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Research Article

Biodegradable Polymeric Pharmaceutical Nanoemulgel Coloaded with Eucalyptol-Lornoxicam: Fabrication and Characterizations for Possible Better Pain Management

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Nanoemulgels are considered as potential and ideal drug delivery systems for the transdermal administration of poorly soluble drugs like lornoxicam. Their intrinsic characteristics, such as small globule size as well as excipient type, make transdermal passage of the drug easier. The current study was aimed at developing nanoemulgel based formulation of lornoxicam coloaded with eucalyptol to enhance drug skin permeation and bioavailability in order to promote therapeutic outcome in appropriate time. High shear homogenization technique was utilized to develop optimized lornoxicam encapsulated nanoemulsion followed by its conversion into nanoemulgel formulation by the addition of 1% Carbopol 940 as gelling agent. Different characterization tests were performed on optimized formulation such as surface charge, particle size and size distribution, viscosity, spreadability, pH, drug content determination, drug entrapment efficiency, in vitro drug release, and drug permeation analysis. The optimized formulation had globule size of 143.7 ± 2.18 nm. The results of drug entrapment efficiency, in vitro drug release, and skin penetration experiments were found satisfactory. Based on the formulation type, permeability parameters such as permeability constant (Kp), enhancement ratio (Er), and steady state flux (Jss) revealed greater values and satisfactory outcomes. When compared to other formulations (LRX nanoemulgel and EU nanoemulgel), the LRX-EU nanoemulgel formulation produced improved skin permeation profile (flux, $189.63 \pm 7.68\ \mu g/cm^2/h$; permeability coefficient, 2.09 ± 0.067 cm/h; and ER, 4.274). This work clearly proved that lornoxicam coloaded with eucalyptol nanoemulgel formulations could be developed to generate stable drug delivery systems. Thus, LRX-EU NEG formulation seems promising in terms of physicochemical properties, enhanced bioavailability, and high skin penetration profile as compared to LRX NEG formulation.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDS) have gained enormous popularity in the management of inflammatory conditions such as rheumatoid arthritis and osteoarthritis [1]. Inflammatory diseases can be effectively relieved by taking NSAIDS orally. However, drug-associated side effects, such as gastrointestinal mucosal irritation and ulceration, have restricted its clinical usage. The administration of NSAIDS via transdermal route is one method to prevent toxicity and utilizing them for a longer period of time [2]. Transdermal systems, which carry medication via the skin

into the bloodstream, counteract changes in the rate of absorption and metabolism as well as gastrointestinal side effects brought on by oral drug delivery [3]. Chronic diseases are the perfect fit for it. It enables the delivery of potent drug moieties with the advantages of self-administration and improved therapeutic effectiveness [4]. Lornoxicam (LRX), member of oxicam class, is quite an effective NSAID. It is frequently recommended for illnesses that are chronically inflammatory and painful in nature. Similar to other NSAIDS, oral administration of LRX has a number of negative renal, gastrointestinal, and hematological consequences. In addition to this, it requires repeated administration due to its short half-life of 3-4 h. Additionally, parenteral administration is not suggested for treating chronic diseases (R. U. [5]). Eucalyptol (1,8-cineole), a terpenoid oxide that is extracted from eucalyptus leaves, is found in high concentrations in a wide range of plants as essential oil. It improves drug permeation profile by disruption of skin intracellular lipids owing to its lipophilicity. It inhibits proinflammatory cytokines (TNF- and IL-1), as well as chemotactic cytokines, when evaluated on human monocytes and lymphocytes in vitro, making it an effective analgesic as well as anti-inflammatory drug. This inhibition results in its effectiveness in neurodegenerative illnesses [6].

An effective strategy to address all the issues with LRX administration is the controlled distribution of LRX via transdermal delivery [7]. In the current investigation, to increase the drug's bioavailability, LRX was developed as a nanoemulgel formulation coloaded with eucalyptol. Numerous studies have demonstrated that penetration enhancers can promote better skin permeation than isolated moieties when used in conjunction with cosolvents [8]. Cosolvents are often employed in transdermal formulations as penetration enhancers and carriers. These substances affect both drug release and permeation because they not only improve drug solubility but also change the skin topology and increase penetration rates [9]. In order to increase the transdermal permeability of LRX, the current study uses eucalyptol as a penetration enhancer and PEG 400 as a cosolvent. Taken altogether, Carbopol-decorated LRX nanoemulgel formulation coloaded with eucalyptol was produced by high shear homogenization method to portray constant drug release rate for a period of 24 h.

2. Materials and Methods

Lornoxicam was kindly gifted for research by Wilshire Pharma Pvt. Limited, Lahore, Pakistan; eucalyptol provided by DDCL was used as active ingredient as well as penetration enhancer; olive oil and almond oil (Marhaba Laboratories, Pakistan) were used as oil phase in nanoemulsion formulation; Carbopol 940 (BDH Industries) was employed as gelling agent in the formulation of nanoemulgel; Tween 80 and PEG 400 (Sigma-Aldrich, Germany) were used as surfactant and cosurfactant, respectively; triethanolamine (Merck, Germany) was employed as pH adjuster, and distilled water was used as vehicle. The chemicals used in this project were of analytical grade and purified.

2.1. Lornoxicam Solubility Studies. Solubility tests are used to screen surfactants, cosurfactants, and oils for nanoemulsion formulations that have a high rate of dissolution as well as possess high degree of skin penetration. In this study, first lornoxicam powder in excess amount was mixed with 10 ml olive oil, almond oil, PEG 400, Tween 80, propylene glycol, ethanol, phosphate buffer saline (pH 5.8, 6.8, and 7.4), and water in separate conical flasks. The flasks were subjected to continuous stirring by means of shaking water bath (1217.2E, Sheldon, USA) for 72 h time period. Finally, the separation of insoluble drug particles was made sure by subjecting the contents of the flasks to centrifugation process for 15 minutes at 5000 rpm. An aliquot was taken and subjected to filtration with the help of a Millipore filter (0.45 μm). The dilution was then subjected to UV-spectrophotometer (UV-1800, Shimadzu, Japan) by taking wavelength at 376 nm. Triplicate readings of concentration were determined [7].

2.2. ATR-FTIR Studies. The efficacy, stability, and success of solid dosage form are heavily dependent upon the rigorous excipient selection. The compatibility of the drug with excipients is critical for the efficacy, stability, and safety of the product [10]. ATR-FTIR spectra were produced by ATR-FTIR spectrometer (Spectrum 100, Perkin Elmer, USA) using MIRacle ATR accessory (PIKE Technologies, Madison, USA). The samples include pure lornoxicam, eucalyptol, Carbopol 940, Tween 80, PEG 400, lornoxicam nanoemulsion formulation, eucalyptol nanoemulsion formulation, lornoxicam cum eucalyptol (LRX-EU) nanoemulsion formulation, and their respective nanoemulgel formulations. Scans were recorded at a range of 4000 cm^{-1} to 625cm^{-1}. The scan lasted 12 min, and the resulting spectra were examined for any spectral alterations [11].

2.3. Nanoemulsion and Nanoemulgel Development. Nanoemulsion formulations were prepared by high-speed homogenization technique [12]. An oily phase comprising olive oil, almond oil, PEG 400, and lornoxicam, either alone or in conjunction with eucalyptol, was subjected to stirring at 700 rpm for 1 h by hot plate magnetic stirrer (VELP Scientifica, Italy) at 70°C temperature as represented in Figure 1. Likewise, aqueous phase comprising Tween 80 and distilled water was subjected to constant stirring for 1 h at same temperature, i.e., 70°C. Drop by drop, the oil phase was injected into the aqueous phase to create the final formulation. Thermodynamic stability of the prepared nanoemulsion formulation was determined by centrifuging the optimized formulation. In this method, nanoemulsion formulation was centrifuged at 3500 rpm for 30 min. The process was carried out to investigate the chances of phase separation or formation of turbidity.

