https://doi.org/10.31351/vol33iss4pp126-140

# Formulation and Development of Ethosomal Drug Delivery System of Silymarin for Transdermal Application

**Hassanien Sagban Taghi[1], Anmar A. Issa[2] and Yasir Qasim almajidi [*,3]**

[1]Department of Pharmaceutics, College of Pharmacy, Al-Bayan university, Baghdad, Iraq.
[2] Department of Pharmaceutics, College of Pharmacy, Esraa university, Baghdad, Iraq.
[3] Department of Pharmaceutics, College of Pharmacy, Al-Nahrain university, Baghdad, Iraq, Baghdad, Iraq.

[*]**Corresponding author**
Received 1/8/2023, Accepted 17/10/2023, Published 20/12/2024



## Abstract

Silymarin (SM), a natural polyphenolic flavonoid, shows antidiabetic and lipid-lowering characteristics with poor aqueous solubility and bioavailability. The present research aimed to develop an anti-psoriatic gel formulation of silymarin. This research work would be an effort towards minimizing the pain and agony of people with psoriasis. In the current investigation, SM-incorporated ethosomes (ETO) were designed and optimized by the cold method applying $3^2$ full factorial design to overcome these pitfalls. The SM-ETO were synthesized and evaluated to determine the physical appearance, percent drug entrapment, size distribution, negative charge potential, morphology study, powder crystallinity and phase transition behaviour. Following optimization, SM-ETO were added to a gel containing carbapol 934p and examined for pH, rheology study, drug content and *in-vitro* drug release study. The results manifested that SM-ETO batches did not show phase separation at 2-8°C. The batch E8 exhibited 89.67% drug entrapment, 168 nm vesicular size, 0.367 polydispersity index and -0.49 mV zeta potential. A morphological study revealed elongated spherical vesicles. X-ray diffraction study exhibit the amorphous nature of SM powder. A formulated gel revealed significant pH range of 6.94 to 7.18. It also displayed 9.187 (cp) viscosity, and 96.32 to 98.45% drug content. *In vitro* drug release showed 96,97,94, and 98 % SM release from gel batches. The comprehensive findings explored the enhanced solubility and bioavailability of the developed gel suggesting its potential as a nanocarrier in delivering SM for future clinical applications. Conclusively it can be stated that: With the aid of formulation development technology ethosomal gel formulation of Silymarin has been successfully developed

**Keywords: Ethosomes, Gel, Silymarin, Topical application, Transdermal drug delivery.**

## Introduction

Transdermal drug delivery systems (TDDS) have been invented to deliver medications for many years. The transdermal bioactive agent penetrates in systemic circulation after passing through the epidermal layer [(1)]. The drug is then transported through the bloodstream to the entire body to exert its pharmacological action [(2)]. The transdermal route of administration has advantages over other methods, including the ability to avoid first pass hepatic metabolism, prolong the period of the drug's action, reduce side effects, improve pharmacological action, reduce fluctuations in drug ratios, and increase patient convenience [(3)]. Most importantly, TDDS can be successfully used when drug therapy is required for long-term or chronic use. Consequently, it is a viable option to develop TDDS for the treatment of a number of pathological conditions, including diabetes. However, because the stratum corneum acts as a barrier to the permeating substances, transdermal therapy is only effective with certain kinds of bioactive agents [(4)].

The purpose of nano-formulations has evolved as a feasible method of circumventing the constraints of transdermal treatment [(5)]. Nano-formulations have been regarded as the best TDDSs because of their advantages in terms of small particle dimension, improved drug absorption, and targeting. As a result, a variety of strategies have been developed to improve the TDD of bioactive compounds employing nanoparticulate delivery systems like liposomes, transferosomes, ETO, dendrimers, and microemulsions [(6)]. Since the 1980s, liposomes have been extensively studied as one of the TDS. Nevertheless, liposomes are restricted to the top layer of rat skin and do not permeate deeply [(7)]. Ethosome (ETO), from the other hand, are a creative liposome variant that contain phosphatidylcholine and a significant amount of water when combined with ethanol (20–45% v/v). Increased malleability, solubility, prolonged stability, bioavailability, and the capacity to entrap insoluble molecules are all benefits of the high ethanol content [(8)]. ETO may

have a smaller size and more stability due to their relatively high negative surface charge.There has been a recent rise in significance in using herbal medicines to treat a variety of diseases. Silymarin (SM) a polyphenolic flavonoid that originates naturally from milk thistle seeds (Silybum marianum L.); its primary active component is silibinin (Silybin)[9]. SM is very well hepatoprotective drug that has demonstrated antioxidant, anti-inflammatory/immunomodulatory, and antifibrotic characteristics in innumerable *in-vitro and in-vivo* animal models [10, 11]. Recent research on animal models has suggested that SM might have prospective lipid-lowering and anti-diabetic properties [13, 13]. Indeed, numerous studies have demonstrated that the ineffectiveness of SM as a therapeutic agent for some diseases may be hampered by both its low intestinal permeability and poor aqueous solubility/bioavailability in conjunction with both extensive metabolism, damage by gastric enzymes, and rapid excretion [14]. Hence to overcome these pitfalls in this study, attempts have been devoted towards increasing the solubility, bioavailability, and permeability of SM by synthesizing SM-ETO and incorporating them into gel formulation for maximum drug release in TDDS. SM-ETO was initially designed and optimized by the cold method applying $3^2$ full factorial design. The word "optimization" comes from the verb "optimize," which means to make anything as excellent, useful, or efficient as possible. When referring to pharmaceutical preparations or pharmaceutical processes, the term "optimized" was previously used to imply that a product had been improved to meet the desired goals of a development scientist. Optimization is a technique for finding the ideal composition or experimental conditions. The use of systematic methods to achieve the best possible combination of a product's and/or process's features under a certain set of constraints is what is commonly referred to as optimization. It may also be said that optimization involves selecting the best component from a range of accessible alternatives [15]. The most popular technique is factorial design (FD), often known as experimental designs for first degree models. A design of experiments (DOE) is most easily set up by taking two or more variables (n) and testing them at various levels. All factors are joined with one another on all levels in a full factorial approach, and the number of experiments is $f^n$, where f is the factor and n is the level. Nine experiments are used in the $3^2$ full factorial design, 16 in the $4^2$ and 25 in the $5^2$. The number of experiments increases to $3^3$, $4^3$, and $5^3$ when the level is 3. Of course, the number of experiments grows and exceeds what is reasonable. To reduce the number of tests, the levels often considered are 2 levels. The design is said to be symmetric if each factor has the same number of levels, for example, $2^2$, $3^3$, etc. The design is referred to as asymmetric if the number of levels differs from the factor, for example, $2^3$, $3^2$, etc [16]. Afterwards, formulated SM-ETO batches were characterized for physical appearance, percent drug entrapment, size distribution, negative charge potential, morphology study, powder crystallinity and phase transition behaviour. Based on these evaluations and the desirability value obtained from Design-Expert software version-7, the optimized batch was selected and subjected to further study. The formulation and development of novel drug delivery systems have revolutionized the field of pharmaceutical research and development. One such innovative approach is the use of ethosomal drug delivery systems, which have garnered considerable attention for their potential in enhancing the therapeutic efficacy of various drugs. Silymarin, derived from milk thistle, has shown promise in treating various skin conditions, including psoriasis. The ethosomal system's unique lipid-based nanocarriers enable efficient skin penetration, ensuring improved drug delivery and bioavailability. This introduction sets the stage for a comprehensive exploration of the formulation and development of silymarin-loaded ethosomal drug delivery systems for topical application, highlighting the potential benefits and contributions of this research in the realm of pharmaceutical science and dermatology. An essential perspective in developing a transdermal delivery system is the permeation of formulation from the application site. Hence the developed ETO formulation was loaded in a gel formulation and evaluated for pH, rheology study, drug content and *in-vitro* drug release study. The details of the adopted methodology and outcomes of the study are explored in the subsequent section.

## 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 ℃ 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 ℃, 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℃ 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.

