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Development of Etoricoxib Cubosomal Transdermal Gel as An Alternative to Systemic Administration

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Abstract: Etoricoxib (Et), a new potent COX-2 anti-inflammatory drug that suffers from severe side effects after oral administration. The present study is concerned with preparation and characterization of transdermal gel formulations containing Et cubosomes nanoparticles. Et cubosome dispersions were formulated using different concentrations of lipid phase Glycerol Monooleate (GMO), nonionic surfactant Poloxamer 407 (P407) and water as aqueous phase. The prepared Et cubosomal dispersions were characterized for % drug content, % entrapment efficiency (EE), particle size, and in vitro drug release. Formulation with highest EE (92.0 ± 1.6 %), small particle size (63.5 ± 1.8 nm) and acceptable drug release profile (S9) was further evaluated for zeta potential and investigated for its internal structure using the transmission electron microscope (TEM). Results confirmed formation of cubic to spherical shaped particles with acceptable zeta potential (- 20± 0.379 mV). Selected formulation was incorporated in different gel formulations using CMC-Na (1, 1.5 % w/w) as gelling agent and glycerol (2.5, 5, 7.5 % w/w) as penetration enhancer. The cubosomal gels were characterized along with drug loaded gel for macroscopic, physical properties and in vitro drug release. Results confirmed acceptable macroscopic, physical properties with variable drug release profiles depending on gelling agent concentration as well as glycerol concentrations. Selected gel formulation with favorable drug release profile (G3) was further evaluated for ex vivo permeation. Results showed enhanced permeation for G3 compared to Et gel. The developed Et-loaded cubosomal gel offers a promising transdermal method for treating arthritis that will be applied directly to the skin.

Keywords: Etoricoxib; cubosomes; nanoparticles; transdermal delivery.

1. INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) represent the most typically used treatment for pain and inflammation by inhibition of cyclooxygenase (COX) enzyme [1]. Non-selective COX inhibitors suffer high risk of gastrointestinal (GIT) complications including minor discomfort up to severe symptoms as life-threatening peptic ulcers which limit its use. This is due to COX-1 inhibition which exerts gastroprotective functions through protection of stomach lining from eroding action of gastric acid. So, COX-2 selective inhibitors would be preferred over nonselective COX inhibitors since COX-2 enzyme is highly expressed in inflammatory conditions [2]. Et is one of the potent new classes of COX-2 inhibitors that is used for treatment of several inflammatory conditions such as osteoarthritis, rheumatoid arthritis, acute gout and chronic musculoskeletal pain [4]. Et has cardiovascular, respiratory, and hepatic side effects if taken orally. So, to avoid these adverse effects of this drug, development of site specific drug delivery carriers would be recommended and more effective than the systemic delivery [2]. The mentioned side effects can result from the high oral dosage required to achieve therapeutic concentrations in the affected joints. Transdermal delivery could be a successful alternative taking into account that efficient transdermal delivery requires good percutaneous

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absorption. This is not applicable for Et due to its very low aqueous solubility and poor dissolution, which limits its percutaneous absorption [3]. For transdermal drug delivery, several vesicular systems were proposed to improve the drug permeation through the stratum corneum. Vesicular systems contain ingredients that enhance the drug permeation possibly via opening tight junctions in the stratum corneum. There are different lipid-based delivery systems such as niosomes, bilosomes, ethosomes, transferosomes, liposomes [4], and cubosomes which have been used to boost poorly soluble drugs permeation and absorption [5,6]. Cubosomes are vesicular systems containing reversed bicontinuous cubic phases with unique physicochemical properties. P407 stabilizes these crystalline isotropic lipidic nanoparticles. The biodegradable and biocompatible lipid excipient monoolein is considered as safe drug delivery carrier for these cubic nanoparticles. Additionally, the stable cubic shape for theses vesicles allows for a slower drug release, improved drug retention, as well as site-specific drug delivery [7].

Among previous work focusing on Et transdermal delivery, to the best of our knowledge, no research has taken the advantage of cubosome to enhance Et transdermal delivery. So, this work was targeted to improve therapeutic efficacy and reduce side effects of oral Et administration by development of Et cubosomal gel as a promising option for delivery of Et through the skin. So, the present study aimed to formulate and characterize Et cubosomal transdermal delivery system. The effect of GMO and P407 concentrations on the particle size, EE and in vitro release was studied to obtain cubosomes with low particle size, high EE, and suitable drug release profile. Transdermal gel formulations were subsequently developed using the selected cubosomal formulation. These formulations were assessed for pH, Et content, viscosity, spreadability, and in vitro Et release. Further investigations included ex vivo Et skin permeation studies for the gel formulation that exhibited acceptable physical properties and a favorable drug release pattern.

2. METHODS

2.1. Materials

Etoricoxib was gifted by Al- DEBAIKY (D B K) Pharmaceutical Industries (Egypt). Sodium Carboxy Methyl Cellulose (CMC-Na) was received as a gift sample from Pharco Pharmaceutical Industries (Egypt). Poloxamer 407 (P407; Pluronic acid F127) and Glycerol monooleate (GMO) were obtained from Sigma–Aldrich Chemie GmbH (Germany). Potassium dihydrogen phosphate, disodium hydrogen phosphate, methylparaben, and Methanol were purchased from El Nasr chemical co., (Egypt). Dialysis tubing cellulose membrane (molecular weight cutoff 12,000 g/mole) was purchased from Sigma Chemical Company Sigma-Aldrich Corp., St. Louis, MO, (USA).

2.2. Compatibility study of Etoricoxib and excipients

Fourier-transform infrared (FTIR) spectra were obtained using FTIR Spectrophotometer (perkin-Elmer, FTS-1710, Beaonsfeild, UK) for samples of Et powder, P407 powder, GMO, Sodium Carboxy Methyl Cellulose (CMC-Na), glycerol, and Et: excipients mixture in the ratio of 1:1. Samples were prepared in KBr discs. The IR spectra were collected in the spectral range of 450-4000 cm^{-1} for analysis [8].

2.3. Preparation of Etoricoxib -loaded cubosomal dispersions

Cubosomal dispersions containing Et were prepared using the melting method. In brief, the accurately weighed amounts of GMO and P407 (Table 1) were melted at 60 ºC using a magnetic stirrer (MSH basic, IKA-WERKE, Germany) until they were homogenous and then 200 mg Et was dissolved in the resultant molten mixture by continuous stirring. Deionized water (5 mL) was added dropwise to the molten mixture containing Etoricoxib while stirring at 100 rpm at room temperature to achieve a homogeneous state. The mixture was left to equilibrate for 48 hr at room temperature. For the preparation of homogenous cubosomal dispersion, the remaining amount of deionized water was added followed by homogenization at 13500 rpm for 3 min using high speed homogenizer (Ultera-turrax T18, IKA, Germany). Then, water bath 60 ºC sonicator (Sonix IV, Model ss101H, USA) was used to sonicate the prepared cubosomal dispersions for 15 min [5]. After cooling, the prepared Et-loaded cubosomal dispersions were held in glass vials for further investigation. Differential scanning calorimetry (DSC) would be performed later to ensure incorporation of Et into cubosomes nanoparticles.

