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Development And Characterization Of Pharmacosomal Nanoemulsion-Based Etodolac Gel For Topical Anti-Inflammatory Therapy

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Abstract
For the most part, our studies center on developing new topical nanoemulsions for etodolac to get around its drawbacks. A combination of ultrasonication and high shear homogenization is used to create etodolac nanoemulsions.The formulation components are optimized in a methodical manner employing a design of experiments approach. The modified formulation's zeta potential (ZP), polydispersity index (PDI), and droplet size (DS) were evaluated using the DLS method. To investigate the drug-excipient interactions, we used Fourier transform infrared spectroscopy (FTIR) analysis, and to investigate the surface topology, we employed electron microscopy. Researchers also looked at permeability and in vitro release. A rat model of carrageenan-induced paw edema was used to evaluate the effectiveness in reducing inflammation. A PDI of 0.141, a DS of 163.5 nm, and a ZP of 33.1 mV are the parameters of the ideal formulation. The in vitro release profile was evaluated as sustained release by employing a non-Fickian drug transport strategy. The coarse dispersions had a flux of 59.7 15.2 g/cm2 h, while the etodolac nanoemulsions had a flux of 165.7 11.7 g/cm2 h. At6,8, and 24 hours, the inhibition of edema improved by 13.4%, 36.5%, and 50.65%, respectively. The findings indicate that nanoemulsions are an excellent vehicle for the topical administration of etodolac.
Keywords: *etodolac, nanoemulsions, topical drug delivery, paw edema, permeation*

1. INTRODUCTION

Topical medication administration is a promising alternative to oral and parenteral methods because to its targeted therapy, less systemic adverse effects, longer therapeutic efficacy, and enhanced patient compliance [1]. Despite these advantages, the Stratum corneum, the outermost layer of skin, blocks medication penetration [2]. Drugs with large molecular sizes, hydrophilicity, or high melting temperatures may not be suitable for topical administration. A lot of research is being done to overcome these limitations and enhance medication delivery to the skin. This encourages formulation and delivery research. Nanoemulsions are utilized to apply drugs to the skin. The platform's adaptability and efficiency make it easier for medication components to penetrate skin and be released precisely. These novel delivery systems of tiny oil or water droplets stabilized by surfactants or co-surfactants have good topical physicochemical properties [3]. The solubility and dissolution of hydrophobic drugs are enhanced by the nanoemulsions' increased interfacial area [4]. By allowing lipophilic drugs to penetrate the skin's lipid barrier, this property enhances their absorption and therapeutic efficacy. Therefore, nanoemulsions may be able to address the drawbacks of the current approaches to the administration of topical drugs.

Etodolac, an NSAID used to treat rheumatoid arthritis, has topical delivery issues [5]. It is a medication that falls under the Biopharmaceutical Categorization System (BCS) class II and has high permeability and low solubility in water [6, 7]. However, the skin's tough barrier prevents it from being applied topically. Oral administration of the medication necessitates frequent dosing due to its short duration of action in the body, which can result in cardiovascular effects, fluid retention, edema, and gastrointestinal issues like bleeding, ulceration, and perforation [8–9]. Nano-sized systems are being used in local or topical formulation techniques to address these issues [10]. It increases etodolac's solubility, skin penetration, and therapeutic efficacy when it is encapsulated in a nanoemulsion system. This innovative method demonstrates how nanoemulsion-based medication delivery devices can overcome the limitations of conventional drug administration and enhance patient outcomes. Nanoemulsions, an alternative to etodolac, are designed using several parameters. Nanoemulsions require careful formulation in order to achieve medication integration,

targeting, and release patterns. Optimize parameters like the oil-to-water ratio, surfactant-to-co-surfactant ratio, and mixing methods in order to make a nanoemulsion that is both stable and effective [11]. Design of experiment (DOE) makes this procedure more effective and methodical. DOE allows simultaneous assessment of various components and their interactions, improving formulation key quality attributes (CQAs) [12]. The careful selection and modification of surfactant type and concentration, oil phase composition, and processing parameters is aided by an established experimental design matrix. An ideal formulation can be found by carefully analyzing how these elements affect response properties like droplet size, polydispersity, and physical stability. To make data-driven decisions and optimize the nanoemulsion formulation for desired properties, the formulation scientist employs statistical analysis of trial results. By streamlining experimentation and enhancing formulation optimization, the DOE method produces etodolac nanoemulsions that are both stable and efficient [13].

The goal of this study is to develop the most effective etodolac nanoemulsion for skin penetration. After monitoring important formulation and process parameters, the DOE method was used to improve the nanoemulsion's formulation. When formulation factors interacted, the response variables were droplet size, droplet size distribution, zeta potential, and encapsulation efficiency. Ex vivo and in vivo studies [14] followed the physicochemical characterization of the best nanoemulsion formulation in vitro.

2. MATERIALS AND METHODS

2.1 Materials

Nobel Pharmaceuticals (Istanbul) generously provided Etodolac. Sigma-Aldrich (Schnelldorf, Germany) supplied castor, olive, sesame, Poloxamer® 188, 407, Brij® 35, and tyloxapol. Croda Turkey (Istanbul, Turkey) provided the Caprylic/Capric triglyceride (CrodamolTM GTCC), isopropyl isostearate (CrodamolTM IPIS), isopropyl myristate (Crodamol Evonik Industries (Essen, Germany) supplied Tego Care® 450. Formulation, production, and analysis all made use of chemicals and reagents of an analytical grade.

2.2 Methods

- **Selection of Excipients:** A previously defined method was used to conduct oil screening research. The following was the methodology for this study, with a few minor adjustments: 5 mL screw-capped glass vials were filled with 1 mL of various oils. After that, between 10 and 250 milligrams of etodolac were added to the vials. In an isothermal shaker (Wise Bath, Daihan Scientific Co., Ltd., Wonju, Republic of Korea), the mixtures were allowed to settle for 24 hours at 65 degrees Celsius. After each addition, the eyes were examined at room temperature for drug crystals. The surfactant was selected using the modified method. Several surfactants' emulsifying properties were evaluated by making coarse emulsions. Ten gaqueous dispersions, one percent surfactant, and five percent oil were combined at 80 degrees Celsius. Using a high-shear homogenizer (Silent Crusher M, Heidolph, Schwabach, Germany), the mixture was mixed for one minute at 25,000 rpm. After the liquid had reached room temperature, a visual examination revealed distinct emulsion characteristics.
- **Critical Quality Attributes (CQAs) and Optimization:** Design Expert Software (version 13.0.2.0, State Ease Inc., Minneapolis, MN, USA) and a D-optimal mixture design were used to refine the nanoemulsion's composition. Oil and surfactant, two independent variables, varied by 5–8 percent and 0.5–5 percent, respectively. Key quality attributes (CQAs) for nanoemulsions include droplet size (DS), polydispersity index (PDI), zeta potential (ZP), and encapsulation efficiency (EE) (Table 1) [15]. The Failure Mode, Effects, and Criticality Analysis (FMECA) method was used to identify and record the nanoemulsions' CQA properties [16]. The nanoemulsion's oil-surfactant ratio had the highest risk priority number (RPN) ratings, which were used to rank the failure mechanisms. The focus of the study was on the composition of the nanoemulsion because controlling the temperature during the experimental procedures was difficult. The 13 trials with mixed process variables used to evaluate these characteristics are shown in Table 2. All 13 nanoemulsions were examined using the phase criteria in Table 1.

Table 1. CQAs, specs, and concise testing procedure descriptions

Stage	Parameter	Value / Range	Variation / Notes

I. Computer-Generated Formulations	Droplet Size (nm)	120–180	Target size range
	Polydispersity Index (PDI)	0.2	Uniform distribution
	Zeta Potential (ZP, mV)	±30 mV	Stable colloidal system
	Relative Energy	90%	High formulation stability
II. 90-Day Stability Study	Droplet Size (DS, nm)	Variation < 20 nm	<10% change from initial size
	PDI	0.2	Stable (variation <10%)
	Zeta Potential (ZP, mV)	Variation < 10%	Good electrostatic stability
	Entrapment Efficiency (EE%)	90%	Stable entrapment over time
	pH	6.5–7.5	Within acceptable skin range
	Viscosity	15.5–17.5 cP	<10% change, good spreadability

Size of the droplets, polydispersity index, zetapotential, and efficiency of encapsulation.