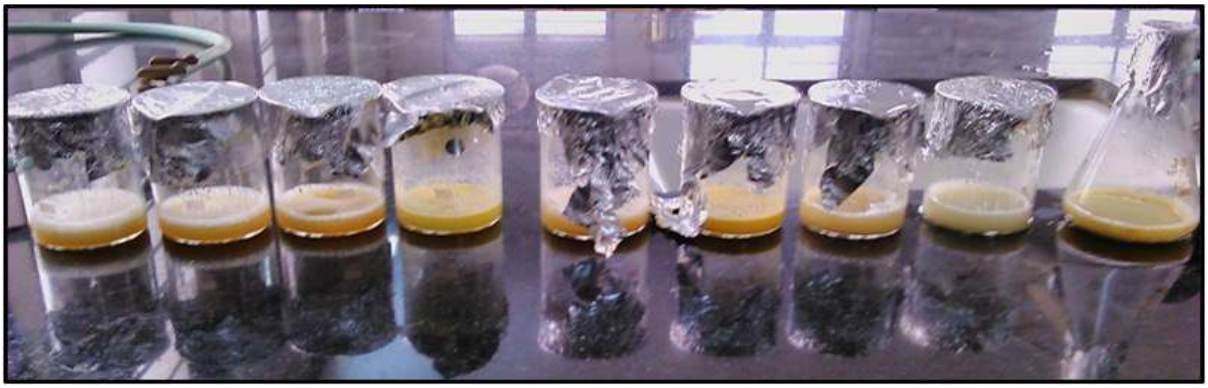

**Figure 1. Appearance of prepared ethosomal batches (E1-E9)**

#### *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].

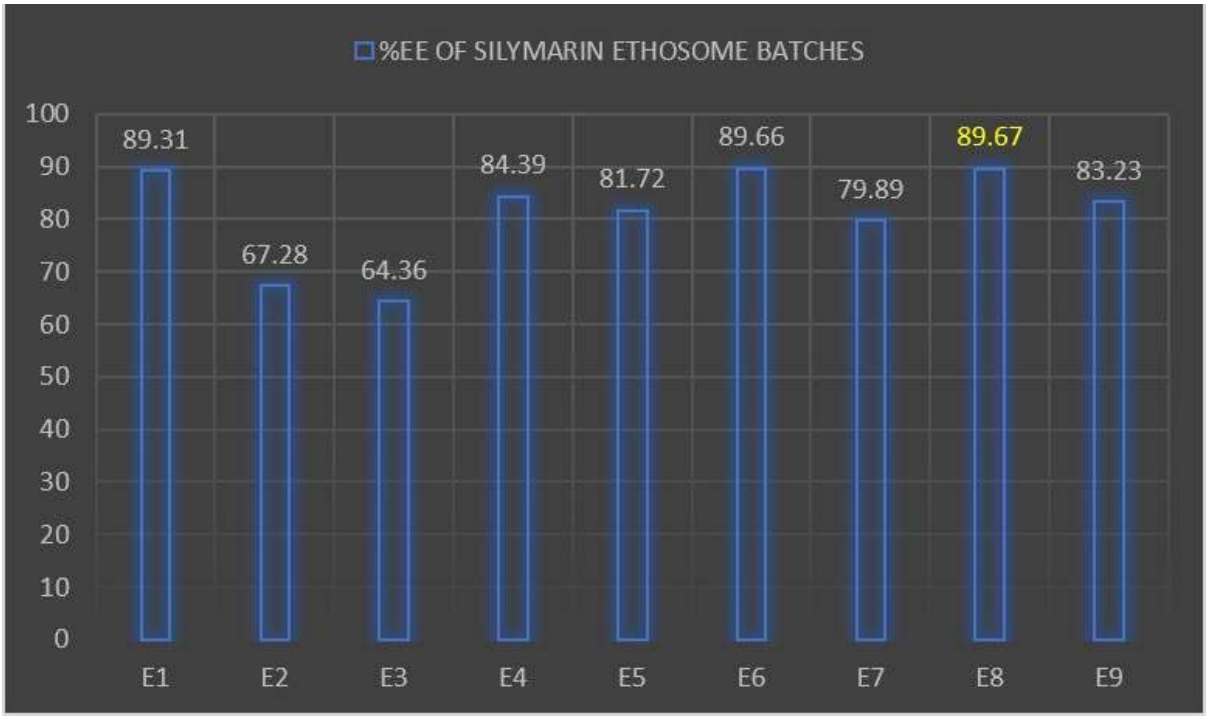


**Figure 2. Percent drug entrapment of ETO batches**

***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.

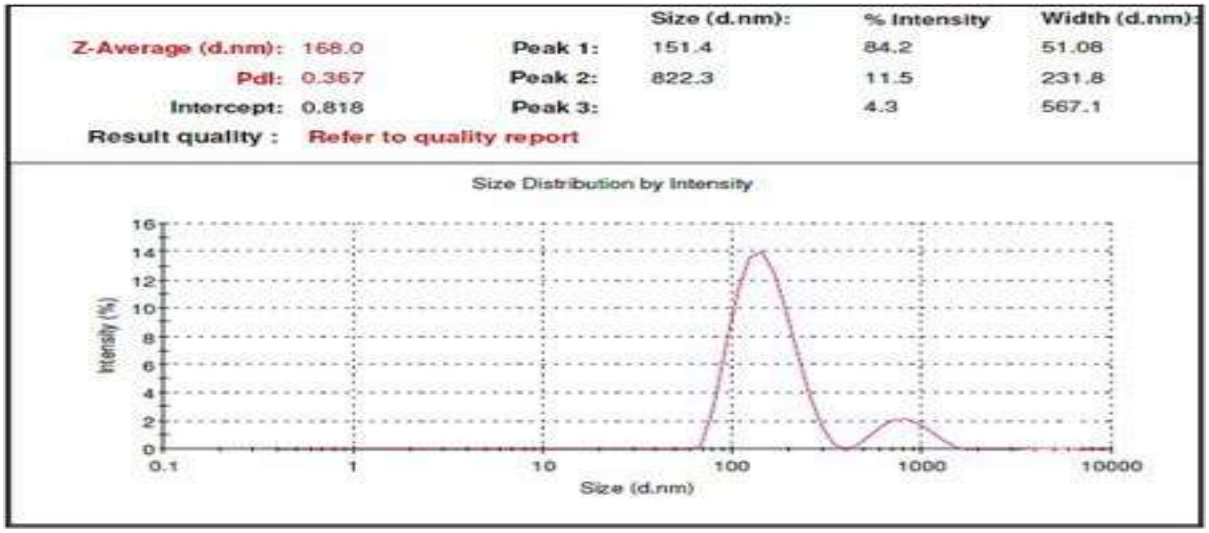


**Figure 3. Particle size distribution curve of ETO batch (E8)**

***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 [31]. 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

.

