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Formulation Optimization and Characterization of Etodolac Loaded Nanoemulgel: A Novel Topical Delivery Approach

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Abstract

The topical drug delivery has a huge beneficial-effect of treating the localized pain and inflammation as it is not subject to first-pass metabolism thus the systemic side-effects are minimal. Nevertheless, poor solubility in water and low skin penetration reduce the clinical application of Etodolac (ED), which is a selective COX-2 inhibitor. The objective of this study was to test and define a nanoemulgel formulation of ED in order to increase its solubility, permeation and efficacy of treatment in a topical application. Screening of ED solubility confirmed oleic acid, Tween 80 and Transcutol P to be the best constituents in formulating nanoemulsion. Pseudo-ternary phase diagrams were used in selecting the most stable region of the emulsion component. Nanoemulsion (NE 3) was found to have the best properties among four tested systems having a small mean size, polydispersity index, high entrapment efficiency, and a highly negative value of zeta potential that proves its physical stability. This nanoemulsion was used to develop a nanoemulgel (NEG 1 to NEG 4) in a Carbopol 934 gel base that was found to NEG 3 have better spreadability, drug content (98.6 1.3%), and prolonged release of the ED in the in vitro release (96.85 % even after 12 h). Franz diffusion testing showed improved permeation through the skin (98.23 percent), and kinetics data evidenced zero order release (R^2 = 0.9675) which was in support of Hixson-Crowell kinetics. During thermodynamic stressing tests NEG 3 did not break up or show signs of degradation, phase separation or recrystallization in a variety of checks. The combination overcame the intrinsic shortcomes of ED, providing a promising vehicle of successful topical anti-inflammatory treatment. Overall, NEG 3 emerged as the most effective nanoemulgel formulation for the dermal delivery of ED.

Keywords: Nanoemulgel, Nanoemulsion, Etodolac, Spradability, Consistency, Phase Diagram.

Introduction:

The topical drug delivery systems have considerable benefits in terms of treatment of the local pain and inflammation mainly because they evade the first-pass metabolism, thereby reducing the overall side effects[1]. ED is a selective cyclooxygenase-2 (COX-2) inhibitor which has been found to have anti-

inflammatory and analgesic effects among many others. Its low water solubility and low skin penetration however limit its clinical use as a topical therapeutic agent. These shortcomings require new formulation strategies that would guarantee a significant improvement in solubility, absorption, and skin penetrability. Owing to small droplet size, high surface area and thermodynamic stability, nanoemulsions have also come into the limelight as potential carriers of poorly water-soluble drugs[2, 3]. Nanoemulsions may be used in the formation of nanoemulgels, a second-generation hybrid system, by embedding the nanoemulsion in a gel delivery system to provide favorite application properties of gels with the permeation enhancing advantage of nanoemulsions. Such systems are capable of enhancing drug retention at the area of application and at the same time releasing yielded drug in a more controlled and sustained manner[4, 5]. Among the present study aims is the development and characterization of nanoemulgel formulations containing ED to make it more suitable and enhanced, so it could be used topically. Examination originates by identifying the λmax of ED by UV-Visible spectrophotometry and considering its solvability with an assortment of solvents and excipients[6]. Appropriate oils, surfactants and co-surfactants were chosen according to their ability to solubilize[7]. Pseudo-ternary phase diagrams were composed to determine the favorable range of composition of nanoemulsion[8]. Picked up formulations were also tested on entrapment efficiency, droplet size, polydispersity index (PDI) and zeta potential[9]. These stable nanoemulsions were next included in Carbopol 934-based gel matrices to develop nanoemulgels[10]. The characterized properties of these formulations were pH, rheological, drug content, in vitro drug release as well as skin permeation using Franz diffusion cells were carried out extensively[11-13]. The release of drugs was measured through kinetic modeling to find out the mechanism of release[14]. Moreover, thermodynamic stability testing was done to ensure how well the formulations will stand up against varying stresses[15]. The holistic trial will focus on designing a stable, effective and skin tolerated ED nanoemulgel system to support the delivery route through the skin so that it has a prospect over its traditional oral or injectable anti-inflammatory treatment.

2. Materials and Methods

2.1 Materials:

ED was received as a gift sample from Lupin Ltd. (Pithampur, Madhya Pradesh, India). Oleic acid, Almond oil, Anise oil and Isopropyl myristate were selected from the Qualikems Lifescience Pvt. Ltd. (Vadodara, Gujrat, India). Tween 80, Tween 20, Propylene glycol, PEG 400, and Transcutol-P obtained from Molychem India LLP (Mumbai, Maharashtra, India) Carbopol 934, Triethanolamine, Methyl paraben, Potassium dihydrogen phosphate, and sodium hydroxide were procured from Loba chemie Pvt. Ltd. (Mumbai, Maharashtra, India). Methanol, Ethanol, Chloroform, Dimethyl sulfoxide (DMSO) and Dimethyl formamide (DMF) were sourced from Changshu Hongsheng Fine Chemical Co. Ltd. (Changshu City, China).

2.2 Methods

2.2.1 Determination of λmax of ED:

The substance ED in the amount of 20 to the nearest mg was weighed using an analytic scale (Model MX204, Mettler-Toledo India Pvt. Ltd., Mumbai) and transferred to the volumetric flask of 50 to the nearest mL. A warm mixture was put in the flask and the excess amount of methanol was added to the flask slowly to make sure that the drug was perfectly dissolved and the volume was brought to 50 mL to

form the stock solution that has the concentration of 400 ug/mL. A working achievable solution of 20ug/mL was prepared by diluting this stock in right amount of methanol. The ultraviolet (UV) absorbance spectrum of diluted solution was measured with a double beam, UV-Visible (model 1700, Shimadzu analytical pvt. ltd., Mumbai) spectrum, wherein the methanol served as a blank[16]. The obtained spectrum is depicted as Figure 1.

2.2.2 Solubility study of ED:

Solubility of ED was measured using a variety of solvents in the determination of equilibrium solubility profile. Each solvent (5 mL) was put in 10 mL glass vials where an excess quantity of ED was added gradually. The vials were subsequently put in a mechanical shaker(Orbital shaker, Lab solution, (Mumbai), India) and given a shaker operation at ambient temperature of 12 hours. After this, the samples were incubated in an extra 24 hours on water bath shaker (IG-15-SWB, iGene Labserve Pvt. Ltd., New Delhi India). To enable a balance to be struck, it has been staged in Transfer of the mixtures into the centrifuge tubes was there after done and centrifugation (Thermo Fisher Pvt. Ltd.-Mumbai India) at 2000 rpm carried out at 5 minutes. Whatman filter paper No. 41 was to be used to filter the supernatants. The filtrates were diluted properly and then analyzed with the help of a double-beam U V-Visible spectrophotometer (Shimadzu 1700, Shimadzu Analytical Pvt. Ltd., Mumbai, India)[17, 18].

2.2.3 Screening of Oils, Surfactants, and Co-surfactants:

To determine the most appropriate excipients in the nanoemulgel formulation of ED, its solubility was evaluated in different oils, surfactants and co-surfactants. The oils contained oleic acid, almond oil, anise oil and isopropyl myristate. Surfactant Tween 20 and Tween 80 were used, and PEG 400, propylene glycol and Transcutol-P were tested as co-surfactants. In every solubility experiment, 5 mg of ED was weighed with great care (Model MX204, Mettler Toledo India Pvt. Ltd., Mumbai) and diluted with 5 mL of respective vehicle in different 25 mL stoppered glass vials. To arrive at saturation equilibrium, the samples were constantly stirred (Secor India research testing instrument, (Mumbai), India) at a regulated temperature of 25+/- 0.50 Celsius degrees in a controlled ambiance of 48 hours. The equilibrated samples were then centrifuged (Thermo Fisher Pvt. Ltd -Mumbai India) at 3000 rpm in 15 minutes to get rid of undissolved particles of the drug. The supernatants were then harvested properly, filtered using Whatman No. 41 filter paper and appropriately diluted with blank. The amount of ED present in individual samples was determined by a double-beam UV-Visible agen spectrophotometer (Shimadzu 1700, Shimadzu Analytical Pvt. Ltd., Mumbai, India) at 225 nm. The oil, surfactant, and co-surfactant with maximum solubilizing ability was, therefore, chosen as the best compounds to be used to solidify further in formulating the product[19, 20].