Table 2. The design matrix comprises formulation factors and response variables dependent on oil concentration

Formulation	Oil (%)	Sorptive (%)	Potential (mV)	PDI	Droplet Size (nm)
F1	8.0	1.5	42.4	0.233	198.6
F2	6.6	3.3	39.4	0.351	249.8
F3	8.0	3.1	33.7	0.150	164.6
F4	5.0	2.8	34.4	0.184	158.9
F5	8.0	5.0	36.0	0.176	159.2
F6	5.6	3.2	36.7	0.355	224.7
F7	5.3	5.0	38.1	0.407	240.3
F8	6.8	4.7	39.2	0.178	171.6
F9	5.0	1.0	33.4	0.239	196.8
F10	6.5	1.0	34.7	0.193	188.7
F11	5.0	4.0	31.6	0.182	176.8
F12	6.6	4.2	31.8	0.190	173.8
F13	6.5	1.0	34.9	0.249	221.5

- **Preparation of Nanoemulsions:** High-shear homogenization and ultrasoni cation generated nanoemulsions. First, a SilentCrusherM (Heidolph, Germany) was used to disperse oil in an aqueous surfactant solution for one minute at 20,000 rpm to produce coarse emulsions. The coarse emulsions were reduced by probe ultrasonication (Bandelin,SonoplusHD 2070,Germany). A previously described approach identified the process parameters, with no significant changes in application durations and amplitude levels; consequently, the formulation preparation was done for 5 minutes at 75% amplitude.
- **Quantification of Etodolac:** An Agilent1100, Santa Clara, California, United States, HPLC instrument was used for the quantification. With only minor modifications, an established analytical approach was utilized[17]. Sonicated before use, the mobile phase (acetonitrile, water, and glacial acid) was 61:38:1, v:v:v, filtered through a 0.45mm membrane filter. The preparation used a 4.6 250mm column with 5 m particles (ThermoScientific, BDSHypersil,C18, Waltham,MA,USA) at 40 0.5 C. The sample injection volume was 40 L, and etodolac was identified at 280nm by isocratic elution at 1.2mL/min. Theretentiontimeofetodolacwas4.53min. Data collected using Agilent ChemStation® Software (Rev. B. 04.03 SP2 (105)). Standard methods were used to validate the approach prior to trials [18].

- **Characterization Studies**

1. **Determination of Droplet Size, Polydispersity Index and Zeta Potential:** A Zetasizer Nano ZS device from Malvern, Pennsylvania, USA, was used to assess the formulations' droplet size (DS) and polydispersity index (PDI). The samples were quantified in a cuvette after being diluted 1:10. All measurements were taken at room temperature, and the dates and numbers of the measurements were automatically updated. The device was operated in zeta mode and the zeta potential (ZP) was measured at 25 C using a capillary electrode cuvette rather than a conventional cuvette.
2. **Morphological Analysis:** The droplets of the na noemulsion were examined using SEM. As previously stated, water-diluted (1:20, v/v) samples were dried in a vacuum oven at room temperature on a glass slide. Gold and palladium were sputter-coated onto the dehydrated nanoemulsions (Bal-tec, Lübeck, Germany). A Zeiss EVO 40 SEM was then used to examine the coated samples. At various magnifications, nanoemulsion micrographs were captured using the apparatus.
3. **Measurement of Encapsulation Efficiency (EE) and Loading Capacity (LC):** The encapsulation efficiency (EE) and loading capacity were measured using the previously reported technique. An aliquot of the formulation was centrifuged at 5000 rpm for 20 minutes. The free drug could be identified thanks to the HPLC quantification of the supernatant. EE and LC were computed using equations (1) and (2):

$$EE = \frac{\text{initial amount of etodolac} - \text{free etodolac}}{\text{initial amount of etodolac}} \times 100 \quad (1)$$

$$LC = \frac{\text{initial amount of etodolac} - \text{free etodolac}}{\text{amount of lipid in the formulation}} \times 100 \quad (2)$$

4. **Fourier Transform Infrared (FTIR) Analysis:** A Fourier transform infrared (FTIR) spectrometer (NICOLET iS50, Thermo Scientific, USA) evaluated formulation component interactions. As previously reported, the active ingredient, medicated, and unmedicated nanoemulsions (lyophilized) were independently evaluated in the wavelength range of 4000 400 cm 1 using the transmission mode of the FTIR instrument with Omnic version 9.0.0 software. To achieve sufficient transmittance for each sample, multiple scans and force adjustments were made.
5. **Measurement of Viscosity and pH:** A spindleS-7 (DV-II viscometer, Brookfield, WI, USA) at room temperature and a shear rate of 100 cm 1 were used to measure the preparations' viscosities. Using a digital pH meter (MP220, Mettler Toledo, Hessen, Germany), the preparations' pH was determined.
6. **Stability Study:** Stability was studied using a standard approach [19]. In a nutshell, the formulations were kept for 90 days in a Binder (Germany) controlled humidity chamber at temperatures of 25 and 40 degrees Celsius. Samples were taken at regular intervals to measure DS, PDI, ZP, EE, pH, and viscosity.
7. **In Vitro Drug Release Study:** In vitro Franz diffusion cell experiments (Permegear, Hellertown, PA, USA) assessed formulation release. With a diffusion area of 2.88 cm2, each cell had 20 mL in the receptor chamber. Under sink conditions, 2 grams of formulations were used in the release investigation in the donor chamber. The donor and receiver chambers were separated by a synthetic membrane with a 3.5 kDa molecular weight cutoff (Spectra/Por Dialysis membrane, Thermo Scientific, Waltham, MA, USA). A total of 0.7 mL of the release medium (PBS, pH 7.4) was replaced with new media on a regular basis. Samples were evaluated using validated HPLC. The zero-order, first-order, Higuchi, Hixon–Crowell, and Korsmeyer–Peppas models used to analyze in vitro release patterns are shown in equations (3) through (7) [20]:

$$Q_t = Q_0 + k_0 t \quad (3)$$

$$Q_t = Q_\infty (1 - e^{-k_1 t}) \quad (4)$$

$$Q_t = Q_0 + k_H t^{1/2} \quad (5)$$

$$W_0^{1/3} - W_t^{1/3} = Kt \quad (6)$$

$$\log [Q_t/Q_\infty] = \log k + n \log t \quad (7)$$

where Qt and Q0 are the drug amounts released into the release medium at t and t = 0, respectively. The release rate constants k0, k1, and kH are used in the Higuchi, zero-order, and first-order models, respectively. W0 and Wt represent the initial dosage of the formulation as well as a specific time (t). The surface–volume connection is covered by this constant K. The ratio Qt/Q represents the fractional drug release. Diffusional release exponent and kinetic constant are k and n, respectively.

8. **Ex Vivo Permeation Study:** Nanoemulsion penetration on porcine skin was evaluated using Franz diffusion cells. Permeation research parameters were taken from the prior study. After trimming hair with an electric trimmer, abdomen skin was carefully removed. For diffusion experiments, skin samples were carefully removed of fat and particles and stored at 25 degrees Celsius. Skin samples were submerged in isotonic saline for 30 minutes prior to diffusion testing. After that, skin samples were placed with the epidermal side facing the donor chamber and the dermal side facing the receptor medium using Franz diffusion cells. Franz cells received two grams of the formulation in their donor chambers. In addition, a control group was created using a coarse suspension of etodolac (1%, w/w) in a 0.5% carboxymethyl cellulose (CMC) dispersion. PBS at pH 7.4 was used as the receptor phase, which was kept at 37± 0.5 C and stirred at 160 rpm. At selected time periods throughout 24 h, 0.7 mL receptor phase samples were replaced with an equivalent amount of fresh PBS at the same temperature and stirring. Permeation was studied in a sink. The samples were tested using validated HPLC. The ex vivo permeation investigation yielded the values for the coefficient constant, steady state flux (Jss), and permeability coefficient (Kp). Tape stripping procedures were modified to measure Stratum corneum etodolac [21]. Skins were removed from Franz cell receptor chambers after the ex vivo examination. Using cotton buds and PBS, excess formulation was gently removed from the skin. After rinsing the skins in PBS to remove excess formulation, normal sellotape (VeGe®, Istanbul, Turkey) was lightly applied and removed. Because they collect formulation residue on the skin, the first two tape applications were skipped. 15 strips of tape were used to separate the stratum corneum from the epidermis and dermis. In tubes containing 10 milliliters of acetonitrile and 75:25 PBS (v/v), these tape strips were inserted. They were taken away for a whole day. The tape strips were removed after 24 hours in a shaker. The residue was reconstituted with 2 mLofPBS after rotary evaporation (Heidolph, Germany) of the extracted solution. After 5 min of vortexing, the sample was quantified by HPLC.
9. Following the tape stripping study, sections of the skin were split. They were then placed in homogenization tubes with 5 mL of 75:25 (v/v) acetonitrile-PBS. The mixture was homogenized for 5 min at 10,000 rpm in a tissue homogenizer (Silent Crusher S, Heidolph, Germany). After homogenization, the mixture was vortexed for 1 min, sonicated for 20 min, then centrifuged at 3000 rpm for 5 min. HPLC was used to measure etodolac concentration after filtering through a 0.45 m pore membrane.