Differential scanning calorimetry was utilized to confirm Et cubosomal formation by studying changes in the physical state of Et entrapped in the cubosomal nanoparticles. DSC was performed on pure Et powder, GMO, P407, and chosen cubosomal dispersion. Thermal behaviors of the samples (5 mg each) were determined using DSC Calorimeter (DSC60, Shimadzu, Japan) at the heating rate of 10 °C min^{-1} from room temperature to 200°C under a N_2 atmosphere at the flow rate of 50 mL min^{-1} under ambient atmospheric pressure [9].

Table 1. Composition of Etoricoxib -loaded cubosomal dispersion (S1-S9).

Formulation	GMO (%)	P407 (%)	Et (mg)	Water to (mL)
S1	0.1	0.01	200	100
S2	0.1	0.1		
S3	0.1	0.2		
S4	0.5	0.01		
S5	0.5	0.1		
S6	0.5	0.2		
S7	1	0.01		
S8	1	0.1		
S9	1	0.2		

2.4. Characterization of the formulated Etoricoxib -loaded cubosomal dispersions

Cubosomal dispersions containing Et were prepared using the melting method. In brief, the accurately weighed amounts of GMO and P407 (table 1) were melted at 60 ºC using a magnetic stirrer (MSH basic, IKA

2.4.1. Drug content

For determination of drug content, a volume of cubosomal dispersions equivalent to 10 mg of Et was taken in a 100 mL volumetric flask and diluted to 100 mL with methanol. The solution was then filtered, and absorbance was measured spectrophotometrically at 235 nm using UV-Vis spectrophotometer (Jenway LTD, Model 16405, UV-VIS, UK). Et content was calculated from calibration curve previously performed (See figure (1S) at supplementary data). The test was performed in triplicate [10].

2.4.2. Entrapment Efficiency (E.E)

Et-loaded cubosomal dispersions (1 mL) were centrifuged at 5300 rpm for 15 minutes (Centriron Scientific, Warminster, Pennsylvania). Free Et contained in the collected supernatant was measured spectrophotometrically at 235 nm after suitable dilution. The entrapment efficiency (EE %) was calculated according to the following equation:

$$\% EE = Ct - Cf / Ct \times 100$$

where, Ct is total Etoricoxib concentration and Cf is free Et concentration in the supernatant. The EE % test was carried out in triplicate [5].

2.4.3. Particle size and polydispersity index (PDI) determination

The average particle size and the size distribution indicated by polydispersity index (PDI) of cubosomal nanoparticles were measured by a particle analyzer (Malvern Ltd., UK) based on dynamic light scattering at an angle of 173º and at a temperature of 25 ºC. Immediately before measurements, samples were diluted with deionized water. The experiment was carried out in triplicate [6].

2.4.4. In vitro drug release

In vitro drug release of Et-loaded cubosomes was studied using a thermostated shaking water bath (Gallent kamp, UK) maintained at 37 ºC and 100 rpm. Cubosomal dispersion equivalent to 10 mg of Et was transferred to the dialysis bag which previously soaked overnight in pH 6.4 phosphate buffer and sealed. The sealed bag was then placed in a beaker filled with 250 mL of phosphate buffer at pH 6.4. Aliquots of four mL of the fluid was periodically withdrawn every one hour up to 6 hrs and immediately replaced by fresh buffer of equal volume to maintain sink conditions. The drug release was analyzed spectrophotometrically at 235 nm after suitable dilution using pH 6.4 phosphate buffer as a blank [11].

The in vitro release data were fitted to different kinetics models. The suitable kinetic model was considered by determination the highest correlation coefficient [12].

2.5. Characterization of the selected Et -loaded cubosomal formulation

The selected Et -loaded cubosomal formulation was evaluated for its zeta potential and investigated for its internal structure using Cryo-TEM.

2.5.1. Zeta potential

The stability and aggregation behavior of the nanoparticles were investigated by considering their surface charge [13]. Zeta potential of the selected Et loaded cubosomal formulation was measured by Zetasizer (Malvern Ltd., UK). One mL of the selected Et- loaded cubosomal formulation was diluted with 29 mL deionized water and measured at 25 ± 0.5 °C in triplicate [5].

2.5.2. Transmission electron microscope (Cryo-TEM)

The morphology of cubosomal formulation was examined by transmission electron microscope. Cubosomes nanoparticles were placed onto a copper grid, stained with 2 % phosphotungstic acid and viewed using TEM (Philips-CM200, SAIF, Bombay, Mumbay, India) [14].

2.6. Preparation of Etoricoxib cubosomal gel

Etoricoxib cubosomal gel formulations were formulated using selected Et-loaded cubosomal formulation that was chosen for appropriate particle size, high EE %, and acceptable in vitro drug release profile. Etoricoxib cubosomal gel formulations were prepared using CMC-Na as gelling agent, and

glycerol as penetration enhancer. The required quantity of CMC-Na was weighed and added slowly to the beaker containing hot (80 ºC) distilled water (30 mL) with continuous stirring for 1 hr at 400-600 rpm. Cold water (10 mL) was then added slowly under continuous stirring. To this, the selected cubosomal dispersion equivalent to 200 mg Et was added and mixed properly. Then, weighed amount glycerol (table 2) was added to the prepared gel. Methylparaben was finally added as preservative. The final quantity was made up to 100 gm with distilled water. For comparison, Et gel was prepared where 200 mg Et powder was added instead of cubosomal dispersion to plain CMC-Na gel. The prepared gel formulations were kept for 24 h in refrigerator at 4 ºC for complete polymer desolvation [15].

Table 2. Composition of Et gel and Et cubosomal gel formulations (G1- G6).

Formulation	CMC-Na (gm)	Glycerol (gm)	Et. eq. to (mg)	Water to (gm)	Methylparaben (gm)
Et gel	1.0	7.5			0.5
G1	1.0	2.5			0.5
G2	1.0	5.0			0.5
G3	1.0	7.5	200	100	0.5
G4	1.5	2.5			0.5
G5	1.5	5.0			0.5
G6	1.5	7.5			0.5

2.7. Characterization of the prepared Etoricoxib cubosomal gels

2.7.1. Macroscopic evaluation of prepared gels

The visual examination of the developed Et cubosomal gel formulations included assessing their color, homogeneity, and texture [16].