2.2.4 Construction of Pseudo-Ternary Phase Diagram

Pseudo-ternary phase diagrams were created through aqueous titration method to study the appropriate area of nanoemulsion formation in establishment of an ED-loaded nanoemulgel. Oleic acid as the oil solvent, Tween 80 as the emulsifying agent and Transcutol-P as the co-emulsifying agent were chosen according to the previous screening of solubility. The mixtures of surfactants and co-surfactants (Smix) were also prepared in various size ratios, that is, 1:1, 2:1, 3:1, and 4:1. The oil phase was subsequently added to these Smix blends in weight ratio of 1:2 to 4:5 (oil:Smix) at different proportions. All of the oil-

Smix solutions were homogenized in presence of a vortex mixer (IG-DMS, iGene Labserve Pvt. Ltd., India) to make sure that there is equal mixing. After homogenization, dropwise addition of double-distilled water was done in each mixture with constant magnetic stirring (15-MLH Plus, Remi Elektrotechnik Ltd., Maharashtra, India) at a room temperature. The titration procedure was carried on up to the point when the preparation turned turbid, defining the boundary of the nanoemulsion range. Any mixture left having low viscosity, clear and transparent was deemed as a successful case of oil-in-water (o/w) nanoemulsions formation. The data acquired of all the combinations were represented on a triangular coordinate graph to create the pseudo-ternary phase diagrams with the help of appropriate plotting software. These plots allowed determining which compositional zones are favorable in the stable formation of nanoemulsion and assisted in the choice of the optimal component ratios to be used in the further formulations studies[21-23].

2.2.5 Preparation of ED Nanoemulsion

The weight-accurate ED (1% w/w) was added to a pre-warmed mixture (75 (C)) of oleic acid (oil phase) and Smixa of Tween 80 and Transcutol-P. This mixture was vortex mixed (IG-DMS, iGene Labserve Pvt. Ltd., India) and vortex mixing was carried out for 5 minutes to make sure that solubilization of drug into the oil-Smix system was complete. The mixture to which the drug was incorporated was subsequently added gradually to dropwise volumes of 2X concentrated (2X, hereafter) double-distilled water under continuous vortex (IG-DMS, iGene Labserve Pvt. Ltd., India) for 2 minutes to bring about the emulsification process, droplet form. This was followed by a cool down of the resulting pre-emulsion at room temperature resulting in a homogenous nanoemulsion of a clear translucent nature. To further alleviate the size of the droplets and to bring about a greater uniformity, the formulation was ultrasonicated in a probe ultrasound system (LI-PS 650, Lasany International, Haryana, India). The kinetic stability and potential bioavailability of the nanoemulsion were enhanced with the help of this high-energy technique in realising nanoscale droplet sizes[24-26].

Table 1. Formulation preparation of nanoemulsion

Formulations	S.mix(ratio)	Oil/S.mix (ratio)	%w/w of components inNanoemulsionformulation			Drug %w/w
			Oil	Smix	Water	
NE 1	1:1	1:2	10	20	70	1
NE 2	1:2	1:4	20	30	50	1
NE 3	1:3	1:6	30	40	30	1
NE 4	1:4	1:8	40	50	10	1

Smix is the combination of the surfactant (Tween 80) and a co-surfactant (Transcutol-P) in the well-defined weight proportions. ED is in all the formulations by 1% w/w. Oleic acid was oil phase used. For standardization, the total weight of every formulation was held at 100 g.

2.2.6 Optimization of Nanoemulsion by Droplet Size, Polydispersity Index and Zeta Potential and Entrapment efficacy.

Dynamic light scattering (DLS) was also carried out to determine the droplet size, polydispersity index

and zeta potential in the optimised nanoemulsion formulations using a 170 o back scattering angle. The required diluting of the formulations with purified water was done before the analysis. The measurements of surface charge were carried out with a zetasizer-nano (Malvern instruments model no. Zen 3600 Zetasizer) and provided with a special cell. Each reading was done in total of three times[27]. To better understand % entrapment efficiency, the ED nanoemulsion dispersion was mixed with 1.0 mL of chloroform (CHCl3) and sonicated within 1 min. The dispersions were analyzed by adding 3.9 mL of methanol to them then sonicating the mixture in 10 min. These dispersions were centrifuged (Thermo Fisher Pvt. Ltd.-Mumbai India) at 14000 rpm and 10 min with the use of an Eppendorf Centrifuge 5424[28]. Afterwards, an analysis of UV-Vis spectroscopy (Shimadzu 1700, Shimadzu Analytical Pvt. Ltd., Mumbai, India) with Eq 1 was taken to detect the presence of ED in the supernatant at 225 nm.

$$\%\ Entrapment\ efficiency = \frac{Initial\ Weight\ of\ etodolac - Free\ etodolac}{Initial\ weight\ of\ etodolac} \times 100 \ldots . Eq\ 1$$

2.2.7 Formulation of Nanoemulgel

The creation of ED nanoemulgel followed a sequential method of three steps whereby variable concentrations of Carbopol 934 taken as the most suitable gelling agent in topical formula. Step 1: An optimized nanoemulsion formulation of ED (NE 3) was developed by aqueous phase titration method. This formulation which had been completed after preliminary testing was used as the drug loaded dispersion medium to prepare further gel. Step 2: To make the gel base, Carbopol 934 was dissolved in distilled water in a rotating motion within 30 minutes using a magnetic stirrer (15-MLH Plus, Remi Elektrotechnik Ltd., Maharashtra, India) in order to mix thoroughly. The dispersion was subsequently left to swell and hydrate during a 6 hour duration at ambient temperature. Step 3: The nanoemulsion was added to the gel base (nanoemulsion to gel) with gentle stirring (Secor India research testing instrument, (Mumbai), India) to make a homogenous and a smooth mixture. Triethanolamine was used to bring the pH within the range of skin utilization after obtaining the ensuing nanoemulgel. This resulted in a clear, stable and therapeutically viable, topically deliverable, ED nanoemulgel[29-31].

Table 2: Formulation of Nanoemulgel

Formulations	Nanoemulsion (mL)	Carbopol 934 (g)	Water(mL)	Methyl Paraben (mL)	Glycerin (mL)	Triethanol amine(mL)
NEG1	50	1	50	0.1	5	Q.S.
NEG2	50	1.5	50	0.1	5	Q.S.
NEG3	50	2	50	0.1	5	Q.S.
NEG4	50	2.5	50	0.1	5	Q.S.

All of the formulations (F1-F4) were then made as 50 mL of the optimised ED nanoemulsion. Carbopol 934 is a gelling agent which was utilized in different concentrations (1- 2.5 g) to determine its role in gel consistency and spreadiness. Methyl paraben was used as a preservative and glycerin to add a humectant and the PH was fixed with the help of tri ethanol amine (Q.S.: quantum satis i.e., adequate amount to adjust pH to a skin-friendly level). Dispersion medium was done with double-distilled water.

2.2.8 pH Determination of Nanoemulgel Formulations:

Before the analysis, it is necessary to calibrate the pH meter (Mettler Toledo India Pvt. Ltd. amar hill Mumbai (Maharashtra), India) by the standard buffer solutions (pH = 4.0, 7.0, and 10.0)[32]. Take about 5 grams of the ED nanoemulgel preparation. Clean the pH electrode using distilled water and be careful to absorb dry using a cotton tissue or a blotting paper ensuring contact is maintained on the sample. Insert the electrode in the nanoemulgel and note the pH level. You may then take a temperature probe and determine the temperature of the sample and log it. Measure the quantities three times to insure the accuracy and reproducibility.