- **Preparation of Etodolac Nanoemulsion-Based Gel:** A secondary carrier mechanism was required in order for etodolac-loaded nanoemulsions to be easily administered topically. Etodolac was applied topically with the help of carboxymethyl cellulose (CMC) in a study on penetration enhancement. Thus, the lowest CMC content (0.5%, w/w) was chosen for application uniformity and minimal nanoemulsion diffusivity interference. CMC was a good choice for the necessary consistency because it was biocompatible and bioadhesive [22,23]. In summary, 0.5 g of CMC was added to 99.5 g of etodolac-loaded nanoemulsion. This gel system based on etodolac nanoemulsion, ETD-NE-CMC,

was shortened by subsequent research. Prior to the investigations, the formulations were kept at room temperature in the dark for one night to eliminate air.

- **In Vivo Study**
  1. **Animals:** The Yeditepe University Institutional Animal Care and Use Committee accepted the 2021-08/6 decision and the in vivo experimental protocol 2021-043. The European Community's guidelines for animal care and welfare were followed in the study [24]. 24 male Wistar albino rats weighing between 240 and 250 g were provided by the Istanbul, Turkey-based Yeditepe University Animal Reproduction Center (YUDETAM). The temperature and humidity of the rats' environment were controlled. The rats were divided into four groups of six at random. The rats were fed a standard diet and had unlimited access to water throughout the study. Every day, weight, food, and water intake were recorded.
  2. **Induction of Inflammation:** The formulations' anti-inflammatory efficacy was evaluated in rats with carrageenan-induced edema of the hind paws. Six rats were randomly assigned to the innovative, control, placebo, and traditional administration groups. Carrageenan (type 1, Santa Cruz, Dallas, TX, USA) was dissolved in saline. Etodolac (20 mg/kg) and placebo gel were applied to the rats' right hind paw plantar area in a gentle massage. Each rat received 100 L of physiological saline subplantarly in the left hind paw and 100 L of 1%w/v carrageenan intraplantarly in the right paw after 30 minutes. The right and left paw volumes were measured prior to, 1, 3, 4, 6, and 24 hours after the carrageenan injection using a digital plethysmometer from Panlab Harvard Apparatus in Barcelona, Spain. The paw edema volume was the volume difference between the left and right paws. As previously stated, the percentage of edema inhibition was calculated using the following formula:

$$Inhibition\ of\ edema = \left(1 - \left(\frac{Vt}{Vc}\right)\right) \times 100 \qquad (8)$$

  where Vt is the paw edema volume of rats treated with the formulations and Vc is the control group's mean.
  3. **In Vivo Experimental Group Design:** The following criteria were used to divide the rats into groups after causing inflammation: 4. There are four groups: unmedicated nanoemulsion-loaded CMC gel (n = 6 animals), conventional (n = 6 animals with coarse etodolac powder in CMC gel), etodolac-loaded CMC gel (n = 6 animals), and control (n = 6 animals with produced inflammation).
- **Statistical Analysis:** Following a one-way ANOVA, the Newman–Keuls multiple comparison test was used to statistically analyze the in vivo edema scores, and the Chi-square test was used to assess the scoring value. A Student t-test comparison test and a one-way ANOVA test were also used to investigate the bioavailability, diffusion, and release characteristics of etodolac. In both cases, the significance level was set at = 0.05 (p 0.05).

3. RESULTS AND DISCUSSION

3.1 Selection of Excipients

The selection of oil and surfactant is an essential part of the research that goes into the pre-formulation. The types of oil and surfactant were identified using both conventional and specialized methods. A number of oils were tested to ascertain the compatibility and solubility of etodolac. In isopropyl isostearate (IPIS), etodolac dissolves more effectively (Table S1, Supplementary Data). Non-ionic surfactants (Poloxamer® 188, 407, Brij® 35, tyloxapol, and Tego Care® 450) were selected because to their low cytotoxicity and outstanding biocompatibility. GRAS compounds are safe. Non-ionic surfactants are little affected by pH or ionic strength. The emulsification properties of a number of non-ionic surfactants were evaluated. TegoCare®450 produced non-viscous, phase-separation-free nanoemulsions, as shown in the screening study (Table S2, Supplementary Data).

4. Optimization Of Nanoemulsions

FMECA was used to identify potential failure modes, effects, and criticalities prior to an optimization study. Noemulsion key quality attributes (CQAs) can be identified and documented using this methodical strategy. In the initial tests, careful evaluations were made of the formulation's composition (API, surfactant, oil, and water ratio) and process parameters (temperature, sonication amplitude and duration, and homogenization rate and duration). The formulation's risk priority number (RPN) was higher than the processing parameters. Based on their highest RPNvalue, the CQAs that were discovered were selected as responses. A D-optimal mixture design was used to calculate the excipient amounts for formulations in an initial optimization study (Figure 1). In this study, the independent variables were IPIS (5%–8%), TegoCare®450 (1%–5%), and the oil and surfactant. The DS, PDI, and ZP all had R2 values of 0.988, 0.954, and 0.976 following the creation of nanoemulsion formulations (Table S3, Supplementary Data). Linear compatibility existed between the model and the design of the placebo nanoemulsions.

The formulations' surfactant ratio was found to have an opposite relationship with DS and PDI in the initial optimization studies (F1–F3 and F9–F11). The placebo nanoemulsions' DS and PDI decrease with emulsifier concentration is explained by surface activity. As emulsifier concentration rose, system surface tension increased. Energy conditions favored oil phase partitioning, enabling excellent interaction between oil and aqueous phases. Surfactant concentrations ranging from 0.5% to 5% w/w were the subject of a study [25]. A similar trend was seen in the investigation, however process conditions limited droplet size reduction, requiring modification to minimize excessive surfactant consumption.

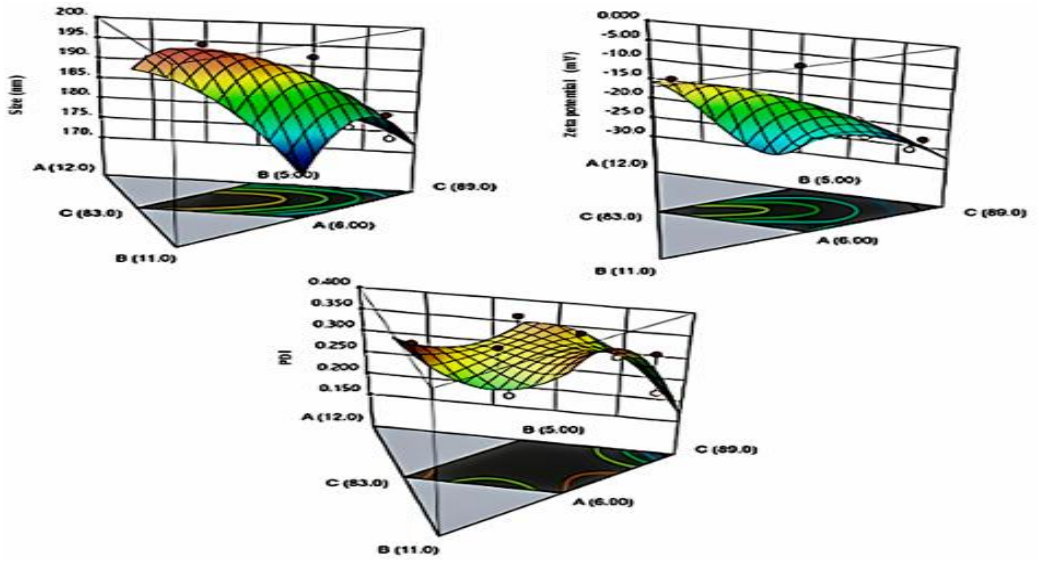

Figure 1. Surface plots in three dimensions of unmedicated nanoemulsion formulations created with the D-optimal mixture design model (version 13.0.2.0) of Design Expert Software

