	30	30	40	40	40
DW	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Evaluation of ethosomal batches

Physical appearance

The developed batches were observed visually for phase separation after 24 h at the storage of 2-8° C [18].

Percent drug entrapment efficiency (% EE)

The EE evaluated the quantity of drug enveloped inside the developed spherical vesicles. Further SM content in the ethosomal formulation was estimated by ultracentrifugation employing a (Bachman Coulter USA) 2 hours at 15,000 rpm. After incorporating the clear supernatant, an aliquot was appropriately diluted with 1:10 (v/v), and its spectrophotometric absorbance was recorded (UV 1700, Shimadzu, Japan) at 286 nm. The following equation (1) calculated the % EE [19].

$$\%EE = \frac{Amount\ of\ drug\ added - Amount\ of\ drug\ in\ supernatant}{Amount\ of\ drug\ added} \times 100 \quad (1)$$

Vesicle size (VS) and polydispersity index (PDI) of ETO formulation

Vesicle size is a crucial parameter for efficient ETO formulation since it has the potential to improve drug permeability and absorption via the transdermal route [20]. The average VS and PDI were ascertained by employing a (Malvern Instrument Ltd. UK) Nano ZS90 and a 5mW neon laser. The analysis was conducted at room temperature of 25 °C, at an angle of 90°, using an expandable polymeric cell with a diameter of 10 mm and a run time of 180 s. The optimized batch E8 sample was examined at room temperature after being diluted at 1:10 (v/v) with distilled water [21].

Zeta Potential (ZP)

Determining the charge present on the SM-ETO is essential for the stability of the formulation. The optimized batch sample was examined at room temperature after being diluted at 1:10 (v/v) with distilled water using (Nano ZS90, Malvern instrument Ltd. UK) [22].

Transmission Electron Microscopy (TEM)

The specimen for TEM analysis was developed by extending 20 µL of the SM-ETO solution on the top of formvar-coated 300 mesh grids for 1 min. These grids were then stabilised using evaporated carbon film. Utilizing filter paper section, the superfluous sample was eliminated. After accumulation of 20 µL an aqueous solution containing 2% uranyl acetate and permitting it to stand for a few seconds, the samples were adversely stained. The samples were dried at room temperature overnight before being examined using a TEM (JEOL 100CX, Tokyo, Japan) performed at 80 KV [23].

X-ray Diffraction study

The X-ray scattering was measured on lyophilized ETO with a Philips PAN analytical expert (X-ray diffractometer D8A, Bruker), utilizing a filtered Cu Ka α energy source (1.542 Å); the scanning rate was 5°C/min. The sample was investigated among the angle of 2 and 50° (2θ) Using 30 kV and 30 mA voltage and current, respectively [24].

Differential Scanning Calorimetry (DSC)

A (DSC, Mettler Toledo Ltd. USA) was used to establish an optimized ETO thermal analysis. For the calibration of the instrument, an indium standard was used. The 2 mg sample was weighed precisely before being placed in a DSC aluminium pan with a volume of 50 µL and a surface of 0.1 mm. The empty pans were utilised as a reference point, while the incorporated pans were enclosed with an aluminium cover pressurised over them. The lyophilized ETO and reference were heated from 25°C to 165 °C at a rate of 10°C/min after being equilibrated at 25 °C for 5 min The material was purified using a stream of dry nitrogen flowing at a rate of 20 mL/min [25].

Fourier-Transform Infrared Spectroscopy (FTIR) Study

The chemical interference between the excipients and the medicament incorporated in the ethosomal formulation was determined using FTIR. The 3 mg of formulation was precisely weighed, combined with IR-grade potassium bromide, compacted into discs, and examined on a (FTIR-Alpha Bruker, Berlin, Germany). The scanning was done at a resolution of 0.48-1.93 cm^{-1} in 4000-400 cm^{-1}[26].

Optimization and reformulation of ethosomal batches using Factorial design

All batches of ethosomal formulation were further optimized on the basis of the above evaluations and with the help of software Optimization design expert version 8.0.7.1. The optimized batch as reformulated by the cold method and finally, it was integrated into a gel base to get the ethosomal gel discussed in the below section. Optimized batch of ethosomes was prepared using Soya-lecithin and the amount of Silymarin (1%w/v) was kept constant.

Integration of optimized ETO into the gel base

The gel was prepared using the cold technique (Table 2), which included continuously stirring a given quantity of carbapol 934p into cold distilled water. The mixture became a clear solution overnight by storing the dispersion at 4°C in the refrigerator. Then after, 5-15 mL of glycerine was incorporated to the above solution, and the pH was balanced to 6.8 – 7.4 by adding 0.5 mL of Triethanolamine. After forming a transparent gel base, and after lyophilization of ETO dispersion, 12 mg the optimized ETO powder (equivalent to 10 mg SM) was mixed in gel-based prepared from carbapol 934p (0.5 % w/v) to get 1:10 ratio of SM: gel (0.1% w/w SM gel). Simultaneously, Propyl Paraben 0.05 gm. and methyl Paraben 0.18 gm were added as a preservative into the gel formulation and stored at 4°C for further evaluation [27].

Table 2. Formulas of the ethosomal gel.

Ingredients	G_1	G_2	G_3	G_4	G_5	G_6
ETO (mg) = 10 mg SM	12	12	12	12	12	12
Carbopol (gm)	0.5	0.5	0.5	0.5	0.5	0.5
Glycerin (ml)	5	5	10	10	15	15
Triethanolamine (ml)	0.5	0.5	0.5	0.5	0.5	0.5
Propyl Paraben (gm)	0.05	0.05	0.05	0.05	0.05	0.05
Methyl Paraben (gm)	0.18	0.18	0.18	0.18	0.18	0.18
Water	q.s	q.s	q.s	q.s	q.s	q.s

Characterization of ethosomal gel

The pH of the ethosomal gel was ascertained on a digital pH meter (Mettler Toledo Ltd. USA). The spreadability of the ethosomal gel was measured by pressing 0.5 gm of the gel using a glass plate for 5 min, where the diameter of the gel after spreading was documented. The drug content was performed to ensure its homogenous distribution in the developed gel. The gel was diluted adequately with phosphate buffer pH 7.4 1:10 (v/v), and its spectrophotometric absorbance was examined at 286 nm [28].

Determination of viscosity

The rheological performance of the ethosomal gel was explored to detect its appropriateness for application. A (Brookfield DV+II model LV) viscometer was employed to test the gel viscosity at 1.5 rpm and 25 °C [29].