Numerous types of gel bases were employed for effective distribution of optimized nanoemulsion formulation in order to promote local drug accumulation in the skin while minimizing blood penetration. Carbopol 940 in different concentrations, i.e., 0.5, 1, 1.5, 2, and 2.5%, was used as gelling agent for the preparation of optimized nanoemulgel formulation. On the basis of physical appearance of a simple

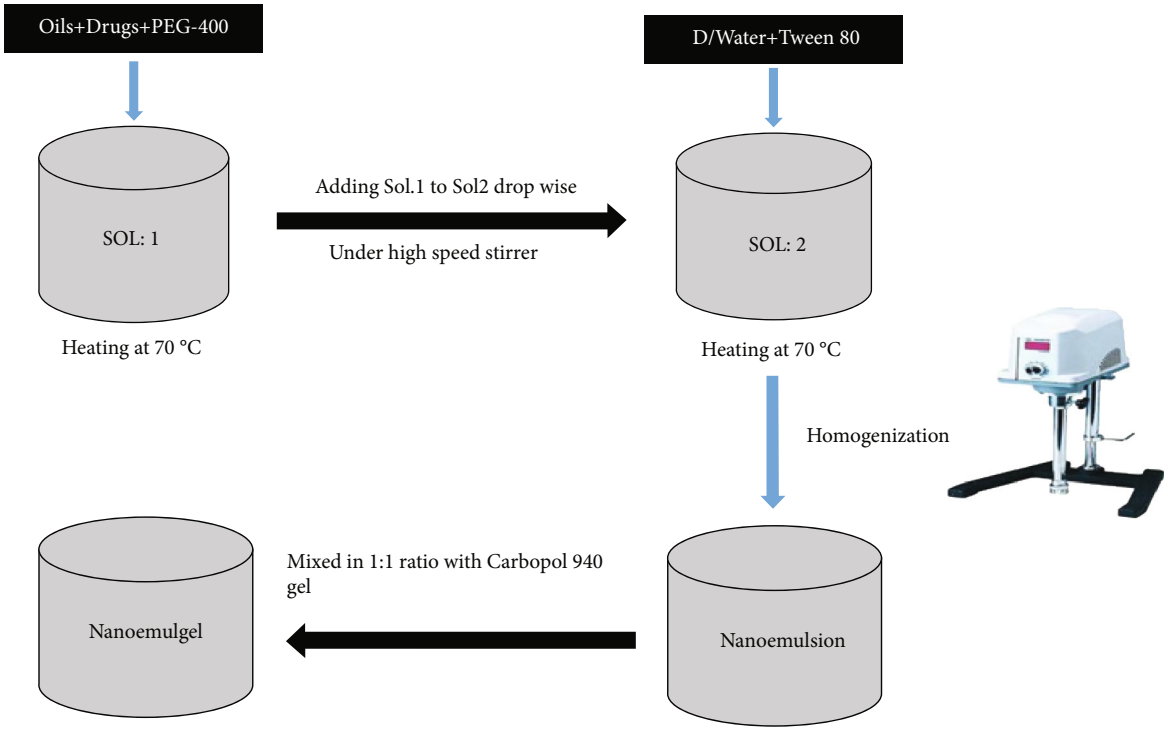

Figure 1: Schematic representation of preparation of nanoemulsion and nanoemulgel.

lornoxicam gel, Carbopol 940 having 1% concentration was selected for the development of lornoxicam/eucalyptol nanoemulgel. To develop a skin friendly formulation, the lornoxicam/eucalyptol-loaded nanoemulsion was mixed with Carbopol gel (1% *w*/*w*). Carbopol was gradually incorporated into distilled water by continuous stirring for 2-3 h time period at 700 rpm until the formation of a clear dispersion. The Carbopol dispersion was kept at room temperature for effective hydration and swelling for a time period of 3 h. The optimized lornoxicam/eucalyptol nanoemulsion formulation was mixed with Carbopol dispersion by means of gentle stirring. Finally, the mixture was made neutral (pH 7.0 ± 0.5) by the addition of 4-5 drops of triethanolamine in order to develop a transparent gel. The produced gel formulation was kept overnight to allow trapped air to escape *[13]*. A similar process was utilized to create lornoxicam nanoemulgel and eucalyptol nanoemulgel formulations. The composition of LRX NEG, EU NEG, and LRX-EU NEG are shown in Table 1 [14].

2.4. Characterization of LRX-EU Nanoemulsion and Nanoemulgel Formulations

2.4.1. pH Determination. The optimized formulation of LRX-EU nanoemulsion and nanoemulgel was subjected to pH determination. The test was carried out at 25 ± 1°C by means of pH meter (Denver, USA). Initially, the instrument was calibrated with the help of buffer solutions having different known concentrations (pH 3, 7, and 9). An average of three readings were taken, and the results were shown as mean ± SD [15].

2.4.2. Size and Size Distribution Analysis. Particle size and particle size distribution of the optimized formulations were determined with the help of Zetasizer 90 (Malvern Instruments; Worcestershire, UK) by employing dynamic light scattering mechanisms. The Zetasizer is equipped with 6.34 version software having laser of wavelength 635 nm and a 90° detect angle. Mixtures of LRX-EU nanoemulsion as well as LRX-EU nanoemulgel (10 μl) in deionized water were vortexed for a time period of 2 min in order to obtain a clear homogenous dispersion. The experiment was carried out in triplicates by maintaining the temperature at 25 ± 1°C. The results were shown in the form of mean ± SD [16].

2.4.3. Determination of Polydispersity Index and Zeta Potential. Polydispersity index (PDI) and zeta potential of LRX-EU nanoemulsion and LRX-EU nanoemulgel were investigated using Zetasizer 90 (Malvern Instruments; Worcestershire, UK). The test was executed by taking formulation (700 μl) in a capillary tube, equipped with a gold electrode. The procedure was repeated three times at 25 ± 1 °C, with the results represented as mean ± SD [17].

2.4.4. Surface Morphology Analysis. A simple light microscope (CX41RF, OLYMPUS, Japan) coupled with a 5 megapixel photographic camera was used to evaluate the surface morphology of the optimized nanoemulsion as well as nanoemulgel formulations of LRX-EU. A drop of the developed formulation was put on the slide and viewed under the microscope. After that, they were photographed.

Table 1: Composition of % *w*/*w* LRX-loaded nanoemulgel, EU-loaded nanoemulgel, and LRX-EU-loaded nanoemulgel formulations.

Components	LRX-loaded nanoemulgel	EU-loaded nanoemulgel	LRX-EU-loaded nanoemulgel
Carbopol 940	1	1	1
Triethanolamine	1	1	1
Optimized LRX-loaded nanoemulsion	50	—	—
Optimized EU-loaded nanoemulsion	—	50	—
LRX-EU nanoemulsion	25	25	1: 1
Distilled water	48	48	48

2.4.5. Entrapment Efficiency. Entrapment efficiency of optimized nanoemulsion and nanoemulgel formulations of LRX-EU was determined by indirect method. This involved centrifugation of formulations at a temperature of 20°C until the nanoemulsion formulation occupying filter unit and the aqueous phase relating to filtrate were completely separated. After the completion of separation process, the resultant filtrate was utilized in order to estimate the quantity of nonentrapped lornoxicam using a UV-spectrophotometer (UV-1800, Shimadzu, Japan) set at maximum wavelength (λ_{max}) of 376 nm. Then, lornoxicam was quantified using a standard curve in PBS with a pH of 7.4, and the findings were represented as mean ± SD ($n = 3$). The findings were compared to a blank control formulation. By subtracting the free drug (lornoxicam) left in the filtrate from the drug initially incorporated into the nanoemulsion formulation, the following equation was used to calculate the quantity of incorporated drug in the nanoemulsion formulation and thus the entrapment efficiency [18]:

$$\text{Entrapment efficiency} = \frac{\text{added drug} - \text{free drug}}{\text{added drug}} * 100. \quad (1)$$

2.4.6. Viscosity Determination. The nanoemulsion and nanoemulgel formulations of LRX-EU were subjected to Brookfield viscometer (dv2t, Ametek Brookfield, USA) at room temperature for the determination of consistency [15]. By employing spindle no. 63 at different rotations, 0.5 ml of the sample was used for viscosity determination at 25 ± 0.5°C. The experiment was repeated three times.

2.4.7. Spreadability Determination. The spreadability of optimized formulation of LRX-EU nanoemulgel was investigated by means of drag and slip assembly. The assembly is made up of a block of wood that is attached to the pulley. It also possesses two equal size glass slides, in which one is firmly attached to the wooden block while the other is moving. Between the two slides, sample is compressed, and a known weight is added onto it. About 2 g of optimized LRX-EU nanoemulgel was added on static slide and then compressed by movable slide. On the upper surface of movable slide, approximately 50 g weight was put. The time period in which upper slide move to 8 cm distance was calculated (M. K. [19]). The spreadability of nanoemulgel was determined by the following equation:

$$S = M * \frac{L}{T}, \quad (2)$$

where S represents the spreadability of nanoemulgel, M represents the weight introduced on movable glass slide, L represents the glass slides length, and T represents the time in which slides cover the distance.