2.7.2. Determination of pH

The pH was determined, by firstly mixing of 1 gm of the prepared gel with 5 mL distilled water. Then, pH was recorded by immersing of the electrode of digital pH meter (MODEL 420, Orion, USA) in the diluted gel formulation. The pH measurement was done in triplicate [3].

2.7.3. Drug content

Drug content of 1 g of Etoricoxib cubosomal gel was determined using the same procedure previously described for Et cubosomes [10].

2.7.4. Viscosity

Viscosity of the formulations was assessed in millipascal second (mPa·s) using rheometer (Physica MCR 502 Anton Paar GmbH, Austria) under controlled shear rate mode. All measurements were performed in triplicate at 25 °C where formulations were subjected to rotational shear for 60 s at shear rates of 1 to 100 s^{-1}. [17].

2.7.5. Spreadability

For determination of Et cubosomal gel spreadability, 0.5 g of gel was spread out within a pre-marked, 1 cm circle on a 20 × 20 cm glass plate. Another glass plate was then placed over the first one. A weight of 500 g was allowed to rest on the upper glass plate for a period of 5 min. The increase in the diameter due to gel spreading was observed [18].

2.7.6. In vitro Etoricoxib release from the prepared gel

The 5 g of drug loaded cubosomal gel equivalent to 10 mg of Etoricoxib was transferred to the dialysis bag (previously soaked overnight in phosphate buffer pH 6.4) and sealed. The sealed bag was then placed in a beaker filled with 250 mL of phosphate buffer at pH 6.4 and stirred using a thermostated shaking water bath (Gallent kamp, UK) maintained at 37 °C and 100 rpm. Four mL of the release medium was periodically withdrawn every one hour up to 10 h and immediately replaced by an equal volume of fresh buffer solution to maintain sink conditions. The Et release was determined spectrophotometrically at 235 nm after suitable dilution. Each data point represents the average of three measurements [2].

To determine kinetic of drug release, the in vitro release data were fitted to different kinetics models. The suitable kinetic model was considered by determination of the highest correlation coefficient [12].

2.8. Ex vivo skin permeation

The study was approved by the ethical committee of faculty of pharmacy Al- Azhar university, Cairo, Egypt under registration number 352/12-6-2022. The abdominal Wistar rat skin was used to investigate the Et skin permeation. In this study, Et permeation through abdominal Wistar rat skin was evaluated for Et cubosomal gel with acceptable physical properties and favorable in vitro drug release profile, in comparison to Et gel. A locally fabricated Franz diffusion cell with a 1.76 cm^2 diffusion area was employed. A quantity of 0.5 g of test gel (equivalent to 1 mg of Et) was applied in an occlusive manner on the skin in the donor compartment. The receptor compartment contained 10 mL of pH 6.4 phosphate buffer solution, maintained at 37°C and agitated at 400 rpm. Samples were withdrawn at various time intervals (1, 2, 4, 8,

12, 18, and 24 hr) and replaced with an equal volume of fresh receptor medium. The drug concentration was determined spectrophotometrically at 235 nm after appropriate dilution with pH 6.4 phosphate buffer solution, using the same solution as the blank [14].

The results of Et permeated were kinetically treated to determine the best order of drug permeation from the formulations. The steady-state flux (J) was calculated from the slope of the linear part of the plot of the cumulative amount of Et permeated per unit area (gm/cm^2) against a time (hr) plot. The permeability coefficient (P) of Et through the rat skin was calculated according to the following equations:

$P = J/ C0$

Where, C0 is the initial Et concentration [19].

3. RESULTS

3.1. Compatibility study of Etoricoxib and excipients

Etoricoxib spectrum figure (1a) shows characteristic peaks of aromatic C=C stretching at 1543.06 cm^{-1}, and 1496.76 cm−1. Et spectrum also shows characteristic peak at 1141.86 cm^{-1} which indicated S=O stretching vibrations and additional peak at 779.24 cm^{-1} due to C–Cl stretching vibration [20]. The FTIR spectrum of GMO figure (1b) exhibits a peak at 2924.99 cm^{-1} which can be explained by presence of GMO lipid poly ester. The most intensive peak at 1739.79 cm^{-1} belongs to the C=O valence vibration of GMO [21]. The FTIR spectrum of P407 figure (1c) is characterized by major absorption peaks at 2889.37 cm^{-1} (C-H stretch aliphatic) [22]. As illustrated in figure (1 d), the FTIR spectrum of pure glycerol shows a sharp and intense peak at 995 cm^{-1} corresponding to C-O stretching [23]. CMC-Na spectrum (figure 1e) illustrates peak at 1584 cm^{-1} due to the C=O bond of the carboxylate ionic groups. A peak at 1410 cm^{-1} represents a symmetric bending vibration of the methyl group appears in CMC-Na spectrum (figure 1e) [24]. Figure (1f) shows FTIR spectrum of physical mixture of Et and excipients showing no observed changes in either main characteristic Et bands (1034 and 851 cm^{-1}) or in the characteristic peaks for excipients. The observed slight decrease of peaks intensity in the spectrum of physical mixture was due to the weight % of ingredients [25]. Results indicated absence of physical and chemical interaction between Et and excipients.

3.2. Characterization of the Etoricoxib -loaded cubosomal dispersions

3.2.1. Differential scanning calorimetry (DSC)

The DSC thermogram for ET (Figure 2) shows an endothermic peak at 137.5 ºC, which is ascribed to drug melting indicating highly Et crystalline nature [10]. DSC thermograms of the used materials shows endothermic peak at 36 ºC due to GMO melting [26]. An endothermic peak at 56.28 ºC due to P407 melting [14]. The thermograms of the Et-loaded cubosomes shows a lower shift in Et melting to 111 ºC which ensures molecular dispersion of amorphous form of Et within lipid-polymer matrix [27]. The peaks observed at 44 ºC and 54.5 ºC in the thermogram of Et- loaded cubosomes were due to GMO and P407 respectively [14].

3.2.2. Drug content

As presented in table (3), the drug content of cubosomal dispersions was found in the range of 88.0 ± 1.40 to 98.9 ± 0.92.

3.2.3. Entrapment efficiency

The mean EE values for Et-loaded cubosomal dispersion ranged from 80.2 ± 0.5 % to 92.0 ± 1.6 % (table 3). It can be observed that at the same GMO content, EE values increased with elevated P407 content (Table 3). In the same manner, increasing GMO level resulted in higher EE values at the same P407 level (Table 3).

3.2.4. Particle size determination and polydispersity index

As presented in table (3), the mean particle size values for Et- loaded cubosomal dispersions ranged from 50.1 ± 1.3 to 155 ± 1.0 nm. It was found that increased P407 level yielded smaller sized particles at constant GMO concentration.