ZP, PDI, and DS served as the dependent variables. A second formulation design was carried out with the fifth formulation (Table 2) in order to estimate the quantity of the active ingredient (etodolac) that needed to be loaded after achieving the ideal oil-to-surfactant ratio. The recommended dosage for chronic arthritic pain is 400–1200 mg of etodolac daily, divided into two doses. Additionally, a consistent dose of 200–400 mg is recommended for the treatment of severe arthritic pain [26]. The lowest documented dose of etodolac was 1% (w/v) [17], while the highest topical dose of etodolac was 5% [26]. A second experimental design was used within the designated ratio range (see Figure 2). The design matrix for the nine software-generated trial runs is shown in Table 3.

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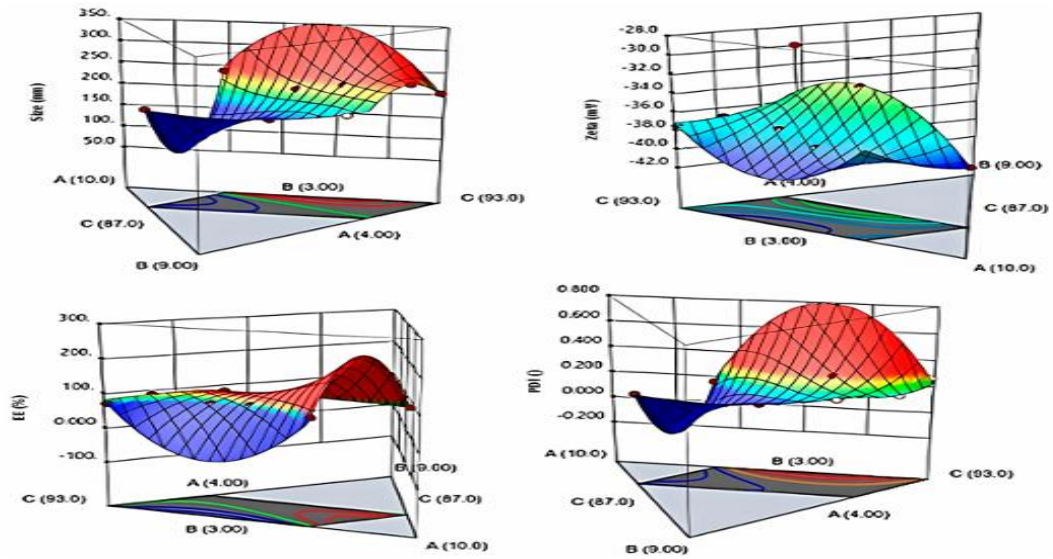

Figure 2. Using Design Expert Software (version 13.0.2.0) and the D-optimal mixture design model, 3D surface plots of medicated nanoemulsion formulation (distilled water, oil, and surfactant).

Of the evaluated formulations, only Formulation 2 satisfied the CQA requirements (Table 3). The optimal formulation was identified using numerical optimization, resulting in an improved oil composition and a significant proportion of etodolac. The formulation with 8 percent oil, 5 percent surfactant, and 1 percent etodolac (F2) had a desire factor (Df) of 0.9877, which prompted additional nanoemulsion characterisation research.

Table 3. Create variables and answers for a matrix

Formulation	ETD (%)	Droplet Size (nm)	Zeta Potential (mV)	PDI	Relative Energy (%)
F1	3.2	175.5	40.1	0.176	73.01
F2	1.0	163.5	33.1	0.141	92.30
F3	2.0	179.5	39.4	0.204	91.02
F4	1.8	184.7	29.6	0.218	92.00
F5	4.0	230.0	37.5	0.315	73.52
F6	3.0	215.6	38.7	0.291	80.19
F7	4.1	239.3	37.2	0.167	73.51
F8	5.0	203.8	37.4	0.264	72.46
F9	2.6	235.1	36.9	0.184	88.70

For nanoemulsions, the CQAs are the PDI value and the DS. This number ought to fall within a narrow range, typically less than 0.2. When bigger droplets expand and smaller droplets contract in nanoemulsions, this is known as Ostwald maturation. This phenomenon is caused by oil molecules flowing between droplets in the continuous phase. The process is driven by the Kelvin effect, which states that because of differences in Laplace pressures, smaller droplets in an emulsion have more oil solubility than larger droplets. A thorough analysis reveals that the lowest ETD concentration of one percent and the highest surfactant concentration of five percent produce the best droplet size distribution for the production of nanoemulsions [27]. Because of its vital role in guaranteeing the physical stability of nanoemulsions, the ZP was selected as a CQA in this study. An emulsifier system may achieve exceptional stability at 60 mV if it has a ZP larger than 30 mV and a low molecular weight. As can be seen in Tables 2 and 3, the formulations have ZP values ranging from -30 to -42 mV. This range indicates that nanoemulsions maintain their physical stability throughout time. In this optimization study, the EE was a CQA. The EE figures out how much drug is in each batch of nanoemulsion relative to the total amount used. The experimental errors for the best design batches are shown in Table 3. The EE values of the nanoemulsion batches ranged from 72.46 to 92.3%. A formulation with 1% etodolac, 8% isopropyl isostearate, and 5% Tego Care® 450 had a much higher EE of 92.46 percent.

5. Quantification Of Etodolac And Analytical Method Validation

The analytical procedure must be validated with sample data that is understandable and accurate. Creating a reliable and consistent analytical procedure is the first step in method validation during development. HPLC was used to collect chromatographic data after an anetodolac-loaded nanoemulsion was produced. It is simple to differentiate between the various components because there is no interference between the etodolac and excipient peaks in the chromatograms. The duration of etodolac was 4.53 minutes (Figure S1, Supplementary Data). Using the regression equation y=0.2292x+0.824 and a high coefficient of determination (R2=0.9993), the analytical method showed a linear connection between 0.05 and 5 g/mL. The method's intra-day and inter-day accuracy, as well as its relative standard deviations (RSDs), were less than 2%. This demonstrates the method's precision in measuring the desired chemical. It recovered by 0.26 percent between 101.78 and 98.55. The limit of detection (LOD) was 5.28 ng/mL, while the limit of quantification was 15.26 ng/mL.

6. Morphological Evaluation

The optimal formulation's surface morphology was seen through SEM. Figure 3 displays nanodroplet micrographs. The photographs show that the nanoemulsions were mostly spherical and free of etodol. The droplet size measurement of heimages is in agreement with the DLS findings. The surface shape of a nanoemulsion is supported by research.

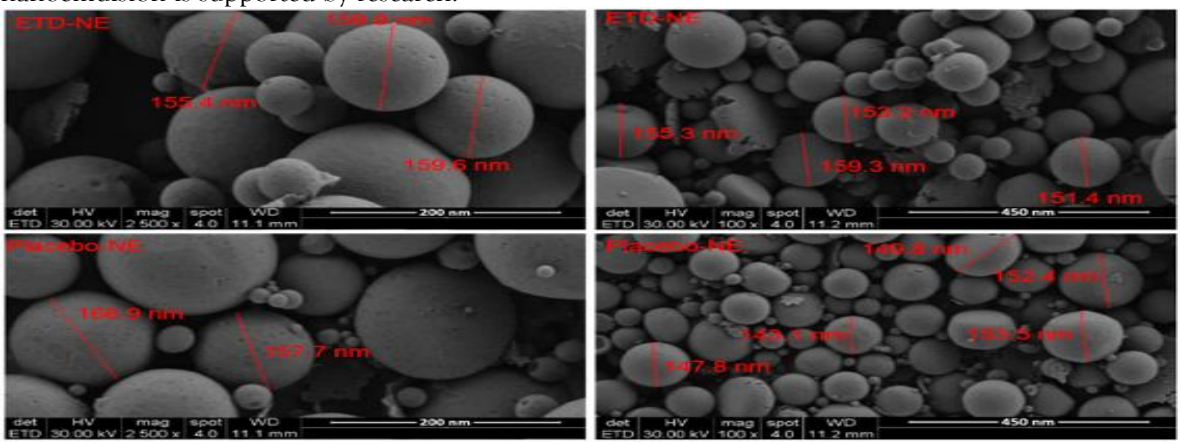

Figure 3. Medicinal and unmedicinal nanoemulsion SEM images (optimal formulation). Right picture is 2500 magnification, left image is 100.