2.4.8. Drug Content Determination. The nanoemulsion and nanoemulgel formulations of LRX-EU were subjected to UV-spectrophotometer (UV-1800, Shimadzu, Japan) for analysis of drug content. In this test, the optimized nanoemulsion formulation of LRX-EU was taken in the Eppendorf tube (Cat no 037108; SCILOGEX, USA) and centrifuged at 13,000 rpm for a time frame of 15 min (D3024, SCILOGEX, USA). It was followed by subsequent collection of supernatant. Dilution of supernatant (0.5 ml) was done with phosphate buffer having pH 7.4. The solution was subjected to constant stirring by means of magnetic stirrer at 1,000 rpm for 10 min time period. By employing UV-spectrophotometer, absorbance of solution was taken at λ_{max} 376 nm.

For the extraction of entrapped drug in LRX-EU nanoemulsion formulation, mixture of methanol (1 ml) and sediment was vortexed for 5 min. Phosphate buffer, pH 7.4, was used to dilute the combination. After that, it was subjected to stirring for 10 min. Using a UV-spectrophotometer, the absorbance was estimated at a λ_{max} 376 nm. To determine the drug content, both sediment and supernatant drug loads were used [20].

$$\text{Drug content} = \text{Drug in supernatant} + \text{drug in sediment}. \quad (3)$$

2.4.9. Drug Release Studies. For the determination of drug release profiles of LRX-EU nanoemulsion and nanoemulgel formulations, the Franz diffusion cell (PermeGear, Inc. No. 4G-01-00-15-12; India) equipped with Tuffryn membrane was employed. The small pore size (0.45 μm) of Tuffryn membrane plays the role of partitioning medium between the two compartments (donor and recipient) of the Franz diffusion cell. Approximately 7 ml of freshly prepared phosphate buffer, pH 5.5 (simulated skin fluid), was incorporated into receptor compartment, and the temperature was maintained at 32 ± 2°C. After predetermined time intervals, 1 ml

samples were taken from the receptor compartment. The same amount of phosphate buffer was substituted. UV-spectrophotometer (UV-1800, Shimadzu, Japan) was employed for the analysis of the samples. An average of three readings were taken, and the results were represented as mean ± SD. A plot of time versus concentration was obtained for the data. The responsible mechanism for drug release was modelled using a power law kinetic equation [12].

$$\frac{M_t}{M_\infty} = \mathrm{Ktn}, \tag{4}$$

where M_t and M_∞ represent the drug fraction release after time t, K represents the rate constant, n represents the value of exponential release.

When n value is 0.5, drug release occurs by Quasi Fickian diffusion mechanism; when $n > 0.5$, then drug release occurs by zero order or non-Fickian, or anomalous mechanism, and n value equal to 1 indicates zero order drug release.

2.4.10. In Vitro Drug Permeation Analysis of LRX-EU Nanoemulsion and Nanoemulgel Formulations

(1) Animal Skin Harvesting. From *in vivo* research facility of Gomal Centre of Pharmaceutical Sciences, healthy male rabbits approximately weighing 2-2.5 kg were obtained. All operations involving the use of experimental animals were carried out in compliance with the Gomal University's ethics and regulations, which were adapted from the worldwide standards (OECD Environment, Health and Safety). For the goal of acclimatization, they were fed a regular diet for seven days. All of the animals were killed with an intravenous injection of sodium pentobarbital ketamine and cervical dislocation. Shaving blades were used to shave the abdomen, and the skin was gently excised. 0.9% NaCl solution (normal saline) was used to cleanse the skin. A knife was used to remove the attached fats. The skin was wrapped in aluminium foil and kept in a deep freezer at -20°C until needed. Before usage, frozen skin was kept at room temperature for 3 hours.

(2) Skin Permeation Determination. For skin permeation tests, a Franz diffusion cell with a vertical diffusion cell (PermeGear, Inc. No: 4G-01-00-15-12; India) was used. Magnetic stirring and a heating circulation system with a programmable temperature control mechanism were included in the Franz diffusion cell assembly. The goal of the skin permeation investigation was to measure the drug transfer rate across the skin and forecast the quantity of localized drug in different skin layers. Skin hydration technique was utilized to precondition the rabbit skin before the experiment using PBS for 30 min. A magnetic stirrer was used to gently agitate the receptor fluid. The purpose of this stirring is to avoid boundary layer effects. 7 ml PBS, with pH 7.4, was added to the receptor compartment, which was then continuously stirred at 500 rpm. To keep the temperature at 37 ± 0.5°C, a heating circulation system was used. The rabbit skin was positioned between the donor and receptor compartments of the Franz diffusion cell with the epidermis facing the formulation and the dermis facing the receptor compartment. The upper surface of the mounted skin was allowed to dry and exposed for 1 hour. Both the receptor media and dermal side of the skin were in touch. The donor compartment was filled with about 1 ml formulation. At predefined time intervals, 1 ml of the samples was withdrawn from the sampling port. The receptor phase was immediately refilled with the same amount of freshly prepared solution and kept at the same temperature. Until the completion of the trial, the sink conditions were maintained. After samples dilution with PBS, pH 7.4, the obtained samples were analyzed for lornoxicam concentration using UV-spectrophotometer (UV-1800, Shimadzu, Japan). The data was presented in the form of mean ± SD. The quantity of lornoxicam penetrated through rabbit skin as a function of time was shown. The following equation was used to compute the total quantity of lornoxicam penetrated per unit area:

$$\mathrm{Qn} = \mathrm{CnVr} + \sum_{i=0}^{n-1} \frac{\mathrm{CiVs}}{A}, \tag{5}$$

where Qn represents the total quantity of drug penetrated per unit area relating to nth sample time, Cn represents the drug concentration in receptor fluid at nth sample, Ci represents the drug concentration in receptor fluid at ith sample $(n-1)$, A represents the diffusion cell effective permeation area (1.767 cm^2), Vr represents the volume of receptor solution (7 ml), and Vs represents the volume of the sample withdrawn (0.5 ml).

The steady state flux, represented by Jss, was shown as $\mu\mathrm{g/cm}^2\mathrm{/h}$. Permeability coefficient, represented by Kp, was calculated by dividing Jss by initial drug concentration in the donor compartment. By dividing flux of test formulation by standard control formulation, enhancement ratio was estimated.

$$\begin{aligned} \text{Enhancement ratio} &= \frac{\text{Jss of LRX} - \text{EU NE}}{\text{Jss of LRX NE}}, \\ \text{Enhancement ratio} &= \frac{\text{Jss of LRX} - \text{EU NEG}}{\text{Jss of LRX NEG}}. \end{aligned} \tag{6}$$

A comparison was made between the results obtained from permeation study analysis to that of lornoxicam nanoemulsion as well as nanoemulgel [21].

2.5. Stability Studies. The optimized nanoemulgel formulation of LRX-EU was subjected to accelerated stability testing. This was done by storing the formulation at three different temperatures (4°C, 25°C, and 45°C) [7]. The samples were examined for drug content, physical appearance, zeta potential, pH, PDI, and droplet size at regular intervals, i.e., 0, 30, 60, and 90 days. An average of three readings were taken.

2.6. Statistical Analysis. The variations between the two groups (control and treatment) in *in vivo* investigations were examined using a one-way ANOVA with Tukey's multiple comparison post hoc test. It was determined that a probability level of $P < 0.05$ was statistically significant. SPSS was used to conduct statistical analysis (version 20).

3. Results and Discussion

3.1. Lornoxicam Solubility Studies. When it comes to achieving optimum bioavailability, a drug's solubility is crucial. Low water solubility is the most common obstacle in the formulation of novel drug moieties. The majority of the drugs exhibit either acidic or basic nature, with negligible water solubility. Lornoxicam also displays a low solubility in water. Olive oil, almond oil, PEG 400, Tween 80, propylene glycol, ethanol, phosphate buffer saline (pH 5.8, 6.8, and 7.4) and water were used to conduct solubility tests for the chosen drug. According to Table 2, lornoxicam exhibited least solubility in water and excellent solubility in phosphate buffer saline with pH 7.4. These findings matched those of Hashmat et al. [7], who found that highest drug solubility was exhibited in phosphate buffer, pH 7.4. Despite the fact that several oils have better lornoxicam solubility, olive oil was selected as the oil phase for nanoemulsion formulation. The optimum drug solubility and influence on skin permeability are two of the reasons [22]. An extensive literature review aided in the selection of the appropriate oil phase for producing the transdermal formulation. The choice of a good surfactant is difficult and needs careful consideration. Tween 80 was chosen as the surfactant in the nanoemulsion formulation. The optimal drug solubility and function in skin permeability are two of the reasons. Another reason is that it is a nonionic surfactant, which is less hazardous and more miscible with the components in the formulation. The solubility of the drug was also examined in cosurfactants, with PEG 400 being chosen as the cosurfactant molecule. It has been utilized as a cosurfactant in emulsion formulations, with the added benefit of acting as a cosolvent. The selection of an appropriate surfactant is difficult and necessitates extensive testing. For the formulation of nanoemulsions, Tween 80 was employed as surfactant because of optimum drug solubility as well as skin penetration profile [23]. Another advantage is that it is a nonionic surfactant, which is less toxic and has greater miscibility with other formulation components. The solubility of the drug was also examined in cosurfactants, and PEG 400 was selected as cosurfactant. It has been utilized in emulsion formulations as a cosurfactant with the added advantage of cosolvency. It not only prevents the formation of stiff structures such as precipitates, liquid crystals, and gels, but it also increases emulsion area [24].