In-vitro diffusion study

In-vitro diffusion test was performed utilising Franz diffusion cells (orifice diameter 0.9 cm; Perme Gear Inc. Hellertown, PA, USA). Before assembling in Franz-type diffusion cells, Synthetic cellophane membrane with molecular weight cut off 10,000–12,000 was rehydrated by immersing in phosphate buffer (PB), pH 6 (60 mL). A glass chamber's membranes were situated in the space between its two cell halves. A clamp maintained the two chambers interconnected. To ensure sink conditions, the receptor chamber of the cell comprised 5 mL) of PB pH 6 that was agitated by a magnetic pole at 500 rpm and thermostated at 32±1 ℃ throughout the studies. At periods up to 450 min, 3 mL of the specimen was evacuated from the receiver chamber, and the same volume of new buffer solution was added to retain the receptor compartment at sink circumstances. The specimens were diluted with phosphate buffer to a concentration of 10 mL, then spectrophotometrically examined at a wavelength of 286 nm to determine the cumulative percent drug release (SM) over time [30].

In-vivo percutaneous permeation study

Confocal laser scanning microscopy (CLSM) experiment was accomplished employing a confocal laser scanning microscope (Leica, DMIRE2, Germany). The study's protocol was approved by the Iraqi Center for Cancer Research/Mustansiriyah University's animal ethical council (approval no. ICCMGR2020-016). Wistar rats were used in the *in-vivo* drug permeation investigation to examine how drugs permeate the body. A contrasting study was conducted between SM ethosomal gel 0.1 % w/w (1:10 ratio of SM: gel) with a conventional market product (Clindamycin Phosphate Gel® USP 1%).

The mechanism of skin penetration of different formulations was assessed using CLSM. Before applying the gel to the rat's skin, dye 6-carboxyfluorescein was employed as a marker and added to the gel mixture. Rats' dorsal skin was exposed to gel formulations for a total of 6 h. The rat was then sacrificed, and dorsal epidermis was removed, washed with a fine stream of pH 7.4 phosphate buffer solution to remove any excess product and transdermal tissue. After that, the skin sample was cut up into smaller pieces. After being dried, the skin was dipped into Carney's solution. After that, CLSM was used to see the fixed skin segments (Leica TCS SP8) [31, 32].

Results and Discussion

Evaluation of ethosomal batches

Physical appearance

The nine batches were observed visually after 24 h. No phase separation was observed at 2-8°C (Figure. 1). Hence developed ethosomal formulation was found to be stable and subjected to further study.

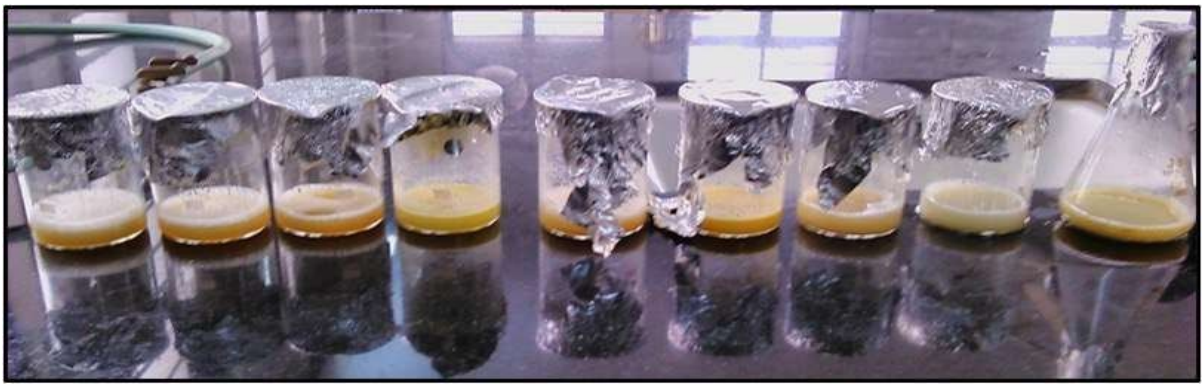

Figure 1. Appearance of prepared ethosomal batches (E1-E9)

Percent drug entrapment efficiency (EE %)

Nine distinct batches of ETO were assembled by varying the excipients concentrations, which affected the drug entrapment. The % EE of all the batches ranges from 64.36 to 89.67 % (Figure. 2). In all batches, the E8 batch showed 89.67 % drug entrapment; hence, it was considered an optimized batch and further evaluations were conducted for E8 batch. The lowest phospholipid concentrations and the highest alcohol deposition (as mentioned in Table 1) exhibited impacts in % EE (E8>E5>E2). The advanced formulations demonstrated that the optimum drug entrapped and greater solubility in phospholipids. As a co-solvent, ethanol makes SM more soluble, allowing for greater SM entrapment in the hydroalcoholic core and vesicle membrane. Additionally, ethanol makes the bilayer membrane more fluid, which improves SM encapsulation [33].

Figure 2. Percent drug entrapment of ETO batches

Vesicle size (VS) and polydispersity index (PDI) of ETO batch (E8)

Photon correlation spectroscopy (PCS) is a technique used to determine the mean VS or Z-average and the width of the VS distribution expressed as PDI. It was discovered that the optimised batch's VS was 168 nm. The lower concentrations of excipients lead to low VS, and higher concentrations result in maximum VS. The PDI was determined using the vesicular average diameter and the distribution variance. A low PDI value assumes high levels of homogeneity within the sample, whereas a high PDI value reflects a diverse size distribution or multiple populations. The PDI of all batches were less than one, indicating a narrow size distribution. The VS and PDI of optimized batch E8 were found to be 168 nm and 0.367 respectively, which were average in all batches (Figure. 3). The vesicles with higher VS indicated higher PDI; thus, reducing VS lead to more homogenous dispersions, which might get efficiently permeated.

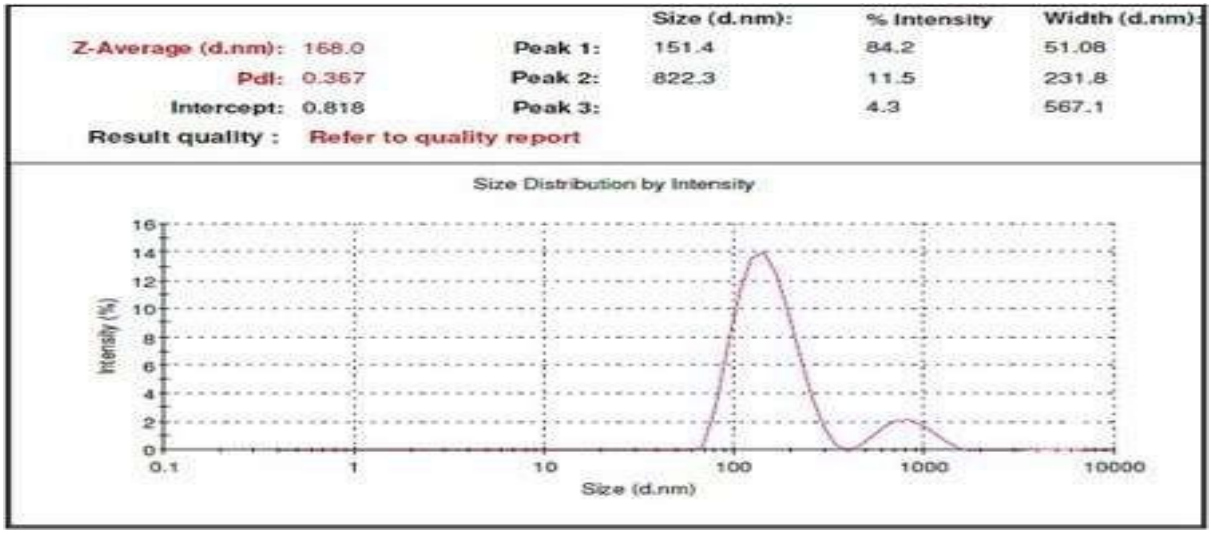

Figure 3. Particle size distribution curve of ETO batch (E8)