2.2.9 Rheology Assessment of ED-loaded nanoemulgel (NEG1-NEG4)

Spreadability of ED nanoemulgel formulations was determined by means of Texture Analyzer (TA.XT Plus) equipped with conical spreadability rig, which is composed of two strictly aligned male and female Perspex cones (Perspex 90)[33]. A fixed platform was fitted with the female cone whereby the formulation was loaded with a spatula. The instrument was calibrated in terms of distance by pointing an empty female holder and accurate distance was taken before calibration using an empty female holder. In the analysis, the male cone was folded into the sample slowly up to 2 mm so as to resemble spreading. The amount of force it takes to go through the gel (firmness) was noted at this depth and the properties of the force and time plot were used to determine spreadability. The probe could automatically get back to its initial position after taking measurement. Multireplicates were done to get average values of maximum force and area under the curve. The consistency of the formulations was determined on the same Texture Analyzer but a back extrusion setup[34]. The experiment was carried out in a cylindrical container with 50 mm diameter like a test tube, which was filled to about 75 percent with the nanoemulgel. It had a 40 mm disc at the centre and it was calibrated with a 30 mm fixed calibration distance to determine the accuracy of the instrument. The disc was then inserted in to the sample, until coming into contact with the densest section of the gel. By now, the instrument would record the maximal force (firmness), whereas the area between the curve and the x-axis would imply the overall consistency of the formulation the bigger the area, the more ordered and dense the formulation. The value of cohesiveness was also derived based on the back extrusion data using the negative part of the force time curve. The higher the negative peak force, the greater the bonding of the internal structure and the amount under this section (work of cohesion) was the amount of energy needed to overcome the internal forces. On the whole, a larger magnitude of both positive and negative sides of the graph represented a greater level of firmness, consistency, and cohesiveness of the ED nanoemulgel formulations.

2.2.10 Drug Content Analysis of Nanoemulgel Formulations (NEG1–NEG4)

To determine the presence of the drug in the formulated nano-emulgel (NEG1-NEG4) containing about 500mg of each preparation was weighed precisely and poured into the petri dish. The sample was mixed with a 5 ml of methanol (65 v/v) and shake gently with a glass rod to make a complete combination. The solution thus obtained was placed in a 10 mL volumetric flask and given 10 minutes of sonication to increase the extraction of the drug. It was then added to 10 mL of methanol. The solution so prepared was then filtered using Whatman filter paper (grade no. 41) to eliminate the particulate matter[35]. UV analysis was done using a UV - visible spectrophotometer (Shimadzu 1700, Shimadzu Analytical Pvt.

Ltd., Mumbai, India) at 225 nm. The amount of drugs in the nanoemulgel samples are determined by the equation 2.

$$\%\ Drug\ Content = \frac{Practical\ amount\ of\ etodolac\ determined\ in\ nanoemulgel}{Theoretical\ amount\ of\ etodolac\ present\ in\ nanoemulgel} \times 100 \ldots . Eq\ 2$$

2.2.11 In Vitro Drug Release Study

A modified dissolution instrument was used to evaluate the in vitro release profile of the formulations of the nanoemulgel. Whatman filter paper (grade no. 41) was cut to suitable size and then attached in a stainless-steel basket assembly along the inner surface and lower spectrum. This newly developed basket was consequently submerged into the 50 mL glass beaker with 30 mL of phosphate buffer set at pH 7.4 and removed as the release medium. The whole apparatus was kept on a magnetic stirrer (15-MLH Plus, Remi Elektrotechnik Ltd., Maharashtra, India) and constant agitation was provided at a predetermined temperature of 32+/-0.5 ^{o}C by using a Teflon magnetically coated stir bar. An amount of nanoemulgel of 5 mg of ED was precisely weighted and uniformly placed on a thin layer on the basket. At fixed time points, 3 mL samples of the release media was taken out and replaced with an equivalent amount of new buffer to keep the sink conditions. The samples thus acquired were filtered with Whatman filter paper (grade no. 41) and the concentration of drug was calculated by absorbance spectrophotometrically measured at 225 nm with a UV-visible double beam spectrophotometer (Shimadzu 1700, Shimadzu Analytical Pvt. Ltd., Mumbai, India)[36-38].

2.2.12 Permeation study by Franz diffusion cell

The nanoemulgel formulations containing ED were analysed in vitro by a Franz diffusion assembly to determine the permeation. An apparatus involves a donor chamber and a receptor chamber, where donor chamber is to the outer environment and is in the nature of a closed vessel. Effective area of diffusion of the cell was 1.43 cm 2. The receptor compartmenting was filled with phosphate buffer (pH 7.4) and 0.0025% w /v sodium azide was added to prevent the growth of microbes. A magnetic stir bar of rice-like shape was added to the receptor chamber such that it would mix it uniformly. A cellophane sheet was firmly attached over donor chamber and the assembled diffusion cell was arranged between two chambers and a clamp was affixed in it to lock it down. The whole set was put on magnetic stirrer (15 - MLH Plus, Remi Elektrotechnik Ltd., Maharashtra, India) and kept at constant temperature (37+/-0.5 ^{o}C). The membrane had to be hydrated before the experiment by being incubated in the receptor medium over a period of 2 h. After the membrane had been hydrated, the nanoemulgel formulation was added to the donor chamber and an equal amount (5 mL) was spread evenly on the surface of the membrane. At given time elapses, 1 mL of the sample in the receptor chamber was sampled and replaced instantaneously with a similar volume of the fresh buffer to keep the sink conditions. Sample analysis was done spectrophotometrically as the filtered samples were analyzed at 225 nm using double-beam UV Visible spectrophotometer (Shimadzu 1700, Shimadzu Analytical Pvt. Ltd., Mumbai, India)[39-41].

2.2.13 Release Kinetics Modeling

The release kinetic of ED in sustained-release formulation of nanoemulgel was tested by several mathematical models to explain the release mechanism, which include diffusion, erosion and osmosis. The next kinetic models were used:

Zero-Order Kinetics: It is a constant rate of drug release and it does not depend on the concentration. The release profile is drawn as percentage of drug released cumulatively with time. The equation that describes the model is:

$$M_0 - M_t = k_0 t \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

M_t = amount of drug released at time t,
M_0 = initial drug concentration in the formulation,
k_0= zero-order release rate constant (concentration/time).

First-Order Kinetics: Assumes that rate of release is a concentration-dependent process and is usually used to describe drug absorption and elimination. The profile of release can be plotted as log cumulative percentage of drug remaining against time and the equation is as follows:

$$k_1 t = \ln(M_t/M_0) \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

k = first-order rate constant ($time^{-1}$).

Higuchi Model: It is a mathematical equation that explains drug release via the matrix system through the Fickian process of diffusion. The release is plotted as percentage cumulative drug release against square root of time and is calculated utilizing:

$$Mt = kHt \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

In which, kH = Higuchi dissolution constant.

Korsmeyer Peppas Model: Applied in the analysis of drug release of the polymeric system when the mechanism itself is not well understood or when there is more than one type of release behavior. The model takes the form:

$$Ktn = Mt / M \quad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Where: Mt / M = investigated fraction of the drug released at time t, K = a kinetic constant that includes structural and geometric attributes to the system, n = the exponent of the release that describes the drug release process.

Hixson Crowell Model: It is appropriate in the case of systems that experience a shift in shape of the surface area and diameter of the particle as a result of drug release. The model is characterised by:

W01/3 -Wt 1/3 =kt ……(7)

Where: W 0 = original weight of drug, Wt = un-used drug at t, k = Hixson Crowell rate constant. The most appropriate kinetic model plotted was elaborated by considering the highest value of regression coefficient (R^2) after construction of the data plot. This has been done to determine the prevailing ED release pattern of nanoemulgel system[42, 43].

2.2.14 Thermodynamic stability of the developed nanoemulgel formulations:

In order to better determine the thermodynamic stability of the formulated nanoemulgel preparations, a freeze-thaw cycle study was performed. Formulations were first kept in -20 ^{o}C and incubated during 24 hours. They were then returned to ambient temperature and visually assessed in regards to their possible changes. Formulations which reformed to their original clear, homogeneous state in 2 to 3 minutes were regarded as stable under freeze conditions. Subsequently the formulations were further centrifuged on a centrifuge (Thermo Fisher Pvt. Ltd.-Mumbai India) at 5000 rpm and 30 minutes to test the physical stability of all the preparations. A physically stable nanoemulsion was also represented by the lack of phase separation, creaming, or turbidity. Further experimentations were conducted by exposing all the formulations to six heating and cooling cycles consecutively in order to stress them on their stability. In each cycle, 48 h at 4 ^{o}C and 40 ^{o}C was used. Formulations, which did not display any visual evidence of precipitation, phase separation, or any physical instability over all cycles, were classified has thermodynamically stable and as potentially eligible for continued development[44, 45].