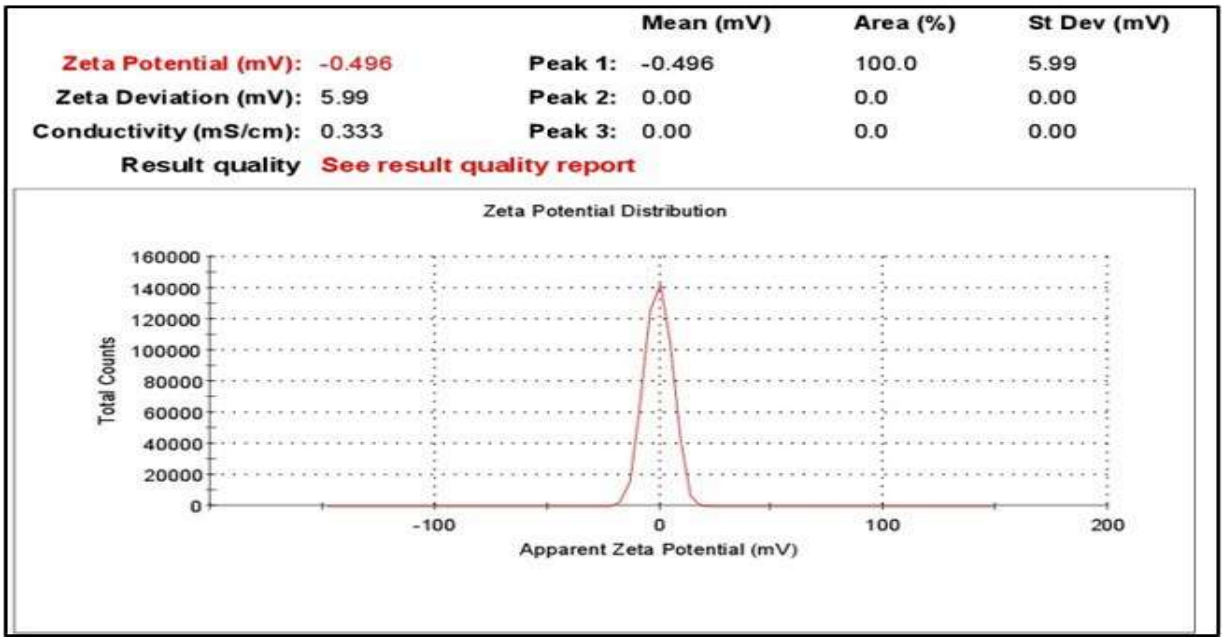


**Figure 4. Zeta potential curve of ETO batch (E8)**

***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].

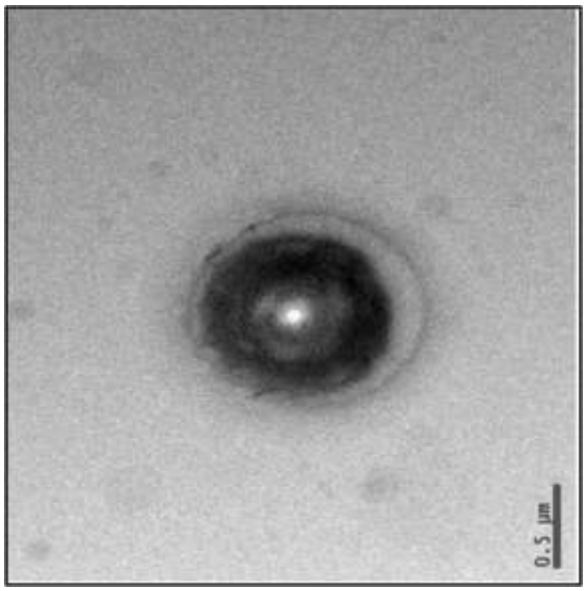


**Figure 5. Transmission electron micrograph of ethosomal batch (E8)**

***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).

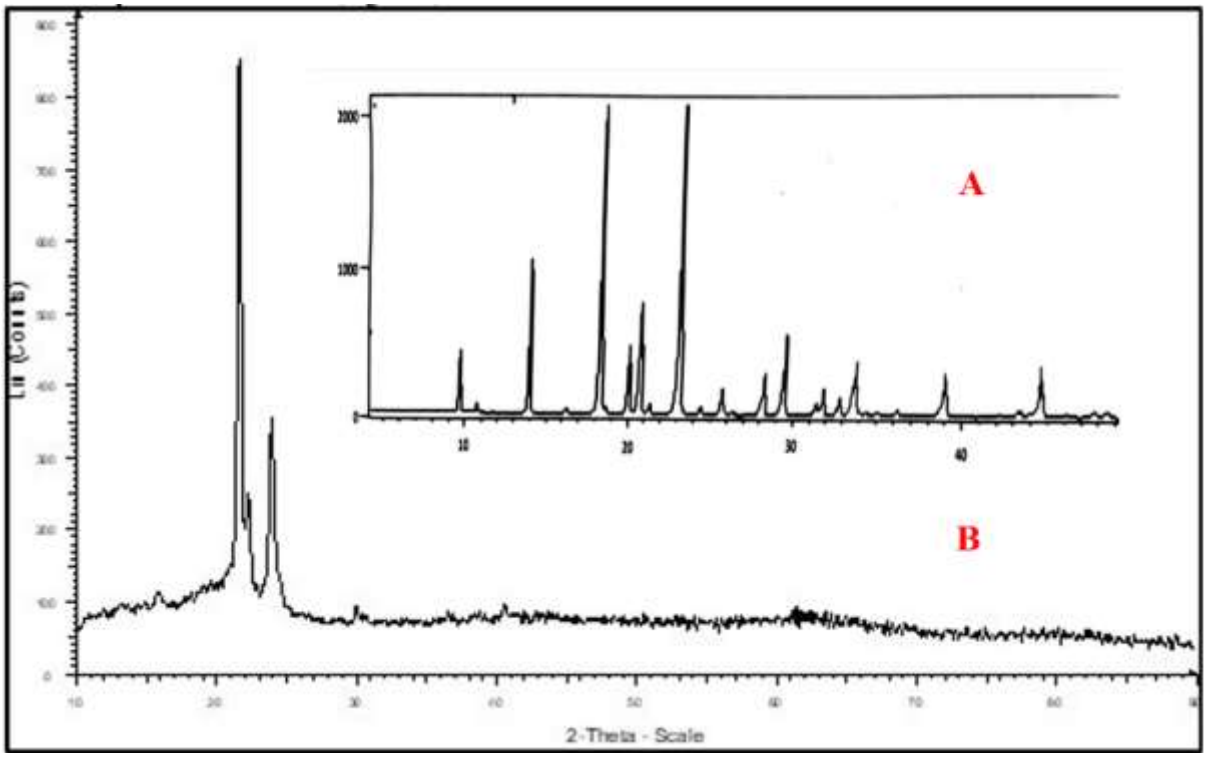


**Figure 6. X-Ray diffractogram of (A) SM, and (B) SM-ETO batch (E8)**

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

The broad endothermic peaks of pure SM at 75 °C and 146 °C, which correspond to the melting temperatures of SM and its crystalline nature [33] (Figure. 7A). The thermotropic behaviour of SM-ETO was ascertained by DSC examination. The optimized batch E8 exhibited two major broad peaks at 179.57° C and 229.76° C and two small peaks at 93.06° C and 145.09 ° C (Figure. 7B). The thermal image of SM-ETO revealed that the distinctive peaks of SM were not present. This may be explained by the fact that SM and soya lecithin interact physically in ETO, most likely by hydrogen bonding between SM and the polar head of the soya lecithin [36].