7. Ftir Analysis

Figure 4 depicts the FTIR spectra of etodolac, medicated nanoemulsion-loaded gels, and etodolac-loaded nanoemulsions. N-H stretching vibrations of the secondaryamine group were detected at 3338 cm 1 in the FTIRspec trumofetodolac. C-H stretching vibrations have been observed in 3060–2810 cm 1 [28]. The aromatic C-H stretching of etodolac was linked to the FTIR peaks at 3026 cm 1 and 2850 cm 1. The carbonylC=Ovibration was predicted to range from 1750 to 1600 cm 1 [28]. Due to the stretching of the carbonylgroup of carboxylic acids, etodolac's FTIR spectra had a peak at 1736 cm 1. Peaks in the 1500–1400 cm 1 range were interpreted as C–C stretching vibrations, which was consistent with previous research. The chemical proof of pure etodolac's structure and purity is provided by this study. Both medicated nanoemulsions and gels readily display the peaks associated with etodola. The carbonyl stretching vibrations at 1750–1600 cm 1, the aromatic stretching vibrations at 3060–2810 cm 1, and the N-H stretching vibrations at 3340 cm 1 all remained unchanged. Compared to ureetodolac, peak intensities in etodolac-loaded nanoemulsions were significantly lower. The carbonyl peak at 1736 cm 1 was weaker, and the peaks in the C-H stretching region (3060–2810 cm 1) were flattened, indicating a change in the etodolac's structure. This change was referred to as an etodolac amorphization or complexation in a research paper [29]. In this manner, oildroplets can effectively encapsulate etodolac.

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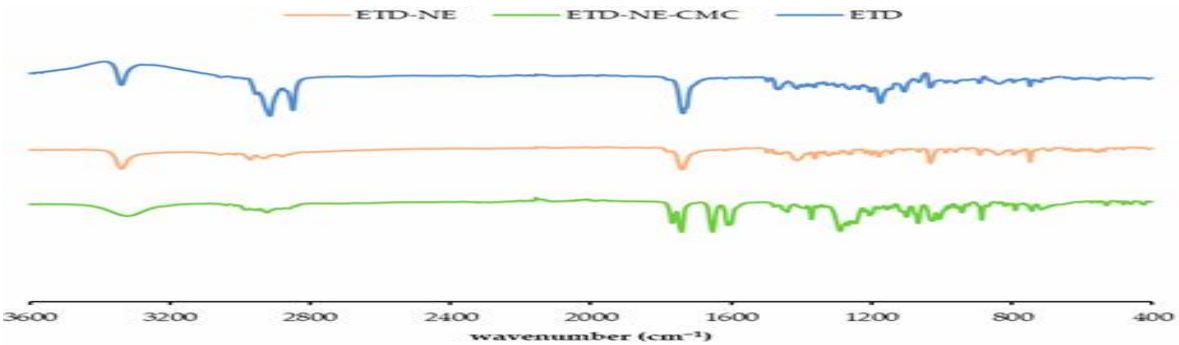

Figure 4. Etodolac and optimum formulations' FTIR spectra.

8. In Vitro And Ex Vivo Release Studies

Before conducting in vitro drug release studies, etodolac-loaded nanoemulsions were dispersed in water. An in vitro release test on the finished formulation was possible because the inclusion of carboxymethyl cellulose (CMC) resulted in the formation of a gel structure that served as a secondary carrier. According to the indicated solubility of etodolac (30.2 g/mL), the release medium and volume were modified to preserve sink conditions. [30].

A Franz diffusion cell and phosphate buffer (20 mL, 0.05 M, pH 7.4) were used as the receptor media in the in vitro drug release investigation. Previous articles provided information on the experimental conditions and critical parameters for these investigations. The profiles of the releases are depicted in Figure 5. The preparations had the greatest etodolac dispersion for the first eight hours. Permeation profiles for gel formulations require early morning medication penetration. Furthermore, applying gel to the skin for an extended period of time is not feasible. Eight hours is sufficient for semi-solid formulations to replicate gel contact time on the skin's surface. The first eight hours of release profiles were the focus of this investigation since the skin has the ability to retain and release active chemicals. Following an initial burst effect during the first hour of the in vitro release experiment, the nanoemulsion-loaded gel (ETD-NE-CMC) exhibited a pattern of sustained release. This result is caused by etodolac adsorption on droplet surfaces or dispersion into surfactants, which is in line with previous evidence [31]. The controlled release pattern of the control group and the coarse dispersion group differed significantly in the first hour (p 0.05). This variation may have resulted from the surface area and particle size of the pharmaceutical ingredient.

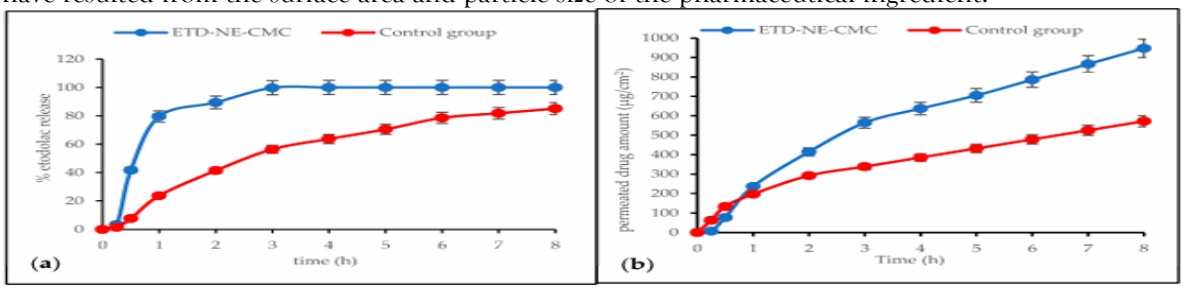

Figure 5. A) cumulative release profiles of the control group and nanoemulsion-loaded gel (ETD-NE-CMC) in vitro (n = 6); b) ex vivo skin permeation of etodolac preparations (n = 3). The data are a mean ± SD.

Using zero order and Ko-rsmeyer Peppas kinetic models, the release patterns of the nanoemulsion-loaded gel (ETD-NE-CMC) and the coarse dispersion (control group) were theoretically modeled (Table 4). In zero-order kinetics, the rate at which an active agent is released is constant, independent of concentration, and time-dependent only. The zero-order release pattern was observed in accordance with the specification, as depicted in Figure 5a. With a release exponent (n) of 0.769, the nanoformulation's release kinetic fits the Korsmeyer Peppas model, indicating non-Fickian transport. Drug release is controlled by diffusion and swelling, and 0.5 n 1 indicates abnormal or non-Fickian transport [32]. In nanoemulsion delivery systems, this situation may be utilized for a diffusion-type release mechanism. Published data on etodolac nanoformulations show similar results. A study's etodolac nanostructured lipid carriers had n-values between 0.558 and 0.749, indicating that the medication diffused through the lipid matrix. According to a different study, the "n" values of etodolac cubosomes ranged from 0.61 to 0.82, suggesting that drug release is controlled by diffusion [33].