Zeta Potential (ZP) of ETO batch (E8)

The ZP represents the degree of vesicular system stability because of the repulsive force between charged vesicles. A greater ZP value indicates that the vesicle has a low ability to aggregate. Zeta potentials greater than +30 mV or more than -30 mV are often regarded as stable formulations [34]. The ZP of the optimized E8 batch was found to be -0.496 mV (Figure. 4), demonstrating the stability of the dispersion of ethosomal formulation

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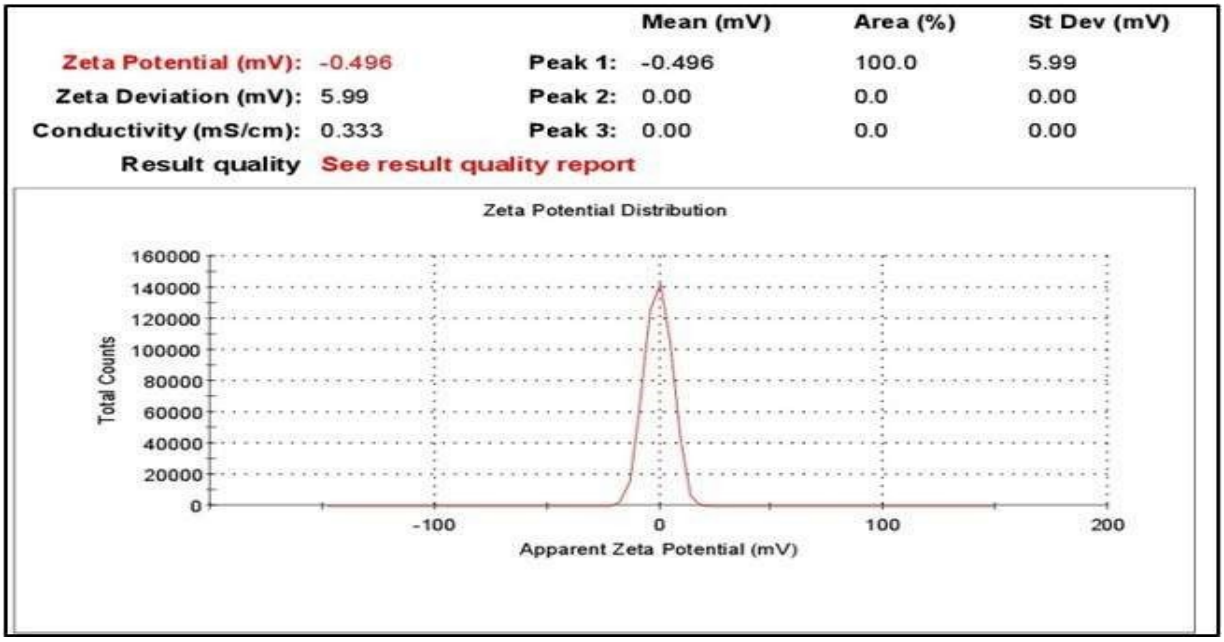

Figure 4. Zeta potential curve of ETO batch (E8)

Transmission Electron Microscopy (TEM) of optimized ETO (E8)

The TEM was employed to achieve the desired morphology and size of the optimized ETO. The TEM micrograph of optimized ethosomal formulation was observed to be unilamellar and multilamellar elongated, and spherical vesicles (Figure. 5). The inclusion of ethanol contributes to a smaller ETO, while the presence of phospholipids contributes to forming a layer enveloped around the drug molecule. The absence of drug crystals in the TEM picture suggested that the drug was encapsulated entirely within the vesicles. It demonstrated nano vesicular properties, which may facilitated its penetration through the epidermis [35].

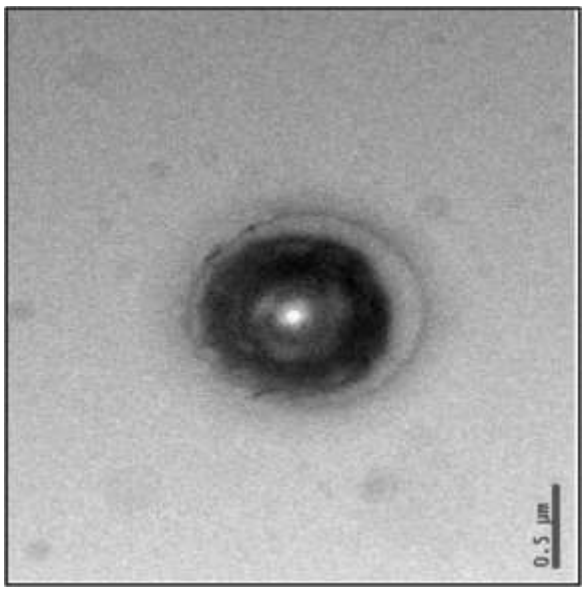

Figure 5. Transmission electron micrograph of ethosomal batch (E8)

X-ray Diffraction study of optimized ETO (E8)

Silymarin's X-ray diffraction pattern reveals its crystalline structure. However, the lack of distinctive drug peaks in the ETO' XRD diffractogram suggests that the drug has been trapped and exhibited a typical XRD pattern of amorphous materials because SM powder was converted into an amorphous form during the ETO formation process as shown in (Figure. 6).