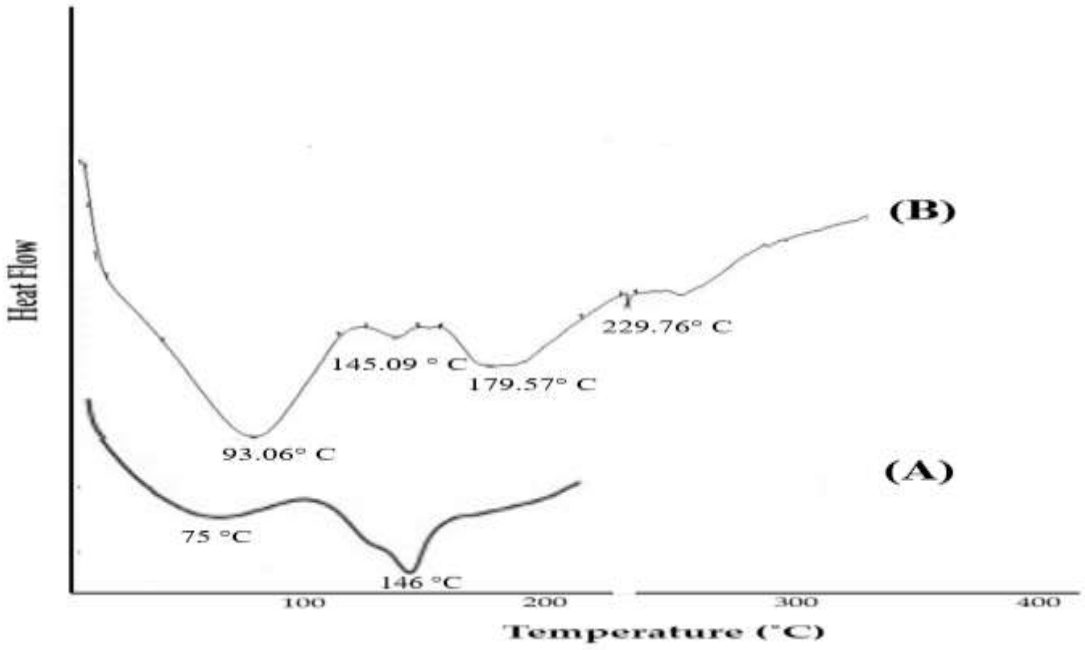


**Figure 7. DSC thermogram of (A) SM, and (B)ETO batch (E8)**

***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.

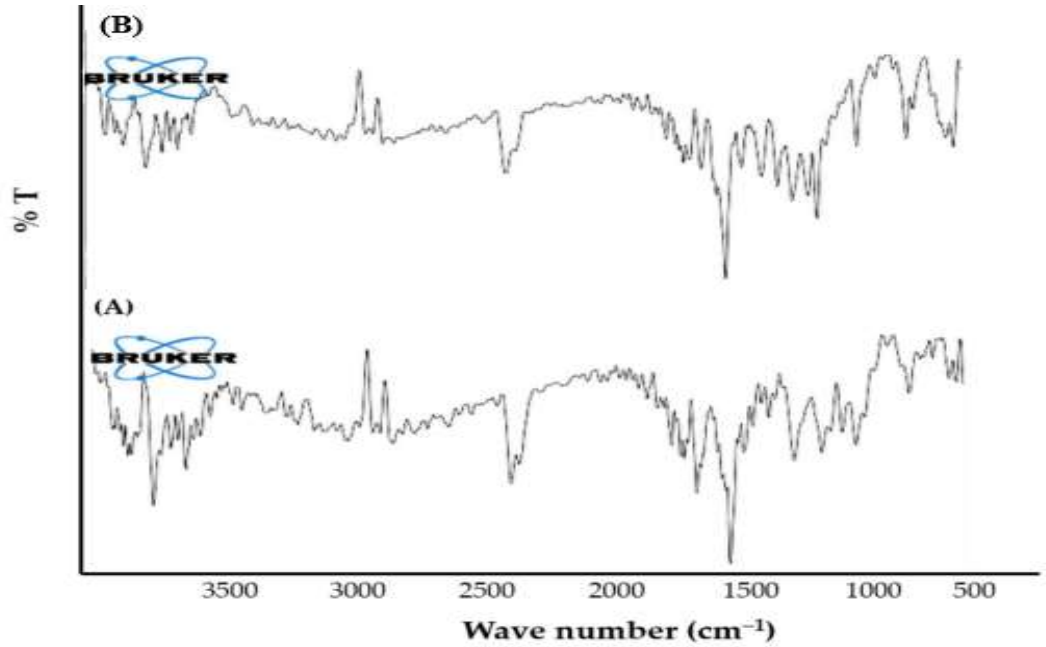


**Figure 8. FTIR spectrum of (A)SM, and (B) Optimized SM-ETO (E8)**

***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. A 2-factor and 3-level factorial design was used to examine the impact of the three independent variables (factors, X)—the sonication intensity (X1), the sonication time (X2) for the synthesis (as mentioned in Table 1) on the dependent variables (responses, Y)- % drug release (Y1), Vesicle size (Y2), and entrapment efficiency (Y3). The impacts of independent variables were investigated at three real and coded levels. Each factor's greater, lower, and intermediate levels are denoted by the codes +1, 1, and 0, respectively. The regression coefficient ($R^2$) and analysis of variance were two statistical metrics for which Design-Expert software gave the model with the greatest fit for comparison. All the batches of ethosomal formulations $E_1$-$E_9$ were compared based on the above evaluations, hence batch $E_8$ was considered as the optimized batch, since batch $E_8$ showed the observed (actual) value for; drug release (Y1=75%), uniform droplet size (Y2=168 nm), and entrapment efficiency (Y3=89.67), while the predicted (theoretical) values of batch $E_8$ as follows: Y1 of 77.122% as shown in Figures. 9a and 9b, Y2 of 169 nm, Y3 of 91. All findings showed that there was good agreement between the actual and expected outcomes. Additionally, the relative error percentage between observed and predicted values being less than 5% supported the validity and reproducibility of the generated models.