3. Results and Discussion

3.1 Determination of λmax of ED

The UV-visible absorption of ED was identified in 225 nm. The UV-visible spectrums (Shimadzu 1700, Shimadzu Analytical Pvt. Ltd., Mumbai, India) were pictorially presented in figure 1.

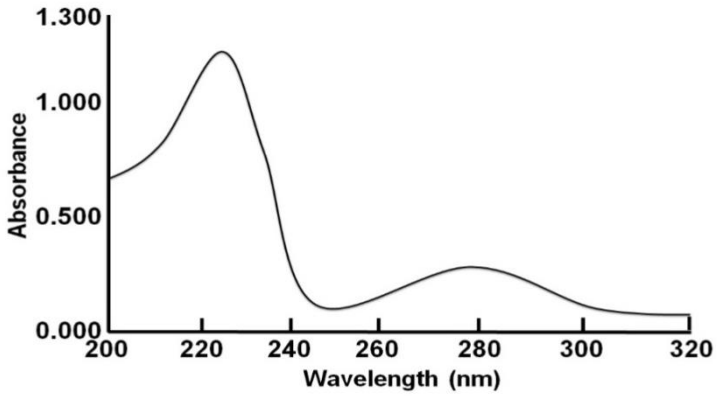

Figure 1: UV visible Spectra of ED

3.2 Solubility study of ED:

The solubility of ED was determined in various solvent systems in order to obtain the most suitable medium of its formulation and possible drug delivery usage. Table 3 gives the results.

Table 3: Solubility of ED in different solvent system

S. No.	Solvent	Solubility (mg/mL)
1.	DM Water	0.01±0.001
2.	Ethanol	22.8±0.03
3.	Methanol	27.5±0.07
4.	Chloroform	11.5±0.08
5.	DMSO	32.6±0.91
6.	DMF	28.8±0.65
7.	Phosphate buffer pH 7.2	0.52±0.01

The data denotes the amount of drug (mg/mL) which dissolved in a solvent at ambient temperature (25 ± 2 °C).

Its findings show a clear indication that ED has poor aqueous solubility and this thus shows the importance of nanoemulgel systems in enhancing its utility in topical or systemic drug delivery system.

3.3 Screening of Oils, Surfactants, and Co-surfactants:

The drug solubility was determined in various oils, surfactants and co-surfactants to know most suitable excipients to use in nanomulsion formulation. Table 4 gives the findings.

Table 4: Screening of Oils, Surfactants, and Co-surfactants:

S. No.	Solvent	Solubility (mg/mL)
1.	Oleic acid	48.6±0.2
2.	Almond oil,	18.4±0.5
3	Anise oil	6.3±0.1
4.	Isopropyl myristate	28.9±0.7
5.	Tween 20	37.1±0.3
6.	Tween 80	64.4±0.2
7.	PEG 400	47.6±0.1
8.	Propylene glycol	28.5±0.5
9.	Transcutol P	88.2±0.9

Oleic acid, isopropyl myristate and anise oil had the highest, medium and lowest drug solubility of 48.6 mg/mL, 28.9 mg/mL and 6.3 mg/mL respectively. The reported solubilizing capacity of Tween 20 in the case of surfactants (37.1 mg/mL) was lower than that of Tween 80 (64.4 mg/mL). Transcutol P was the highest soluble co-surfactant (88.2 mg/ mL) revealed among the co-surfactants tested and therefore

highly recommended among the co-surfactants to enhance drug incorporation and emulsion stability. Based on these results, oleic acid, Tween 80, and Transcutol P were selected as oil phase, surfactant and cosurfactant respectively, upon which the nanoemulsion formulation development would continue considering their high solubilization capacity.

3.4 Construction of Pseudo-Ternary Phase Diagram

Determine the most appropriate area in which nanoemulsion could be formed, a pseudo-ternary phase diagram was established in figure 2, which makes use of oleic acid as a source of oil, a mixture of tween 80 and transcutol-P as surfactant and cosurfactant (Smix), and with double-distilled water serving as the aqueous phase. progressively varying the concentration of oil Smix and water to determine their effect on emulsification process.

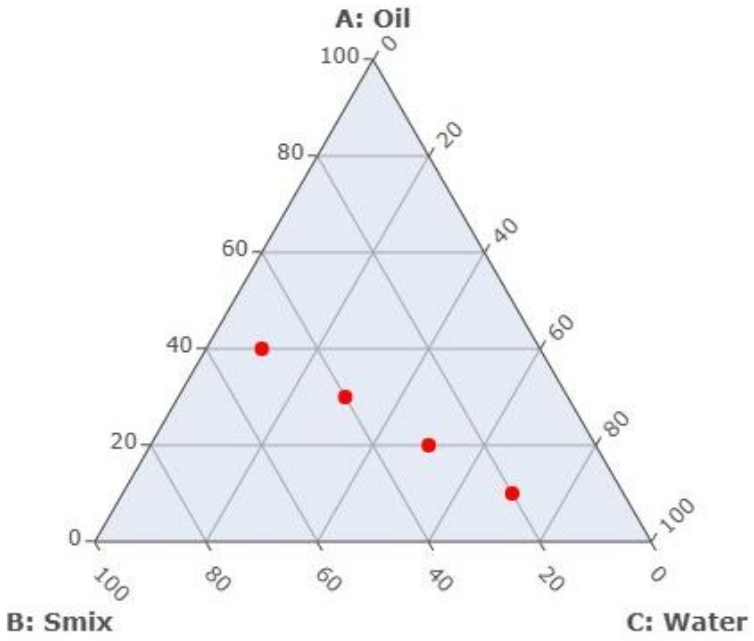

Figure 2: **Pseudo-ternary phase diagram** shows the area which is conducive to the formation of nanoemulsion with oleic acid as oil phase, a Smix system made up of Tween 80 and Transcutol-P and double-distilled water as the aqueous phase. The red markers denote various formulations which were made using different Oil:Smix ratios but using the same amount of water. The aqueous titration approach was adopted in the development of the diagram obtaining stable and clear nanoemulsion compositions in the form of composition diagrams.

One of the data points that were plotted, the formulations with 30 percent oil and 40 percent Smix (3:4 ratio) was almost in the middle part of the nanoemulsion area. Such compositions showed better emulsification and higher formulations stability. It indicates that a growth in proportion of Smix up to an optimum level augments nanoemulsions formation probably because of better oil-water interface stabilization and decreased droplet diameter. On the contrary, the compositions that contained low Smix levels and large amount of water positioned at the water-rich side of the diagram were more likely to

leave the desirable emulsification area. This could be due to the poor concentration of the surfactant that might destabilize the interfacial film integrity resulting in phase separation or phase instability.

3.5 Optimization of Nanoemulsion by Droplet Size, Polydispersity Index and Zeta Potential and Entrapment efficacy:

A comparative analysis of the nanoemulsions formulations (NE1 to NE4) showed an observable difference in the physicochemical characteristics of the formulations such as droplet size, polydispersity index (PDI), zeta potential, and entrapment efficiency. The results were recorded in Table 5. NE 3 exhibited the best properties out of formulation.

Table 5. Showing different formulations and their respective analysis. Data are expressed as mean values ± SD (n = 3).

Formulations	Droplet Size (nm)	PDI	Zeta Potential (mV)	Entrapment efficiency (%)
NE1	178±2.2	0.254±0.08	-38.14±4.1	68.58±1.25
NE2	217±3.5	0.187±0.05	-28.89±2.7	70.98±2.62
NE3	158±1.8	0.112±0.07	-36.21±3.1	94.85±0.84
NE4	250±2.7	0.212±0.03	-35.76±2.1	57.66±5.09

It had the best droplet size of 158 ± 1.8 nm which would be advantageous in increasing the surface area, and the better permeation of drugs. Along with that, NE 3 also showed a very low PDI at 0.112 ± 0.07, reflecting great homogeneity and formulation stability. The value of zeta potential of NE 3 was determined as -36.21 ± 3.1 mV which is considered good in regard to electrostatic stabilization with an aim of reducing the tendency of particle aggregation. Notably, the formulation used obtained the best entrapment efficiency which was 94.85 ± 0.84% thus this formulation was the best with regard to entrapment efficiency as compared to the rest of the samples. In contrast, NE4 had a much higher droplet size 250±2.7 nm nm that indicates poorer formulation stability. NE3 which had been optimized as an ideal formulation because it provided an optimal balance between particle size reduction, stability, non-agglomerated distribution and high entrapment capacity and as such is a good formulation candidate in future development of nanoemulgel based drug delivery systems.