Hence, formulations containing 0.2 % P407 yielded smaller particles than that containing 0.01 % and 0.1 % P407 (Table 3). On the other hand, at constant P407 concentration, the particle size of cubosomes was directly proportional to the increase in GMO concentration (Table 3). PDI values of cubosomal formulations ranged from 0.146 ± 0.02 to 0.428 ± 0.01 (Table 3).

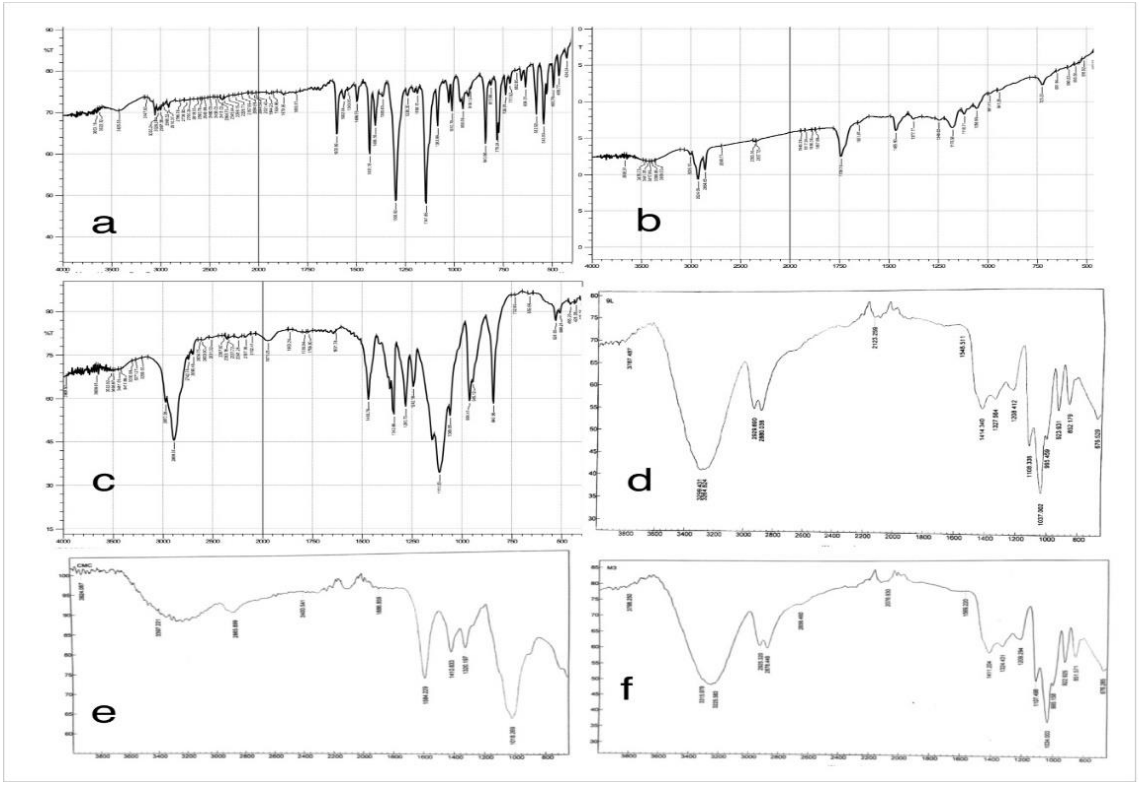

Figure 1. FTIR spectrum of (a) Et, (b) GMO, (c) P407, (d) glycerol, (e) CMC-Na, and (f) physical mixture of Et- excipients.

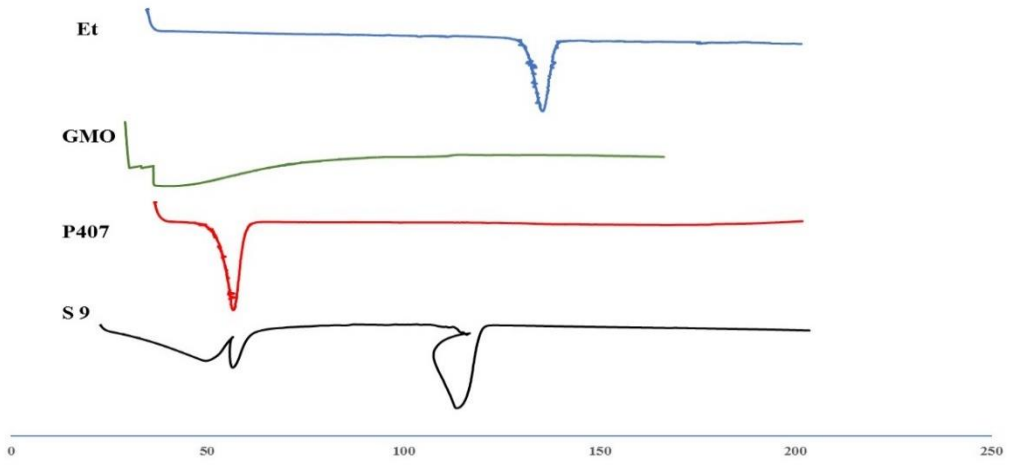

Figure 2. DSC thermogram of Et, GMO, P407, Et- loaded cubosomes.

3.2.5. In vitro drug release

Et cubosomal formulations showed complete Et release ranging from 4 to 6 hr (Figure 3). It was found that drug release increased upon increasing P407 concentration at constant GMO concentration (Figure 2). That's why formulation S6 (0.2 % P407) had somewhat higher Et release after 5 hours compared to S4 (0.01 % P407). For GMO effect, it was observed that at the same concentration of P407, higher GMO concentration led to slower drug release (figure 2). That could be seen in S1 (0. 1% GMO) which showed 100 % Et release that decreased to 81 % in S7 (1% GMO) at the end of 5th hr.

Kinetic analysis of Et release showed that Et release from the developed cubosomes followed diffusion –controlled release as per Higuchi model because it showed higher r^2 values (0.979 – 0.998) as compared to other models (See table 1S in supplementary data).

Table 3. Drug content, particle size, and entrapment efficiency % of Etoricoxib -loaded cubosomal dispersion (S1-S9).