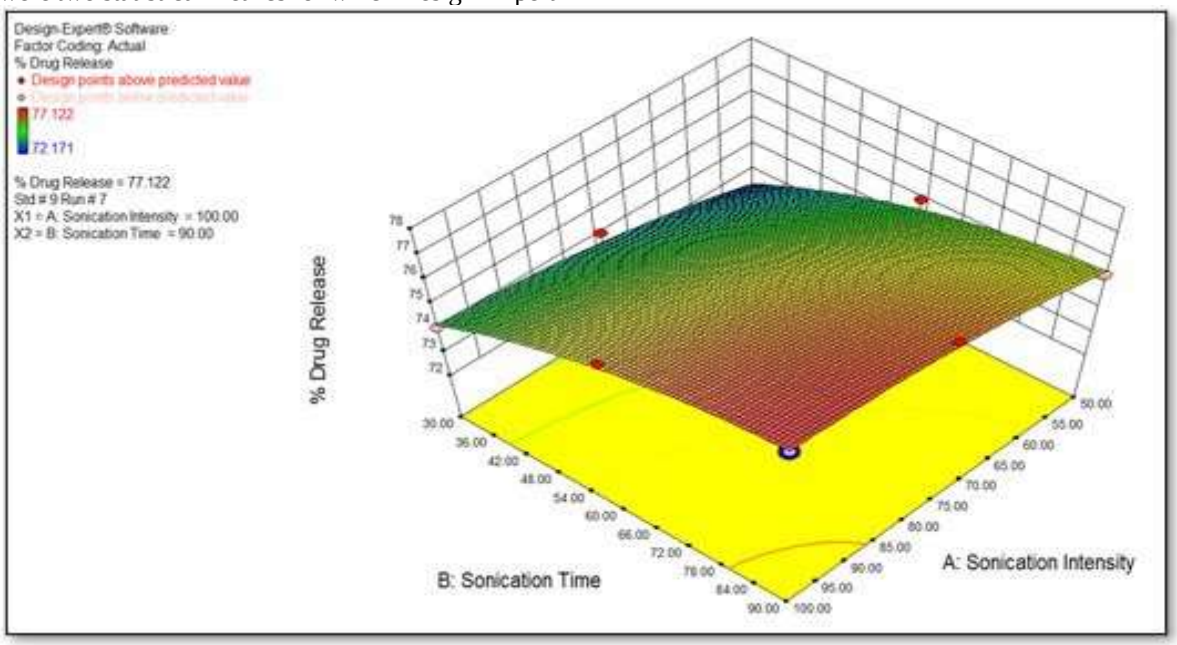


**Figure 9a. Contour Plot (Response surface plot) of % Drug Release (Y1)**

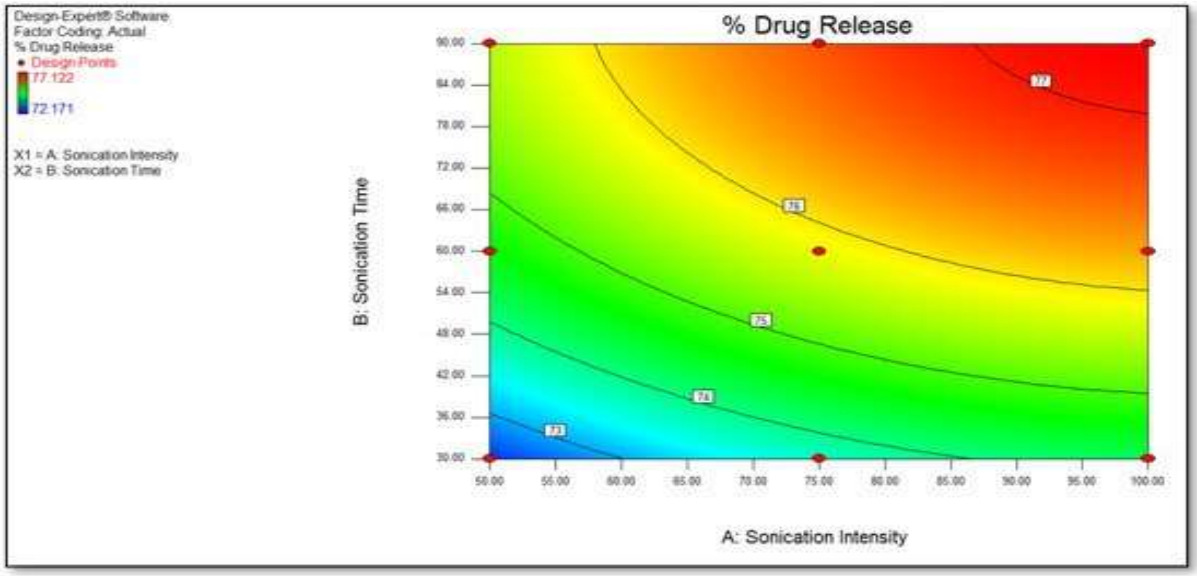


**Figure 9b. Response surface plot showing the effect of formulation variables on % Drug Release (Y1)**

***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).

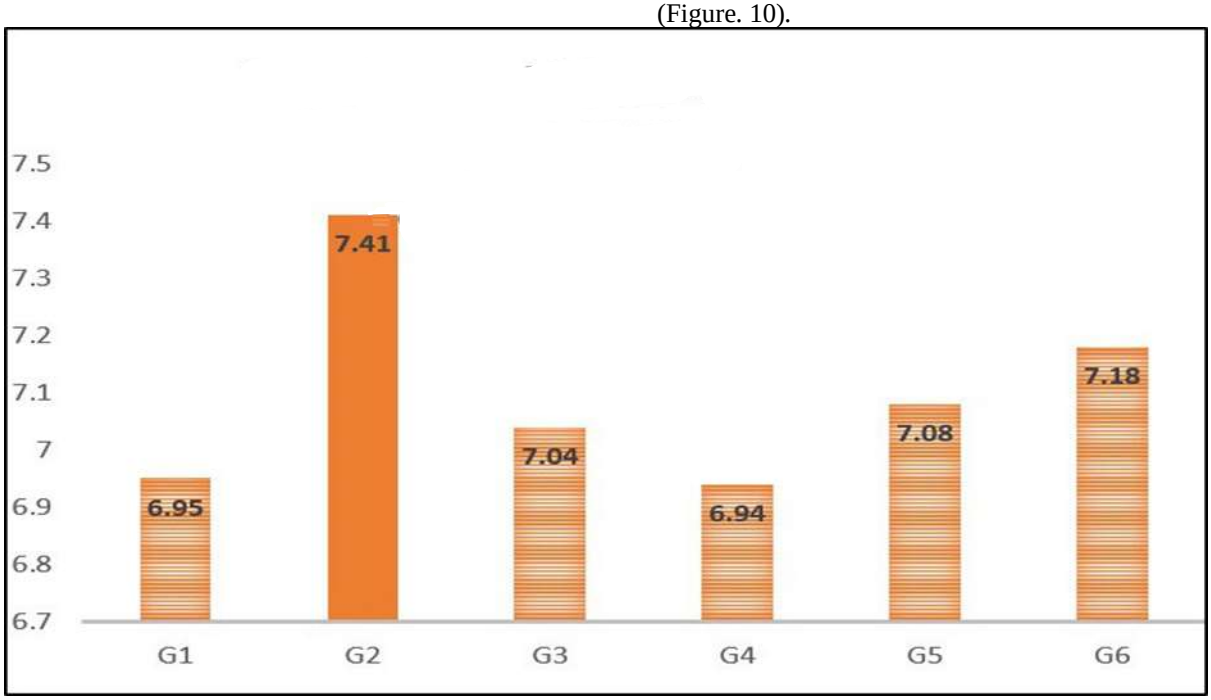


**Figure 10. pH of ethosomal gel batches**

The spreadability of all given formulations was found to be the best for application & within limits (20.00 to 30.00 g.cm/sec) [3Y] 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.

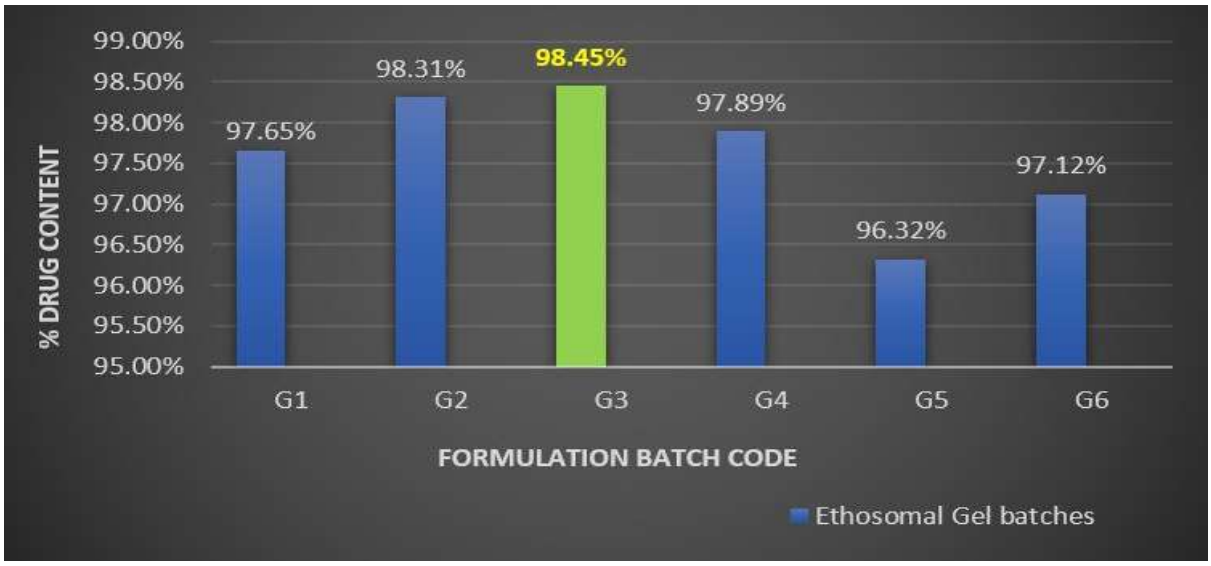


**Figure 12. Percent drug content of ethosomal gel batches**

***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) [3A] (Figure. 13). From all batches, G4 showed maximum viscosity as 9.187 (pa.s); hence it was considered as ideal viscous batch.

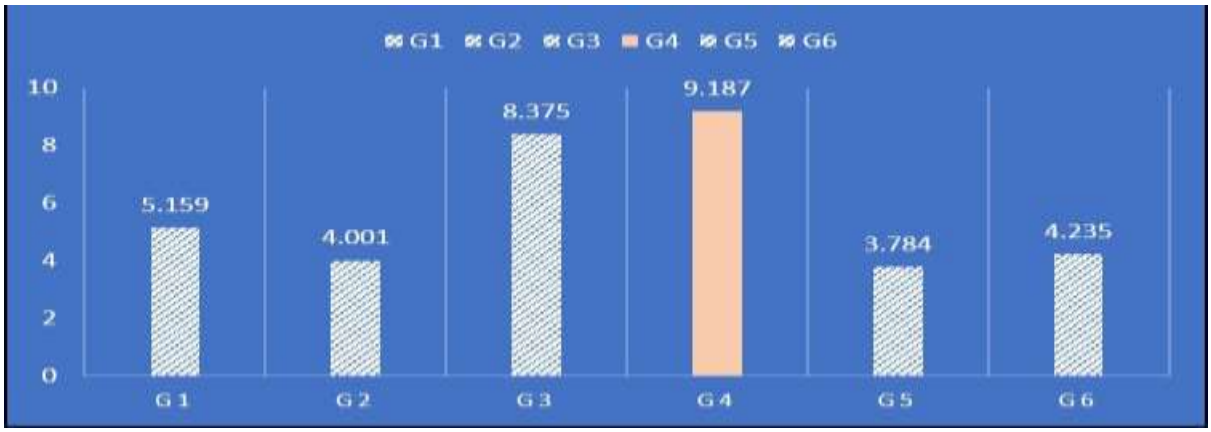


**Figure 13. Viscosity of ethosomal gel batches**

***In-vitro diffusion of SM-ETO-loaded gel***

The efficacy of the drug release from SM-ETO gel employing synthetic membrane was compared by adopting an *in-vitro* Franz cell system. When developing a topical drug delivery system, IVRT was used as a screening method to evaluate the efficiency characteristics of multiple formulations. This helps ensure that the final product has the desired effects. The percentage of SM from ETO-loaded gel through the synthetic membrane was evaluated for up to 450 min (Figure. 14). The finding demonstrated that $G_1$, $G_2$, $G_5$, and $G_6$ batches exhibited a more significant percentage of drug release than other batches in 450 min with attributes of 96, 97, 94 and 98, respectively, which was satisfactory. The obvious increase in the release may attributed to the presence of ethanol, which plays a vital role in enhancing drug transport through deep skin layers [39].