3.2. Nanoemulsion and Nanoemulgel Development. In this work, a high-speed homogenizer was utilized to make various formulations of lornoxicam nanoemulsion as well as eucalyptol nanoemulsion. Lornoxicam coloaded with eucalyptol (LRX-EU) nanoemulsion was fabricated from optimized nanoemulsion formulations of lornoxicam and eucalyptol by mixing equal volume of both nanoemulsion formulations, with Tween 80 acting as a surfactant. Tables 3 and 4 list the formulation components. Tween 80 is a nonionic surfactant, having HLB value 15 and exhibiting a lower CMC value than ionic surfactants. Surfactants reduce the tension between the aqueous and oily phases of emulsions. As a result, oil in water type of emulsions is formed very easily because of quicker and rapid oil dispersion in an aqueous medium. Tween 80 was chosen as an emulsifier because of a variety of reasons such as its nonionic nature, optimum HLB, capacity to reduce surface tension, steric ability of nanoemulsion formulations, and better permeation enhancer characteristics [25]. In vivo stability is also great for topical surfactants of nonionic nature. In this work, polyethylene glycol (PEG) was utilized as a cosurfactant to minimize interfacial stress and also provide flexibility to the interfacial layer. The droplets of nanoemulsion formulation undergo rapid and easy deformation due to the flexible nature of interfacial film. The responsible factor behind the deformation phenomenon is the decrease of polar groups between the surfactant molecules [26].

For additional research purpose, an optimized LRX-EU nanoemulsion was chosen on the basis of superior skin permeability characteristics. Many studies were conducted in order to find a suitable vehicle for applying LRX-EU nanoemulsion to the skin. Because of its nonirritant nature, great storage stability, high bioadhesive qualities, and capacity to produce translucent and pharmaceutically elegant gels, Carbopol (1% *w*/*w*) was chosen as a gelling agent [27]. Preliminary studies were carried out to identify the optimum concentration of Carbopol for required gel viscosity for topical application while avoiding the danger of the formulation being drained off (Table 1).

3.3. Nanoemulsion and Nanoemulgel In Vitro Characterization

3.3.1. Thermodynamic Stability. Nanoemulsions should be stable at varied temperatures and be able to preserve their spontaneous emulsification nature when diluted. As a result, thermodynamic stability investigations were carried out. Optimized nanoemulsion formulations of lornoxicam, eucalyptol, and LRX-EU were subjected to numerous thermodynamically stressed environments. Any differences in color, odor, or overall appearance were observed and compared to a blank nanoemulsion formulation. Nanoemulsion formulation passed the required thermodynamic test, such as centrifugation. There were no signs of turbidity, creaming, or phase separation (Table 5). The phenomenon of centrifugation results in creaming or sediment formation. Because oil is less thick than aqueous phase, O/W emulsions are suitable choices. This approach is an indirect parameter that depicts emulsion stability against the force of gravitation. The developed nanoemulsion formulation of LRX-EU passed the centrifugation test, indicating that the overall system was more homogeneous and stable. Tween 80, when used as a surfactant and emulsifying agent, often prevents oil globules from moving due to centrifugal forces. Aggregation, creaming, and phase separation are all impeded as a result [15].

Table 2: Lornoxicam solubility studies at 25°C ($n = 3$).

S. no.	Solvents	Solubility (mg/ml) Mean ± SD	Role
1.	Olive oil	0.385 ± 0.011	Oil phase
2.	Almond oil	0.032 ± 0.003	Oil phase
3.	PEG 400	1.354 ± 0.018	Cosolvent
4.	Tween 80	4.05 ± 0.047	Surfactant
5.	Propylene glycol	1.145 ± 0.021	Cosolvent
6.	Ethanol	0.073 ± 0.012	Cosurfactant
7.	Phosphate buffer saline (pH 5.8)	0.145 ± 0.008	Solvent/vehicle
8.	Phosphate buffer saline (pH 6.8)	0.298 ± 0.067	Solvent/vehicle
9.	Phosphate buffer saline (pH 7.4)	0.363 ± 0.003	Solvent/vehicle
10	Water	0.031 ± 0.001	Solvent/vehicle

Table 3: Composition of 1% *w*/*w* LRX O/W nanoemulsion formulation.

Formulation code	Lornoxicam	Olive oil	Tween 80	PEG 400	Water
LRX 1 (blank)	_____	7.5 g	7.5 g	5.5 g	q.s
LRX 2	1 g	7.5 g	7.5 g	5.5 g	q.s
LRX 3	1 g	6.5 g	6.25 g	6.25 g	q.s
LRX 4	1 g	5.5 g	7.75 g	7.75 g	q.s
LRX 5	1 g	7.5 g	8.50 g	7.50 g	q.s

LRX 2 = optimized nanoemulsion formulation.

Table 4: Composition of 1% *w*/*w* EU O/W nanoemulsion formulation.

Formulation code	Eucalyptol	Olive oil	Tween 80	PEG 400	Water
EU 1 (blank)	_____	7.5 g	7.5 g	5.5 g	q.s
EU 2	1 g	7.5 g	7.5 g	5.5 g	q.s
EU 3	1 g	6.5 g	6.25 g	6.25 g	q.s
EU 4	1 g	5.5 g	7.75 g	7.75 g	q.s
EU 5	1 g	7.5 g	8.50 g	7.50 g	q.s

EU 2 = optimized nanoemulsion formulation.

Table 5: Physical properties of blank and LRX-, EU-, and LRX-EU-loaded nanoemulsion at 4°C, 25°C, and 45°C.

Formulation codes	Temperature	Color	Odor change	Phase separation	Centrifugation stability	Thermodynamic test
Blank	4°C	White	No change	Nil	Stable	Passed
	25°C	White	No change	Nil	Stable	Passed
	45°C	White	No change	Nil	Stable	Passed
LRX NE	4°C	Pale yellow	No change	Nil	Stable	Passed
	25°C	Pale yellow	No change	Nil	Stable	Passed
	45°C	Pale yellow	No change	Nil	Stable	Passed
EU NE	4°C	White	No change	Nil	Stable	Passed
	25°C	White	No change	Nil	Stable	Passed
	45°C	White	No change	Nil	Stable	Passed
LRX-EU NE	4°C	Yellow	No change	Nil	Stable	Passed
	25°C	Yellow	No change	Nil	Stable	Passed
	45°C	Yellow	No change	Nil	Stable	Passed