Table 4. Release kinetics math release exponent

Kinetic Model	Control Group (R^2)	ETD-NE-CMC (R^2)
Zero-Order	0.9975	0.9340
First-Order	0.8519	0.9707
Higuchi	0.9666	0.9865
Hixson-Crowell	0.7995	0.9600
Korsmeyer-Peppas (R^2 / n)	0.9554 / 0.201	0.9914 / 0.769

There are a variety of methods for determining skin integrity. Examples of conventional methods include transepider malelectrical resistance, water loss, and water flow [34]. The limitations for TEER, TEWL, and TWF were 1k, 2.5 10-3 cmh 1, and 10gm 2h 1. There are a variety of commercial tools that can be used to measure TEWL. For the purpose of measuring TEWL in vivo, these instruments are used in dermatology and formulation development. The commercial launch of an invitroTEWL probe for early assessments using excised skin samples is one recent innovation [35]. The majority of laboratories lack this probe, so this research is limited. Researchers may also use visual microscopic examination to evaluate the integrity of the skin. A visual evaluation method from a previous study [36] showed that the integrity of the skin was satisfactory. The preparation permeability profiles are depicted in Figure 5b. In accordance with previous research, the flow and permeability coefficients were calculated. The flux was divided by the concentration of the formulation donor to get the permeability coefficient. The slope represented flux (Jss) when the total amount of medication that was penetrated was plotted against time, which was measured in hours. According to Table 5, the flow and permeability coefficients of the dialysis membrane were significantly higher than those of pig skin (p 0.05). Due to its intricate structure, pig skin was more resistant to drug molecule penetration. As shown in Table 5, nanoemulsions penetrated porcine skin and dialysis membranes more readily than the control group. The increase in permeation can be attributed to a number of factors, including the increased saturation solubility, the diffusion and concentration gradient, the substantial surface area of nanoemulsions, and the rapid dissolution of etodolac by nanosized droplets. Cubosomes, solid lipid nanoparticles, and etodolac nanosuspensions all had comparable outcomes.

Table 5. Preparation permeability and flux (n = 3). The results are the mean standard deviation

Parameter	Control Group	ETD-NE-CMC
Quantity Permeated (Dialysis Membrane)	783.4 ± 56.2	59.7 ± 15.2
Quantity Permeated (Porcine Skin)	1068.4 ± 71.5	165.7 ± 11.7
Steady-State Flux (Jss, g/cm²/h)	0.069 ± 0.005	0.004 ± 0.001
Permeability Coefficient (Kp, cm/h)	1.03	0.011 ± 0.001
LogKp	1.99	0.72
Flux Ratio	0.06	2.55
Enhancement Ratio	0.03	0.09
Permeability Coefficient ± SD	8793.6 ± 630.0	1449.3 ± 357.2
Amount Permeated After 8h (g/cm²)	14,336.9	45.4
Total Permeated (μg)	613.1	18.7

After the ex vivo study, pork skins were removed from the Franz cell receptor chambers. The percentage of skin penetration, the amount of drug in the stratum corneum, and the amount in other skin layers were all determined using the Material and Methods technique. Figure S2 (Supplementary Data) shows the results. For both the coarse dispersion and the nanoemulsion-loaded gel, the absorption rate was 19.0 0.95%. The drug concentrations in the stratum corneum layer of the nanoemulsion-loaded gel and coarse dispersion skins were 2.03 0.08% and 65.01 0.49%, respectively. The drug was left in the other skin layers by the nanoemulsion-loaded gel, 48.23 0.16%, and the coarse dispersion, 15.27 0.09%, respectively. The stratum corneum successfully occluded the coarse etodolac dispersions powder, according to the tape striping test. Additionally, additional layers prevented etodolac from entering any of the preparations. However, nanoemulsions increased etodolac skin penetration 2.5 times more than coarse dispersion.

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9. Stability Studies

At 25 C (65% relative humidity) and 40 C (65% relative humidity), the enhanced final formulation's DS, PDI, ZP, EE, pH, and viscosity were measured for 90 days. The literature has suggested a variety of test conditions and time intervals, from four to forty degrees Celsius, to assess the physical stability of a nano delivery system [37, 38]. The stability test was conducted at 25 C (60 percent relative humidity, 5 percent) and 40 C (75 percent relative humidity, 5 percent) to examine actual environmental effects. The findings regarding stability are presented in Supplementary Data in Table S5. While DS demonstrated a significant difference (p 0.05) after the 30th day at 40 C, physical stability measurements did not alter substantially (p > 0.05) between the production day and the 90th day at 25 C. At 40 C, there was a significant shift in viscosity (p 0.05) after 30 days of stability. In addition, after 60 days, EE showed a significant change (p 0.05). The stability test found that after 30 days at 40 C, the size of the nanoemulsion droplets increased from 167.4 1.3 nm to 193.9 1.4 nm. This is explained by Ostwald ripening and coalescence. Size distributions can be mostly or completely homogeneous when coalescence is a degradation process. Ostwald ripening destabilizes nanoemulsions, whereas coalescence destabilizes coarse emulsions. Ostwald matures faster at higher temperatures. A recent study found that nanoemulsions stored at 40 C mature faster than those stored at 25 C. The physical stability and skin-droplet interactions of a nano droplet are influenced by its electrostatic potential (ZP). The nanoemulsion had a negative charge of 35 mV because the sugar in Tego Care® 450 has carboxylic and hydroxyl groups that are negatively charged under neutral conditions. Due to strong mutual repulsion, the large absolute value of ZP stabilized the nanoemulsions. The literature states that exceptional stability is provided by a zeta potential around 60 mV, whereas excellent stability is provided by a ZP larger than 30 mV. Due to the high absolute ZP value, the zeta potentials of nanoemulsions maintained at 25 and 40 degrees Celsius were comparable. The nanoemulsion's EE (%) dramatically dropped at 40 C after the 60th day of the stability study (from 92.69 1.40% to 70.83 1.59%). Active ingredient leakage was thought to be caused by the increased diffusion rate at 40 C. Instability was also experienced by nano carriers. The CQAs for the final formulation are viscosity and pH. For topical treatments, the ideal pH range is 5–7 and the ideal viscosity range is 14–19 cP. Both conditions were met by the final formulation. The viscosity of the nano formulation significantly decreased (p < 0.05) during 30 days of stability testing. Furthermore, after 60 days of stability examination, the pH of the nano formulation significantly decreased (p < 0.05). The dispersion medium may become more acidic as a result of the droplets' release of etodolac, a carboxylic acid moiety. Similar changes in pH and viscosity were noted in earlier research.

10. In Vivo Studies

Carrageenan-induced rat paw edema is a common method for studying anti-inflammatory properties in vivo. Injections of carrageenan trigger an immediate, multistage, localized inflammatory response. Histamine, bradykinin, serotonin, and substance P are released during the first phase (one hour), while pro-inflammatory mediators are produced during the second phase (more than an hour) [39]. The control and placebo rats' edema volumes peaked eight hours after induction, having increased for the previous hour (Figure 6). When compared to the control and placebo groups, the formulation's excipients had no effect on the volume of edema (p > 0.05). After eight hours, the pharmacological preparations significantly decreased the amount of edema (p < 0.05).

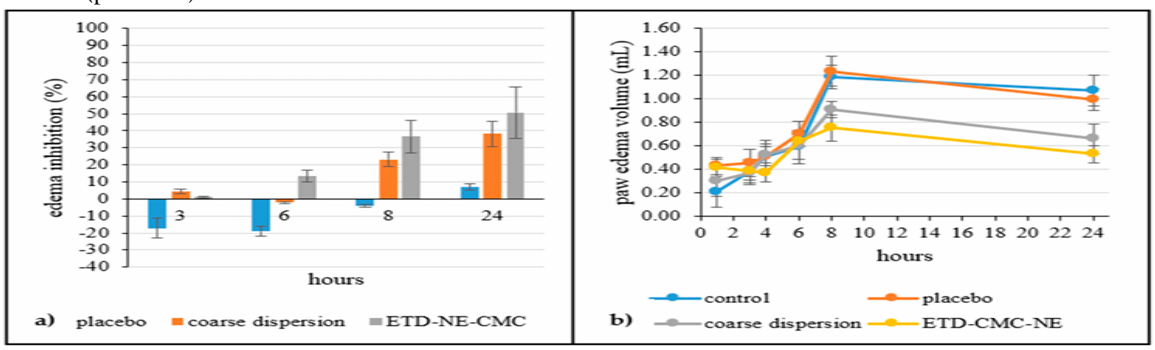

Figure 6. % preparation edema inhibition (a) and paw edema volume (mL) (b) in rats (n = 6). The data are a mean ± SD.