Formulation	Drug content % ± SD	Particle size (nm) ± SD	(PDI) ± SD	EE % ± SD
S1	89.0 ± 0.01	78.4 ± 1.5	0.203 ± 0.02	80.2 ± 0.5
S2	88.0 ± 1.40	55.1 ± 3.3	0.384 ± 0.01	81.0 ± 1.0
S3	90.0 ± 1.90	50.1 ± 1.3	0.280 ± 0.03	83.2 ± 0.8
S4	90.6 ± 1.00	82.2 ± 3.6	0.192 ± 0.01	84.8 ± 2.5
S5	88.4 ± 0.50	63.5 ± 1.8	0.146 ± 0.02	85.5 ± 1.3
S6	89.2 ± 2.00	56.6 ± 2.0	0.336 ± 0.03	88.0 ± 0.9
S7	92.0 ± 0.60	155 ± 1.0	0.259 ± 0.02	90.0 ± 0.2
S8	91.9 ± 0.80	89.9 ± 2.0	0.428 ± 0.01	90.5 ± 0.7
S9	98.9 ± 0.92	63.5 ± 1.8	0.299 ± 0.08	92.0 ± 1.6

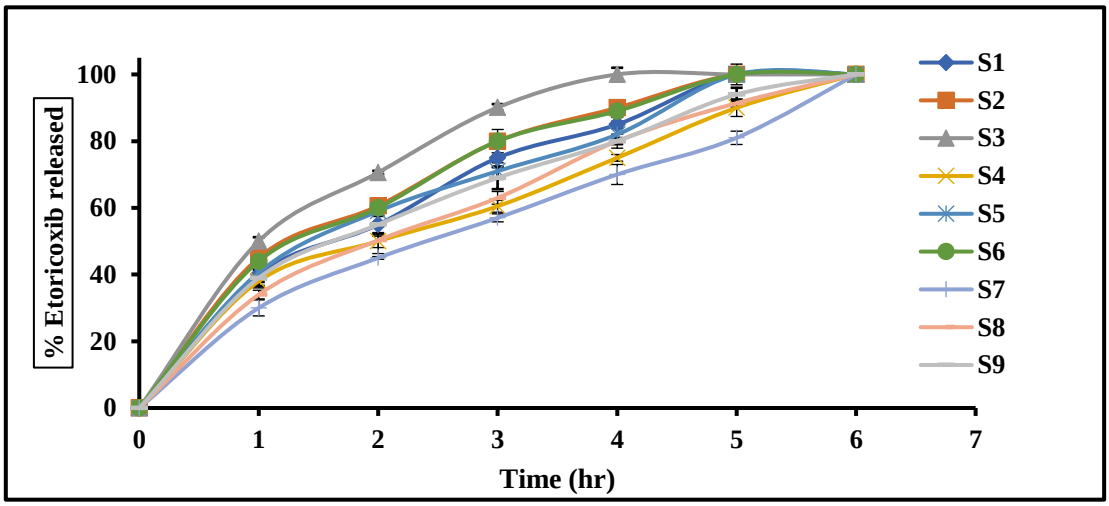

Figure 3. Percentage of Et released from Et- loaded cubosomal dispersions (S1-S9).

3.3. Characterization of the selected Et -loaded cubosomal formulation

3.3.1. Zeta potential

Zeta potential value of the selected Et -loaded cubosomal dispersion was found to be -20.1 ± 0.379 mV indicating high stability for the selected formulation (Figure 4a).

3.3.2. Transmission Electron Microscope (TEM)

To confirm the formation of cubic structures in the prepared dispersions, morphology was examined using TEM, and the obtained photomicrographs are presented in figure 4b.

3.4. Characterization of the prepared Etoricoxib cubosomal gel formulations

3.4.1. Macroscopic evaluation of prepared gel formulations

Visual observation of the prepared Et cubosomal gel formulations showed homogenous white colored gels with smooth texture.

3.4.2. pH

The pH values of the prepared Et cubosomal gel formulations were between 6 ± 0.23 and 7 ± 0.11 (Table 4). Such results indicate the suitability of the developed gel formulations for application to the skin since the acceptable pH value of transdermal formulation ranges from 4 to 7 [28].

3.4.3. Drug content

As presented in table (4), the prepared Et cubosomal gel formulations had drug content in the range from 96 ± 0.99 to 99.3 ± 1% (Table 4).

3.4.4. Viscosity

Table (4) showed that range of viscosity value of the prepared Et cubosomal gel formulations were from 3527 to 6923 mPa·s. The viscosity of the prepared gel formulations was dependent on CMC-Na concentration. Viscosity was also slightly affected by glycerol concentrations. In the context of CMC-Na, higher viscosity formulations were obtained with increasing CMC-Na concentration.

That's why G6 (1.5 % CMC) had somewhat higher viscosity compared to G3 (1 % CMC) (Table 4). Additionally, the prepared gel formulations showed mild increase in viscosity with increasing glycerol concentration. That's why G5 (5 % glycerol) had somewhat higher viscosity compared to G4 (2.5 % glycerol) (Table 4).

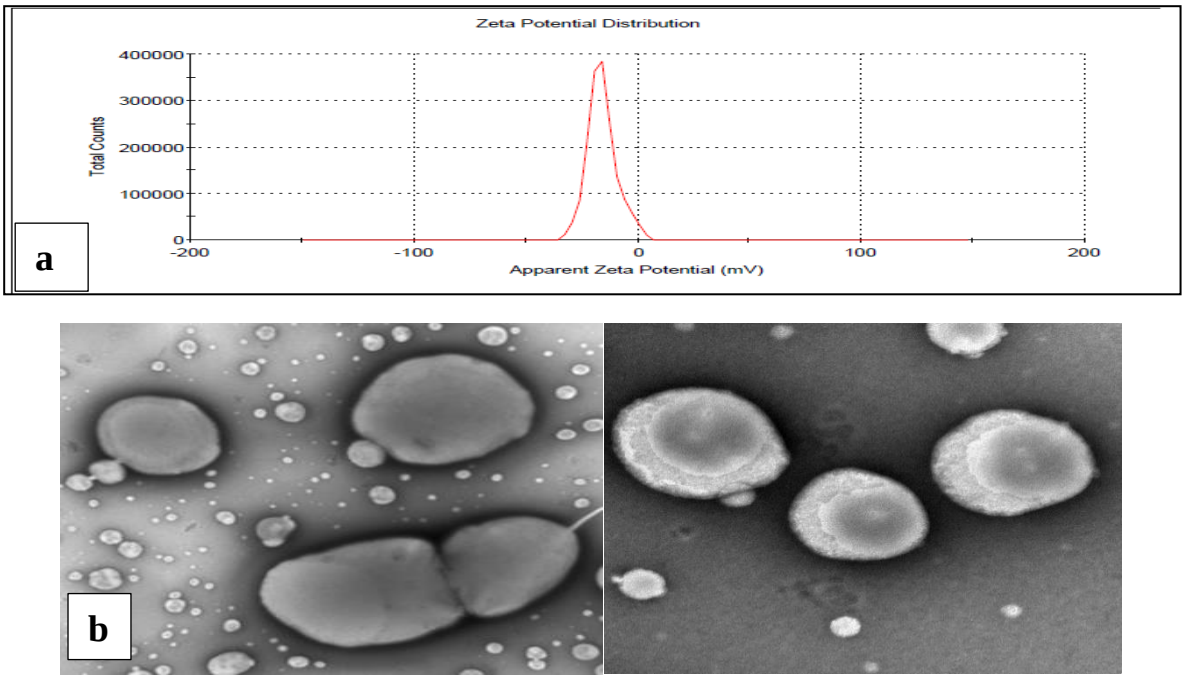

Figure 4. Zeta potential (a) and Typical TEM images (b) of Et-loaded cubosomal dispersion S9.