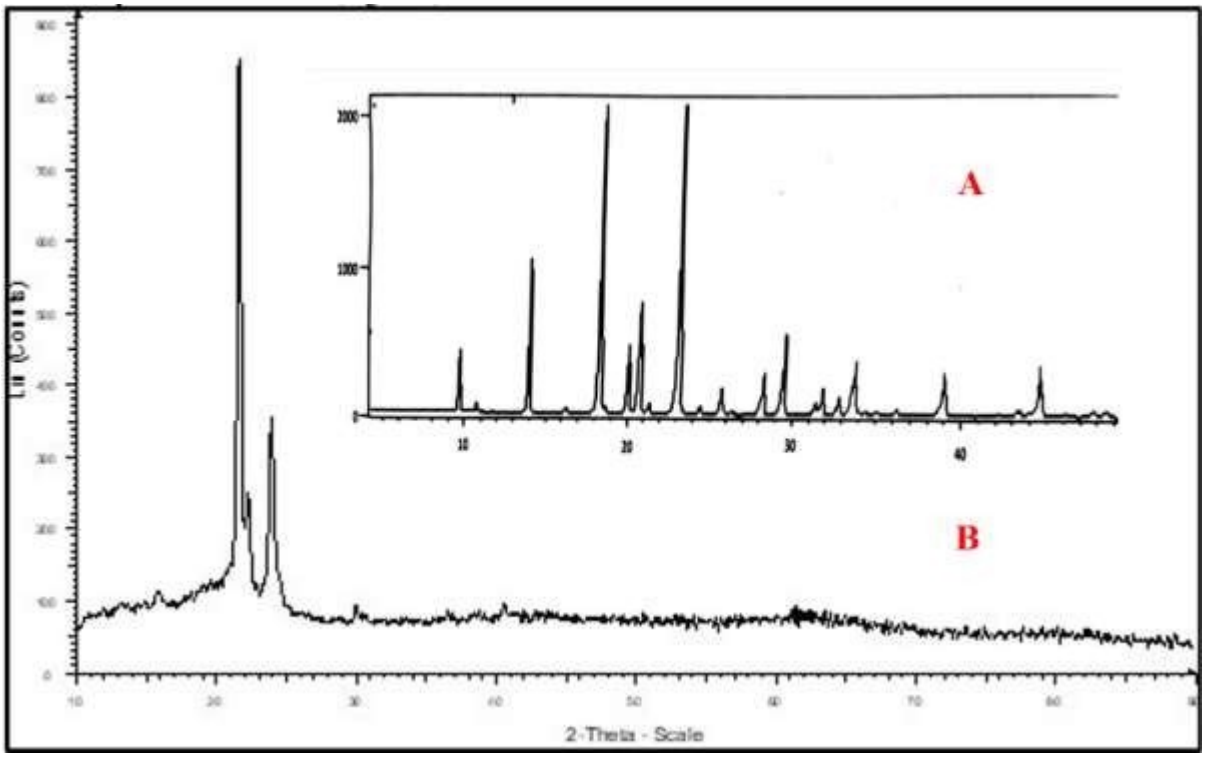

Figure 6. X-Ray diffractogram of (A) SM, and (B) SM-ETO batch (E8)

Differential Scanning Calorimetry (DSC) of SM-ETO (E8)

The melting temperatures of SM and its crystalline character are shown by the large endothermic peaks of pure SM at 75 °C and 146 °C (33) (Figure 7A). By using DSC analysis, the thermotropic behavior of SM-ETO was determined. Two prominent wide peaks were seen in the optimized batch E8. two minor peaks at 93.06° C and 145.09° C, as well as at 179.57° C and 229.76° C (Figure 7B). The characteristic peaks of SM were absent from the SM-ETO thermal picture. The physical interaction between SM and soy lecithin in ETO, most likely due to a hydrogen connection between SM and the soy lecithin's polar head, may help to explain this (36).

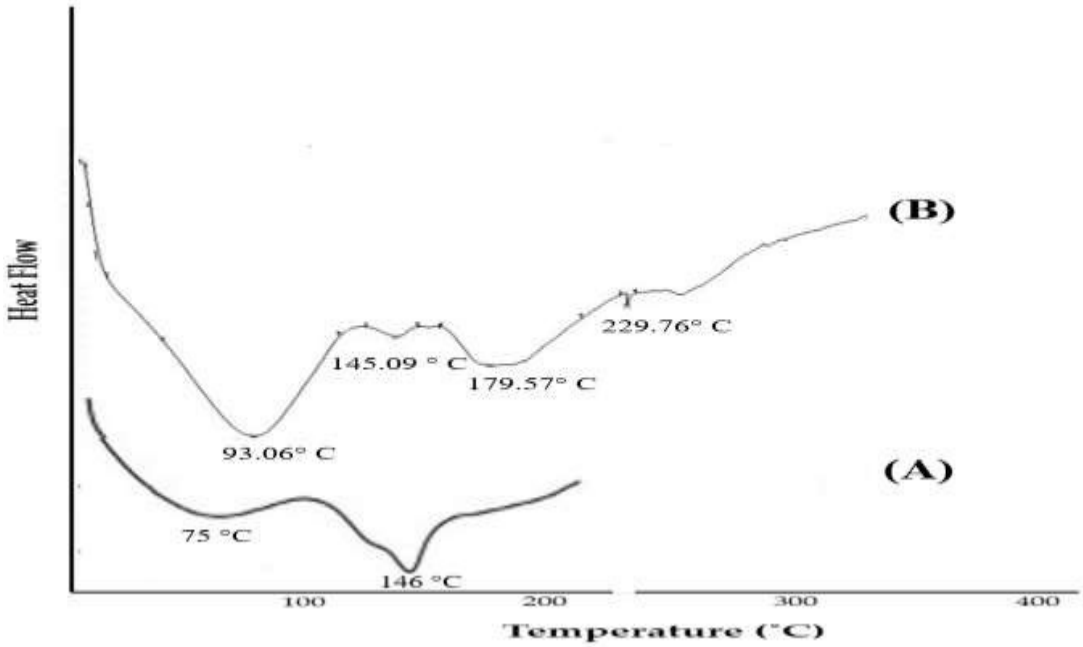

Figure 7. DSC thermogram of (A) SM, and (B)ETO batch (E8)

Fourier-Transform Infrared Spectroscopy (FTIR) of optimized SM-ETO (E8)

The FTIR analysis of an optimized E8 batch was utilized to evaluate any interaction between SM and excipients during ethosomal preparation. The FTIR spectrum of ethosomal formulation of SM shown in (Figure. 8) was characterized by aromatic ring present and 3426.89-2932.23 cm^{-1} (Alcoholic – OH bonds present) and the absorption peak between 3426.89-2932.23 cm^{-1} due to presence of alcoholic - OH, was found to be at lower intensity which may be due to entrapment of SM by phospholipids. This FTIR spectra of ETO suggested that there was not any interaction among the excipients of formulation.