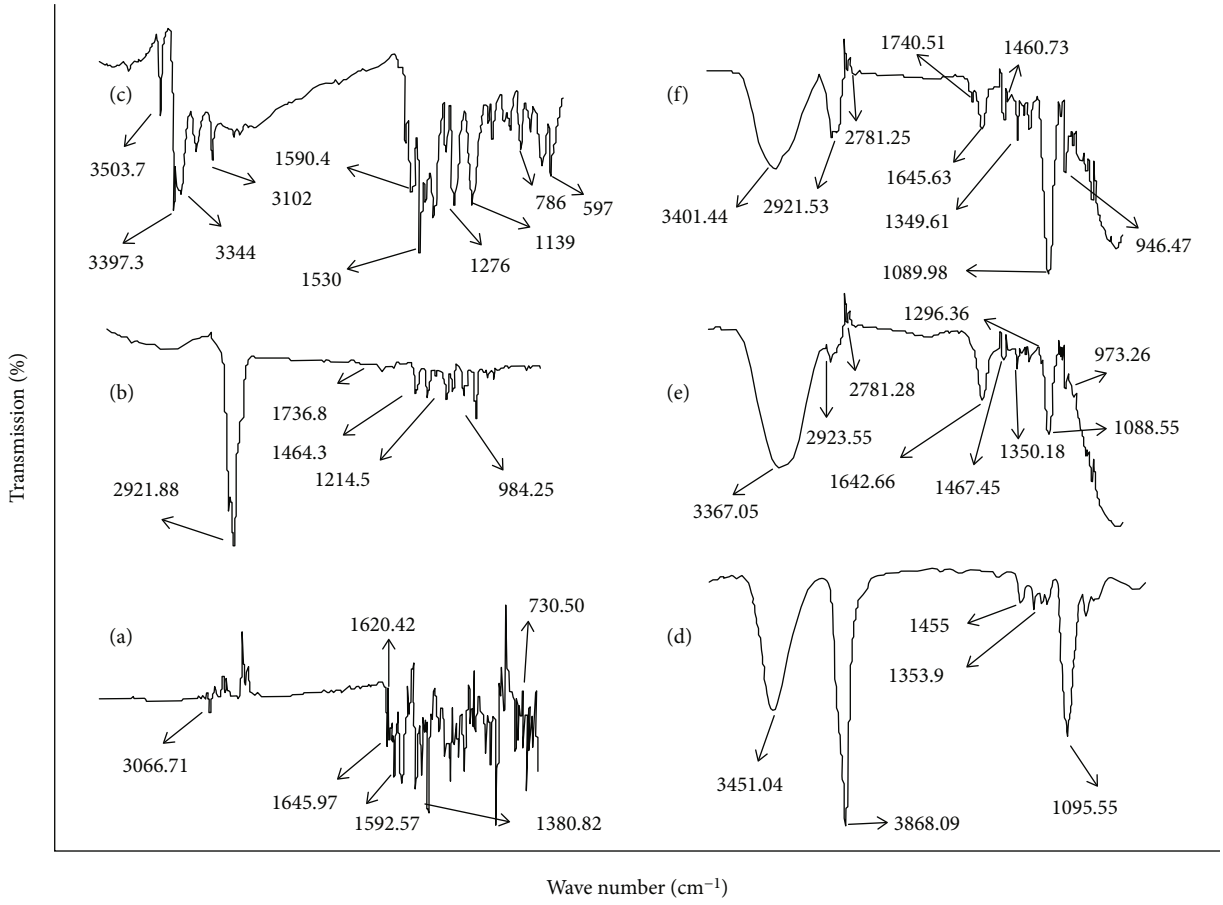

Figure 2: ATR-FTIR spectra of (a) lornoxicam, (b) eucalyptol, (c) Carbopol, (d) PEG 400, (e) LRX-EU NE, and (f) LRX-EU NEG.

3.3.2. ATR-FTIR Studies. The investigation of possible interactions taking place between lornoxicam, eucalyptol, and polymers in nanoemulsion and nanoemulgel formulations was carried out by means of ATR-FTIR spectrometer (Spectrum 100, Perkin Elmer, USA) using MIRacle ATR accessory (PIKE Technologies, Madison, USA). The ATR-FTIR spectra of lornoxicam, eucalyptol, and LRX-EU nanoemulsion and nanoemulgel formulations are presented in Figure 2. ATR-FTIR spectrum of lornoxicam exhibited characteristic band peaks at 1644 cm^{-1} (primary amide stretching vibration), 1618 cm^{-1} and 1590 cm^{-1} (N-H group bending vibration), 1143 cm^{-1}-1380 cm^{-1} (O=S=O stretching vibration), and 788 cm^{-1} (C-Cl stretching vibration) [28]. The presence of band peak at 3064 cm^{-1} (C-H aromatic ring stretching vibration) was also exhibited. Similarly, ATR-FTIR spectra of eucalyptol exhibited characteristic band peaks at 1464.3 cm^{-1} and 1214.5 cm^{-1} (C-C band) and 984.25 cm^{-1} (C-O-C stretching vibration), and the peak at 1736.8 cm^{-1} corresponded to carbonyl group [29]. ATR-FTIR spectra of LRX-EU nanoemulsion and nanoemulgel formulations displayed characteristic band peaks of drug without the presence of blue or red shifts, but with accelerated intensities, confirming the absence of any possible interaction of drug (lornoxicam) with polymers. However, the drug peaks with less intensities confirmed the encapsulation of lornoxicam in nanoemulsion and nanoemulgel formulations.

3.3.3. pH Determination. The pH of lornoxicam, eucalyptol, and LRX-EU nanoemulsion and nanoemulgel formulations was found to be within the acceptable range of transdermal application as described in Table 6. The physiological skin pH of 4.9–5.9 clearly demonstrates that any change in this pH range might promote bacterial development, especially Staphylococcus aureus. The development of inflammation, irritation, and enzymatic abnormalities is possible [30]. Since the pH values of all formulations were within the defined range scale of physiological skin pH, as a result, it is envisaged that the composition would be skin friendly and cause no irritation when applied topically. Furthermore, it is predicted that the drug will be present in a more unionized form inside the formulation, with a pH comparable to that of the skin, resulting in increased drug absorption via the skin. As a result, the pH of the developed optimized formulations of LRX-EU nanoemulsion and nanoemulgel is suitable for transdermal administration. Compatibility profile with biological tissues and efficacy are other features of the formulation [31].

3.3.4. Size and Size Distribution Analysis. The values of both particle size and size distribution of blank as well as lornoxicam, eucalyptol, and LRX-EU nanoemulsions and nanoemulgels are shown in Table 7. Droplet size has a critical role in case of topical or transdermally delivered

Table 6: Physicochemical parameters of LRX, EU, and LRX-EU nanoemulsion and nanoemulgel formulations.

Formulations	pH	Viscosities (cps)	Drug content (%)
LRX NE	5.88 ± 0.03	6378 ± 3.6	94.75 ± 1.37
EU NE	5.91 ± 0.06	6280 ± 3.4	92.43 ± 0.56
LRX-EU NE	5.96 ± 0.05	6837 ± 11.7	98.29 ± 1.37
LRX NEG	6.52 ± 0.04	11860 ± 9.9	91.96 ± 2.41
EU NEG	6.36 ± 0.01	10250 ± 10.3	93.16 ± 1.88
LRX-EU NEG	6.71 ± 0.03	12384 ± 11.9	96.63 ± 0.64

Table 7: Physicochemical characterization tests of different formulations.

Formulations	Particle size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)
Blank NE	51.64 ± 0.2	0.195 ± 0.3	−3.69 ± 2.78	—
LRX NE	61.28 ± 1.3	0.389 ± 0.2	−3.94 ± 3.66	76.11 ± 1.8
EU NE	63.57 ± 1.04	0.267 ± 0.3	−4.18 ± 3.10	80.23 ± 2.1
LRX-EU NE	62.21 ± 2.11	0.351 ± 0.1	−11.6 ± 2.83	81.65 ± 1.7
Blank NEG	103.76 ± 0.3	0.242 ± 0.01	−7.82 ± 1.93	—
LRX NEG	136.65 ± 1.7	0.430 ± 0.21	−11.85 ± 2.19	75.32 ± 1.8
EU NEG	155.45 ± 1.17	0.361 ± 0.11	−16.9 ± 1.52	79.56 ± 1.9
LRX-EU NEG	143.7 ± 2.18	0.374 ± 0.12	−19.95 ± 4.78	81.67 ± 2.3

formulations. This parameter has a drastic impact on numerous prominent parameters like drug release, permeation, and biodistribution. The particle size of a nanoemulsion determines the drug's absorption and bioavailability. Because small particle size offers a large surface area, drug release into the aqueous media is increased, resulting in increased drug absorption. It has been claimed that integrated drug particles interact with the system's microstructure, lowering particle size, especially if the drug is amphiphilic in nature [32]. The blank nanoemulsion exhibited lowest droplet size (51.64 ± 0.2 nm) as compared to drug-loaded nanoemulsion formulations. Same trend was exhibited by blank nanoemulgel (103.76 ± 0.3 nm) formulation when compared to respective drug-loaded nanoemulgel formulations. It has been explored by Razzaq et al. [33] that drug loading can influence the size of droplet in the formulation as revealed in case of drug-loaded nanoemulsion as well as nanoemulgel formulations in comparison to their blank counterparts. Our results are in agreement with the previously published work that reported increased droplet size upon addition of curcumin to the blank formulation [34]. Carbopol 940 addition as gelling agent significantly increased formulation particle size. Our results are in accordance with the findings of other authors who described that polymer incorporation into the nanoemulsion systems resulted in larger droplet size owing to increased formulation viscosity caused by higher degree of cross-linking [35].