Table 4. Values of pH, drug content, viscosity, and spreadability values of Et gel and Et cubosomal gel formulations (G1-G6).

Formulation	pH	Drug content %	Viscosity (mPa·s)	Spreadability (cm)
Et gel	6.0 ± 0.02	97.9 ± 0.62	4144	9.00 ± 0.01
G1	6.6 ± 0.04	97.5 ± 0.62	3527	10.0 ± 0.02
G2	6.5 ± 0.10	97.0 ± 0.17	4000	9.80 ± 0.05
G3	6.4 ± 0.22	98.0 ± 1.50	4547	6.50 ± 0.01
G4	6.7 ± 0.84	97.3 ± 0.25	4232	8.50 ± 0.27
G5	6.8 ± 0.09	97.7 ± 0.71	5628	6.30 ± 1.00
G6	6.6 ± 0.45	98.0 ± 0.91	6923	6.00 ± 0.54

3.4.5. Spreadability

Spreadability values of all prepared gel formulations were found to be between 6 ± 0.54 to 10 ± 0.02 (cm) which indicate suitability of the formulations for application on the skin (Table 4).

3.4.6. In vitro Etoricoxib release

Et cubosomal gel formulations showed sustained release pattern up to 24 hr whereas Et gel showed complete Et release at the end of 7th hr (Figure 5). In vitro Et release was affected by gelling agent (CMC-Na) concentration and permeation enhancer concentration (glycerol). For CMC-Na concentration, slower drug release was observed by formulations having higher CMC-Na concentration at the same glycerol level (Figure 5). This could explain higher Et release (75 %) in G1 (1 % CMC) at the end of the 10th hour compared to the slower release profile for G4 (1.5 % CMC) that showed only 49 % Et release at the end of 10th hr. It was also obvious that elevating glycerol concentration from 2.5 % to 5 % or 7.5 % resulted in positive shift in drug release at the same concentration CMC-Na (Figure 5). Hence, G5 (5%

glycerol) exhibited 63 % Et release that increased to 75 % at the end of 10th hr when glycerol concentration increased to 7.5 % in G6.

Kinetic analysis of the in vitro Et release data showed that the prepared Et cubosomal gels (G1 - G6) followed diffusion– controlled release kinetics because it showed higher r^2 values (0.980 – 0.995) as compared to other models (See table 2S in supplementary data).

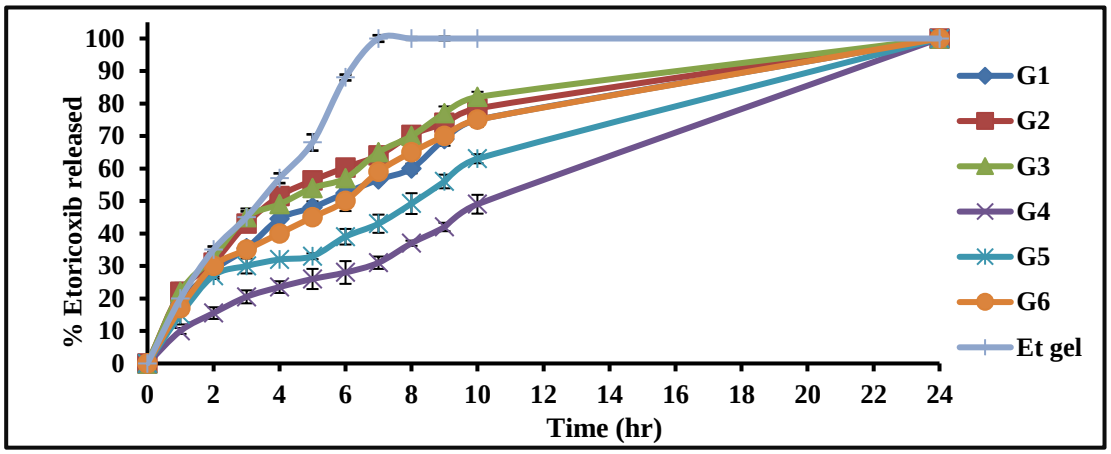

Figure 5. Percentage Et released from Et gel and Et cubosomal gel formulations (G1- G6).

3.5. Ex vivo skin permeation

Based on the previous results, it is evident that G3 (1% CMC, 7.5% glycerol) exhibited favorable physical characteristics and highest 82 % drug release at the end of the 10th hour, making it the preferred choice for further ex vivo studies. Permeation studies for G3 were conducted using abdominal rat skin, and the results are presented in Figure (6). These results revealed that, at the end of the 24th hour, approximately 44.5 ± 1.2% of the drug permeated from the Et gel, whereas approximately 92.5 ± 1.4 % of the drug permeated from Et cubosomal gel (G3). These findings indicate a higher Et permeation rate from Et cubosomal gel compared to Et gel.The Et permeation data from Et gel and Et cubosomal gel (G3) were subjected to mathematical permeation modeling by calculating steady-state flux (J) , permeability coefficient (P) and coefficient value (r^2) (See table 3S in supplementary data). The steady-state flux (J) and permeability coefficient (P) were 4.361 µg cm^{-2} hr^{-1} and 0.0043 hr^{-1} respectively for Et gel while Et cubosomal gel (G3) showed steady-state flux (J) of 4.507 µg cm^{-2} hr^{-1} and permeability coefficient (P) of 0.0045cm hr^{-1}.

Kinetic treatment of ex vivo permeation data indicated that Et permeation from Et gel and Et cubosomal gel followed zero-order mechanism since that zero-order permeation model had the highest coefficient values (0.935 and 0.867 for Et gel and Et cubosomal gel (G3) respectively.

4. DISCUSSION

The present study aimed to formulate transdermal gel containing Et loaded cubosomes. Compatibility study between drug and excipient is needed to detect any possible interaction that may affect performance of drug product. Compatibility between Et and the selected excipients was studied using FTIR spectroscopy [29]. From the FTIR spectra, the essential absorption bands for Et were identified unchanged in the FTIR spectrum of physical mixture (Figure 1f). Therefore, no interactions would be suspected between Et and the used excipients.

Different Et cubosomal formulations were fabricated by melting method using three different concentrations of GMO and Poloxamer 407. Drug content was (88.0 ± 1.40 to 98.9 ± 0.92) which ensure uniform Et distribution into the developed cubosomal formulations [30].