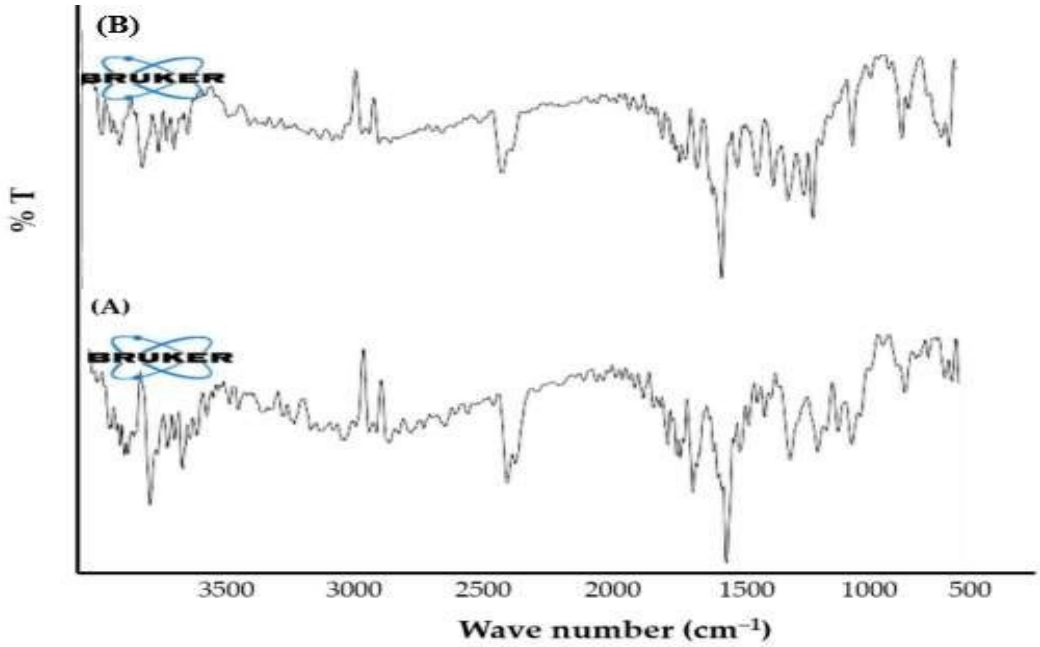

Figure 8. FTIR spectrum of (A)SM, and (B) Optimized SM-ETO (E8)

Optimization and reformulation of ethosomal batches using factorial design

Design Expert Software version 8.0.7.1 makes it easier to plan and analyse multi-factor tests. There are many different designs in the software, including factorials, fractional factorials, and composite designs. In situations where standard designs are not appropriate or we want to modify an

existing design to match a more flexible model, Design-Expert offers computer-generated D-optimal designs. Based on initial experimental batch results where the concentration of soya lecithin (SL) and of SM (1%) was kept constant. The effects of the three independent variables (factors, X)—the synthesis's sonication intensity (X1), sonication time (X2), and % drug release (Y1), vesicle size (Y2), and entrapment efficiency (Y3)—on the dependent variables (responses, Y) were investigated using a 2-factor, 3-level factorial design. Three actual and coded levels of the effects of independent variables were examined. The codes +1, 1, and 0 represent the larger, lower, and intermediate values of each component, respectively. Two statistical measures for which Design-Expert was consulted were the regression coefficient (R2) and analysis of variance.

The best-fitting model was provided by the program for comparison. Since batch E8 displayed the observed (actual) values for drug release (Y1=75%), uniform droplet size (Y2=168 nm), and entrapment efficiency (Y3=89.67), all of the batches of ethosomal formulations E1–E9 were compared based on the aforementioned evaluations. In contrast, batch E8's predicted (theoretical) values were as follows: Y1 of 77.122% as shown in Figures 9a and 9b, Y2 of 169 nm, and Y3 of 91. All of the results demonstrated that the predicted and actual results agreed well. Furthermore, the created models' validity and repeatability were reinforced by the relative error percentage between observed and projected values being less than 5%.

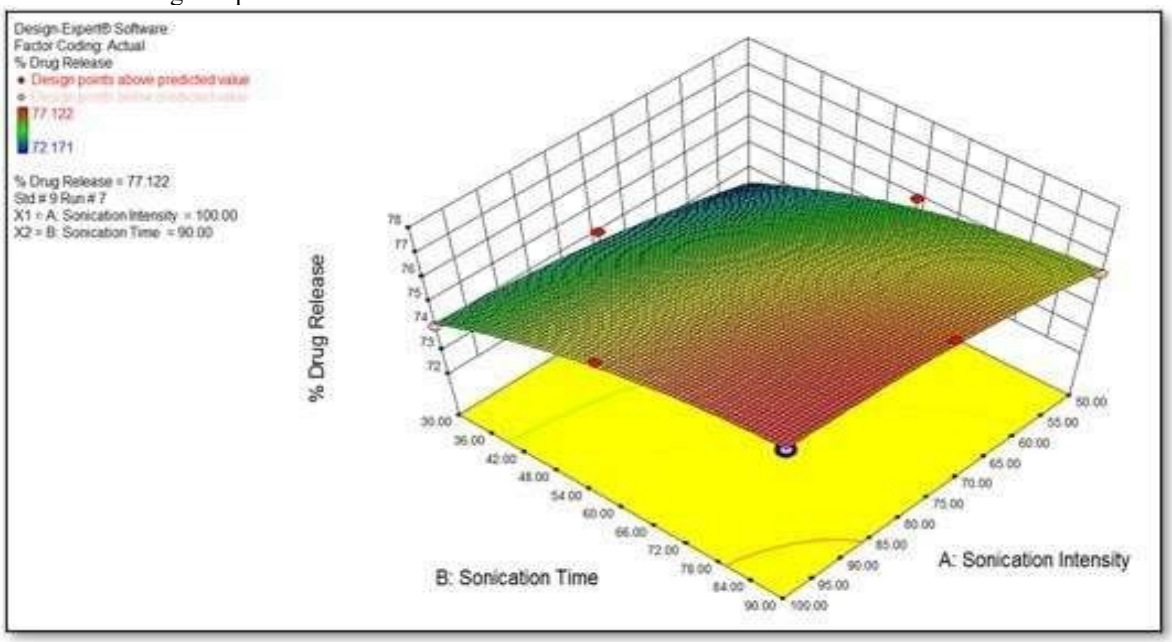

Figure 9a. Contour Plot (Response surface plot) of % Drug Release (Y1)

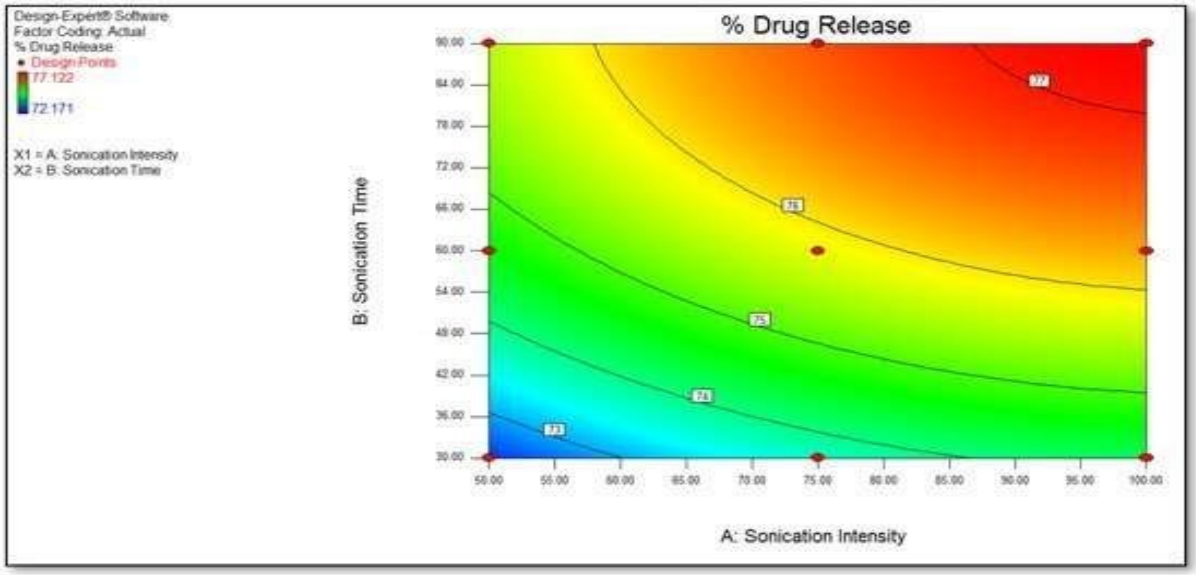

Figure 9b. Response surface plot showing the effect of formulation variables on % Drug Release (Y1)