3.6 pH Determination of Nanoemulgel Formulations:

The pH of ED nanoemulgel preparing formula was determined on a calibrated pH meter (Mettler-Toledo India Pvt. Ltd., Amar Hill, Mumbai, Maharashtra). Skin compatibility is a main factor in any topical formulation. As shown in the table, the values of the pH were recorded (Table 6) by taking three separate samples and averaged out as a mean.

Table 6: Determination of pH of blank and loaded emulsion

Formulations	pH of Blank Nanoemulgel	pH of Blank Loaded Nanoemulgel

NEG 1	5.2±0.2	4.5±0.3
NEG 2	5.4±0.1	4.8±0.2
NEG 3	6.2±0.2	5.6±0.2
NEG 4	5.8±0.3	5.0±0.3

All values were set as a mean (standard deviation = SD; n = 3). The pH of blank and ED-loaded nanoemulgel formations was estimated with a calibrated pH meter. All formulas were also tested regarding their compatibility to the skin and the pH was between the recommended limits to put on the skin.

3.7 Rheology Assessment of ED-loaded nanoemulgel (NEG1-NEG4)

The spreadability of the ED-loaded nanoemulgel formulations was determined using texture analyzer which was equipped with a conical spreadability rig. Each of the formulations was tested to find their maximum force 2 mm penetration (firmness) on the scale of that and area under the force time curve as indicated in the table. The mean values with standard deviation after three times replicate are reported as mean The original formulation NEG 3 presented the lowest values of firmness and work; this indicates that this formulation has a higher spreadability, and ease of application. Comparatively, NEG 4 was found to be more firm, therefore, having a more limited spreadability. These findings point at the influence of formulation composition on the mechanical properties, as well as application characteristics of the nanoemulgel. The rheology characteristic of nanoemulgel showed in the Table 7 and spradability graphically represent in the figure 3.

Table 7: Assessment of Rheology Characteristics in ED Nanoemulgels

S. No	Formulation Code	Mean Max. +ve Force (Firmness) (g)	Mean +ve Area (Consistency) (g·sec)	Mean Max. -ve Force (Cohesiveness) (g)	Mean -ve Area (Viscosity Index) (g·sec)
1	NEG 1	120.4 ± 2.3	310.6 ± 5.4	-85.3 ± 2.1	-290.7 ± 4.7
2	NEG 2	132.7 ± 2.7	328.1 ± 6.2	-90.9 ± 1.6	-312.3 ± 3.4
3	NEG 3	95.2 ± 1.8	245.3 ± 4.1	-72.6 ± 1.9	-220.4 ± 3.9
4	NEG 4	148.9 ± 3.0	355.7 ± 5.9	-96.4 ± 1.4	-335.1 ± 3.1

Values are expressed in the form of mean + standard deviation (n=3). With a conical Perspex spreadability rig and a Texture Analyzer (TA.XT Plus) measurements made. The reduced value of force and shear work depicts improved spreadability of nanoemulgel formulation.

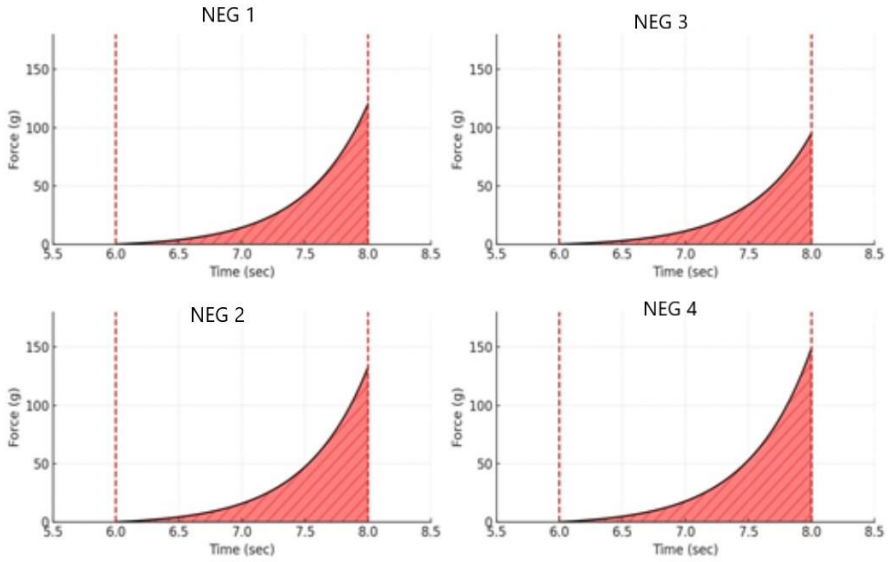

Figure 3: Graph illustrating the spreadability of ED-loaded nanoemulgel formulations.

The spreadability outcomes revealed that NEG 3 was the least firm (95.2 g), and work of shear (245.3±4.1 g·sec) and therefore, the best spreadable and less resistant to application. Under comparison, NEG 4 was associated with a maximum firmness (148.9 ± 3.0 g) and work of shear (355.7 ± 5.9 g·sec), which meant that there is less spreadability. NEG 1 and NEG 2 proved to be intermediary in terms of values with NEG 1 proving to have slightly improved spreadability compared to NEG 2. The ED nanoemulgel formulations (NEG1, NEG2, NEG3 and NEG3) were evaluated using texture profile analysis to determine texture features. The parameters considered were firmness, consistency, cohesiveness and index of viscosity. Firmness (mean largest positive power) and consistency (mean positive area) give inference on resistance of the formulation to deformation and its structural strength. In the meantime, cohesiveness (mean maximum negative force) and viscosity index (mean negative area) are connected with the processes of internal binding of the formulation and ability to receive shape after deformation. NEG 4 exhibited the greatest firmness (148.9 ± 3.0 g) and consistency (355.7 ± 5.9 g·sec), compared to any sample which suggests a fine grained, strong, and highly organized gel. It too proved to be more cohesive (-96.4 ± 1.4 g) and viscos index (-335.1 ± 3.1 g·sec), which lends itself to a more internal integrity and resistance when going through compression and retraction cycles. The firmness and consistency, namely 132.7 ± 2.7 g and 328.1 ± 6.2 g·sec, and the comparatively high values of cohesiveness and viscosity index placed NEG 2 second in terms of overall mechanical stability. NEG 3 on the other hand was the softest (95.2 ± 1.8 g) and consistency (245.3 ± 4.1 g·sec), and the values of cohesiveness and viscosity were lower, which shows it is weaker structure. The values of all parameters were moderate, and firmness was 120.4 ± 2.3 g and consistency of 310.6 ± 5.4 g·sec, that characterize equilibrium texture and moderate viscosity.

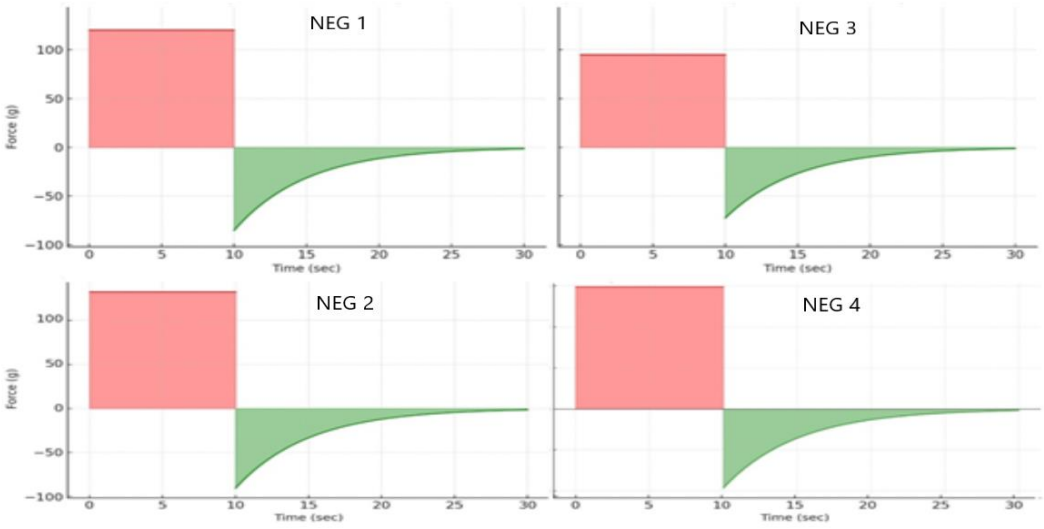

Figure 4: Consistency graph of nanoemulgel of ED, Compression phase, Orange color (0-10 sec): It indicates the corresponding firmness of every formulation. Retraction phase, Green color (10 30 sec): Symptomatic of the behavior of cohesiveness and viscosity of both.