Up until the eighth hour, the volume of oedema in the control group increased. Paw oedema was lessened by the medicated nanoemulsion-incorporated gel (ETD-CMC-NE) (Figure 6; ETD-NE-CMC group: 13.4%, 36.5%). Six rats with pawedema volume (mL) (b) and preparation inhibition percentage (a). The results demonstrate SD. The volume of oedema in the control group continued until the eighth hour. During the first six, eight, and twenty-four hours, ETD-CMC-NE prevented the development of pawoedema by 13.4%, 36.5%, and 50.65%, respectively. Similar results were observed by ibuprofen nanoemulsions in a 24-hour anti-inflammatory activity study [40]. Another study found that topical etodolac microemulsions reduced edemainan's anti-inflammatory bioactivity by 66.8% [41] and were effective for administration. In contrast to other nanosystems, etodolacnanosuspensions prevented edema for 24 hours. Numerous prior investigations have shown that medications may enter the skin by a number of pathways, including an intercellular route, through keratin-rich corneocytes, hair follicles, sweatducts, and across the lipid bilayer. The stratum corneum pores, which might serve as entrance sites to the deeper layer of skin, are thought to be 20–200 nanometers broad. Drug penetration is also improved by stratumcorneum development caused by hydration. Penetration enhancers may also alter the lipid and hydrocarbon chains of the stratum corneum, thereby increasing drug permeability. Nanoemulsions made the tiny droplets of etodolac larger, more charged, and more viscous, thereby enhancing its penetration. These chemical and physical characteristics may provide an explanation for the investigation's suppression of pawedemain.

11. Comparison Of Current Work To Previous Reports

The current study was compared to other published studies that were similar. The first experiment employed a standard hydrogel strategy to increase topical etodolac penetration. Etodolac and penetration enhancers were dissolved in CMCgel with the help of sodium lauryl sulfate, ethanol, and PEG400 in this study. There was no discernible difference between the formulations and the control group in in vitro (artificial membrane) tests. In ex vivo tests, however, only a formulation with anethol as a penetration enhancer demonstrated a twofold increase in permeability. In comparison to the current investigation, etodolac-loaded nanoemulsions had twice as much penetration in vitro and ex vivo without the use of organic solvents or penetration enhancers. This could have been brought on by the minute size of the formulation's droplets. In a different study, solid lipid nanoparticles (SLN) loaded with etodolac were dispersed throughout a carbopol 934 gel system for in vitro, ex vivo, and in vivo release assays. The nanoemulsions' ex vivo release patterns were comparable to those of other studies of a similar nature, while the current study's in vitro trend slightly increased due to the smaller particle size. The anti-inflammatory effects in this study were comparable, despite the fact that formulations with SLNs performed better in vivo. This could be because the SLN carrier mechanism released more slowly than nanoemulsions did. To investigate etodolac-loaded nanostructured lipid carriers (NLCs), a different study utilized a design of experiments (DOE) strategy that was comparable to ours. Particle size, PDI, ZP, and EE (percent) were carefully examined during characterization in accordance with current study standards. NLCs were larger and had PDI values that differed from a unimodal distribution (PDI > 0.2), according to comparisons. The ZP value (30 mV) of our experiment was comparable to that of the NLCs with 80% EE. The ex vivo release characteristics of the NLCs and the nanoemulsions in this investigation were different, with the NLCs releasing more slowly. A solid matrix in NLCs might be the source of this behavior.

In one work, etodolac nanosuspensions with a PDI of less than 0.2 were created by physically shrinking the particle size to 500 nm. Similar to the current study, hydroxypropylmethylcellulose (HPMC) and hydroxyethylcellulose (HEC) were used to create a topical gel system. Experiments were conducted in vitro, in vivo, and ex vivo. In this study, the ex vivo penetration rate of nanosuspensions was marginally lower than that of nanoemulsions. This is because nanodroplets are so small and flexible. According to in vivo anti-inflammatory characteristics, both investigations decreased the amount of paw edema.

12. CONCLUSIONS

In this study, etodolac-loaded nanoemulsions were developed and optimized utilizing a DOE method. Improving topical medication delivery and changing the administration route were the main goals. The improved formulation was characterized in accordance with the CQAs of the study. CMC gels coated with nanoemulsions improved etodolac penetration more effectively, as demonstrated by studies of ex vivo

permeation and in vitro release. In addition, the finished product exhibited stability for three months due to its high ZP. The medicated nanoemulsion-loaded gels outperformed the coarse etodolac dispersion in terms of anti-inflammatory action, and the difference was striking. As a result, gels filled with etodolac nanoemulsion provide a potential and efficient alternative delivery method for inflammatory therapy.

REFERENCES

[1]. Prausnitz, M.R.; Langer, R. Transdermal Drug Delivery. Nat. Biotechnol. 2008, 26, 1261–1268.
[2]. Kováˇcik, A.; Kopeˇcná, M.; Vávrová, K. Permeation Enhancers in Transdermal Drug Delivery: Benefits and Limitations. Expert Opin. Drug Deliv. 2020, 17, 145–155.
[3]. Rai, V.K.; Mishra, N.; Yadav, K.S.; Yadav, N.P. Nanoemulsion as Pharmaceutical Carrier for Dermal and Transdermal Drug Delivery: Formulation Development, Stability Issues, Basic Considerations and Applications. J. Control. Release 2018, 270, 203–225.
[4]. Shaker, D.S.; Ishak, R.A.H.; Ghoneim, A.; Elhuoni, M.A. Nanoemulsion: A Review on Mechanisms for the Transdermal Delivery of Hydrophobic and Hydrophilic Drugs. Sci. Pharm. 2019, 87, 17.
[5]. Jones, R.A. Etodolac: An Overview of a Selective COX-2 Inhibitor. Inflammopharmacology 1999, 7, 269–275.
[6]. Madhavi, N.; Sudhakar, B.; Reddy, K.V.N.S.; Ratna, J.V. Design by Optimization and Comparative Evaluation of Vesicular Gels of Etodolac for Transdermal Delivery. Drug Dev. Ind. Pharm. 2019, 45, 611–628.
[7]. Karakucuk, A.; Tort, S.; Han, S.; Oktay, A.N.; Celebi, N. Etodolac Nanosuspension Based Gel for Enhanced Dermal Delivery: In Vitro and in Vivo Evaluation. J. Microencapsul. 2021, 38, 218–232.
[8]. Patel, D.K.; Kesharwani, R.; Kumar, V. Etodolac Loaded Solid Lipid Nanoparticle Based Topical Gel for Enhanced Skin Delivery. Biocatal. Agric. Biotechnol. 2020, 29, 101810.
[9]. Czajkowska-Ko´snik, A.; Szyma´nska, E.; Czarnomysy, R.; Jacyna, J.; Markuszewski, M.; Basa, A.; Winnicka, K. Nanostructured Lipid Carriers Engineered as Topical Delivery of Etodolac: Optimization and Cytotoxicity Studies. Materials 2021, 14, 596.
[10]. Shilakari Asthana, G.; Asthana, A.; Singh, D.; Sharma, P.K. Etodolac Containing Topical Niosomal Gel: Formulation Development and Evaluation. J. Drug Deliv. 2016, 2016.
[11]. Özdemir, S.; Celik, B.; Sümer, E.; Acar, E.T.; Üner, M. Eplerenone Nanoemulsions for Treatment of Hypertension. Part II: Physical Stability Assessment and in Vivo Study. J. Drug Deliv. Sci. Technol. 2018, 45, 287–295.
[12]. Yu,L.X. Pharmaceutical Quality by Design: Product and Process Development, Understanding, and Control. Pharm. Res. 2008, 25, 781–791.
[13]. Cirri, M.; Maestrini, L.; Maestrelli, F.; Mennini, N.; Mura, P.; Ghelardini, C.; Di Cesare Mannelli, L. Design, Characterization and in Vivo Evaluation of Nanostructured Lipid Carriers (NLC) as a New Drug Delivery System for Hydrochlorothiazide Oral Administration in Pediatric Therapy. Drug Deliv. 2018, 25, 1910–1921.
[14]. Özdemir, S.; Çelik, B.; Türköz Acar, E.; Duman, G.; Üner, M. Eplerenone Nanoemulsions for Treatment of Hypertension. Part I: Experimental Design for Optimization of Formulations and Physical Characterization. J. Drug Deliv. Sci. Technol. 2018, 45, 357–366.
[15]. Uner, B.; Ozdemir, S.; Tas, C.; Uner, M.; Ozsoy, Y. Loteprednol-Loaded Nanoformulations for Corneal Delivery by Quality-by Design Concepts: Optimization, Characterization, and Anti-Inflammatory Activity. AAPS PharmSciTech 2023, 24, 92.
[16]. Herneisey, M.; Liu, L.; Lambert, E.; Schmitz, N.; Loftus, S.; Janjic, J.M. Development of Theranostic Perfluorocarbon Nanoemul sions as a Model Non-Opioid Pain Nanomedicine Using a Quality by Design (QbD) Approach. AAPS PharmSciTech 2019, 20, 65.
[17]. Tas, C.; Ozkan, Y.; Okyar, A.; Savaser, A. In Vitro and Ex Vivo Permeation Studies of Etodolac from Hydrophilic Gels and Effect of Terpenes as Enhancers. Drug Deliv. 2007, 14, 453–459.
[18]. Borman, P.; Elder, D. Q2(R1) Validation of Analytical Procedures. In ICH Quality Guidelines; Wiley: Hoboken, NJ, USA, 2017; pp. 127–166. ISBN 9781118971147.
[19]. Üner, B.; Özdemir, S.; Ta¸s, Ç.; Özsoy, Y.; Üner, M. Development of Lipid Nanoparticles for Transdermal Loteprednol Etabonate Delivery. J. Microencapsul. 2022, 39, 327–340.
[20]. Özdemir, S.; Üner, B.; Karaküçük, A. Preparation and Characterization Studies of Etodolac Suppositories: Investigation on Oleaginous Blends of Witepsol®H15. J. Res. Pharm. 2023, 27, 837–847.
[21]. Kahraman, E.; Ne¸seto˘glu, N.; Güngör, S.; Ünal, D.¸S.; Özsoy, Y. The Combination of Nanomicelles with Terpenes for Enhancement of Skin Drug Delivery. Int. J. Pharm. 2018, 551, 133–140.
[22]. Marchianti, A.C.N.; Sakinah, E.N.; Elfiah, U.; Putri, N.K.S.; Wahyuliswari, D.I.; Maulana, M.; Ulfa, E.U. Gel Formulations of Merremia Mammosa (Lour.) Accelerated Wound Healing of the Wound in Diabetic Rats. J. Tradit. Complement. Med. 2021, 11, 38–45.
[23]. Simmons,P.A.; Carlisle-Wilcox, C.; Chen, R.; Liu, H.; Vehige, J.G. Efficacy, Safety, and Acceptability of a Lipid-Based Artificial Tear Formulation: A Randomized, Controlled, Multicenter Clinical Trial. Clin. Ther. 2015, 37, 858–868.
[24]. Smith, A.J.; Clutton, R.E.; Lilley, E.; Hansen, K.E.A.; Brattelid, T. PREPARE: Guidelines for Planning Animal Research and Testing. Lab. Anim. 2017, 52, 135–141.
[25]. Uluata, S.; Decker, E.A.; McClements, D.J. Optimization of Nanoemulsion Fabrication Using Microfluidization: Role of Surfactant Concentration on Formation and Stability. Food Biophys. 2016, 11, 52–59.