Properties of ethosomal gel

The six batches of gel formulation containing carbapol 934 P were successfully synthesised with the integration of optimized SM (E8) (1% w/w). The gel was analysed for the corresponding attributes. The pH of ethosomal gel was essential for the application to the target site because the formulation's high pH may damage the skin. The pH of ETO incorporated gel was found to be 6.94 to 7.41. The G_1 and G_4 showed ideal pH ranges of 6.95 and 6.94, respectively, correlated with skin pH (Figure. 10).

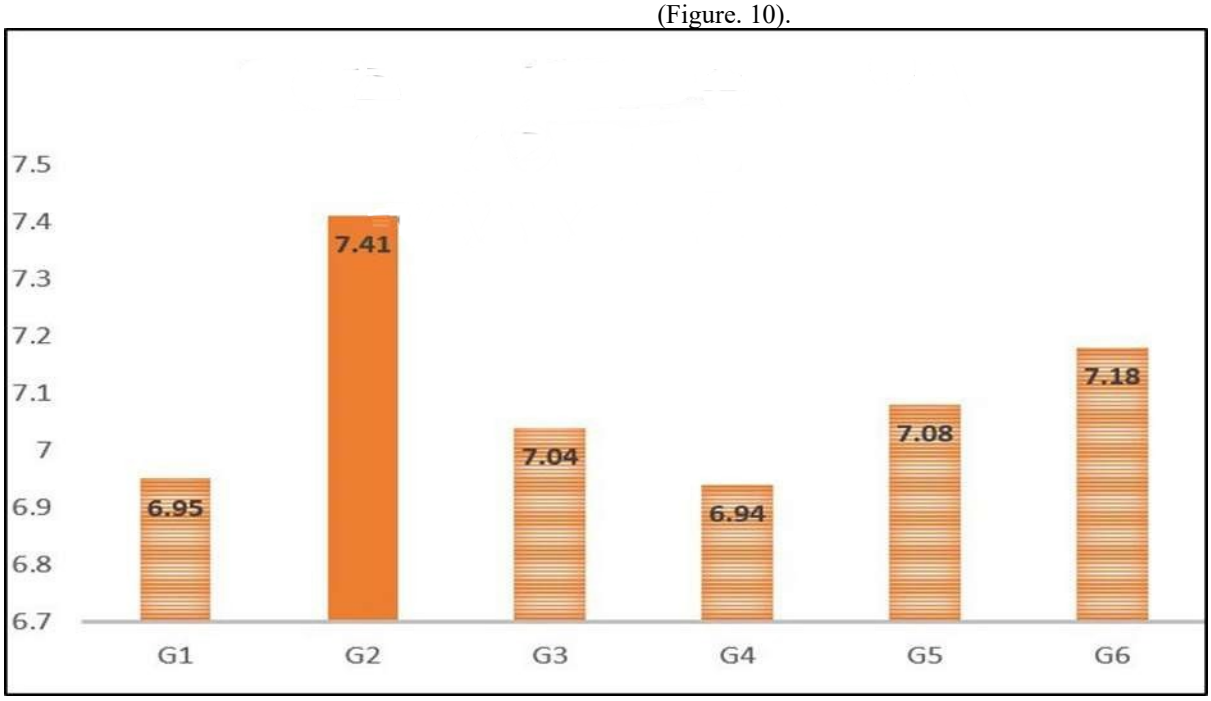

Figure 10. pH of ethosomal gel batches

The spreadability of all given formulations was found to be the best for application & within limits (20.00 to 30.00 g.cm/sec) [37] as shown in the Figure. 11. Ethosomal gel spreadability exhibited a significant spread time of 25.71 gm.cm/sec for G6, while better spreadability will result from faster slide separation and G3 showed ideal spreadability in less time (22.14 gm.cm/sec) which was determined to be satisfactory for topical application.

Figure 11. Spreadablity of ethosomal gel batches

In addition, gel formulation displayed good drug content in the range of 96.32% to 98.45% (Figure. 12). This demonstrated that optimized ETO was dispersed homogenously throughout the gel formulation.

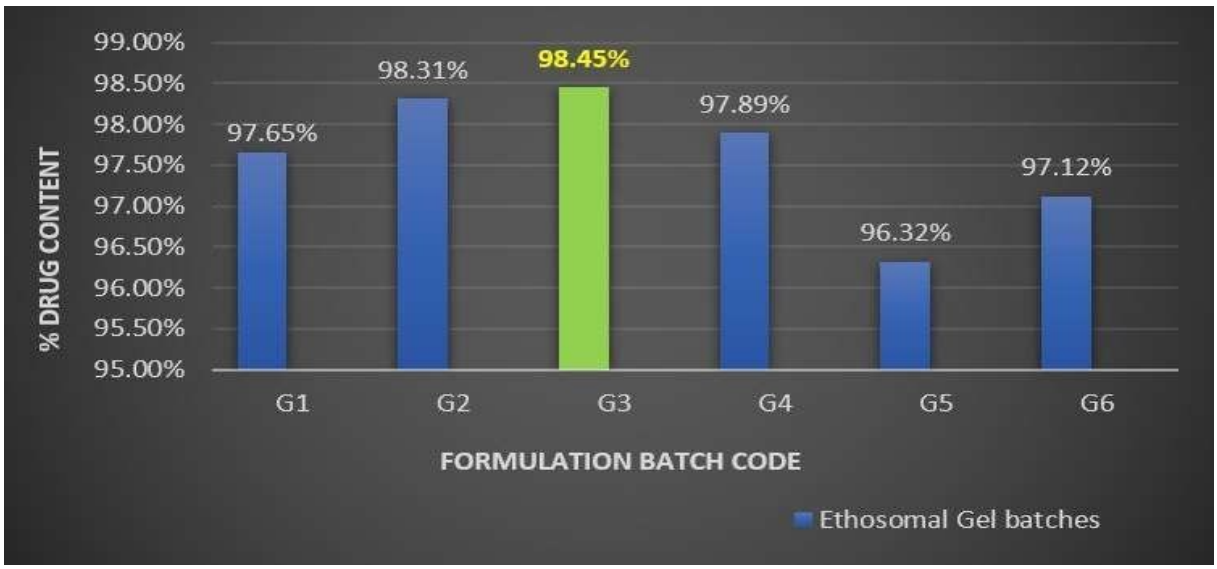

Figure 12. Percent drug content of ethosomal gel batches

Viscosity of SM-ETO gels

The determination of the rheological properties of the SM-ETO gel is required for TDDS since it is a prerequisite for the ability of the gel to adhere to the skin. The viscosities of ethosomal gels were found to be within the limits (0-10 pa. s) [38] (Figure. 13). From all batches, G4 showed maximum viscosity as 9.187 (pa.s); hence it was considered as ideal viscous batch.