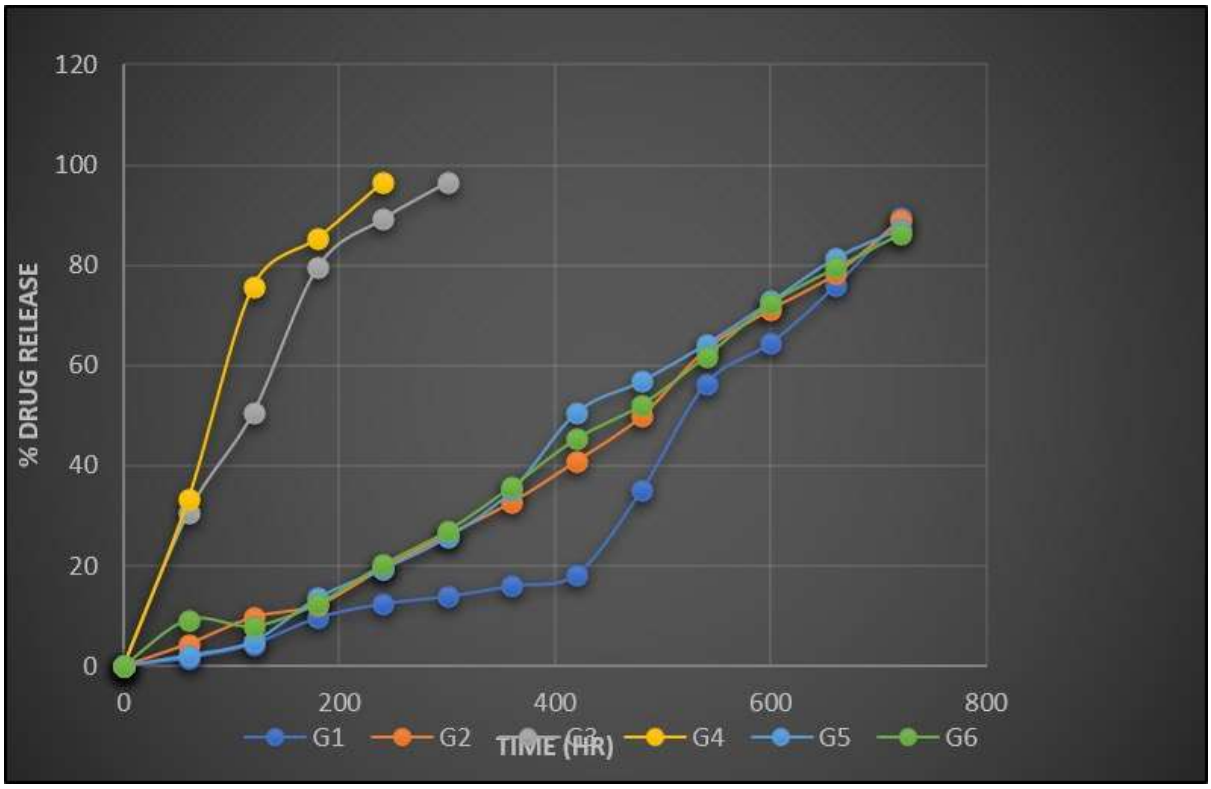


**Figure 14. *In-vitro* drug diffusion from ethosomal gel**

***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.

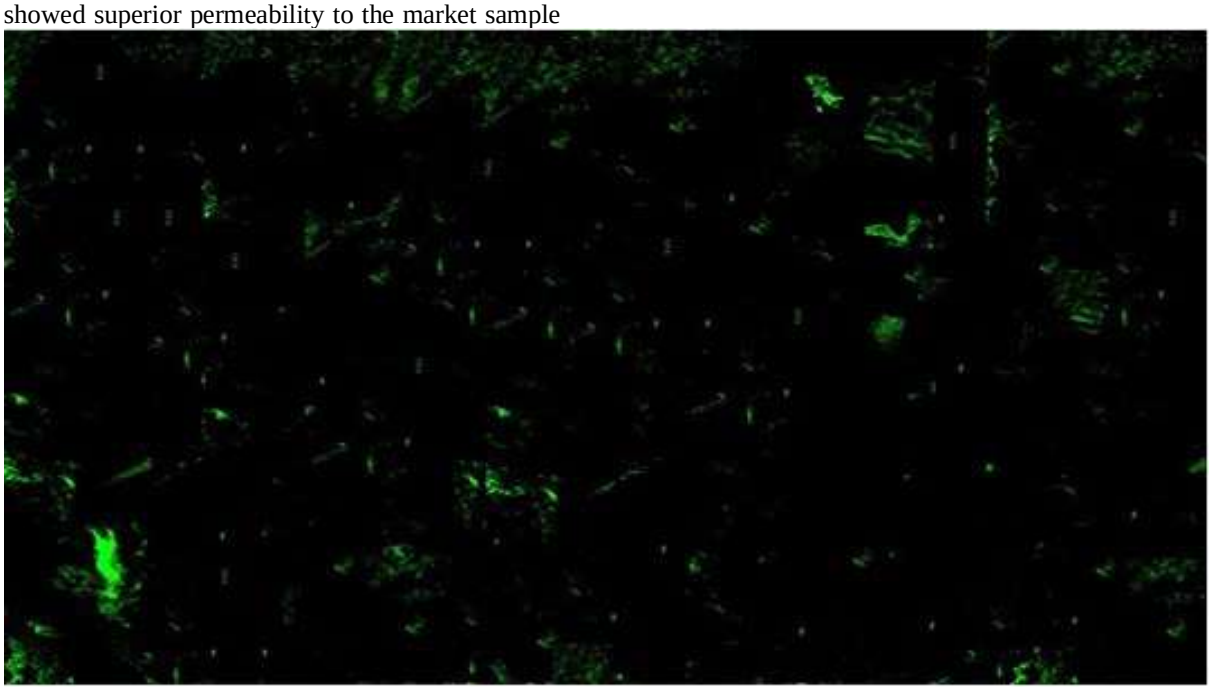

**Figure 15a. Confocal Laser Scanning Microscopy (CLSM) image of 0.1 % w/w ethosomal gel formulation treated Wistar rat skin**