[26]. Barakat, N.S. In Vitro and in Vivo Characteristics of a Thermogelling Rectal Delivery System of Etodolac. AAPS PharmSciTech 2009, 10, 724–731.

[27]. Alvarado, H.L.; Abrego, G.; Souto, E.B.; Garduño-Ramirez, M.L.; Clares, B.; García, M.L.; Calpena, A.C. Nanoemulsions for Dermal Controlled Release of Oleanolic and Ursolic Acids: In Vitro, Ex Vivo and in Vivo Characterization. Colloids Surfaces B Biointerfaces 2015, 130, 40–47.

[28]. Amul,B.; Muthu, S.; Raja, M.; Sevvanthi, S. Spectral, DFT and Molecular Docking Investigations on Etodolac. J. Mol. Struct. 2019, 1195, 747–761.

[29]. Sherje, A.P.; Kulkarni, V.; Murahari, M.; Nayak, U.Y.; Bhat, P.; Suvarna, V.; Dravyakar, B. Inclusion Complexation of Etodolac with Hydroxypropyl-Beta-Cyclodextrin and Auxiliary Agents: Formulation Characterization and Molecular Modeling Studies. Mol. Pharm. 2017, 14, 1231–1242.

[30]. Karakucuk, A.; Celebi, N. Investigation of Formulation and Process Parameters of Wet Media Milling to Develop Etodolac Nanosuspensions. Pharm. Res. 2020, 37, 111.

[31]. Ali, F.R.; Shoaib, M.H.; Ali, S.A.; Yousuf, R.I.; Siddiqui, F.; Raja, R.; Jamal, H.S.; Saleem, M.T.; Ahmed, K.; Imtiaz, M.S.; et al. A Nanoemulsion Based Transdermal Delivery of Insulin: Formulation Development, Optimization, in-Vitro Permeation across Strat-M®Membrane and Its Pharmacokinetic/Pharmacodynamic Evaluation. J. Drug Deliv. Sci. Technol. 2022, 71, 103338.

[32]. Bruschi, M.L. Strategies to Modify the Drug Release from Pharmaceutical Systems; Bruschi, M.L., Ed.; Woodhead Publishing: Cambridge, UK, 2015; pp. 63–86, ISBN 978-0-08-100092-2.

[33]. Salah, S.; Mahmoud, A.A.; Kamel, A.O. Etodolac Transdermal Cubosomes for the Treatment of Rheumatoid Arthritis: Ex Vivo Permeation and in Vivo Pharmacokinetic Studies. Drug Deliv. 2017, 24, 846–856.

[34]. Guth, K.; Schäfer-Korting, M.; Fabian, E.; Landsiedel, R.; van Ravenzwaay, B. Suitability of Skin Integrity Tests for Dermal Absorption Studies in Vitro. Toxicol. Vitr. 2015, 29, 113–123.

[35]. Schoenfelder, H.; Liu, Y.; Lunter, D.J. Systematic Investigation of Factors, Such as the Impact of Emulsifiers, Which Influence the Measurement of Skin Barrier Integrity by in-Vitro Trans-Epidermal Water Loss (TEWL). Int. J. Pharm. 2023, 638, 122930.

[36]. Klang, V.; Schwarz, J.C.; Haberfeld, S.; Xiao, P.; Wirth, M.; Valenta, C. Skin Integrity Testing and Monitoring of in Vitro Tape Stripping by Capacitance-Based Sensor Imaging. Ski. Res. Technol. 2013, 19, e259–e272.

[37]. Uner, B.; Ozdemir, S.; Yildirim, E.; Yaba, A.; Tas, C.; Uner, M.; Ozsoy, Y. Loteprednol Loaded Nanoformulations for Corneal Delivery: Ex-Vivo Permeation Study, Ocular Safety Assessment and Stability Studies. J. Drug Deliv. Sci. Technol. 2023, 81, 104252.

[38]. Çelik, B.; Sa˘gıro˘glu, A.A.; Özdemir, S. Design, Optimization and Characterization of Coenzyme Q10- and D-Panthenyl Triacetate Loaded Liposomes. Int. J. Nanomed. 2017, 12, 4869–4878.

[39]. Morris, C.J. Carrageenan-Induced Paw Edema in the Rat and Mouse. Methods Mol. Biol. 2003, 225, 115–121.

[40]. Salunkhe, S.S.; Bhatia, N.M.; Thorat, J.D.; Choudhari, P.B.; Bhatia, M.S. Formulation, Development and Evaluation of Ibuprofen Loaded Nanoemulsion Prepared by Nanoprecipitation Technique: Use of Factorial Design Approach as a Tool of Optimization Methodology. J. Pharm. Investig. 2014, 44, 273–290.

[41]. Goindi, S.; Kaur, R.; Kaur, R. An Ionic Liquid-in-Water Microemulsion as a Potential Carrier for Topical Delivery of Poorly Water Soluble Drug: Development, Ex-Vivo and in-Vivo Evaluation. Int. J. Pharm. 2015, 495, 913–923.