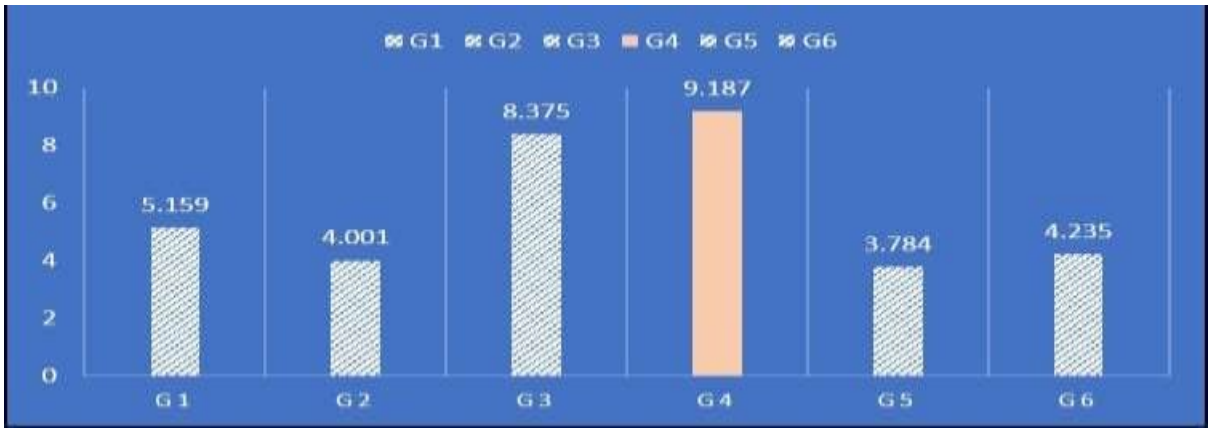

Figure 13. Viscosity of ethosomal gel batches

In-vitro diffusion of SM-ETO-loaded gel

By using an in-vitro Franz cell system, the effectiveness of drug release from SM-ETO gel using a synthetic membrane was compared. IVRT was used as a screening technique to assess the efficiency features of many formulations while creating a topical medication delivery system. This makes it more likely that the finished product will have the intended effects. The proportion of SM that passes through the synthetic membrane from the ETO-loaded gel was assessed for a maximum of 450 minutes (Figure 14).

With characteristics of 96, 97, 94, and 98, respectively, the results showed that the G1, G2, G5, and G6 batches had a higher significant proportion of drug release than the other batches in 450 minutes, which was good. The presence of ethanol, which is essential for improving drug transport into deep epidermal layers, may be the cause of the apparent increase in release (39).

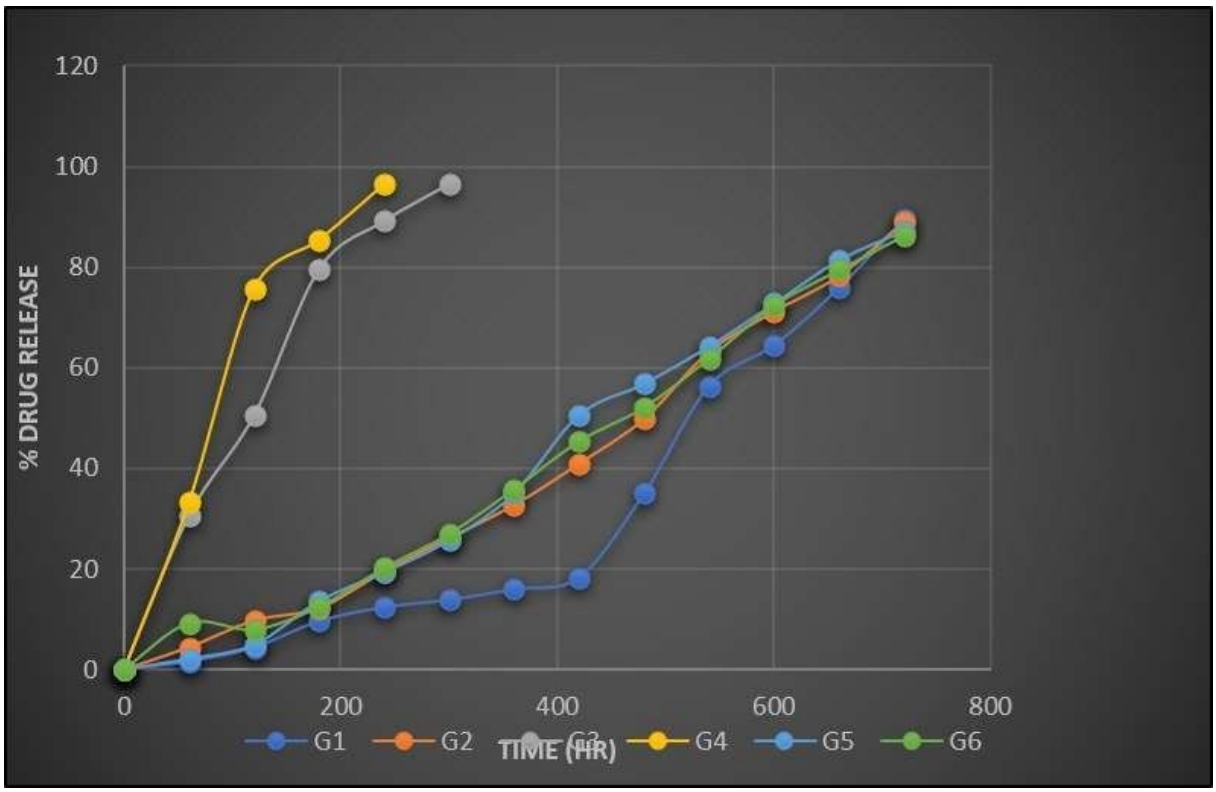

Figure 14. ***In-vitro*** **drug diffusion from ethosomal gel**

In-vivo percutaneous permeation of ETO gel by CLSM

The development of topical formulations must consider the drug's ability to penetrate the skin because the intended impact is to affect the skin's outermost layers. The CLSM result showed that ETO gel of 0.1 % w/w (1:10 ratio of SM: gel) showed superior permeability to the market sample gel (1% w/w clindamycin® gel) and the prepared formulation was evenly dispersed in the deeper layer of skin and readily penetrable by deeper layers. However, because of skin deposition, the traditional gel was unable to penetrate the stratum corneum barrier. The CLSM images are showed in Figures. 15a and 15b.