3.8 Drug Content Analysis of Nanoemulgel Formulations

Formulations developed through the use of nanoemulgel displayed the maximum drug content in NEG 3 (98.6 ± 1.3%)**,** indicating high incorporation levels and lack of high losses of the drug during preparation of the formulations. The level of drug content of NEG 2 was also satisfactory (91.3 ± 0.2%), which means a drug distribution in the gel phase did not differ. Conversely, (88.7 ± 0.9%) and especially NEG 4 (78.3 ± 1.0%) had lower values of the drug content. The low quantity of NEG 4 can be explained by the imperfect solubilization of the medicine, possible interaction of the drug molecules with the gelling agent, or ineffective entrapment during the gelation process. Such observations indicate that there is a difference in the efficiency of formulations, and NEG 3 was the most efficient to maintain the theoretical drug concentration in formulation. The results of drug content of ED loaded nanoemulgel were gives in Table 8.

Table 8: Drug contents of ED- loaded nanoemulgel formulations

S. No.	Formulation code	Drug content (%) (n=3)
2	NEG1	88.7 ± 0.9
4	NEG2	91.3 ± 0.2
5	NEG3	98.6 ± 1.3
8	NEG4	78.3 ± 1.0

Drug content is important to determine to assess topical dosage form performance. The results are presented as Mean±SD (n=3).

3.9 In Vitro Drug Release Study

Determine the sustained release profile of ED using various nanoemulgel formulations a 12 h. in vitro drug diffusion test was conducted. Percentage of drug through release was measured cumulatively at a given intervals of time. In vitro ED release results were recorded in Table 9. Of all the formulations, NEG 3 recorded the highest release of drugs after 12 hours at 96.85±0.7% which implies very efficient and sustained diffusion capacity. The prevalent and gradual delivery implies the optimised proportion of nanoemulsion and gel-base permits the easy movement of the drug. NEG 1 released more rapidly (29.69±1.9% at 0.5 hour) and reached an overall release of 92.45±1.1% in 12 hours. This implies a gel structure with moderate viscosity, which allows the rapid onset and subsequent slow drug release. Conversely, NEG2 showed slow onset of release (9.59± 1.5% at 0.5 hour) and it steadily got increased up to 88.29±1.5% by the conclusion of the study. There is the possibility that the slower release rate is attributed to the denser network of the gel at the initial phase that limits the diffusion but favors long release in the long run. NEG 4 with the largest amount of gelling agent again exhibited a long release pattern of 90.14±0.9% after 12 h. The results data were graphically represent in figure 5.

Table 9: In Vitro release studies of ED nanoemulgel formulations

Time (h)	In Vitro ED release of Nanoemulgel Formulations			
	NEG 1	NEG 2	NEG 3	NEG 4
0	0.00	0.00	0.00	0.00
0.5	29.69±1.9	9.59± 1.5	23.87±0.9	16.07±1.2
1	38.35±0.9	13.78±1.1	28.09± 0.7	22.98±1.6
2	42.87±0.6	22.16±1.1	31.95±1.6	26.74±0.7
3	46.78±1.2	26.40±1.9	37.08±1.1	32.85± 1.8
4	51.23±1.7	31.38±1.4	47.67±1.8	37.92±1.3
5	57.18±1.9	38.59±1.2	54.10± 0.9	45.39±0.9
6	60.74±0.7	40.98±1.7	58.93±1.4	51.53± 1.8
7	67.85±1.4	49.96±1.5	67.12±0.8	57.61±0.7
8	74.86±1.8	54.90±0.5	75.85±0.5	64.28±1.2
9	82.62±1.7	69.82± 0.4	85.07±1.2	81.35±1.6
10	85.94±1.8	70.47±1.8	89.42±1.9	84.02±1.3
11	89.32±1.3	74.19±0.9	93.06±1.3	87.17±0.8
12	92.45±1.1	88.29±1.5	96.85±0.7	90.14± 0.9

The table shows the cumulative release of Etodolac as an in vitro release of four nanoemulgel formulations (NEG 1-4) across a 12 hour period. Means are given as standard deviation (n=3).

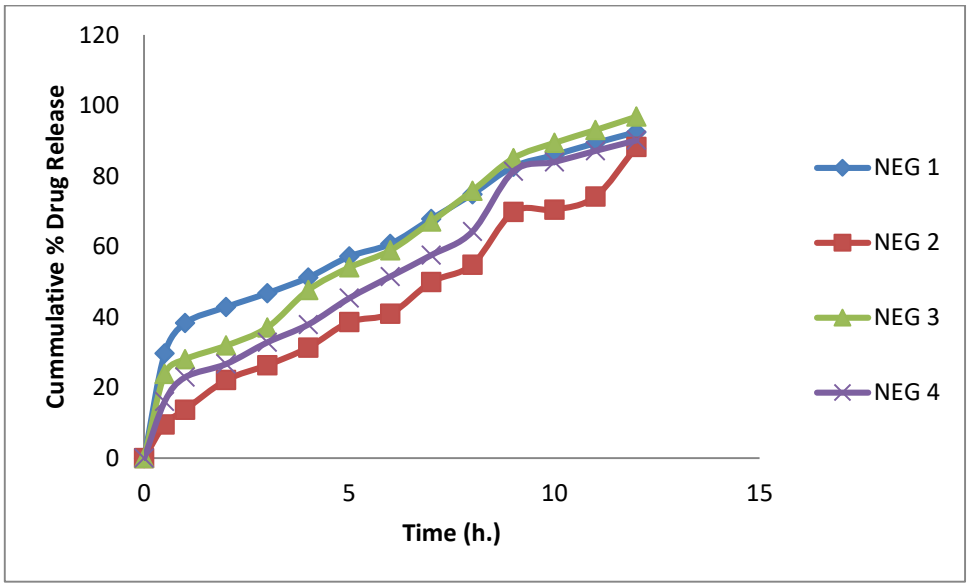

Figure 5: In Vitro release profile of prepared nanoemulgel of ED. A graph was plotted to depict a cumulative drug release of ED nanoemulgel formulations (NEG1 to NEG4) in vitro and 12-hours.

3.10 Permeation study by Franz diffusion cell

Compared to the other tested formulations NEG 3 showed the best drug permeability and the fastest pattern of release which can be explained by the optimized set of surfactant-to-co-surfactant ratio and ambivalent properties of droplets. Conversely, NEG 2 exhibited relatively lower rates of diffusion of the drug implying its possible application to where a prolonged or controlled release of the drug is desirable. Formulations NEG 3 had the highest cumulative drug permeation of 98.23% at 12 hour time slot, that would indicate an exchange of greater efficiency in release. Contrastingly, NEG 2 showed the lowest release (90.31%) indicating reduced and longer route of diffusion of the drug. In the case of NEG 1 and NEG 4, the permeation levels were compared within an intermediate range, and the cumulative releases accounted to 95.28% and 95.61% accordingly. The results of in vitro permeation of ED loaded nanoemulgel were showed in Table 10 and graphically represented in figure 6.