3.3.5. Determination of Polydispersity Index and Zeta Potential. The values of both PDI and zeta potential of blank, lornoxicam, eucalyptol, and LRX-EU nanoemulsion and nanoemulgel formulations are shown in Table 7. Within the formulation, PDI provides globule size consistency. If PDI value of any formulation is <0.5, the formulation possesses homogenous and uniform size globules. Smaller size of globules is an important feature for topical administration of the formulation because it allows the drug to penetrate deeper into the skin. Our results of PDI values of both nanoemulsion as well as nanoemulgel formulations clearly reflect droplet size uniformity within each formulation [36]. The zeta potential values of blank, lornoxicam, eucalyptol, and LRX-EU nanoemulsion and nanoemulgel formulations are shown in Table 7, indicating that the system is stable physically. Electrostatic repulsion between the particles is represented by the zeta potential value. Zeta potential values of formulations larger than ±30 mV are deemed stable without aggregation phenomenon [37]. The electrostatic repulsive interactions between oily globules were validated by higher values of zeta potential, avoiding coalescence and the formation of a dispersion with homogenous globule size as well as greater stability was resulted. The free fatty acids provide the oil globules a negative charge, and the OH^- adsorption from the water onto the interface of oil-water gives the system an overall net negative charge [38].

3.3.6. Entrapment Efficiency. The average entrapment efficiency of lornoxicam, eucalyptol, LRX-EU nanoemulsion and nanoemulgel formulations is presented in Table 7. The amount of drug entrapped within a nanoformulation in contrast to the original drug concentration in the formulation is known as entrapment efficiency. The entrapment efficiency and system's homogeneity are based upon higher drug solubility in conjunction with the specified oily phase and also drug compatibility with other constituents. The insoluble

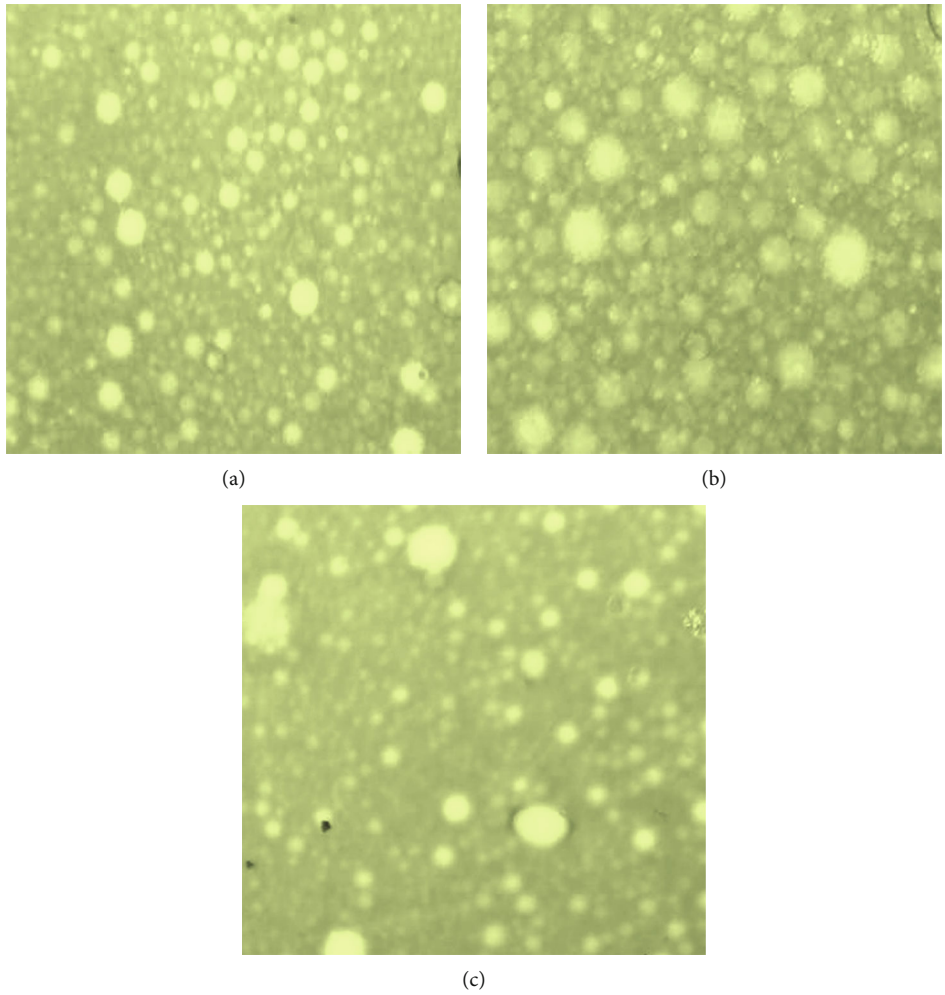

Figure 3: Microscopic images of (a) lornoxicam NE, (b) eucalyptol NE, and (c) LRX-EU NE.

nature of drug in water is responsible for its entrapment by oil globules, which can then be stabilized by means of employing surfactant and cosurfactant. The surfactant concentration, which has an inverse connection with encapsulation of drug, is also a highly important element that affects drug encapsulation. This might be because a higher surfactant concentration leads to smaller particle size, resulting in less entrapment of drug within nanoemulsion and nanoemulgel formulations [39]. Furthermore, drug partitioning resulted in increased solubilization of drug from the oily to the aqueous phase, resulting in a reduction in formulation viscosity, which improved the diffusion phenomenon during the process of self-assembly, demonstrating conclusive reason behind lesser entrapment efficiency of drug in the formulation.

3.3.7. Surface Morphology Analysis. The microscopic pictures presented in Figure 3 reveal the globules' homogeneity. The globules shape must be taken into account while formulating nanoemulsions since it has an impact on the formulation's performance [40]. A simple light microscope connected with a camera (5 MP) was used to investigate the surface morphology of the formulated nanoemulsion.

3.3.8. Viscosity Determination. The gels had viscosities ranging from 10250 ± 10.3 to 12384 ± 11.9 (Table 6). Viscosity signifies prominent role in topical or transdermal delivery of drugs. Literature revealed the direct impact of viscosity upon numerous formulation parameters such as spreadability, application ease, release data, and stability profile [41]. There is also a drastic impact of various gelling agents, emulsifiers, various oils, and cosurfactants present in the formulation upon viscosity. The spreadability of a formulation onto the skin is also determined by its viscosity. This is also necessary for proper skin penetration following its topical application [42]. Similar range of viscosity values has also been established by stable nanoemulgels [15], implying that produced nanoemulsion formulations possess the necessary viscosity for spreadability, stability, and transdermal absorption.

3.3.9. Spreadability Determination. Table 8 shows the average spreadability values for lornoxicam, eucalyptol, and LRX-EU nanoemulsion and nanoemulgel formulations. The spreadability of a topical formulation refers to how far it spreads across the skin. The therapeutic effectiveness of topical formulations is based on the spreadability of the

Table 8: Average spreadability values of nanoemulsion and nanoemulgel formulations at different temperatures (mean ± SD, $n = 3$).

Formulations	Spreadability (8°C)	Spreadability (25°C)	Spreadability (40°C)
LRX NE	15.93 ± 1.53	19.62 ± 1.11	24.56 ± 1.06
EU NE	16.10 ± 1.32	19.99 ± 1.17	25.35 ± 1.23
LRX-EU NE	16.59 ± 1.71	20.63 ± 1.12	26.11 ± 1.72
LRX NEG	11.71 ± 1.98	13.37 ± 1.09	14.84 ± 1.17
EU NEG	12.02 ± 1.31	13.93 ± 1.14	15.03 ± 1.07
LRX-EU NEG	12.46 ± 1.82	14.37 ± 1.39	16.09 ± 1.19

formulation [43]. For a formulation to expel from a container with optimal spreadability, a less amount of shear is necessary. The spreadability coefficient of a topical preparation is affected by a variety of factors, such as high and low temperatures. Generally, the viscosity of formulations increased at reduced temperatures. As a result, spreadability of formulation decreased. On the other hand, the viscosity of topical preparations reduced at high temperatures, resulting in greater spreadability [44]. When a comparison was made between the identical formulations stored at low temperatures, the formulations maintained at high temperatures demonstrated increased spreadability. Furthermore, as compared to nanoemulgel formulations, nanoemulsion formulations exhibited higher spreadability values, which can be attributed to the absence of the gelling agent and depicted statistically significant difference ($P < 0.05$).