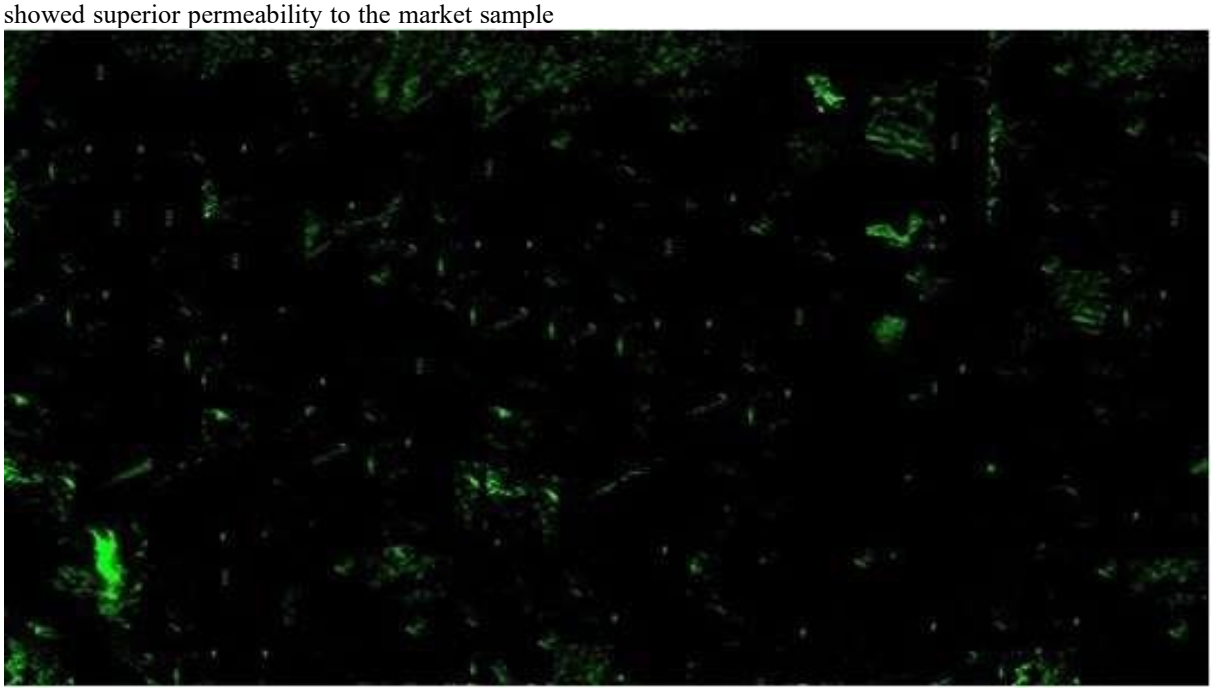

Figure 15a. Confocal Laser Scanning Microscopy (CLSM) image of 0.1 % w/w ethosomal gel formulation treated Wistar rat skin

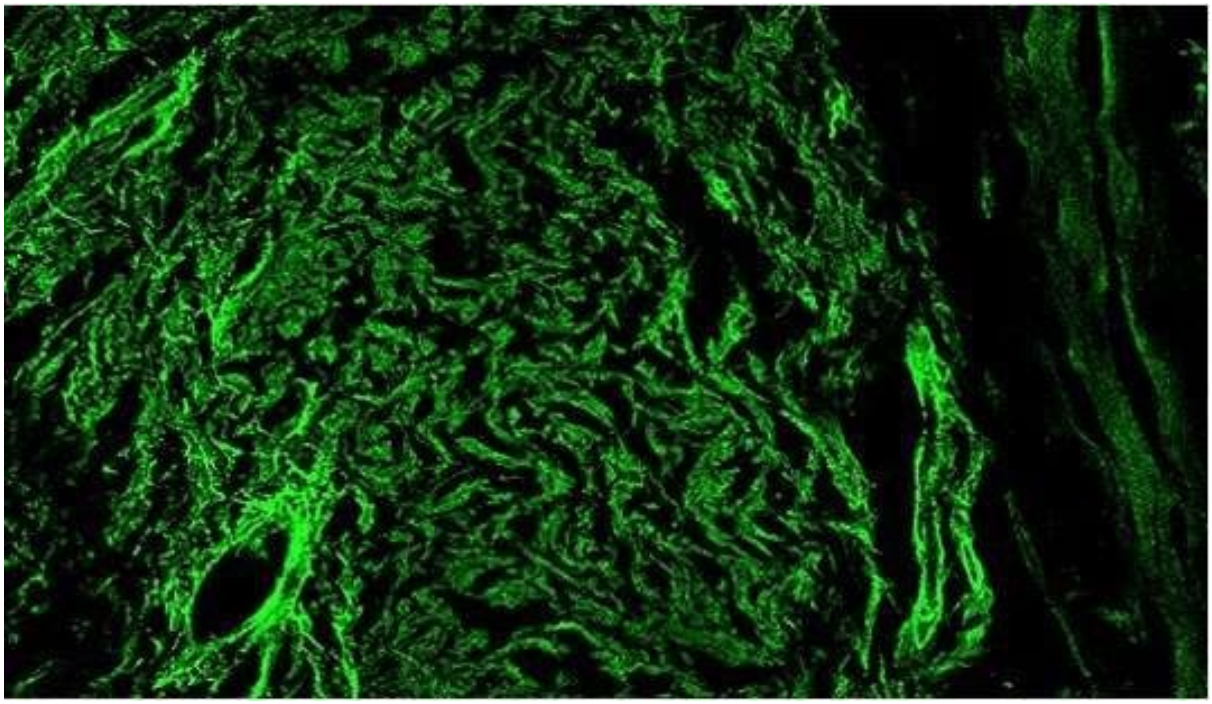

Figure 15.b. Confocal Laser Scanning Microscopy (CLSM) image of 1% w/w Clindamycin market sample gel treated Wistar rat skin

Conclusions

The SM-ETO was successfully created and refined to create nanovesicles with increased entrapment efficiency. The Carbopol 934p gel for transdermal distribution successfully integrated the optimized SM-ETO. The capacity of SM-ETO-based gel to maintain SM release for 450 minutes (7.5 hours) was shown by drug diffusion tests. An in-vivo pharmacokinetic release study might potentially generalize this conclusion. With the continuous release, this novel method may effectively provide SM in a non-invasive manner. Furthermore, when it came to medication penetration into the epidermis, ethosomal gel outperformed a traditional formulation. The SM-ETO loaded gel is a novel approach that is highly practical, reasonably priced, and time-efficient, according to the successful and similar results of the present study. Future studies are required to examine the possible use of this formulation in an in-vivo model.

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