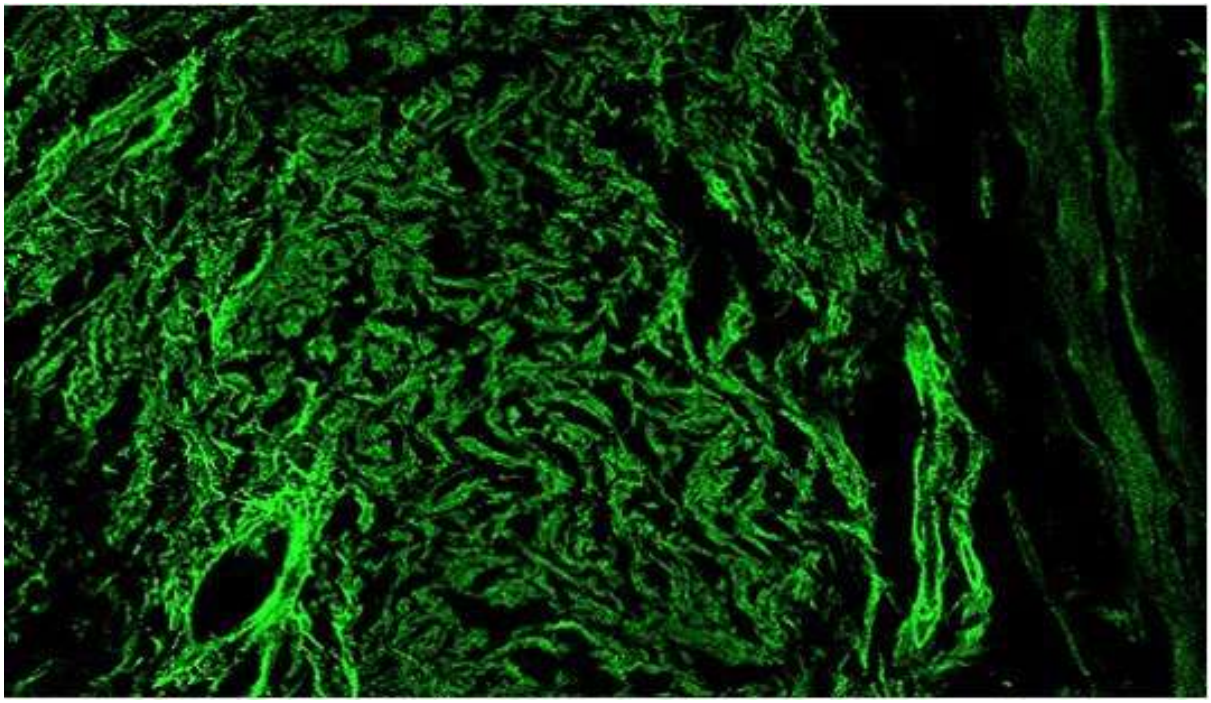

**Figure 15.b. Confocal Laser Scanning Microscopy (CLSM) image of 1% w/w Clindamycin market sample gel treated Wistar rat skin**

## Conclusions

The SM-ETO was effectively developed and optimised to synthesise vesicles in the nano form with higher entrapment efficiency. The optimised SM-ETO were effectively incorporated into the Carbopol 934p gel for transdermal delivery. Drug diffusion studies revealed the capability of SM-ETO-based gel sustained release of SM for 450 min (7.5 h). To an *in-vivo* pharmacokinetic release research, this finding may be generalised. The success of this new approach can deliver SM efficiently through a non-invasive route with the continuous release. Further, ethosomal gel showed the best results compared to a conventional formulation for penetration of drugs into the epidermis. Based on the effective and comparable outcomes of the current investigation, it can be concluded that the SM-ETO loaded gel is a new strategy that is quite feasible, affordable, and time-efficient. To investigate the potential application of this formulation *in-vivo* model, future research is needed.

## Acknowledgment

The authors would like to thank each of Al-Bayan University, Esraa university, and College of Pharmacy, Al-Nahrain university, Baghdad/Iraq for supporting this work.

## Conflicts of Interest

Declared none.

## Funding

Declared none.

## Ethics Statements

The authors declared the Iraqi Center for Cancer Research/Mustansiriyah University's animal ethical council (approval no. ICCMGR2020-016).

## Author Contribution

All the authors have contributed equally.

## References

1. Nainwal N, Jawla S, Singh R, Saharan VA. Transdermal applications of ethosomes–a detailed review. Journal of liposome research. 2019;29(2):103-13.
2. Sabbagh F, Kim BS. Recent advances in polymeric transdermal drug delivery systems. Journal of controlled release. 2022;341:132-46.
3. Latif MS, Nawaz A, Shah MKA, Iqbal A. A Review on Transdermal Drug Delivery: Design, Evaluation and Approach towards Painless Drug Delivery System. Pharmaceutical Communications. 2022;1(01):31-45.
4. Witika BA, Mweetwa LL, Tshiamo KO, Edler K, Matafwali SK, Ntemi PV, et al. Vesicular drug delivery for the treatment of topical disorders: Current and future perspectives. Journal of Pharmacy and Pharmacology. 2021;73(11):1427-41.
5. Jafari A, Daneshamouz S, Ghasemiyeh P, Mohammadi-Samani S. Ethosomes as dermal/transdermal drug delivery systems: applications, preparation and characterization. Journal of Liposome Research. 2023;33(1):34-52.
6. Pandey V, Golhani D, Shukla R. Ethosomes: versatile vesicular carriers for efficient

transdermal delivery of therapeutic agents. Drug delivery. 2015;22(8):988-1002.

7. Abdulbaqi IM, Darwis Y, Khan NAK, Assi RA, Khan AA. Ethosomal nanocarriers: The impact of constituents and formulation techniques on ethosomal properties, in vivo studies, and clinical trials. International journal of nanomedicine. 2016:2279-304.
8. Moolakkadath T, Aqil M, Ahad A, Imam SS, Praveen A, Sultana Y, et al. Fisetin loaded binary ethosomes for management of skin cancer by dermal application on UV exposed mice. International journal of pharmaceutics. 2019;560:78-91.
9. Hanafy NA, El-Kemary MA. Silymarin/curcumin loaded albumin nanoparticles coated by chitosan as muco-inhalable delivery system observing anti-inflammatory and anti COVID-19 characterizations in oleic acid triggered lung injury and in vitro COVID-19 experiment. International journal of biological macromolecules. 2022;198:101-10.
10. Karimi G, Vahabzadeh M, Lari P, Rashedinia M, Moshiri M. "Silymarin", a promising pharmacological agent for treatment of diseases. Iran J Basic Med Sci. 2011;14(4):308-17.
11. Archoo, Sajeeda, Shahid H. Naikoo, and Sheikh A. Tasduq. "Role of herbal products as therapeutic agents against ultraviolet radiation-induced skin disorders." Herbal Medicines. Academic Press, 2022. 345-360.
12. Chang L-W, Hou M-L, Tsai T-H. Silymarin in liposomes and ethosomes: Pharmacokinetics and tissue distribution in free-moving rats by high-performance liquid chromatography–tandem mass spectrometry. Journal of agricultural and food chemistry. 2014;62(48):11657-65.
13. Rahman HS, Othman HH, Hammadi NI, Yeap SK, Amin KM, Abdul Samad N, et al. Novel drug delivery systems for loading of natural plant extracts and their biomedical applications. International journal of nanomedicine. 2020:2439-83.
14. Aroraa S, Dhoke V, Moharir K, Yende S, Shah S. Novel drug delivery system of Phytopharmaceuticals: a review. Current Traditional Medicine. 2021;7(5):73-86.
15. Khanam, Nazia, et al. "A review on optimization of drug delivery system with experimental designs." Int J App Pharm 10.2 (2018): 7-12.
16. Vinod KR, Sandhya S. Factorial Designing for Pharmaceutical Product and Process Development. Research J. Pharma. Dosage Forms and Tech. 2011; 3(5): 199-202.
17. Abdallah MH, Abu Lila AS, Shawky SM, Almansour K, Alshammari F, Khafagy E-S, et al. Experimental Design and Optimization of Nano-Transfersomal Gel to Enhance the Hypoglycemic Activity of Silymarin. Polymers. 2022;14(3):508.
18. Akhtar N, Varma A, Pathak K. Ethosomes as vesicles for effective transdermal delivery: from bench to clinical implementation. Current clinical pharmacology. 2016;11(3):168-90.
19. Paiva-Santos AC, Silva AL, Guerra C, Peixoto D, Pereira-Silva M, Zeinali M, et al. Ethosomes as nanocarriers for the development of skin delivery formulations. Pharmaceutical research. 2021;38(6):947-70.
20. Almajidi YQ, Maraie NK, Raauf AMR. Utilization of solid in oil nanodispersion to prepare a topical vemurafenib as potential delivery system for skin melanoma. Applied Nanoscience. 2023; 13(4): 2845-2856.
21. Sakdiset P, Amnuaikit T, Pichayakorn W, Pinsuwan S. Formulation development of ethosomes containing indomethacin for transdermal delivery. Journal of drug delivery science and technology. 2019;52:760-8.
22. Ascenso A, Raposo S, Batista C, Cardoso P, Mendes T, Praça FG, et al. Development, characterization, and skin delivery studies of related ultradeformable vesicles: transfersomes, ethosomes, and transethosomes. International journal of nanomedicine. 2015:10; 5837-51.
23. Limsuwan T, Amnuaikit T. Development of ethosomes containing mycophenolic acid. Procedia chemistry. 2012;4:328-35.
24. Ma H, Guo D, Fan Y, Wang J, Cheng J, Zhang X. Paeonol-loaded ethosomes as transdermal delivery carriers: design, preparation and evaluation. Molecules. 2018;23(7):1756.
25. Pathan IB, Jaware BP, Shelke S, Ambekar W. Curcumin loaded ethosomes for transdermal application: Formulation, optimization, in-vitro and in-vivo study. Journal of Drug Delivery Science and Technology. 2018;44:49-57.
26. Almajidi YQ, Maraie NK, Raauf AMR. Modified solid in oil nanodispersion containing vemurafenib-lipid complex-in vitro/in vivo study. F1000Research. 2022; 11(841):1-19.
27. Saifee M, Atre M, Toshniwal R. Formulation and In-vitro Evaluation of Ethosomal Gel of Repaglinide for Transdermal Delivery. Int J Pharm Phytopharmacol Res. 2021;11(4):11-7.
28. Kumar B, Sahoo P, Manchanda S. Curcumin loaded ethosomal gel for improved topical delivery: formulation, characterization and ex-vivo studies. Pharmaceutical Nanotechnology. 2021;9(4):281-7.
29. Ismail TA, Shehata TM, Mohamed DI, Elsewedy HS, Soliman WE. Quality by design for development, optimization and characterization of brucine ethosomal gel for skin cancer delivery. Molecules. 2021;26(11):3454.
30. Rossetti FC, Depieri LV, Bentley M. Confocal laser scanning microscopy as a tool for the