3.3.10. Drug Content Determination. Lornoxicam, eucalyptol, and LRX-EU nanoemulsion and nanoemulgel formulations with Tween 80 used as surfactant and PEG 400 employed as a cosurfactant produced by means of high shear homogenization process exhibited homogenous drug distribution within the formulations. The optimized nanoemulsion and nanoemulgel formulations of LRX-EU showed drug content values of 98.29 ± 1.37% and 96.63 ± 0.64%, respectively (Table 6). The findings of drug content experiment confirmed that % drug content was within United States Pharmacopoeia official limit, i.e., 100 ± 10% (M. K. [19]).

3.3.11. Drug Release Studies. Abdur Rashid et al. [14] found that nanocarrier systems incorporate the medicinal product in either dissolved or dispersed state. As a result, drug solubility in lipoidal matrix is a critical element in limiting drug release from nanocarriers. To achieve the criteria, in vitro drug release experiments were used to study the medication's release pattern. A UV-spectrophotometer with a wavelength of 376 nm and 556 nm was utilized for this purpose to estimate drug release as well as drug contents of lornoxicam and eucalyptol, respectively. To analyze in vitro drug release profiles of different formulations, the Franz diffusion cell was employed. To simulate physiological state of the skin, in vitro drug release profiles were evaluated. This experiment made use of a receptor medium with phosphate buffer solution (pH 5.5) at 32 ± 0.5°C. LRX nanoemulsion, EU nanoemulsion, and LRX-EU nanoemulsion were studied in comparison, and the findings are presented in Figure 4. Similarly, lornoxicam nanoemulgel and eucalyptol, nanoemulgel, and LRX-EU nanoemulgel were studied in comparison, and the findings are displayed in Figure 5. All nanoemulsion formulations had a quick initial drug release phase in the first 2 hours, followed by a 22-hour period of delayed release. Surface drug entrapment might be the cause of the initial burst release phenomenon from nanoemulsion formulations. This is aided by cooling caused by the shift from high to room temperature during the formation of nanoemulsion [12]. In nanodermatology, the proven sustained and prolonged drug release behavior is of tremendous interest. This type of drug release pattern may be assumed as the cornerstone of intended topical application. At pH 5.5, around 78.93% of the drug in LRX-EU nanoemulsion is released after 24 hours. In the order of LRX-EU nanoemulsion > EU nanoemulsion > LRX nanoemulsion, the drug release profiles improved producing statistically significant difference between the drug release of LRX-EU nanoemulsion versus LRX nanoemulsion ($P < 0.05$). Similarly in case of nanoemulgels, the drug release pattern enhanced in the order of EU nanoemulgel > LRX nanoemulgel > LRX-EU nanoemulgel. There was statistically significant difference found between the drug release of LRX-EU nanoemulgel and LRX nanoemulgel formulations ($P < 0.05$). Interactions between drug and surfactant molecules, as well as drug

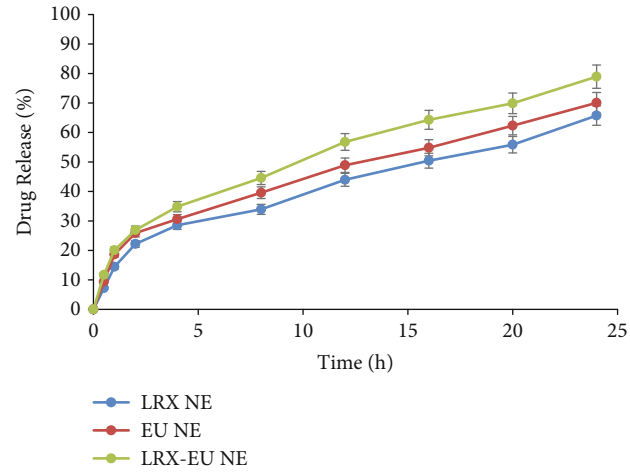

Figure 4: Percentage of drug release from optimized formulations LRX NE, EU NE, and LRX-EU NE. The data is presented as mean ± SD and analyzed using one-way ANOVA. $P < 0.03$ refers to LRX-EU NE which is statistically significant from LRX NE and EU NE.

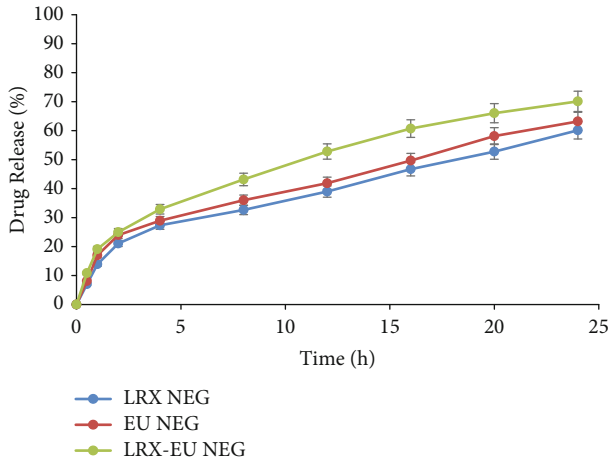

Figure 5: Percentage of drug release from optimized formulations LRX NEG, EU NEG, and LRX-EU NEG. The data is presented as mean ± SD and analyzed using one-way ANOVA. $P < 0.01$ refers to LRX-EU NEG which is statistically significant from LRX NEG and EU NEG.

partitioning between the oil and aqueous phases, are all regulating variables for drug release. The smaller globule size and larger surface area help to regulate optimal drug release from nanoemulsions. The nanosize of the formulation increases the rate of lornoxicam solubility into the aqueous phase. Dissolution rate and drug release are also improved. The release is done in a regulated manner. Slower drug release is advantageous in chronic painful and inflammatory disorders, but quick or burst release is not. Burst release is also responsible to cause toxicity since the medicine is released and is absorbed more quickly in inflamed regions [45]. In order to achieve gradual release of the active moiety as well as better drug retention parameters, the optimized nanoemulsion formulation was transformed into gel form. According to kinetic data analysis, the release mechanism and best fit R^2 value for all nanoemulsion and nanoemulgel formulations are presented in Table 9.

3.4. Ex Vivo Permeation Experiment. The ultimate goal of this study was to investigate the potential of codelivery of lornoxicam and eucalyptol for enhanced bioavailability to attain optimum therapeutic efficacy for the management of painful inflammatory disorders. The Franz diffusion cell was used in this study to assess codelivery of lornoxicam and eucalyptol to evaluate their *in vitro* skin penetration capability. The skin of a rabbit was chosen as the model barrier. When nanoemulsion formulations of lornoxicam, eucalyptol, and coloaded formulation as well as their respective nanoemulgel formulations were compared, and the findings demonstrated a statistically significant difference ($P < 0.05$). Table 10 summarizes the permeation experiment parameters, such as cumulative lornoxicam and eucalyptol penetrated after 24 hours, permeation flux, and enhancement ratio. It is easy to deduce from this that nanoemulsion formulations and nanoemulgel formulations of lornoxicam, eucalyptol, and combination of both play an important role in drug release and drug targeting to the skin. Diffusion of epidermal drugs and aligned carriers is strongly reliant on interactions between formulation and components of this layer, which include enzymes, transporters, and a plethora of membrane components [46]. The diffusion rate is also controlled by the formulation's physicochemical properties. Hydrogen bonding, surface charge, drug loading ability, and drug application manner are among them [47]. The choice of surfactant is a critical component that influences drug flux rate. Surfactants with longer carbon chains allow for more medication penetration into the skin [48]. Surfactants interact with intercellular keratin to aid in the solubilization of lipophilic substances inside the stratum corneum. Due to stratum corneum fluidization, channels are formed, allowing for higher drug permeability [12]. In the formulations, Tween 80 is used as a surfactant. Lipid packing fluidization occurs as a result of Tween 80 incorporation. The skin lipid extraction process also raised the water content of the stratum corneum. The solvent polyethylene glycol has an effect on the drug penetration from nanoemulsion and nanoemulgel formulations. These cumulative effects cause the epidermis' barrier functions to be reduced, resulting in increased transdermal drug transfer. LRX-EU nanoemulsion > EU nanoemulsion > LRX nanoemulsion was the sequence of skin permeation parameters (Figure 6). Similarly, ex vivo permeation parameters also increased in the order of LRX-EU nanoemulgel > EU nanoemulgel > LRX nanoemulgel (Figure 7). Due to their capacity to squeeze themselves through the skin's comparatively smaller pores, nanoemulsion and nanoemulgel formulations of LRX-EU have superior skin penetration characteristics. When compared to LRX-EU nanoemulsion and LRX-EU nanoemulgel, however, LRX and EU nanoemulsion formulations and nanoemulgel formulations have a lesser penetration capacity. This might be due to inherent less permeation capability of lornoxicam [7]. After 24 hours, an average of 73.19% of lornoxicam was permeated from the LRX-EU nanoemulsion with an average flux of $203.13 \pm 6.75\,\mu g/cm^2/h$, according to the permeation data. After 24 hours, the average quantity of drug penetrated from lornoxicam nanoemulsion was 56.16%, and the flux was $44.36 \pm 2.95\,\mu g/cm^2/h$. The average quantity of drug penetrated and flux of LRX-EU nanoemulgel and lornoxicam nanoemulgel were determined to be 67.19%, $189.63 \pm 7.68\,\mu g/cm^2/h$ and 50.69%, $41.25 \pm 3.13\,\mu g/cm^2/h$, respectively. The permeability of topical formulations is increased by systems based on nanoemulsion. The responsible mechanisms include adsorption onto the stratum corneum, vesicle fusion, oil or surfactant penetration across the skin, and targeted site [49]. Surfactant and oil-based nanoemulsions may readily permeate the stratum corneum's pores. Fusion or adsorption onto the stratum corneum occurs, followed by penetration across unbroken skin. The formulation's viscosity allows for homogeneous deposition on the skin and inhibits flocculation due to gravitational separation. Small droplet size improves penetration by increasing the overall surface area that is directly involved in the drug release, drug transfer, drug absorption, and droplets interaction on a given region of the stratum corneum. The introduction of nanoemulsion into the polar