The degree of drug entrapment or encapsulation within the nanoparticles is measured by EE [31]. The values of % EE (Table 3) confirmed efficient Et encapsulation into the prepared cubosomes nanoparticles. High EE values of Et cubosmoes was attributed to its hydrophobic nature which accounts for its higher affinity to the hydrophobic domain of the cubic phase bilayer [43]. Both GMO and P407 showed positive correlation with % EE values of the prepared Et cubosomes (Table 3). For P407, higher P407 concentration facilitated partitioning of the hydrophobic Et molecules into the aqueous phase during cubic gel phase transition to cubosomes [32]. For GMO effect, increasing GMO concentration resulted in higher % EE values at the same poloxamer concentration (Table 3) due to faster solidification of the

cubosomal dispersion by higher GMO concentration. Faster solidification would prevent drug diffusion out to the external phase [33]. It was obvious that formulation S9 containing the largest concentration of P407 (0.2 % w/w) and GMO (1% w/w) obtained the highest % EE value (92.0 % ± 1.6).

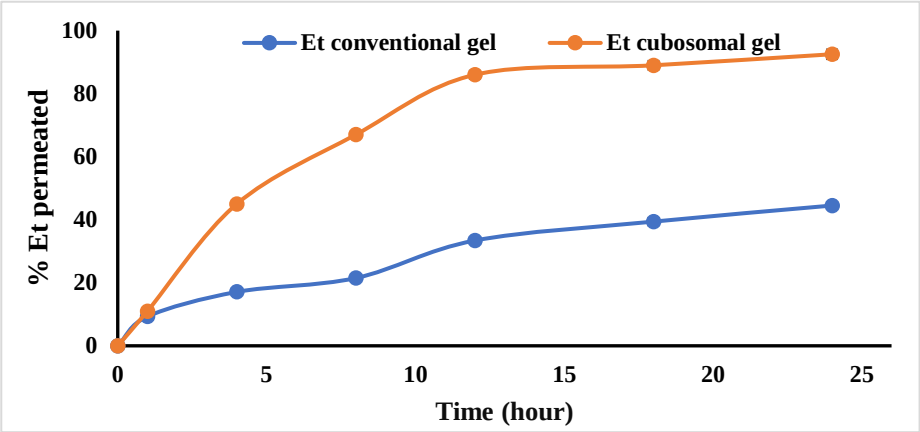

Figure 6. Cumulative permeation of Et gel and (G3) through abdominal rat skin in pH 6.4 phosphate buffer solution at 37 ± 2°C.

Particle size is a critical parameter in skin penetration since penetration into deep skin layers can be assured/confirmed for nanoparticles having particle size lower than 300 nm. Therefore, the developed Et cubosomal formulations were studied for particle size [34]. The results obtained indicated that the prepared Et- loaded cubosomal dispersions were suitable for transdermal drug delivery. As obtained from results, smaller sized particles were yielded upon increasing P407 concentration (Table 3). This could be due to the nonionic surfactant P407 that reduces the interfacial tension between the liquid crystalline nanoparticles and the aqueous surroundings, preventing the particles from aggregating and creating smaller sized cubosomes [9]. In contrast, the particle size of cubosomes would be larger upon increasing GMO concentration at constant P407 concentration (Table 3). This could be explained by GMO tendency to agglomerate at high GMO concentrations producing cubosomes of bigger particle sizes [35]. Particle size distribution was indicated by PDI where higher PDI values are seen in samples having wider range of particle sizes. On the other hand, lower PDI values are seen in samples with equally sized particles. The prepared Et cubosomes formulations showed low values of PDI which indicated uniform particle size distribution [36].

The in vitro Et release from the prepared cubosomal formulations was studied where results showed fast Et release with elevated P407 level and slow Et release with higher GMO conc (Figure 2). This could be explained by the ability of P407 to solubilize Et in the aqueous release medium resulting in higher Et affinity to the in vitro release medium. Furthermore, higher P407 concentration led to smaller sized particle with increased surface area for drug diffusion, which accelerated Et release [14]. In contrast, GMO retarded Et release due its ability to slow down Et (lipophilic drug) partitioning from cubic nanoparticles to the aqueous release medium [37]. Additionally, higher GMO concentration led to larger cubosome particle sizes and a smaller surface area for drug diffusion, which caused Et to be released more slowly [14].

From the results obtained, formulation S9 (1 % GMO and 0.2 % P407) was proved to have high % EE, suitable particle size and appropriate in vitro Et release profile. Furthermore, changes in the physical state of Et as a result of cubosomal formation was evaluated using DSC. According to the DSC findings, pure Et powder, GMO, P407, displayed distinct endothermic peaks that can be attributed to their individual melting points. The shift observed in Et melting point in the thermograms of Et-loaded cubosomes (Figure 3d) ensures molecular dispersion of Et within lipid polymer matrix in its amorphous form [38]. Therefore, DSC provided evidence for Et encapsulation in the developed cubosomes in an amorphous state [27].

Zeta potential is an important indication for the magnitude of the electrical repulsion between the developed particles. High repulsion forces prevent coalescence and aggregation between particles. Zeta potential value of the selected Et-loaded cubosomal formulation was -20.1 ± 0.379 mV (Figure 4a). The presence of a negative charge on the surface of cubosomes is primarily attributed to the ionization of the carboxylic end group of oleic acid, which is derived from GMO, given its lipophilic nature. Furthermore, P407 contributes to the negative charge on the cubosomes through the interaction between the hydroxyl ions of poloxamer 407 and the aqueous medium. Although the zeta

potential in this system was not high enough (>25 mV) to provide effective electric repulsion to avoid the aggregation of particles, poloxamer 407, acting as a steric stabilizer, would not only stabilize the cubic phase dispersions efficiently but also preserve the inner cubic structure of the particles [39].

Cryo-TEM is a powerful supplement to scatter data since it allows for direct visualization and verification of lattice symmetry[26]. The generated cubosomes were nano-sized, as shown by transmission electron micrographs, which supports the findings of particle size analysis. The particles are well separated from one another and appear to be slightly spherical or cube-shaped in micrographs (Figure 4b).

The chosen Et cubosomal formulation was then incorporated into gel using CMC-Na as gelling agent and glycerol as permeation enhancer. The fabricated gels were studied for pH since the skin is highly affected by the pH of the applied preparations. Since higher pH values can lead to skin dryness and lower pH values might irritate the skin, the pH range (6–8) is ideal for transdermal treatments [40].The results of the developed gel formulations (Table 4) indicated their suitability for application to the skin [5].

Drug content was measured to ensure uniform drug distribution in the gel. The results of drug content experiments showed good Et incorporation in the formulated cubosomal gel formulations with no drug loss during preparation.