Table 10: In Vitro permeation study of ED loaded nanoemulgel

Time (h)	In Vitro permeation study of ED loaded nanoemulgel			
	NEG 1	NEG 2	NEG 3	NEG 4
0	0.00	0.00	0.00	0.00
0.5	17.69±1.7	16.98±1.5	19.34±1.2	18.07±0.8
1	23.86±1.2	19.78±1.1	25.54±1.8	24.65±0.5
2	43.98±0.8	24.16±1.6	33.69±1.6	31.50±1.6
3	49.75±0.5	28.25±1.9	42.25±1.2	32.85±1.8
4	51.23±1.6	34.18±0.5	52.34±1.7	40.54±1.4

5	60.62±1.4	40.62±1.6	58.21±1.3	49.53±1.1
6	64.74±1.8	43.58±1.5	62.35±0.5	55.23±1.6
7	71.85±1.1	51.27±1.8	70.87±1.5	62.16±1.8
8	77.68±1.3	60.85±0.5	81.59±1.1	72.80±0.7
9	85.35±1.5	72.87±1.6	89.24±1.3	85.39±0.2
10	87.12±0.8	75.34±1.2	90.54±0.8	86.90±1.3
11	91.32±0.4	81.48±1.5	95.20±0.4	91.70±1.9
12	95.28±0.7	90.31±1.3	98.23±0.9	95.61±1.5

The table shows the cumulative mean of in vitro permeation results of Etodolac nanoemulgel formulations (NEG1-NEG4) through synthetic membrane in a period of 12 hours. The expression of data result is mean ± standard deviation (n=3).

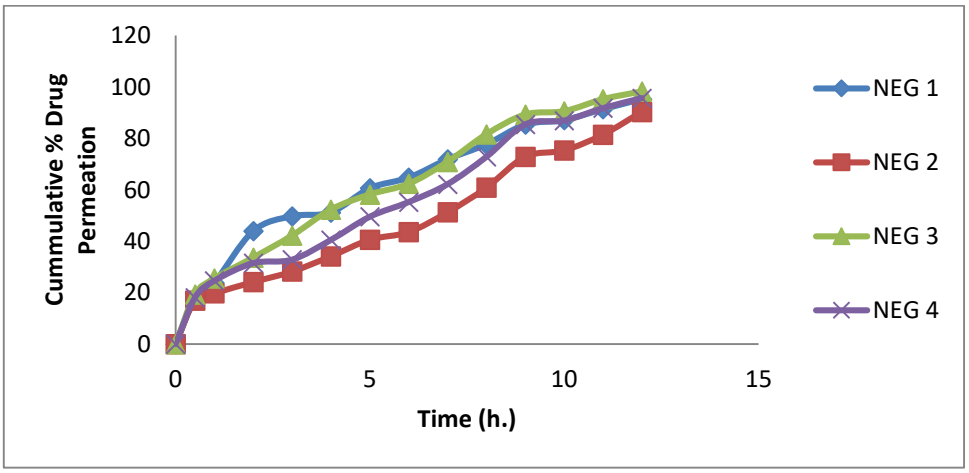

Figure 6: Cumulative in vitro permeation characteristics of Etodolac-loaded nanoemulgel formulations (NEG1-NEG4) were calculated after 12 hours.

3.11 Release Behavior and Kinetics

In order to explain the mechanism of release of the ED, loaded nanoemulgel formulations (NEG 3): in vitro release data were fitted into various mathematical models: Zero-order, First-order, Higuchi, Hixson Crowell, and Korsmeyer Peppas. Figure 7 depicts the summary of the correlation coefficients (R^2) acquired on each model. The Zero-order model (R^2= 0.9675) and Hixson Crowell model (R^2= 0.9659) were most linear and this implies that the Zero-order model would best describe the release of the drug, as this is the model that indicates that the rate of drug release is constant. Also, the agreement with the Hixson Crowell model indicates the important role of the release also depends on the nanoemulgel matrix geometric changes, such as the reduction of the surface area and the reduction in the particle size of the nanoemulgel during dissolution. The First-order (R^2= 0.9045) and Higuchi (R^2= 0.8587) models were fairly correlate, which helps to indicate the possibility of the concentration-dependent release and diffusion mechanisms also determining the drug release distance. On the contrary, the Korsmeyer Peppas model showed a lesser correlation coefficient (R^2= 0.5067), hence the drug release in this

formulation showed that the mode of drug release does not obey anomalous diffusion or non Fickian diffusion. Summarily, the study shows that the experiment results to the drug release performed using the NEG 3 nanoemulgel in the release dynamics are mostly comparable to the Zero-order kinetics with the complementary release dynamics being mainly given to erosion. The results value gives in Table 11 and kinetic model represent in figure 7.

Table 11: Release kinetic model of ED nanoemulgel formulation (NEG 2)

Time (h.)	√t	Log Time	Percent Cumulative Drug Release	Log Percent Cumulative Drug Release	Cumulative % Drug Remain	Cube root of % Drug Remain	Log % cumulative Drug Remain
0	0.00	0.00	0.00	0	100	4.6416	2
0.5	0.707	-0.3010	9.59	1.378	76.13	4.2382	1.882
1	1.0	0.0000	13.78	1.449	71.91	4.1584	1.857
2	1.414	0.3010	22.16	1.504	68.05	4.0827	1.833
3	1.732	0.4771	26.40	1.569	62.92	3.9774	1.799
4	2.0	0.6021	31.38	1.678	52.33	3.7404	1.719
5	2.236	0.6990	38.59	1.733	45.9	3.5804	1.662
6	2.449	0.7782	40.98	1.77	41.07	3.4502	1.614
7	2.645	0.8451	49.96	1.827	32.88	3.2036	1.517
8	2.828	0.9031	54.90	1.88	24.15	2.8905	1.383
9	3.0	0.9542	69.82	1.93	14.93	2.4624	1.174
10	3.162	1.0000	70.47	1.951	10.58	2.1953	1.024
11	3.316	1.0414	74.19	1.969	6.94	1.9074	0.841
12	3.464	1.0792	88.29	1.986	3.15	1.4659	0.498

The kinetic data obtained based on the in vitro release profile of ED nanoemulgel formulation NEG3. It contains converted values to assess drug releases kinetics in accordance with different models.

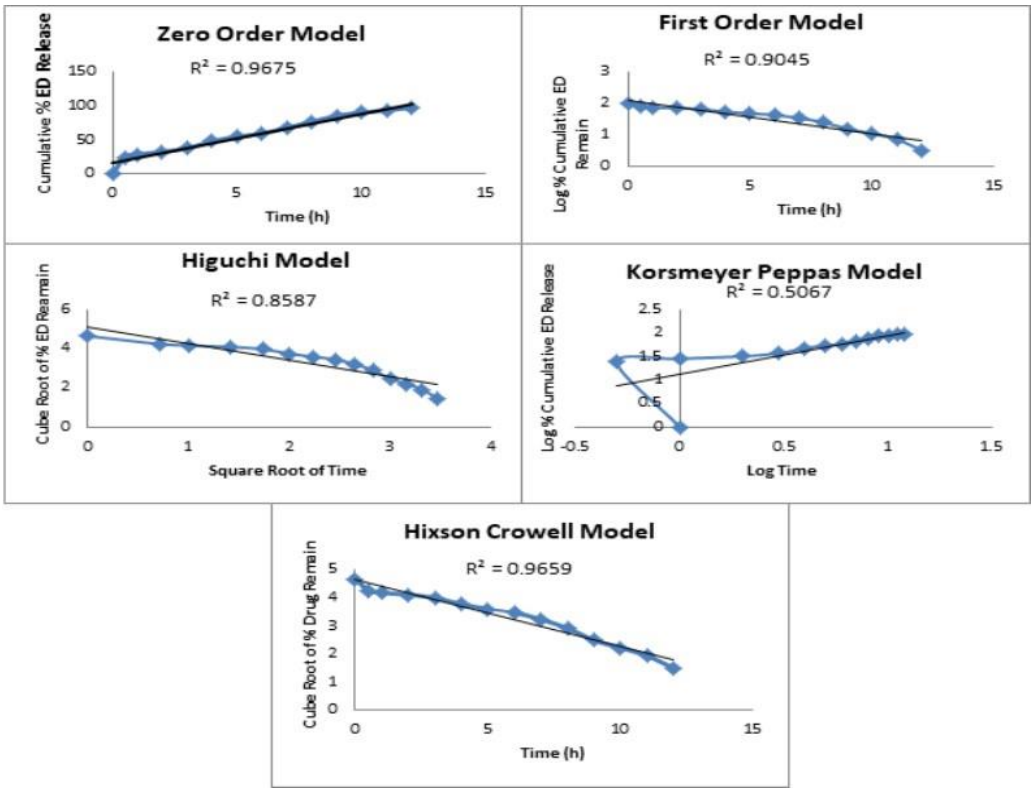

Figure 7: Application of kinetic models: The measurement of in vitro drug release profile of ED-loaded nanoemulgel (NEG 3) by application of kinetic models.