Table 9: Kinetic profiling of drug release data from optimized nanoemulsion and nanoemulgel formulations.

Formulations	Power law kinetic model			
	$K \pm SD$	R^2	n	Release mechanism
LRX NE	0.291 ± 0.143	0.8087	0.436	Fickian diffusion
EU NE	0.011 ± 0.0019	0.9186	0.402	Fickian diffusion
LRX-EU NE	0.045 ± 0.021	0.9765	0.553	Anomalous non-Fickian diffusion
LRX NEG	0.186 ± 0.002	0.9615	0.513	Anomalous non-Fickian diffusion
EU NEG	0.023 ± 0.004	0.9137	0.537	Anomalous non-Fickian diffusion
LRX-EU NEG	0.051 ± 0.131	0.9867	0.568	Anomalous non-Fickian diffusion

Table 10: Permeation parameters of optimized nanoemulsion and nanoemulgel formulations (mean ± SD, $n = 3$).

Formulation codes	Permeation flux ($\mu g/cm^2/h$)	Permeability coefficient (cm/h)$*10^{-2}$	Enhancement ratio
LRX NE	44.36 ± 2.95	0.34 ± 0.014	1
LRX-EU NE	203.13 ± 6.75	2.21 ± 0.132	4.579
LRX NEG	41.25 ± 3.13	0.31 ± 0.018	0.929
LRX-EU NEG	189.63 ± 7.68	2.09 ± 0.067	4.274

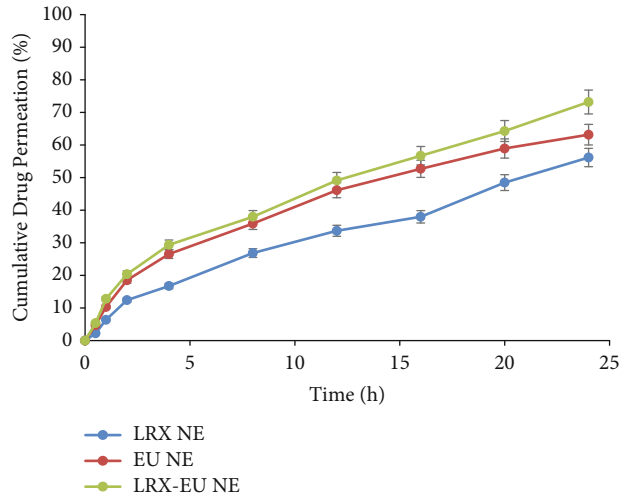

Figure 6: Percentage of drug permeated from optimized formulations LRX NE, EU NE, and LRX-EU NE. The data is presented as mean ± SD and analyzed using one-way ANOVA. $P < 0.05$ refers to LRX-EU NE which is statistically significant from LRX NE and EU NE.

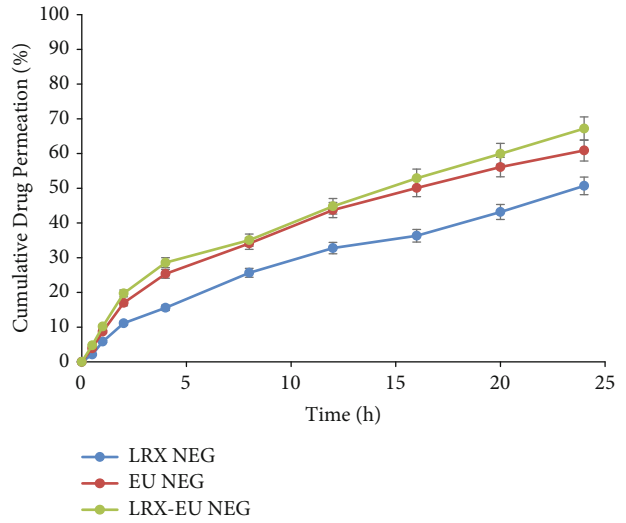

Figure 7: Percentage of drug permeated from optimized formulations LRX NEG, EU NEG, and LRX-EU NEG. The data is presented as mean ± SD and analyzed using one-way ANOVA. $P < 0.05$ refers to LRX-EU NEG which is statistically significant from LRX NEG and EU NEG.

route is predicted to increase the interlamellar volume of the stratum corneum's lipid bilayers. This causes the interfacial structure to break down, increasing drug penetration.

3.5. Stability Determination. For three months, the developed formulation was monitored for changes in drug content, droplet size, pH, PDI, and surface charge under the storage conditions described in Table 11. Stability experiments were performed to assess the stability of the formulations over time under different environmental variables such as temperature, light, and humidity. As a result, determining the proper storage conditions for LRX-EU nanoemulgel is an indirect method. There is no turbidity or phase separation after 3 months of storage at the stated conditions, indicating that the formulation is physically stable. The stored LRX-EU nanoemulgel and freshly manufactured formulations had similar surface charge and pH. Despite a nominal increase in globule size at accelerated and ambient conditions after 3 months, the globule size at selected specified conditions has not changed much, because globule kinetic energy is increased, resulting in collision and globule aggregation [45]. However, the globule size of the formulation

Table 11: Storage stability of LRX-EU NEG at various temperatures for 3 months.

Parameters	4°C	25°C	45°C
Zeta potential (mV)	−15.17 ± 3.87	−19.59 ± 3.93	−19.91 ± 4.41
Particle size (nm)	139.18 ± 3.54	145.88 ± 2.81	154.13 ± 4.76
PDI	0.253 ± 0.12	0.370 ± 0.04	0.461 ± 0.24
pH	6.01 ± 0.87	6.13 ± 0.04	5.97 ± 0.72
Drug content (%)	93.81 ± 2.13	93.92 ± 2.88	96.11 ± 1.83
Clarity	Clear	Clear	Clear
Color change	No change	No change	No change
Phase separation	Nil	Nil	Nil

and freshly developed LRX-EU NEG is almost identical (143.7 ± 2.18 nm), with a PDI of less than 0.3 at refrigerated temperatures over a three-month period, indicating overall formulation stability in line with the method utilized for its production. Because changes in particle size and other characteristics of the produced formulation and freshly synthesized formulation are fewer, these stability test findings represent the storage of LRX-EU NE and NEG at chilled settings [50].

4. Conclusion

From the findings of this study, it can be concluded that the optimized LRX-EU nanoemulgel presented droplet size in nanorange with size uniformity. The formulation was found thermodynamically as well as physically stable. The in vitro drug release and drug permeation were found optimum. The optimized LRX-EU nanoemulgel exhibited controlled *in vitro* release behavior which may lead to enhanced bioavailability of lornoxicam as compared to single-loaded lornoxicam nanoemulgel. The use of eucalyptol and PEG as penetration enhancers was responsible for improved drug flux value through the skin. LRX-EU nanoemulgel can be best substituted to the oral administration of lornoxicam because of producing enhanced therapeutic effect with optimum patient compliance and reduced GIT side effects as well as frequency of drug administration for better pain management.

Data Availability

Data can be available on demand or request from the CA.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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