investigation of skin drug delivery systems and diagnosis of skin disorders. Confocal laser microscopy-principles and applications in medicine, biology, and the food sciences. 2013:99-140.

**31.** Olley RC, Alhaij S, Mohsen BM, Appleton PL, Chadwick RG, Ball G. Novel Confocal-Laser-Scanning-Microscopy and conventional measures investigating eroded dentine following dentifrice dab-on and brushing abrasion. Heliyon. 2020;6(3); 1-8.

**32.** Yücel Ç, Şeker Karatoprak G, Değim İT. Anti-aging formulation of rosmarinic acid-loaded ethosomes and liposomes. Journal of microencapsulation. 2019;36(2):180-91.

**33.** Alkawak, Rasha Saadi Younus, and Nawal A. Rajab. "Lornoxicam-Loaded Cubosomes:-Preparation and In Vitro Characterization." Iraqi Journal of Pharmaceutical Sciences. 2022; 31(1): 144-153.

**34.** Abdulbaqi, Mustafa R., and Nawal A. Rajab. "Preparation, characterization and ex vivo permeability study of transdermal apixaban O/W nanoemulsion based gel." Iraqi Journal of Pharmaceutical Sciences 2020; 29(2): 214-222.

**35.** Lian, Ruyue, et al. "Silymarin glyceryl monooleate/poloxamer 407 liquid crystalline matrices: physical characterization and enhanced oral bioavailability." Aaps Pharmscitech. 2011; 12 (4): 1234-1240.

**36.** Ismail, Muna Y., and Mowafaq M. Ghareeb. "Enhancement of the solubility and dissolution rate of rebamipide by using solid dispersion technique (Part I)." Iraqi Journal of Pharmaceutical Sciences. 2018;27(12): 55-65.

**37.** NGN, Swamy, Mazhar Pasha, and Zaheer Abbas. "Formulation and Evaluation of Diclofenac Sodium Gels Using Sodium Carboxymethyl Hydroxypropyl Guar and Hydroxypropyl Methylcellulose." Indian Journal of Pharmaceutical Education & Research. ٢٠١٠; 44(4):310-314.

**38.** Jelvehgari, Mitra, Mohammad Reza Rashidi, and Hedayte Samadi. "Mucoadhesive and drug release properties of benzocaine gel.200;2(4); 185-194.

**39.** Jain, Shashank, et al. "Recent advances in lipid-based vesicles and particulate carriers for topical and transdermal application." Journal of pharmaceutical sciences. 2017; 106 (2);423-445.

# تحضير وتطوير نظام توصيل الأدوية الايثوسومل لدواء سيليمارين للتطبيق عبر الجلد

**حسنين صكبان طاغي[١] ، أنمار أدهم عيسى[٢] و ياسر قاسم الماجدي*[٣]**

[١] قسم الصيدلانيات ، كلية الصيدلة، جامعة البيان، بغداد،العراق.
[٢] قسم الصيدلانيات، كلية الصيدلة، جامعة الاسراء، بغداد،العراق.
[٣] قسم الصيدلانيات، كلية الصيدلة ، جامعة النهرين، بغداد،العراق.

**الخلاصة**

سيليمارين هو فلافونويد بوليفينوليك طبيعي ، يظهر خصائص مضادة لمرض السكر وخفض الدهون مع ضعف الذوبان المائي والتوافر البيولوجي. يهدف العمل البحثي الحالي إلى تطوير تركيبة هلامية مضادة للصدفية من السيليمارين وتقييم إمكاناتها المضادة للصدفية. في هذه الدراسة, تم تصميم ايثوسوم لدواء السليمارين وتحسينة باستخدام طريقة التبريد بتطبيق خبير التصميم للتغلب على هذه المزالق. تم تصنيع ايثوسوم السليمارين وتعريضه لتحديد المظهر المادي ، ونسبة انحباس الدواء ، وتوزيع الحجم ، وإمكانات الشحنة السالبة ، ودراسة التشكل ، وتبلور المسحوق ، وسلوك انتقال الطور. بعد التحسين ، تمت إضافة ايثوسوم السليمارين إلى الجل المحتوي على الكاربوبول وفحص الأس الهيدروجيني ودراسة الانسيابية ومحتوى الدواء ودراسة إطلاق الدواء في المختبر. أظهرت النتائج أن عينات ايثوسوم السليمارين لم تظهر فصل الطور عند ٢-٨ درجة مئوية. أظهرت عينة (٨) احتباسًا للأدوية بنسبة ٨٩,٦٧ ٪ ، وحجم حويصلي ١٦٨ نانومتر ، ومؤشر تشتت متعدد ٠,٣٦٧ وإمكانية زيتا -٠,٤٩ مللي فولت. كشفت دراسة مورفولوجية عن حويصلات كروية مستطيلة. تظهر دراسة حيود الأشعة السينية الطبيعة غير المتبلورة لمسحوق السليمارين أظهر الهلام المركب مدى حموضة كبير من ٦,٩٤ إلى ٧. كما أظهر لزوجة ٩,١٨٧ سنتي بويس وكان المحتوى الدوائي بين ٩٦,٣٢ إلى ٩٨,٤٥٪ . أظهر إطلاق الدواء في المختبر قابلية تحرر السليمارين من عينات الهلام بنسبة اكثر من ٩٤٪. استكشفت النتائج الشاملة قابلية زيادة الذوبان والتوافر البيولوجي للهلام المطور مما يشير إلى أمكانيته كحامل نانوي في توصيل السليمارين للتطبيقات السريرية المستقبلية. بشكل قاطع يمكن القول: بمساعدة تكنولوجيا تطوير التركيبة، تم بنجاح تطوير تركيبة هلامية إيثوسومالية من السيليمارين تمتلك نشاطًا مضادًا للصدفية.

**الكلمات المفتاحية: الإيثوسومات، الهلام، سيليمارين، توصيل الأدوية عبر الجلد، التطبيق الموضعي.**