3.12 Thermodynamic stability of the developed nanoemulgel formulation (NEG 3):

The determination of thermodynamic stability of the prepared nanoemulgel formulations a number of stresses has been applied. All the samples turned back to transparent uniform sample within 2-3 minutes since they had been thawed to be at -20 °C implying that they are freeze stable. The physical stability was thereby indicated, as centrifuged at 5000 rpm and 30 minutes, no creaming, or phase separation or any turbidity occurred. Moreover, the formulations remained stable in that there was no evident precipitation or instability after undergoing six heating-cooling cycles (4 °C and 40 °C for 48 h each). Overall, all the findings in Table 12 that showed the formulations of nanoemulgel are very well thermodynamically stable and can be developed further.

Table 12: Thermodynamic Stability Evaluation of Nanoemulgel Formulations (NEG 3)

Test Parameter	Applied Conditions	Observation	Conclusion
Freeze thaw Stability	-20 °C (24 h) followed by room temperature	Regained clear and uniform appearance within 2-3 minutes	Freeze stable
Centrifugation Stability	5000 rpm for 30 minutes	No signs of phase separation, creaming, or turbidity	Physically stable
Heating–Cooling Cycle	6 cycles: 4 °C ↔ 40 °C (48 h per cycle)	No visible precipitation, separation, or instability	Thermodynamically stable

Stability		observed	

Evaluation of the thermodynamic stability testing of ED nanoemulsion formulations even under stress, in freeze-thawing stress studies, centrifugation, and heating and cooling stress. The uniformity of appearance and non-separation or turbidity of the formulations shows great physical and thermodynamic stability of the formulations.

Discussion

This study was devoted to a preparation and description of a nanoemulgel of ED as a topical drug with the objective to solve its low solubility, insufficient drug release, and low skin permeability. The detailed evaluation proved that the nanoemulgel system, and, especially, the NEG 3 formulation, had significant benefits regarding the drug delivery efficacy, stability of this system, and bioavailability. ED was found to be highly insoluble in water (0.01 0.001 mg/mL) illustrating the reason why there is need of an improved delivery system. When compared with other test organic solvents, DMSO and DMF relatively showed high solubilization (32.6 ± 0.91 and 28.8 ± 0.65 mg/mL, respectively). When it came to the selection of the phase of lipids used, oleic acid presented the greatest solubility (48.6+0.2 mg/mL) and thus this could be used as an appropriate oil to be developed into a nanoemulsion. Tween 80 and Transcutol P were selected to be used in further formulation due to high levels of solubilization (64.4 ± 0.2 mg/mL and 88.2 ± 0.9 mg/mL, respectively). The phase diagram generated on the basis of the chosen oil-surfactant mixture (oleic acid, Tween 80, and Transcutol P) defined an extensive area of the nanoemulsion with strong emulsification characteristics. An optimum blend was 30% oil and 40% Smix (in a ratio of 3:4), which stabilized thermodynamically with every droplet being reproduced. Increasing surfactant/co-surfactant ratio enhanced droplet stabilization and reduced the mean size of the globule which enhanced system uniformity. Of all the four nanoemulsion compositions tested, NE3 exhibited ideal properties, such as the smallest diameter of (158 ± 1.8 nm), the lowest PDI (0.112 ± 0.07), and a very negative zeta potential of (-36.21 ± 3.1 mV), which shows high physical stability and uniform distribution. Moreover, it had the best entrapment efficiency (94.85 ± 0.84%) which proves that it effectively incorporated the drug in the nanoemulsifier system. All formulations had a pH that falls in the acceptable therapeutic range (4.5 to 6.2) thus applicable to the skin. The texture profile analysis has shown that the NEG 3 was the most not firm (95.2 ± 1.8 g) and work of shear (245.3 ± 4.1 g·sec), which means, that has the best spreadability and is easy to apply. The NEG 4, on the other hand, was stiffer and thicker and this could cost the patient compliance. In NEG 3, the drug loading efficiency was the highest (98.6 ± 1.3%), regarded as effective incorporation during the gel formulation. Comparatively, however, a lower drug level was found in NEG 4 (78.3 ± 1.0%), which was perhaps because of bad drug retention in the gel form or because it was incompatible with the base of higher viscosity. Due to a wide range of increased and slow drug release, about (96.85 ± 0.7%), of NEG 3 indicated a good combination of prolonged dynamics and quick action throughout the 12-hour release operation. The slightly lower rates were followed by NEG 2 and NEG 4 with a release of 88.29% and 90.14%, respectively, respectively. It is probable that NEG 3 has increased release because of its optimized maximum concentration of the surfactant and reduced gel resistance and hence efficient migration of the drug. This was supported by Transdermal permeation results of which NEG 3 recorded the highest percentage cumulative permeation (98.23%). This can be explained by the fact that its droplet size is nanoscale, its viscosity is lower, and

its emulsifier blend is also the best which increases drug mobility and travels through the skin. The release of the drug in NEG 3 was most likely to fit a zero-order model (R^2 = 0.9675), which is characterized by steady and concentration independent release. It was also indicated that release was affected by geometrical transformation and erosion of the system of the matrix (R^2 = 0.9659), which showed a good correlation with the Hixson-Crowell model. Low regression coefficients were considered for Higuchi and Korsmeyer Peppas models which indicated that diffusion was not the main mechanism, showing that the profile of the release was controlled by the erosion of the matrix. All the stress conditions such as repeated freezing and thawing, centrifugation and variations in temperatures were applied in NEG 3 without its suppression. No phase separation, turbidity or precipitation occurred and the formulation was found to be physically stable. This speaks of its effectiveness of handling and storing in the real world without altering its effectiveness or safety. In general, NEG 3 was found the most successful nanoemulgel delivery system to dermally deliver ED product, as it managed to overcome its main limitations concerning low solubility and skin penetration. The formulation showed good entrapment efficiency, desirable spreadability, zero-order controlled release and promising permeation potentials. These findings emphasise the relevance of the formulation as the use of local treatment to treat inflammatory disorders in a clinical environment can be done more efficiently and with patient compliance.

Conclusion

In the current work, a nanoemulgel formulation of ED was effectively developed and optimized to enhance the intrinsic drawbacks of ED due to low solubility in water, rate of skin penetration, and poor release of the drug during topically applied preparations. Of the many formulations studied NEG 3 has been found to be the most promising in that it has shown to have better physicochemical properties and spreadability as well as drug loading which is about 98.6±1.3% and cumulative in vitro release reported as 96.85±0.7 over 12 hours. Optimized formulation embodied by nano-scale droplet size and low viscosity combined with synergistic blend of emulsifiers in comparison with their counter parts was found to exert improved transdermal permeation (98.23±0.9%). The kinetic modeling produced a zero-order release profile (R^2=0.9675) which is in accordance with the Hixson Crowell model in support of the existence of controlled, geometry-dependent release mechanisms. NEG 3 was physically so stable that it could endure extreme thermodynamic stress origins such as rigorous conditions of phase separation or degradation and could hence be handled and stored in the real world. The therapeutic pH range was well below the formulation making it within the range and thus both rheological and textural properties were desirable since the formulation was easily applied and patients adhered to it. On the whole, NEG 3 is a new effective and efficient topical delivery system in treating ED because of its enhanced bioavailability and therapeutic effectiveness.

Conflict of Interest:

The authors declare that they have no known financial or personal conflicts of interest that could have influenced the work presented in this paper.

CRediT authorship contribution statement

Umesh Kumar Atneriya: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Validation, Project administration. **Kajal Shinde:** Resources, Software, Supervision, Dharmendra Solanki: Visualization, Writing – review and editing.

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