The gel viscosity can affect spreadability, skin feel and drug release from transdermal preparation. Therefore, viscosity of the prepared Et cubosomal gels was studied [41]. The viscosity of the prepared Et cubosomal gels was affected by the polymer concentration together with moderate effect for glycerol concentration. From the results presented in (Table 4), it was observed that more viscous formulations were yielded upon increasing CMC-Na concentration at the same concentration of glycerol. The viscosity increase with increase in CMC-Na concentration can be attributed to the presence of more cross linking in polymer upon polymer concentration increasing [42]. Glycerol increasing also resulted in mild increase in the viscosity of the prepared gel formulations. However, glycerol effect was lower than the polymer effect (Table 4). The capability of glycerol to alter water-polymer hydrogen bond characters could explain the glycerol effect on viscosity by altering polymer swelling and gel viscoelasticity [43].

Spreadability was studied for the developed gel formulations to ensure uniform application of the gel to skin which is crucial for patient compliance. A good gel will spread quickly and have good spreadability [44]. It was observed that spreadability was decreased upon increasing viscosity [45]. The prepared gels showed good spreadability values that were suitable for skin application (Table 4).

In vitro Et release study was performed to characterize the release profiles from the developed formulations [46]. The difference in release pattern between Et gel and Et cubosomal gel formulations could be attributed to lipophilic nature of the developed cubosomes nanoparticles [47]. Et release from the fabricated gels was retarded as compared to its release from the selected cubosomal formulation (S9) which showed 100 % at the 6th hr due to gelling agent effect (Figure 5). The in vitro Et release from the formulated gels was dependent on CMC-Na concentration with slower release obtained at higher polymer conc. That could be explained by the fact that the concentration of gelling agent directly affects the viscosity of the preparation. Increased viscosity resulted in lower fluid penetration into the gel formulation leading to slower Et release [48]. In contrast to CMC-Na effect, increasing glycerol concentration resulted in faster drug release. The positive effect of glycerol on Et release from the prepared cubosomal gel formulations could explained by its ability to hydrate the formulated gel. Hence, glycerol enhances water channels formation that resulted in increasing permeation of the release medium into Et cubosomal gel formulations [49]. Although increasing glycerol concentration resulted in increasing viscosity of gel formulation, the lipid nature of nature of Et containing cubosomes incorporated in the gels made the hydration effect of glycerol predominant for its stiffing effect.

Et release from cubosomal gel formulations followed Higuchi diffusion model. So, Et release could be explained by diffusion mechanism. In diffusion model, drug is transported through polymer by molecular diffusion due to concentration gradients [50].

Ex vivo permeation studies are regarded as a useful tool that could provide insight into the performance of transdermal gel under in vivo conditions.[51] As illustrated in figure (6), Et permeated from Et gel and Et cubosomal gel (G3) was 45.0± 1.2% and 95. 0± 1.4% respectively at the end of 24th hr. The enhanced permeation of Et through the skin from Et cubosomal gel could be due to the entrapment of Et in the cubic structured nanoparticle (cubosomes) composed mainly of GMO and P407. GMO and P407 could have interacted with the lipids of the skin to form channels which facilitated their/ drug permeation. Additionally, small sized particles (63.5 nm) incorporated into Et cubosomal gel (G3) formulation could have assisted in the transfer through the

epidermis since these particles had sizes less than 100 nm. The enhanced Etoricoxib concentration in the skin would be beneficial and helpful in treating inflammation at the site of application [32].

The results indicate that Et gel and Et cubosomal gel (G3) exhibit different permeation characteristics (See table 3S in supplementary data). The superior permeation characteristics, with a flux (J) of 4.507 μg cm^{-2} hr^{-1} and a permeability coefficient (P) of 0.0045 cm hr^{-1}, observed in the case of Et cubosomal gel in comparison to Et gel, strongly indicate a more efficient and enhanced Et permeation from Et cubosomal gel than Et gel.

The zero-order coefficient value (r^2 = 0.867) for Et cubosomal gel indicates a predominant zero-order release mechanism, similar to that observed for Et gel. The prevalence of a zero-order release mechanism in both formulations suggests controlled and consistent drug release over time, which may have significant implications for therapeutic applications [52].

From the previous results, G3 (1 % CMC, 7.5 % glycerol) showed suitable physical characters, acceptable sustained release profile, and efficient drug skin permeation. Results presented the developed formulation as an efficient drug delivery system for which in vivo performance would be evaluated in future studies.

5. CONCLUSIONS

In the present study, Et cubosomal gel was successfully developed as a promising transdermal delivery system. The prepared Et cubosomes exhibited satisfactory entrapment efficiency and particle sizes within the nano range. Among the formulations studied, the formulation with satisfactory in vitro drug release, higher drug entrapment efficiency showed appropriate stability with a negative zeta potential (- 20.00 mV), and well defined particles in nanometer range. Consequently, it was chosen for incorporation into a CMC-Na gel base to formulate the transdermal cubosomal gels. The formulated cubosomal gels containing Et exhibited desirable pH values, drug content, viscosity, and spreadability values, along with sustained drug release characteristics. Et cubosomal gel showing acceptable physical properties together with suitable release profile was selected for ex vivo skin permeation studies, where it demonstrated efficient drug permeation through the skin. Efficient Et permeation through skin presents Et cubosomal gel formulation as successful transdermal drug for which in vivo performance would be investigated in future work. This developed Et cubosomal gel holds promise as an effective formulation for Et delivery through the skin, potentially maximizing therapeutic benefits and minimizing adverse effects associated with oral Et administration.

In the present study, Et cubosomal gel was developed successfully as a promising delivery system for transdermal application. The prepared Et cubosomes showed acceptable EE and particle sizes within the nano range. Vesicles with high EE (92.0 % ± 1.6), low particle size (63.5 nm) and adequate stability (-20 ± 0.379 mV) were incorporated into gel formulations. The developed cubosomal gel formulations showed suitable pH, % drug content, viscosity, and spreadability values with sustained drug release. Ex vivo permeation conducted on the formulation showing best sustained release profile confirmed the ability of the developed cubosomes to enhance Et skin permeation. Efficient Et permeation through skin presents Et cubosomal gel formulation as successful transdermal anti-inflammatory drug for which in vivo performance would be investigated in future work. The developed Et cubosomal gel could therefore be considered as a good candidate for Et delivery through skin in order to maximize therapeutic benefit and minimize adverse effects caused by oral Et administration.

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Author Contribution: MHA performed the experiment, collected the data, performed the graphical and statistical analysis, and wrote the manuscript. MAA and MKD designed the research idea, supervised the data analysis, writing, and revised the manuscript. HAE revised the manuscript.

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